



Diatom communities associated with gold mine tailings in the North West province, South Africa

T Brits

 **orcid.org 0000-0003-4449-3508**

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

Supervisor: Prof J Taylor

Co-supervisor: Prof H Bouwman

Co-supervisor: Mr DI Luyt

Graduation: July 2024

Acknowledgments

I want to thank Professors Jonathan Taylor and Henk Bouwman, and lab manager Ruan Koen for all the help and advice they gave me, Ian Luyt and Dr Velesia Lesch for helping with the fieldwork, and my uncle, Robert O'Connell for all the proofreads and advice he gave me.

Abstract

There is a continuous increase in environmental pollution through the release of toxic elements and acid mine drainage (AMD) into the environment by anthropogenic activities. This study aimed to determine the effect of gold mine tailings on diatom community composition and abundance in field-based studies and assess the physical and chemical condition of the waterbodies related to mining activities. Diatoms were used as bioindicators for this study due to their fast response to change in their environment, as well as their wide distribution and important role in the food web.

For this study, 19 sampling sites were selected in the Klerksdorp-Orkney-Stilfontein-Hartebeesfontein region, which included tailings storage facilities, drainage ditches, return water dams, stormwater outlets, the Africa Explosive Limited dam complex, and streams located upstream from the mining activities. Water quality variables such as phosphate, dissolved oxygen, pH, nitrate and electrical conductivity were measured on-site with portable meters. The cyanide concentrations of the sites were measured and provided by the mining company. Sediment samples were collected and analysed for elemental concentrations using inductively-coupled plasma mass-spectrometry (ICP-MS) after microwave digestion. The diatoms were sampled using standard sampling techniques and was identified and counted using differential interference contrast (DIC) light microscopy.

Both the phosphate and nitrate concentrations indicated hypertrophic water conditions which are usually productive but not diverse systems. Often, gold mine effluent has very low pH levels, but the study sites were all weakly acidic to alkaline environments. The results also showed high electrical conductivity at the mining sites. The tailings storage facilities showed adequate removal of cyanide, removing >90% of the total amount of cyanide released by the gold extraction process, although concentrations

found were still toxic to the biological systems in the water. The physico-chemical parameters in the water that may have had the greatest effect, individually and combined, on the biological systems in the water, included cyanide, dissolved oxygen (DO), NO_3^- and PO_4^{3-} . The elements in the sediments which had the greatest toxic potential on the overall biological systems in the water were As, Cr, Cu, Fe, Hg, Mn, Na, Pb, Ni, Se, Sr, and U and Zn, individually, in combination with each other, and with the physico-chemical parameters identified above.

Across all study sites 169 species in 49 genera were recorded, with 98 species tolerant to pollution and 71 species less tolerant. A total of 77% of the diatom species that were most commonly encountered were from the order Pennales, classified as Biraphidae. This is because this group is found generally in systems where there are steep physical and chemical gradients (extreme conditions). The results showed low diatom community diversity and abundances, with *Craticula buderii*, *Nitzschia palea*, and *Achnanthisidium* sp. as the most commonly occurring species. The alkaline conditions in combination with the high phosphate and nitrate concentrations, made it a more favourable environment for pollution tolerant diatoms to grow. These conditions may explain the relatively high diatom abundances in some seasons at several of the sites.

Deformities in diatom cells are morphological changes in the structure of the diatoms, including internal and external alterations, here, presumably caused mainly by high metal concentrations in the sediments but may also be due to other stressors. Most deformities were in the form of irregular striation patterns, abnormal valve outlines, and slightly bent valves. Although the high metal concentrations affected the morphology of the diatom cells, it also caused a change in the composition of diatom communities, with the more tolerant species found at impacted sites and low species diversity and richness. The elements that had the strongest association with deformities in cells were As, Cu, Hg, Ni, and Zn, although the cyanide concentrations might have had a slight effect on the diatom responses.

This study showed that diatoms are suitable bio-indicators for the observation of metal pollution at mining sites, although direct causal associations could not be identified for individual agents. The effects seen is therefore likely attributable to a combination of stressors and toxic chemicals unique to affected sites as sites differed greatly. Further research is necessary to investigate some aspects such as smaller diatom size

associated with toxic metal concentrations, and the occurrence of metal-sensitive diatoms such as *Cyclotella meneghiniana* and *Cyclotella ocellata* found at study sites with high metal concentrations. This study contributed various new questions and research opportunities regarding diatoms at mine-impacted areas.



Raphe canal system alterations in *Nitzschia palea* (twisted disorder, extended fibulae), found at AEL East (North), a normal cell is illustrated in the bottom left-hand corner (Photo by Talitha Brits).

Keywords

Gold mine tailings; Acid mine drainage (AMD); Diatoms; Diatom deformities; Toxic metals

Contents

Chapter 1.....	15
Introduction	15
Diatom community composition and abundance associated with gold mine tailings in the North West province, South Africa	15
1.1 Gold mining	16
1.1.1 A brief history of gold mining in SA.....	16
1.1.2 The influence of gold mining on SA's economy	16
1.1.3 The influence of AMD and gold mine tailings on the environment	17
1.1.4 The cyanide code and the influence of cyanide on the environment	22
1.2 Biomonitoring and bioindicators	25
1.2.1 Diatoms	25
1.2.2 Diatoms as bioindicators.....	26
1.2.3 Diatoms as primary producers.....	28
1.2.4 Influence of heavy metal concentrations and AMD on diatoms	29
1.3 Aims and objectives	32
1.3.1 Aims.....	32
1.3.2 Objectives.....	32
Chapter 2.....	33
2.1 Methods.....	33
2.1.1 Study area	33
2.1.1.1 Study area descriptions.....	35
2.1.2 Physico-chemical factors.....	41
2.1.3 Elemental analysis.....	42
2.1.4 Diatom sampling, preparation, and analysis	44
2.1.5 Statistical analysis	46
Chapter 3.....	47

3.1. Results	47
3.1.1. Physico-chemical parameters and elemental concentrations	47
3.1.1.1 Standard analysis.....	47
3.1.1.2 Multivariate analyses.....	66
3.1.2 Diatom community composition	71
3.1.3 Diatom deformations	86
Chapter 4.....	88
4.1. Discussion	88
4.1.1. Physico-chemical parameters in water	88
4.1.1.1 Electrical conductivity.....	88
4.1.1.2 pH.....	88
4.1.1.3 Nitrate.....	89
4.1.1.4 Temperature.....	90
4.1.1.5 Dissolved oxygen	90
4.1.1.6 Phosphate	91
4.1.1.7 Cyanide	92
4.1.2. Elemental parameters in sediments	94
4.1.2.1 Calcium	94
4.1.2.2 Iron	95
4.1.2.3 Manganese	95
4.1.2.4 Zinc	95
4.1.2.5 Molybdenum.....	96
4.1.2.6 Rubidium	96
4.1.2.7 Selenium	97
4.1.2.8 Sodium	97
4.1.2.9 Copper	98
4.1.2.10 Arsenic	99

4.1.2.11 Aluminium	100
4.1.2.12 Cadmium.....	101
4.1.2.13 Lead	102
4.1.2.14 Chromium.....	102
4.1.2.15 Mercury	103
4.1.2.16 Vanadium	104
4.1.2.17 Beryllium	105
4.1.2.18 Cobalt.....	105
4.1.2.19 Nickel	106
4.1.2.20 Antimony	107
4.1.2.21 Uranium.....	107
4.1.2.22 Barium.....	108
4.1.2.23 Platinum	108
4.1.2.24 Strontium.....	109
4.1.2.25 Summary of the elements	110
4.1.2.26 Other observations.....	110
4.1.3. Diatom community composition.....	111
4.1.4. Diatom deformations	115
Chapter 5.....	119
5.1 Conclusions and recommendations.....	119
Chapter 6.....	122
6.1 References	122
Appendix A	138
Diatom species present at sites from June 2021 to May 2022	138
Appendix B	186
Diatom species present at sites in winter (2 - 4 August 2022) and summer (25 – 26 October 2022)	186

Appendix C	202
Species list of the tolerant and less tolerant species found at the sites.....	202

Figures

Figure 1: A model of a tailings dam and the affected subsurface (Redrawn by Talitha Brits after Rösner and Van Schalkwyk, 2000).	20
Figure 2: Map of the study area and study sites (Google Earth). The study sites are located in the North West Province, South Africa.	33
Figure 3: The Klerksdorp-Orkney-Stilfontein-Hartebeesfontein region's land use and land cover distribution based on the national land cover dataset 2013-2014 (Dudumashe and Thomas, 2020).	34
Figure 4: Graphs A to S indicate the comparison between the EC concentrations, pH values, and temperature in water between the different sites over a period of one year.	53
Figure 5: A comparison between the free, Weak Acid Dissociable (WAD), and total cyanide concentrations, between the different sites, over a period of one year.	59
Figure 6: A comparison between the mean cyanide values of each site of one year.	61
Figure 7: A PCA biplot of the similarities and dissimilarities between sites. The correlation biplot describes 75% of all variation, with 42% on the first axis and 33% on the second axis (Recoverable cyanide = Total cyanide)(Sampling dates: Jun-21, Aug-21, Oct-21, Dec-21, Feb-22, and Apr-22).	62
Figure 8: The column graphs (A-F) indicate the difference between pH, EC, NO_3^- , DO, and PO_4^{3-} concentrations and the temperature at the various sites between winter and summer. The bars indicate standard deviation.	65
Figure 9: The PCA biplot showing the ordination of sites and water quality variables between the sites in the winter, the plots accounts for 66% of all measured variation, with 42% on the first axis and 24% on the second axis.	66
Figure 10: The PCA biplot of the differences between sites in the summer, showing 62% of all variation, with 39% on the first axis and 23% on the second axis.	68
Figure 11: PCA showing the ordination of diatom taxa (presence or absence of diatom species or genus) among the sites in the winter. The two axes show 38% of all the variation, with 20% on the first axis and 8% and 18% on the second axis.	73
Figure 12: A PCA showing the difference in species diversity of the various sites in the summer, is showing 46% of all variation, with 28% on the first axis and 18% on the second axis.	75

Figure 13: PCA illustrating diatom community composition and mean water quality variables for winter and summer during the study period. The plot accounts for 77% of all variation in diatom communities, with 58% explanation on the first axis and 18% on the second axis.	77
Figure 14: PCA plot illustrating the diatom community composition and water quality variables form an entire year (May– 2021 to May- 2022). The two axes accounti for 75% of all variation, with 42% on the first axis and 33% on the second axis (Recoverable cyanide = Total cyanide).	79
Figure 15: A PCA illustrating the diatom community composition and elemental concentrations in the sediments of the different sites for an entire year (Jun- 2021 to May- 2022). The axes account for 55% of all variation, with 34% on the first axis and 21% on the second axis.	82

Tables

Table 1: A description of each sampling site.	35
Table 2: Elements measured for each sample.	43
Table 3: Cyanide concentrations in water of the three reference sites.	60
Table 4: Elemental concentrations (mg/kg DM) in the sediments at the different sampling sites (green indicates the minimum value and grey the maximum value for each element). (The number of minimum or maximum values found of an element per site is indicated in the last two rows). Locations and descriptions of the sites are provided in Figure 2 and Table 2 (Dates of sampling: June 2021, September 2021, January 2022, and May 2022).....	69
Table 5: Classification of the diatoms found at the sites.	71
Table 6: Diatom abundance range of each site for each season.	84
Table 7: The mean diatom abundance range of one year for each site.	85
Table 8: Types of deformities that occurred at the various sites during the study (All photos in table by Talitha Brits).	87

List of abbreviations

AMD	Acid Mine Drainage
AEL	Africa Explosive Limited
CFA	Continuous Flow Analysis
DD	Drainage Ditch
DIC	Differential Interference Contrast
DO	Dissolved Oxygen
EC	Electrical Conductivity
ICP-MS	Inductively-Coupled plasma Mass-Spectrometer
KOSH	Klerksdorp-Orkney-Stilfontein-Hartebeesfontein
PAH	Polycyclic Aromatic Hydrocarbons
PCA	Principal Component Analysis
PHEs	Potential Harmful Elements
PUFA	Polyunsaturated Fatty Acid
RWD	Return Water Dam
SA	South Africa
SAD cyanide	Strong Acid Dissociable Cyanide
TSF	Tailings Storage Facility
TSS	Total Dissolved Solids
USB	Universal Serial Bus
VR	Vaal River
WAD cyanide	Weak Acid Dissociable Cyanide

Glossary

Acidification	This is the means by which acids are included into a water body or soil, resulting in a pH lower than 7. This is a type of water pollution, which can damage aquatic ecosystems below a pH of 6.5, and cause mortality of aquatic species below a pH of 5.5 (Park, 2017a).
Acid Mine Drainage (AMD)	This is wastewater resulting from coal as well as other mineral mining, which normally consists of very high acidity, sometimes even lower than a pH of 2. Due to its contact with sulphide minerals, it causes sulphuric acid (H ₂ SO ₄) to form, which is considered very corrosive and dissolves toxic metals such as Lead, Zinc, Copper, Arsenic, Selenium, Mercury, and Cadmium into groundwater, surface water, and soils (Park, 2017b).
Amalgamation	The fusion of a metal or alloy and mercury to give rise to an amalgam. Usually, the Mercury and alloy are suppressed in an amalgam capsule (Ireland and Yeung, 2020).
Araphid	These species contain a pseudoraphe. They have no raphe but may contain a rimoportula at the apex of the valve (Taylor <i>et al.</i> , 2007b).
Areolae	These are chamber-forming perforations, which are circular to angular, occurring in the cross section, in the cell wall of the diatom valve. They are enclosed by a velum on either the inside or the outside (Taylor <i>et al.</i> , 2007b).
Benthic	Describes organisms occurring at the base of water bodies. Benthic organisms are attached to a substrate or sediment, which limits their movement in proportion to the substrate they are attached to (Mayhew, 2023).
Biomonitoring	This is an environmental evaluation method that uses organisms that are likely to indicate any chemical or physical changes within their environment (Morin <i>et al.</i> , 2008; Kock <i>et al.</i> , 2019).
Biraphid	These species contain a raphe on both sides of the valve. They are defined by their thickened silica ribs surrounding the raphe (Taylor <i>et al.</i> , 2007b).

Costae	This is longitudinal thickenings of the diatoms valve (Taylor <i>et al.</i> , 2007b).
Diatom	This is a protist, belonging to the class Bacillariophyceae, of which the cell wall (frustule) is constructed from silica and contains two halves, where one overlaps the other. The frustules are normally ornamented. Most diatoms are unicellular, but a few are colonial or filamentous. Although most diatoms are photosynthetic, but some lacks chlorophyll, and lives thermophilically in the company of decaying marine algae (Allaby, 2015).
Electrical Conductivity	The manner of how an electrical current will pass through a material, measured in siemens or reciprocal ohms per metre (Allaby, 2020a).
Eukaryotic	This is an organism that consists of a cell nucleus, as well as a cell membrane. This includes all living organisms, except for bacteria and viruses (Eukaryotic, 2020).
Eutrophic	This describes a waterbody that is nutrient rich, with a high formation rate of organic matter due to elevated photosynthesis levels (Eutrophic, 2019a).
Eutrophication	This is the process whereby a water body is enriched by nutrients, normally nitrates and phosphates. This process occurs to such an extent that the productivity of the system fails to be limited by nutrient availability. Normally an increase in photosynthetic activity will occur, following a drastic decrease in dissolved oxygen, as the plants die and are decomposed by the aerobic organisms (Allaby, 2020b).
Fibulae	This is support in the shape of a silica strut, guiding the raphe-bearing keel on the inside of the valve in numerous species containing a canal raphe. These structures can end up in one or more transapical stria, which could be solid, tubular or filamented (Taylor <i>et al.</i> , 2007b).
Frustule	The complete siliceous cell wall of a diatom, comprising of an epi- and hypovalve (Taylor <i>et al.</i> , 2007b).
Hypertrophic	When a waterbody contains an excessive amount of nutrients and constantly displays symptomatic

	changes such as excessive cyanobacterial, algal or plant growth (Park and Allaby, 2017c).
Monoraphid	These taxa only contain a raphe on one side of the valve. They normally consist of a curved or flexed valve face (Taylor <i>et al.</i> , 2007b).
Oligoelement	This are trace elements required in small amounts by plants and animals for growth and development (Oligoelement, 2014).
Oligotrophic	When a waterbody contains deficient nutrient concentrations, as well as a very low formation rates of organic matter, due to a decrease in photosynthesis (Oligotrophic, 2019b).
Periphytic	Aquatic organisms attached to a substrate or plant above the bottom sediments (Periphytic, 2004).
Phototrophic organisms	This includes any organism whose main source of energy is sunlight. Most of these organisms use light energy to synthesize their essential organic nutrients from inorganic precursors. For example, plants and algae (Phototrophic organisms, 2006).
Phytoremediation	This remediation technique uses plants to extract contaminants from soils, sediments, and groundwater (Park and Allaby, 2017d).
Phytotoxic	Damaging or toxic to plants (Park and Allaby, 2017e).
Planktonic	Describing algae or other organisms that drift freely in the water column (Bellinger and Sigee, 2015).
Raphe	This is a slit-shaped opening in the surface of the valve, whose main purpose is movement of the cell. Most valves having raphes, consist of two symmetrical raphe branches (Taylor <i>et al.</i> , 2007b).
Saturated zone	This is the part of a soil or aquifer in which all the pores are filled with water. It is also known as the phreatic zone or zone of saturation (Park and Allaby, 2017f).
Septae	The septum is flat to undulating, and is not attached to the valve itself, but to the copulae (Taylor <i>et al.</i> , 2007b).

Stria	This is rows of puncta or areolae, normally positioned along the transapical axis, which is divided by unornamented ribs (Taylor <i>et al.</i> , 2007b).
Unicellular	Describes tissues, organs, or organisms containing a single cell. For example, the reproductive organs of certain fungi and algae are unicellular (Unicellular, 2019c).
Wetland	This is a piece of land that is seasonally or annually covered with water and can thus shift between terrestrial and aquatic ecosystems. These environments contain aquatic vegetation adapted to waterlogged soil conditions, such as in swamps, marshes and estuaries (Park and Allaby, 2017g).

Chapter 1

Introduction

Diatom community composition and abundance associated with gold mine tailings in the North West province, South Africa

South Africa (SA) is one of the largest gold-producing countries in the world (Ali, 2006; Rösner and Van Schalkwyk, 2000, Belle *et al.*, 2021), which contributes significantly to SA's economy (Belle *et al.*, 2021). It has brought widespread employment and wealth (Belle *et al.*, 2021) for more than a century, but also caused excessive pollution of nearby soils and watercourses, mainly due to past poor management of TSFs (Rösner and Van Schalkwyk, 2000).

Two major influences of gold mining include the production of acid mine drainage (AMD) and the release of toxic metals into the natural environment (Belle *et al.*, 2021; Lusilao-Makiese *et al.*, 2014; Rösner and Van Schalkwyk, 2000; Aucamp and Van Schalkwyk, 2003; Pandey and Bergey, 2016; Simate and Ndlovu, 2014), which affects the physical and chemical characteristics of the influenced areas (Rösner and Van Schalkwyk, 2000). This can cause human and animal health problems (Simate and Ndlovu, 2014), impede the growth and establishment of higher plants (Simate and Ndlovu, 2014; Orlekowsky *et al.*, 2013), and cause either mortality or morphological changes in aquatic organisms (Simate and Ndlovu, 2014).

Algae divisions associated with AMD include Bacillariophyta (Diatoms), Chlorophyta, and Euglenophyta (Orlekowsky *et al.*, 2013). Diatoms are unicellular, phototrophic organisms (Taylor *et al.*, 2007a; Round *et al.*, 1990; Molino and Wetherbee, 2008; Dixit *et al.*, 1992; Wang *et al.*, 2013; Mishra *et al.*, 2017), that are globally used as bio-indicators, due to their quick response to physical and chemical changes within their environment (Sivarajah *et al.*, 2019). They are also essential primary producers in many aquatic environments (Morin *et al.*, 2008; da Silva *et al.*, 2009). Diatoms can attach to substrates, therefore incorporating the actual habitat conditions, allowing them to react more rapidly to environmental changes than organisms higher in the food web, due to their short life cycle (da Silva *et al.*, 2009). Their siliceous cell valves

can be preserved in the sediments. This makes them exemplary paleolimnological indicators to determine a wide range of environmental stressors, including acidification, heavy metal contamination, and eutrophication (Sivarajah *et al.*, 2019; Dixit *et al.*, 1992; Taylor *et al.*, 2005).

Alterations within the diatom communities, including community composition and individual morphological changes (deformities), can be good indicators of metal pollution. The diatom community composition in mining areas is often associated with a combination of low pH and highly dispersible toxic metals (Olenici *et al.*, 2017).

1.1 Gold mining

1.1.1 A brief history of gold mining in SA

The gold mining industry in South Africa (SA) is more than a century old and is considered one of the world's largest gold producers (Rösner and Van Schalkwyk; 2000; Belle *et al.*, 2021; Ali, 2006).

In 1886, the first mining company in the Witwatersrand basin was registered as the Witwatersrand Gold Mining Company. By the end of 1887, there were 68. As larger mining companies started to incorporate smaller ones in the 1900s, new mining strategies were developed, and the basic pick and shovel operations were replaced by mechanised processes. The gold mines were initially opencast and shallow pit mining. However, the first shaft mining was implemented in 1887 to extend extraction to the deeper gold-rich deposits. The nature of this deeper ore changed, and the original extraction process of amalgamation using mercury became less effective. Therefore, gave rise to the MacArthur-Forrest cyanidation process in 1890 where gold is extracted from crushed ore through the solution by cyanide, which remains the main gold extraction method of today (Durand, 2012).

1.1.2 The influence of gold mining on SA's economy

Over the past 100 years, the mining industry has been the foundation of South Africa's economy and its industrial revolution. This fast-growing gold mining industry allowed SA to become one of the most industrialised countries in the world (Belle *et al.*, 2021).

From the late nineteenth century up to and till the 1980s, SA dominated international gold production and sales. In the 1960s, SA already produced two-thirds of the world's gold (Nel and Binns, 2002). The gold-bearing Witwatersrand basin forms one of the most pronounced metallogenic regions of the world. The sediments within this basin are known as the Witwatersrand Supergroup and consist of the West Rand Group and the Central Rand Group. The West Rand goldfields fall within the semi-circular band of the Transvaal Supergroup, which arises south of Johannesburg and extends past Carletonville to Orkney, nearby Klerksdorp, to the west. The Vaal River (VR) mining region includes the following towns: Klerksdorp, Orkney, Kana, Stilfontein, Khuma, Hartebeesfontein, and Tigane. Klerksdorp is considered one of the core gold mining centres in SA, although its' value has been decreasing over the past years (Lusilao-Makiese *et al.*, 2014).

The gold mining industry contributes many benefits for SA, such as widespread employment, wealth, as well as a contribution to the development of several sectors in SA's economy. These sectors comprise infrastructure development, and the establishment of manufacturing and service industries (Belle *et al.*, 2021).

Although gold mining brought great development to SA's economy, it also caused excessive contamination of soils and nearby water sources, due to the poor management of nearly all TSFs (Rösner and Van Schalkwyk, 2000).

1.1.3 The influence of AMD and gold mine tailings on the environment

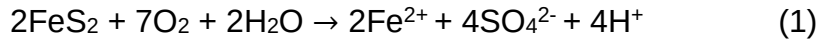
In 1996 alone, 377 million tonnes of gold mine waste were produced, which accounted for 81% of the total mining waste produced in South Africa (SA) (Rösner and Van Schalkwyk, 2000). The gold-bearing rock (ore) is crushed to enable the removal of gold through a wet metallurgical process. The excess that remains in the form of a slurry is then pumped onto a tailings dam, where the water is left to drain or evaporate (Aucamp and Van Schalkwyk, 2003). The Witwatersrand basin is the largest gold and uranium-producing basin in the world, covering 1600 km² (Kamunda *et al.*, 2016).

Currently, this basin contains approximately 270 TSFs and 380 mine excess dumps, which consist of 6 billion tonnes of pyrite tailings and 600 000 tonnes of poor-quality uranium (Kamunda *et al.*, 2016). It is estimated that these TSFs cover a total area of

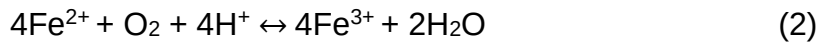
180 km² (Rösner and Van Schalkwyk, 2000). These gold mine tailings may contain a variety of contaminants, which can either derive from the gold ore itself or be added during the chemical separation process of the gold from the ore (Belle *et al.*, 2021). The contaminants derived from the ore itself include sulphide minerals such as pyrite, which is also an acid-producing rock, as well as chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) nickel (Ni), and zinc (Zn) (Belle *et al.*, 2021; Lusilao-Makiese *et al.*, 2014; Rösner and Van Schalkwyk, 2000; Aucamp and Van Schalkwyk, 2003; Pandey and Bergey, 2016). The potential harmful elements (PHEs) added during the chemical process include arsenic (As), cadmium (Cd), cyanide (CN), lead (Pb), and mercury (Hg) (Lusilao-Makiese *et al.*, 2014; Belle *et al.*, 2021). The previously mentioned contaminants can remobilise under acidic conditions (Rösner and Van Schalkwyk, 2000; Petersen *et al.*, 1997) and elevated calcium (Ca) concentrations (alkaline conditions) (Petersen *et al.*, 1997), causing them to move into the underlying soil saturated zone and groundwater system (Rösner and Van Schalkwyk, 2000, Petersen *et al.*, 1997).

As most goldmines are located near highly urbanised areas or important agricultural land (Aucamp and Van Schalkwyk, 2003; Rösner and Van Schalkwyk, 2000), these contaminants and mining activities can cause serious environmental pollution and health problems to humans and biota living near or close to these facilities (Belle *et al.*, 2021; Durand, 2012). The severity of these impacts may depend on a few factors, including the mining and extraction methods, type of metal contamination, and the efficiency of pollution management and restoration (Luís *et al.*, 2011). The condition of water bodies such as wetlands, and river- and groundwater systems have degraded significantly due to the mines in the Gauteng and the North West provinces (Durand, 2012). These water bodies are polluted by mine tailings through run-off, water seepage, and wind erosion (Durand, 2012; Belle *et al.*, 2021), which affects the acidity, salinity, turbidity, toxicity, and radioactive contamination (Durand, 2012). The oxidation of the sulphide minerals, such as pyrite, can lead to the formation of acid mine drainage (AMD) (Rösner and Van Schalkwyk, 2000; Simate and Ndlovu, 2014; Fuelling, 2013). Although this process occurs naturally in the environment, the increased exposure to water and oxygen (O₂) of the exposed tailings and waste accelerates the AMD formation. Therefore, both operational and non-operational mines can cause environmental problems, but the problems caused by non-

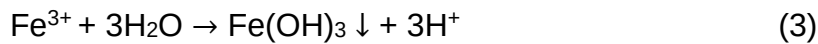
operational mines are more severe (Simate and Ndlovu, 2014). The primary reaction is the oxidation of pyrite into dissolved Fe, sulphate (SO_4^{2-}), and hydrogen (H^+) (Eq. 1). The pace of this reaction is determined by the solid-phase compositional parameters, microbial activity, and the availability of O_2 and water (H_2O).



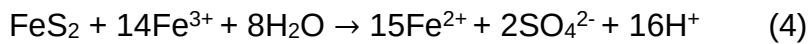
The appearance of SO_4^{2-} in AMD is the initial indicator of sulphide mineral oxidation. Within an environment with an adequate oxidation rate, ferrous iron will be oxidised to ferric iron (Eq. 2).



With inadequate oxygen levels, this reaction will not take place, until a pH of 8.5 is reached. If the pH levels are between 2.3 and 3.5, it can cause the ferric iron to precipitate as iron hydroxide ($\text{Fe}(\text{OH})_3$), which causes a decrease in the pH levels (Eq. 3).



If the pH levels are lower than 2, the hydrolysis process will not occur, and the Fe^{3+} will not precipitate into $\text{Fe}(\text{OH})_3$, but rather be used to oxidise additional pyrite (Eq. 4).



However, this oxidation of pyrite by ferric iron can cause a further decrease in the pH levels (Simate and Ndlovu, 2014).

This AMD dissolves the PHEs to the ground or surface water bodies and soils within the area near the mining activities (Rösner and Van Schalkwyk, 2000; Belle *et al.*, 2021), thereby increasing the acidity, salt concentrations, and toxic metals in the soils and groundwater (Kamunda *et al.*, 2016). Particulate matter from the tailings can also be transported to various nearby soils, water bodies, and vegetation, via wind (Belle *et al.*, 2021). Some of these phytotoxic pollutants such as Cu, Ni, and Zn can have a major effect on future rehabilitation measures since they restrain soil function, and normally exceeding the threshold concentrations of soils. Therefore, they are known to be some of the main sources of soil contamination in mining areas (Rösner and Van Schalkwyk, 2000).

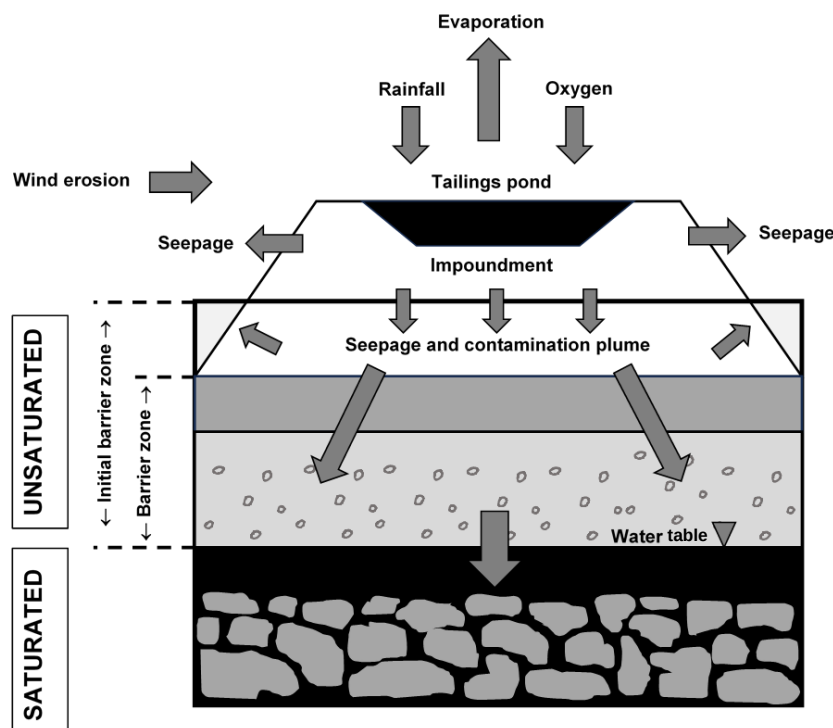


Figure 1: A model of a tailings dam and the affected subsurface (Redrawn by Talitha Brits after Rösner and Van Schalkwyk, 2000).

TSFs consist of whitish or yellowish precipitation on their surfaces due to upward capillary movement of salt-rich liquids. During dry seasons, these precipitates form due to evaporation and are usually soluble and contain high concentrations of metallic elements (Aucamp and Van Schalkwyk, 2003; Simate and Ndlovu, 2014). Thus, these tailings storage facilities are characterised by severe geotechnical and geochemical conditions (Orlekowsky *et al.*, 2013).

Mine tailings can cause chemical (low pH, dissolved metals) and physical (accumulation of metal oxides) stress to associated ecology (Olenici *et al.*, 2017), thus influencing both aquatic and non-aquatic life (Simate and Ndlovu, 2014). Regarding humans and animals, there are two major concerns with toxic metal pollution. Since metals do not break down and may remain concentrated within an environment for a relatively long time, they can accumulate in the food web causing chronic and acute health problems (Simate and Ndlovu, 2014).

Contamination of toxic metals can impede the growth and establishment of higher plants in numerous ways (Simate and Ndlovu, 2014; Orlekowsky *et al.*, 2013). Plants can undergo oxidative stress, which can cause cellular damage and disturbance of cellular ionic homeostasis, therefore agitating the physiological and morphological characteristics of plants (Simate and Ndlovu, 2014).

Aquatic organisms can also undergo oxidative stress and are especially sensitive to Cd, Cu, Pb, and Zn (Simate and Ndlovu, 2014; Marella *et al.*, 2020). Exposure to these metals can either cause mortality or non-fatal effects such as growth reduction, lower production rates, and tetralogies (Simate and Ndlovu, 2014).

Algae divisions associated with AMD, includes Chlorophyta (green algae), Bacillariophyta (diatoms), and Euglenophyta (euglenoids) (Pandey and Bergey, 2016). Green algae thrive in acidic to slightly alkaline post-mining areas, whereas the diatoms prefer a more neutral to alkaline environment and habitats containing high salt and calcium (Ca) concentrations (Orlekowsky *et al.*, 2013). Newly-deposited gold mine tailings are considered to be non-acidic (Orlekowsky *et al.*, 2013) due to high Ca concentrations, because of liming before being added to the TSF (Rösner and Van Schalkwyk, 2000). However, the oxidation of pyrite minerals causes the pH to drop, which then increases the solubility of elements such as aluminium (Al), manganese (Mn), and iron (Fe), which decreases the availability of essential nutrients, such as phosphorus and potassium for plant-life (Orlekowsky *et al.*, 2013). Under these conditions, the aluminium can impede root elongation in plants, which influences water and nutrient uptake (Giannakoula *et al.*, 2008). Whereas excess iron can damage cell structures and impede nutrient uptake by damaging the epidermis surface of the roots (Saaltink *et al.*, 2017). Manganese can damage the photosynthetic apparatus when in excess. Excess manganese can also alter other processes, such as absorption and

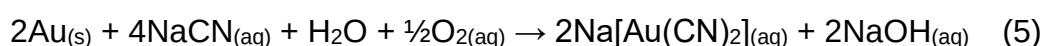
utilisation of mineral elements such as Ca, Mg, Fe and P, which can cause oxidative stress (Millaleo *et al.*, 2010).

Numerous studies have shown that diatoms react to metal pollution not only on a community level but also on an individual level through changes within their frustule morphology, manifesting as deformity (da Silva *et al.*, 2009).

1.1.4 The cyanide code and the influence of cyanide on the environment

Gold mining operations have been using cyanide in their extraction processes for more than ten decades (Kumar *et al.*, 2017), although this chemical is considered very toxic and fatal if consumed (Laitos, 2012). The Cyanide Code is the “International Cyanide Management Code for the Manufacture, Transport, and Use of Cyanide in the Production of Gold”. This code was one of the first standards and certification programs established for the mining industry. The objective of this code is to better the management of the use of cyanide in the mineral extraction processes, enhance human health and safety, and reduce the environmental impact of this chemical, therefore providing a system for cyanide, which includes processing, handling, disposal, transport, and recycling of this chemical at the mining site (International Cyanide Management Institute, 2021). According to Kumar *et al.* (2017), mining operations use hundreds of tons of cyanide annually.

The main use of cyanide in the mining industry is to extract gold and silver from ores, but it is also used as a flotation reagent in removing base metals, such as Cu, Pb, and Zn (Laitos, 2012). Normally NaCN and KCN or a combination of these cyanide complexes are used in the gold extraction process (Bakatula and Tutu, 2016), which are also the main man-made forms of cyanide (Kumar *et al.*, 2017). The process of cyanide solution used in gold extraction can be illustrated in Equation 5 (Kumar *et al.*, 2017).



The one advantage that mining industries gained by using cyanide in the extraction process is that they were now finally able to extract precious metals from low-grade ore, which couldn't be done before with conventional mining methods (Laitos, 2012).

Cyanide can form compounds that are both toxic and chemically inactive. The inactive cyanide has little effect on the environment, whereas the toxic cyanide is considered very poisonous (Laitos, 2012). Cyanide can occur naturally in many forms, such as hydrogen cyanide, salts, and cyanide complexes (Dash *et al.*, 2009).

Cyanide is weakly adsorbed by soils and remain in the aqueous form (Dash *et al.*, 2009) and can therefore create complexes with most transition metals (Fe, Au, Cd, Co, Cu, and Ni), but it forms the most stable complexes with iron and gold (Kumar *et al.*, 2017; Dash *et al.*, 2009). The formation of these complexes can also accelerate the dispersal of cyanide into the environment (Bakatula and Tutu, 2016). Complex cyanides are also considered less toxic than simple cyanides (Laitos, 2012). Even though this is the case, the mining industries prefer using simple cyanides in the leaching processes, due to its capability of dissolving and then combining with metals to produce complex cyanides (Laitos, 2012).

Sodium cyanide is easily converted to hydrogen cyanide and cyanide ion in water, which is also known as free cyanide (Bakatula and Tutu, 2016; Dash *et al.*, 2009; Laitos, 2012). The amount of free cyanide also has an effect on the toxicity of the solution, since the higher the free cyanide concentration, the more toxic the solution (Laitos, 2012).

In low grade ore, containing small amounts of gold compared to other elements, such as sulphur or iron, some of the metal-cyanide complexes are classified as weak acid dissociable (WAD) cyanide (Laitos, 2012; Dash *et al.*, 2009). These are mostly complexes with Cd, Cu, Ni, and Zn (Bakatula and Tutu, 2016; Dash *et al.*, 2009). WAD cyanide can readily form toxic free cyanide at neutral pH levels (Bakatula and Tutu, 2016; Laitos, 2012). This could cause serious damage to the surrounding area since free cyanide is very toxic to humans and the environment. But the formation of this cyanide is mostly avoided, because WAD cyanide readily evaporates through volatilisation, which makes it ineffective for gold leaching, since the cyanide is unavailable in the solution (Laitos, 2012). Other metal-cyanide complexes that can occur are classified as strong acid dissociable cyanide (SAD), which are mostly complexes with Fe, Co, Ag and Au. But these complexes can only dissociate under extreme acidic conditions (Bakatula and Tutu, 2016).

The variables that mainly affect the toxicity of cyanide includes water, pH, dissolved oxygen availability, and complexation. The water affects the dilution and distribution into the environment, whereas the pH determines the toxicity (Laitos, 2012). At a neutral pH of 7, cyanide exists as free cyanide (Dash *et al.*, 2009; Laitos, 2012), and when the pH becomes more acidic (< 7), this state of cyanide becomes more prevalent (Laitos, 2012). Complexation is when other elements bind with the cyanide to produce reactions such as volatilisation, oxidation, and precipitation (Laitos, 2012). Despite these effects of pH on the toxicity of cyanide, the pH of a dilution and the cation present also determines the stability of the cyanide salts that are formed (Bakatula and Tutu, 2016).

In contrast, while lower pH produces more free cyanide, a higher pH increases the leaching ability of gold from the ore. Due to this important role of pH in the leaching process, the mining industries try to keep the pH level between 9.5 and 11, where 10.5 is the optimal pH for leaching. At a pH of 9.36 or below, the dominant form of cyanide is hydrogen cyanide (gas). Thus, mining operations usually try to maintain a high pH level to also protect their workers from the toxic cyanide gas (Laitos, 2012).

Cyanide rarely remains biologically available in soils due to it either being complexed by trace metals, microbially metabolised, or removed by volatilisation. However, cyanide can remain in groundwater, mine tailings, and in deserted leach piles for at least a century under alkaline conditions (Kumar *et al.*, 2017). The TSFs will most likely always have the highest cyanide concentrations since this chemical is part of the gold extraction process (Rösner and Van Schalkwyk, 2000).

The toxic effects of cyanide on living organisms are dependent on factors such as pH of soil or water, temperature, and sunlight. Some research indicates that the main environment that will be affected by an accidental cyanide spill is the aquatic environment. Land animals such as mammals and birds would most likely be exposed to cyanide due to the misinterpretation of a pond on a leach pad or a TSF as an actual food or water source (Laitos, 2012). The lethal concentration of cyanide in some mammals is 0.50 – 3.50 mg/kg body weight (Kumar *et al.*, 2017), but can be much higher, depending on species, for example the lethal cyanide concentrations for a moose is between 18 to 43 mg/kg body weight (Eisler and Wiemeyer, 2004). Due to

the unpredictability of cyanide, good management and remediation of cyanide-infected water and tailings are essential.

1.2 Biomonitoring and bioindicators

1.2.1 *Diatoms*

Diatoms are eukaryotic, unicellular, phototrophic, micro-organisms, belonging to the class Bacillariophyceae (Taylor *et al.*, 2007a; Round *et al.*, 1990; Molino and Wetherbee, 2008; Dixit *et al.*, 1992; Wang *et al.*, 2013; Mishra *et al.*, 2017; Katiyar *et al.*, 2010), containing more or less 200 genera and over 100 000 species (Marella *et al.*, 2020; Katiyar *et al.*, 2010). They occur mostly as single cells, but a few species may form colonies (Taylor *et al.*, 2007a), and their cell sizes extend from 5 µm to 0.50 mm (Singh and Parikh, 2020).

There are two main groups of diatoms which are primarily distinguished by their cell symmetry; pennate diatoms are bilaterally symmetrical and are generally benthic, whereas centric diatoms are radially symmetrical and mostly planktonic (Taylor *et al.*, 2007a; Molino and Wetherbee, 2008; Mishra *et al.*, 2017). Planktonic algae float freely in the water column, whereas benthic algae are attached to a substrate with limited movement relative to their substrate.

The benthic and planktonic algae are in dynamic equilibrium, depending on the depth and flow rate of the water (Bellinger and Sigee, 2015). Diatom communities can be observed by touch (mucilaginous texture) or by a thin gold-brown layer coating the substrate, which can become more recognisable within certain environmental conditions or seasons of the year (Taylor *et al.*, 2007a; Marella *et al.*, 2020). Diatoms have a high photosynthetic productivity and contains numerous bioactive compounds and beneficial pigments (Biswas and Choudhury, 2021).

The morphology of the diatoms is primarily distinguished by an ornamented siliceous cell wall combined with intimately linked organic layers and components, which defines the diatom frustule (Molino and Wetherbee, 2008; Dixit *et al.*, 1992; Wang *et al.*, 2013; Katiyar *et al.*, 2010). This frustule consists of two overlying thecae that are joined together by a cingulum or girdle. Every theca consists of a silica valve and one or more girdle bands to accommodate cell enlargement and growth (Molino and Wetherbee,

2008; Dixit *et al.*, 1992; Wang *et al.*, 2013; Biswas and Choudhury, 2021). This robust cell wall is species-specific (Park *et al.*, 2020).

Diatoms are differentiated from higher plants through size, cell wall structure, cellular organisation (single cells), life-habit, type of photosynthetic pigments and storage products and from photosynthetic bacteria through their biochemistry and the possession of organelles and a nucleus (Bellinger and Sigee, 2015). Diatoms can be found in four major micro-habitats:

- Epipelon: This includes the surface of the sediments.
- Episammon: This consists of the parts between the sand particles.
- Epilithon: This includes substrates such as gravel, bedrock, and stone.
- Epiphyton: This is the surface of macrophyte plants, where the diatoms will attach (Taylor *et al.*, 2007a).

1.2.2 Diatoms as bioindicators

Biomonitoring is a very useful tool to evaluate aquatic ecosystem health. This approach involves organisms that are likely to reveal any physical or chemical changes in their environment, caused by natural or anthropogenic activities (Morin *et al.*, 2008; Kock *et al.*, 2019).

Diatoms can respond rapidly to changes in their environment, including both chemical (nutrients, pH, metal concentrations) and physical (temperature, habitat availability) (Sivarajah *et al.*, 2019). Diatoms are considered to be accurate indicators of certain water quality issues, including organic pollution, eutrophication, metal pollution, and acidification, along with general water contamination (Taylor *et al.*, 2005; Sivarajah *et al.*, 2019; Dixit *et al.*, 1992; Biswas and Choudhury, 2021). Diatoms are good biological indicators due to several factors:

- Diatoms are commonly found in all water sources and damp soils (Taylor *et al.*, 2005; Wassie, 2008).
- Sampling is fast and straightforward (Taylor *et al.*, 2005), and cost-effective in comparison with other organisms (Srivastava *et al.*, 2016).

- They have a very short life cycle which enables them to react swiftly to environmental changes (Srivastava *et al.*, 2016; Wassie, 2008; Taylor *et al.*, 2005).
- They are rather insensitive to physical features in the environment (Taylor *et al.*, 2005).
- Microscopic techniques used for cell counting are fast and precise (Taylor *et al.*, 2005).
- The amount of cells per unit area of the substratum is immense, thus making random counting possible for assessment using diatoms (Taylor *et al.*, 2005).
- The ecological requirements for diatoms are in general better known than those of other aquatic organisms (Taylor *et al.*, 2005).
- Permanent records can be created for each sample (Taylor *et al.*, 2005; Srivastava *et al.*, 2016).
- In contrast to invertebrates, diatoms do not have distinct food demands, distinguished habitat niches, and are not greatly affected by streamflow (Taylor *et al.*, 2005).
- Diatoms are sensitive to certain pollutants, which might not clearly affect other organisms, or may perhaps only affect them at higher concentrations (Wassie, 2008).
- Diatoms are very responsive to nutrient changes especially silica and phosphate. They have a rapid response to eutrophication, since they are photoautotrophic, and are directly affected by changes in the dominant nutrient concentrations and light accessibility (Wassie, 2008).
- They are fast colonisers since they have no physical dispersal barriers, thus there is little lag-time between perturbation and response (Wassie, 2008).
- Since their siliceous valves can be well preserved in sediments, they are exceptional paleolimnological indicators (Sivarajah *et al.*, 2019).
- Diatom indices are also more reliable than for instance benthic macro-invertebrates due to their direct reaction to contaminants (Srivastava *et al.*, 2016).

- Changes within diatom communities are connected to changes in other biotic communities (Dixit *et al.*, 1992).

Thus, diatoms have numerous attributes that make them suitable as indicator organisms due to their wide distribution and their crucial position in the food web (Sivarajah *et al.*, 2019).

1.2.3 Diatoms as primary producers

The algae are one of the main groups of photosynthetic organisms in the aquatic environment due to their considerable role in carbon fixation and biomass production (Bellinger and Sigee, 2015; Dixit *et al.*, 1992). Owing to diatoms' substantial storage vacuole, their production levels are considered to be much higher than other classes of microalgae which enables them to store a large amount of nutrients even when nutrient limitations occur (Marella *et al.*, 2020). Approximately half of the world's net primary production is due to phytoplankton with diatoms as the dominant group (Kroth, 2007) at an estimated growth rate of two to four cell divisions per day (Biswas and Choudhury, 2021). Estimations show that these photosynthetic organisms produce 20-25% of all freshwater primary production (Bozarth *et al.*, 2009) and almost 40% of all marine primary production (Bozarth *et al.*, 2009; Marella *et al.*, 2020) which makes them the most significant group of organisms that fix carbon from the atmosphere (Bozarth *et al.*, 2009) producing 20-25% of the world's oxygen (Singh and Parikh, 2020).

For these reasons, diatoms are considered the main contributor to the aquatic food web. They are not only involved in carbon fixation, but also silica cycling by incorporating it into their cell walls. After death, it is deposited on the bottom of water sources to serve as a silica reserve or may form diatomaceous earth (Kroth, 2007) and limestone (Hallock, 1997). Not only are diatoms the main input into the world's primary production, but they also promote secondary productivity by providing an alternative source of polyunsaturated fatty acid (PUFA) (Singh and Parikh, 2020). This plays an essential role in the phytoremediation of toxic metals (Marella *et al.*, 2020).

Within benthic biofilms, primary production and respiration are based on the physical, chemical, and biotic characteristics of the habitat. Factors such as stream velocity,

temperature, nutrient accessibility, and light availability can strongly affect photosynthesis and respiration rates (Fuelling, 2013; Taylor *et al.*, 2007a). Diatoms are an essential component of the aquatic ecosystem and serve as a fundamental connection between primary and secondary production (Taylor *et al.*, 2007a). For that reason, they are seen as very ecologically successful organisms, in both planktonic and benthic habitats (Bellinger and Sigee, 2015).

1.2.4 Influence of heavy metal concentrations and AMD on diatoms

Since diatoms are primary producers, they are also the first to respond to any environmental changes or pollution (Marella *et al.*, 2020). Numerous studies have shown that diatoms are very useful bioindicators for metal pollution due to their short life cycle and ubiquitous (Luís *et al.*, 2016; Biswas and Choudhury, 2021), as well as taxonomic and morphological alterations it causes in the diatom communities (Luís *et al.*, 2016; Olenici *et al.*, 2017; da Silva *et al.*, 2009; Morin *et al.*, 2008). Although morphological changes have been reported in numerous algae species, they are very apparent in diatoms (Park *et al.*, 2020).

The diatom community composition within mining areas is associated with a combination of low pH and highly dispersible toxic metals (Olenici *et al.*, 2017). The best season to sample diatoms is in the summer, due to their high species abundance and low defined dominance (Morin *et al.*, 2008). The micropollutant changes are also best observed in the summer with lowest discharges but highest metal concentrations (Morin *et al.*, 2008; Olenici *et al.*, 2017).

Many studies have shown that diatom frustule deformities and size reduction are directly associated with high metal concentrations (Luís *et al.*, 2016; da Silva *et al.*, 2009; Olenici *et al.*, 2017; Morin *et al.*, 2008; Pandey and Bergey, 2016; Falasco *et al.*, 2009). However other factors may also cause deformities in diatom communities such as drought, light intensity, UV, salinity, acidification, and nutrients (Olenici *et al.*, 2017; da Silva *et al.*, 2009). Joining these factors are a few other compounds, including pesticides, cyanide, and polycyclic aromatic hydrocarbons (PAH) (da Silva *et al.*, 2009; Falasco *et al.*, 2009). Except for deformities and size reduction, metal pollution can also affect diatom abundance and diversity (Olenici *et al.*, 2017; Pandey and Bergey,

2016). It is seen that with an increase in metal pollution there is a decrease in both species' richness and diversity (da Silva *et al.*, 2009).

The toxicological effects of the toxic metals on the diatoms are dependent on the metal species present, concentration, and exposure time (Luís *et al.*, 2016; Olenici *et al.*, 2017). Previous research showed that the metals with the most influence on diatom communities include zinc (Zn) and copper (Cu) (Falasco *et al.*, 2021; Olenici *et al.*, 2017). However, these elements, among others, such as cobalt (Co), manganese (Mn), molybdenum (Mo), and nickel (Ni), are essential for algal growth and metabolism, they can be toxic at high concentrations (Marella *et al.*, 2020; Biswas and Choudhury, 2021). Whereas non-essential elements, such as mercury (Hg), cadmium (Cd), and lead (Pb) can be harmful even at low concentrations, due to the damage they cause to the metabolic processes of the essential micro-elements (Falasco *et al.*, 2009; Biswas and Choudhury, 2021).

If the metal or metalloid (such as arsenic (As)) is available in a chemical form which can be easily absorbed, then there will be a larger occurrence of valve tetralogies, due to undissolved metals trapped by Fe or Mn hydroxides that cover the cell wall (Luís *et al.*, 2016). These changes in cell morphology include deformities in the cell structure and valve ornamentations (Olenici *et al.*, 2017; Biswas and Choudhury, 2021). It has been observed that heavy metal pollution, especially Cu and Zn, specifically affects the valve outline and not the fine structures, such as the stria and raphe (Falasco *et al.*, 2009; Olenici *et al.*, 2017). Two other abnormalities caused by this pollution include abnormally curved raphe branches and abnormalities in the areolae (Olenici *et al.*, 2017; Luís *et al.*, 2011). Some of the secondary effects may include rearrangement of stria and position of fibulae (Luís *et al.*, 2011; Falasco *et al.*, 2009), as well as the density of these ornamentations (Falasco *et al.*, 2009). Along with all these other morphological changes, size reduction can also cause the outline of the cells to become oversimplified or in some cases more elaborate (Falasco *et al.*, 2009).

The diversity of the diatom communities can be governed by low pH and high metal concentrations (Luís *et al.*, 2016). The low productivity rate in acid mine drainage (AMD) waters is most likely due to the metal stress or the low dispersible reactive phosphate concentrations (Luís *et al.*, 2016). Low pH concentrations alone do not

affect photosynthetic productivity but there are a few tolerant species which can survive under these extreme conditions (Luís *et al.*, 2016).

According to Luís *et al.* (2016), there are two explanations for the formation of tetralogies in diatoms at mine impacted sites. Firstly, the diatom tetralogies are associated with the weak silicification of the individual cells. Some of the metals, such as Cu, silver (Ag), Cd, and Hg (Luís *et al.*, 2011; Luís *et al.*, 2016) can bind to sulfhydryl groups of the cell membranes (Luís *et al.*, 2016) since metals have a high affinity for these groups (Luís *et al.*, 2011). This binding of the metals to the sulfhydryl groups can then govern the silica uptake and result in an impersonation of silica limitation (Luís *et al.*, 2016; Luís *et al.*, 2011).

An additional explanation for the occurrence of deformities is when the ferric hydroxide precipitates on the sediments. This precipitate forms a thick red blanket, which blocks out the sunlight, therefore limiting the photosynthesis process and producing tetralogies (Luís *et al.*, 2016).

The cells that are influenced by abnormalities likely have a disadvantage in the natural environment. The survival capability of irregularly shaped diatoms can also be associated with the kind of teratology involved. For example, the disordering of the raphe can cause a decrease in the motility and reproductive capacities of the diatoms (Falasco *et al.*, 2021).

Previous research has shown that some algae species may become tolerant to toxic metals or acidic conditions (Luís *et al.*, 2016; da Silva *et al.*, 2009). However, this can cause a loss in species diversity (da Silva *et al.*, 2009; Fuelling, 2013) and lead to restrained intracellular transport of metals at a low pH level (Luís *et al.*, 2016). Some metal tolerant species include, *Sellaphora seminulum*, *Eolimna minima*, *Cocconeis placentula* var. *euglypta*, *Fragilaria gracilis*, *Gomphonema parvulum*, *Nitzschia palea*, *Nitzschia sociabilis*, *Pinnularia parvulissima* and *Surriella angusta* (da Silva *et al.*, 2009; Pandey and Bergey, 2016). Although these species are considered to be tolerant to these environmental conditions, they can still undergo morphological changes (Luís *et al.*, 2011).

Although gold mining has a major contribution to the economic development and wealth of SA, it causes major environmental problems and health issues. Seasons and climate play a key role in the toxicity of acid mine drainage (AMD) and metal

contamination because toxic metals have a higher accumulation rate and sulphide minerals have a higher acid-producing rate within warmer climatic conditions. As mentioned, temperature changes alone do not particularly have a negative effect on diatoms, but they can cause changes in the toxicity levels of AMD and toxic metals, which can cause damage to these organisms. These effects include morphological, diversity, and abundance changes within diatom communities, which makes these organisms good biological indicators for environmental changes caused by mining activities.

1.3 Aims and objectives

1.3.1 Aims

Little research on the influence of gold mine tailings on diatoms has been done, especially in South Africa. The aim of the study is to evaluate the impact of gold mine tailings on diatom community composition and abundance. This information will be used to determine the condition of water bodies associated with gold mining activities, using diatoms as indicator organisms.

1.3.2 Objectives

It is necessary to determine the influence of mine tailings on the environment and to distinguish the difference between the highly impacted sites and the moderately impacted sites, for this reason the present study will:

1. Determine the composition of different diatom communities and species distribution at different aquatic (presumed polluted and presumed reference) sites within a large gold mining area.
2. Determine the physical, chemical, and elemental variables at each site.
3. Determine the influence of toxic metals and other contaminants associated with mine effluent on the diatom community composition and productivity.
4. Infer the ecological status of the study sites using diatom community composition as well as the condition of individual diatom cells (deformities).

Chapter 2

2.1 Methods

2.1.1 Study area

The Klerksdorp-Orkney-Stilfontein-Hartebeesfontein (KOSH) district was chosen as the study area for this research. This area comprises four districts, including Klerksdorp, Orkney, Stilfontein, and Hartebeesfontein, with a coverage of 2757 km² (Dudumashe and Thomas, 2020; Thomas, 2020). These districts form part of the Vaal River (VR) catchment. The KOSH region is located 160 km southwest of Johannesburg (Thomas, 2020). The region's climate consists of hot to warm summers with an annual rainfall of between 300-750 mm, and cold, dry winters (Dudumashe and Thomas, 2020).



Figure 2: Map of the study area and study sites (Google Earth). The study sites are located in the North West Province, South Africa.

The KOSH area forms part of the Witwatersrand basin (Dudumashe and Thomas, 2020; Thomas, 2020) which also includes the Far East, Central Rand, Western, and Far Western basin, as well as the Free State gold mines (Thomas, 2020). Many years of continuous gold mining in the KOSH area have led to the underground linkage of all the mines in this area, which caused the formation of a path whereby the underground water moves from the aquifers into the shallower mines and shafts, and

then flows into deeper regions. Thus, mining in the KOSH area contributes largely to the drainage of underground water into the mines which increased the cost of mining operations, due to the need for constant water removal from the mines (Thomas, 2020).

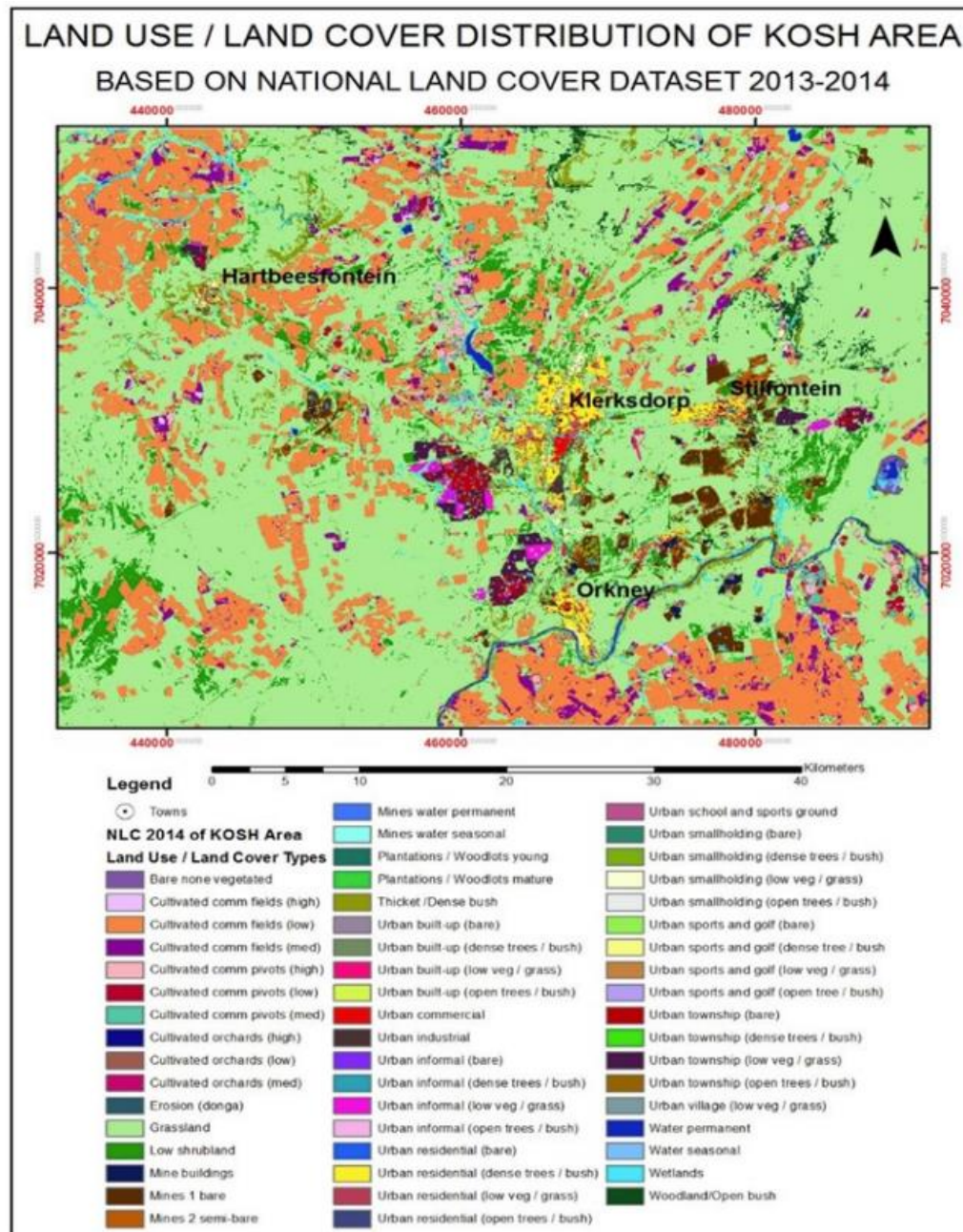


Figure 3: The Klerksdorp-Orkney-Stilfontein-Hartebeesfontein region's land use and land cover distribution based on the national land cover dataset 2013-2014 (Dudumashe and Thomas, 2020).

This area is underlain primarily by an intercalated conglomeration of sedimentary, extrusive-and intrusive rocks (Thomas, 2020; Dudumashe and Thomas, 2020), and mafic lavas, including dolomite, gold-bearing conglomerates, Black Reef quartzite, Ventersdorp lavas, and dykes. Furthermore, this area contains shallow aquifers with unpolluted water (Thomas, 2020).

Figure 3 shows that the main coverage of the KOSH area is grassland and the main land uses include mining, agriculture, and residential areas (Dudumashe and Thomas, 2020).

2.1.1.1 Study area descriptions

Table 1 Presents a description of each sampling site for this study.

Table 1: A description of each sampling site.

	Sampling site	Description	Picture
1	Karee Rand TSF	Uninhibited by animals, with no vegetation surrounding the water body. The estimated amount of slurry deposited per day is 40 000 tons, with the slurry containing silt particles (0.002-0.05 mm in diameter).	 (Photo by: Talitha Brits)
2	Karee Rand DD	Uninhibited by animals, some reeds and grasses surrounding water body. Has a red precipitate on the surface of the water body.	 (Photo by: Ian Luyt)

3	Karee Rand RWD	Uninhibited by animals, with no vegetation surrounding the water body.	 (Photo by: Ian Luyt)
4	Catchment Dam	This is a natural catchment dam. This dam contains a large amount of vegetation, especially reeds.	 (Photo by: Dr Velesia Lesch)
5	AEL East (North)	AEL East serves as a return water dam for Karee Rand. This dam is surrounded by grasses and some trees. The wall and bottom of the dam is lined with rocks.	 (Photo by: Talitha Brits)

6	AEL South	This dam has a small amount of vegetation, mainly consisting of grasses and a small number of reeds surrounding the dam. The wall and bottom of the dam are lined with rocks.	 (Photo by: Talitha Brits)
7	AEL DD	This drainage ditch had a low water level, with high amounts of vegetation. The vegetation mostly consisted of reeds in the ditch and grasses on the banks.	 (Photo by: Talitha Brits)
8	Eye Dam	This dam is located near the Vaal River and is the most vegetated of all sites. Consisting of large amounts of grass and reed species, including other types of vegetation, such as trees and shrubs. This dam provides breeding grounds for numerous aquatic birds. There were also signs of other animals, like horses and cattle drinking at the dam. In the dry seasons the water level is very low and mostly restricted to the	 (Photo by: Ian Luyt)

		middle of the dam, where the water is the deepest.	
9	Bokkamp RWD	This is an artificial dam, which serves as a return water dam for the West Extension Catchment. The bottom of the dam is lined with rocks, with the dominant vegetation in and around the dam consisting of reeds.	 (Photo by: Talitha Brits)
10	Bokkamp DD (West Extension DD)	This is the Bokkamp drainage ditch, which supplies the Bokkamp return water dam of water and white slurry from the TSF. Most of the vegetation surrounding this ditch consisted of grasses and a small number of reeds.	 (Photo by: Ian Luyt)
11	West Extension TSF	This TSF mostly comprises of bare sediment, with a few reeds growing toward the middle of the TSF. Minimal to no vegetation present.	 (Photo by: Talitha Brits)

12	West Complex TSF	This is a large TSF comprising bare sediment, and some small reed patches. After deposition was terminated, vegetation started to grow.	 (Photo by: Talitha Brits)
13	Queen Mary RWD	This is an artificial dam that serves as a return water dam for West complex TSF. This dam has small amounts of vegetation surrounding it, mostly grasses. The walls of this dam comprise of sandbags and wire, which is set at a 30° angle. The water level of this dam is also very variable and may run dry from time to time.	 (Photo by: Talitha Brits)
14	Queen Mary (West complex DD)	This is the Queen Mary drainage ditch, which is the water source of the Queen Mary Return Water Dam. The drainage ditch contained many reeds, with a red/orange precipitate. There were also some grasses and other vegetation types surrounding the banks of the ditch.	 (Photo by: Talitha Brits)

15	West Storm Water	The West Storm Water Dam is lined with rocks, with vegetation in and around the dam. The vegetation in the dam mostly comprises of reeds, and those on the banks of the dam, includes shrubs, trees, and a few grass species. The water level of this dam is season dependent.	 (Photo by: Talitha Brits)
16	Koekemoer Spruit	This stream is located South of Khuma (an informal settlement). This water body contains water with a bad odour and black coloured mud. This narrow stream is filled with reeds and the banks are lined with grasses. A small catchment dam is located further downstream, which contains water most times of the year.	 (Photo by: Dr Velesia Lesch)
17	Ref A (Wildskamp)	This dam is located on a private farm, outside Klerksdorp on the Witpoort gravel road. The dam is surrounded by thick vegetation, including large trees, and serves as main water source for the wildlife on the farm. This dam	 (Photo by: Dr Velesia Lesch)

		receives its water from a stream entering from the north, which flows through private properties, mainly used for agriculture.	
18	Skoon Spruit	This stream is located north of Klerksdorp, which flows through several private properties that are used for agriculture. The water of this stream is also clear and undisturbed.	 (Photo by: Dr Velesia Lesch)
19	Rietgat Spruit	This stream is located north-east of Klerksdorp. Throughout the winter, the water is restricted to a small section that is slightly deeper than the seasonal wetland section. The wetland section mostly comprises different types of grasses. This water serves as a source of water for cattle.	 (Photo by: Ian Luyt)

2.1.2 Physico-chemical factors

Before the sampling of diatoms, conditional parameters were measured at each site (seasonally). The phosphate concentration was measured using a phosphate

photometer (HI97706), with software version v1.01. At some mining sites it proved difficult to measure the phosphate concentrations due to high chlorine (>150000 mg/L), calcium (>10000 mg/L CaCO_3), and ferrous iron (>100 mg/L) concentrations interfering with the photometer. An optical dissolved oxygen meter was used to measure the dissolved oxygen (DO) and temperature at each site, and a LAQUA twin kit was used to measure the following variables: nitrate (Y045), electrical conductivity (EC) (514-22), and pH (514-7). All measurements were done on site.

Cyanide concentrations were measured and provided by the mining company. The cyanide concentrations were measured using Continuous Flow Analysis (CFA) (ISO-14403:2002), with a minimum quantifiable concentration of 0.02 mg/L. Three types of cyanide were measured, including free cyanide (CFA-1D), weak acid dissociable cyanide (WAD)(CFA-1C), and total cyanide (CFA-1D).

Although all these parameters that were measured are important within a study, nitrate and phosphate concentrations were considered particularly important due to their role in algal growth.

2.1.3 *Elemental analysis*

Benthic diatoms occur at the sediment/water interface with motile species often retreating into the sediment at night to avoid predation. Sediments in general would provide a more stable reflection of the accumulation of pollutants at a site when compared to water quality, especially in the highly dynamic mine environment. Thus, it was considered important to compare diatom community composition, as well as deformation of individual cells, to sediment quality in order to determine environmental impact. The sediments were sampled using a plastic scoop, which was cleaned between samplings with distilled water. The top 5 cm of the sediments were sampled and placed into 15 ml plastic tubes.

The sediment samples of each month were analysed for selected elements using the microwave extraction method 3051A, as demonstrated by Element (2007). First the samples were frozen at -80°C for 24 hours, whereafter they were freeze-dried at -52°C and 15Pa. Afterwards approximately 0.5 g (with the precise mass noted for each sample) of the sample was weighed off in a vessel containing a controlled pressure relief mechanism. Then 10 ± 0.1 ml concentrated nitric acid (HNO_3) or, as an

alternative, 9 ± 0.1 ml concentrated HNO_3 and 3 ± 0.1 ml concentrated hydrochloric acid (HCl) was added to the vessel in a fume hood. The samples were pre-digested to eliminate the dangers produced by rapid addition of thermal energy (MW power).

Subsequently, digestion took place. The temperature of each sample was increased to $175 \pm 5^\circ\text{C}$ for roughly 5.5 ± 0.25 minutes and remained at this temperature for 4.5 minutes, or for the remainder of the 10-minute digestion period. The pressure peaked between 5 and 10 minutes for most of the samples.

After digestion, the sample was left to cool to room temperature. Then it was determined if the sample remained sealed through the process. In the case that more than 1% of the sample was lost in the process, the sample was considered compromised. The sample was then decanted to a volumetric flask, diluted to a known volume and analysed using an Agilent 7500ce, inductively-coupled-plasma-mass-spectrometer (ICP-MS). The concentrations in sediments were converted to mg/kg dry mass, and in water to mg/L. Table 2 shows the elements measured for each sample.

Table 2: Elements measured for each sample.

Element	Symbol	CAS Registry No. ¹
Aluminium	Al	7429-90-5
Antimony	Sb	7440-36-0
Arsenic	As	7440-38-2
Barium	Ba	7440-39-3
Beryllium	Be	7440-41-7
Boron	B	7440-42-8
Cadmium	Cd	7440-43-9
Calcium	Ca	7440-70-2
Chromium	Cr	7440-47-3
Cobalt	Co	7440-48-4
Copper	Cu	7440-50-8
Gold	Au	7440-57-5
Iron	Fe	7439-89-6
Lead	Pb	7439-92-1
Magnesium	Mg	7439-95-4
Manganese	Mn	7439-96-5

Mercury	Hg	7439-97-6
Molybdenum	Mo	7439-98-7
Nickel	Ni	7440-02-0
Phosphorus	P	7723-14-0
Platinum	Pt	7440-06-4
Potassium	K	7440-09-7
Rubidium	Rb	7440-17-7
Selenium	Se	7782-49-2
Silver	Ag	7440-22-4
Sodium	Na	7440-23-5
Strontium	Sr	7440-24-6
Thallium	Tl	7440-28-0
Thorium	Th	7440-29-1
Tin	Sn	7440-31-5
Uranium	U	7440-61-1
Vanadium	V	7440-62-2
Zinc	Zn	7440-66-6

¹ Chemical abstract service registry number of each element.

2.1.4 Diatom sampling, preparation, and analysis

The diatom samples were collected, processed, and analysed in accordance with Taylor *et al.* (2005). Periphytic or benthic diatoms were sampled, according to the available substrate, which included vegetation and rocks. Where necessary planktonic samples were collected if periphytic or benthic environments were unavailable or inaccessible. Three sampling methods were used:

1. The first, is where five submerged stems (reeds or grasses), roughly 10-15 cm in length, were sampled and placed into a zip lock bag with approximately 50 ml of water, whereafter it was rubbed vigorously to dislodge the diatoms from the surface of the substrate.
2. In the second method diatoms were scraped from rock surfaces (approximately 5 cm²), using a toothbrush. With both these methods, after sampling, the water

containing the diatoms was transferred to a clean 100 ml plastic bottle with a screw cap.

3. For phytoplankton a water sample was collected from the water surface in a clean 1L bottle with a screw cap. After sampling, 100% ethanol (final concentration not exceeding 20%) was added to preserve the samples.

The diatom samples were cleaned and prepared for the slides, using the hot hydrochloric acid (HCl) and potassium permanganate (KMnO₄) method, which is the preferred method used in SA due to the high organic matter content in the samples (Taylor *et al.*, 2005).

Before cleaning, and the addition of ethanol, each sample was observed under a light microscope as a wet slide (where the coverslip and slide are not mounted together, but a drop of the unprocessed sample is put on the slide). This was done to determine if the diatom cells were in fact alive before processing. This was only done for the samples collected in Sep-2022 and Oct-2022, where there was not already ethanol added to the samples. After this was done, the samples were left to settle out for 24 hours, whereafter the top layer was decanted, and the bottom layer mixed again. Marked test tubes were filled with 2-3 ml of the shaken sample (depending on the material concentration), which was then treated with KMnO₄, and left for another 24 h. Then, 3-5 ml of 32% HCl was added, and the samples boiled on a hotplate for approximately 20 min at 115°C. This was then left to cool to approximate room temperature (25°C), which was afterward treated with three drops of hydrogen peroxide, to ensure the degradation of all organic material. The samples were then washed through centrifuging and decanting the supernatant and resuspending it in distilled water, four to five times (Taylor *et al.*, 2005). This wash process is very important, to remove any traces of acids that will otherwise react with the mountant (Round *et al.*, 1990). After the washing process, the pellet was resuspended once more with distilled water, 400 µl of this solution, was then pipetted onto clean coverslips, air dried and mounted onto the microscope slides, using Pleurax mounting medium.

The prepared slides were then observed under a Nikon 80i compound light microscope equipped with differential interference contrast (DIC) and a 100 x 1.4 N. A. oil immersion objective. The diatoms were identified to species level where possible, or to genus level otherwise, with the help of applicable literature, including

Taylor *et al.* (2007) and Taylor & Cocquyt (2016). Diatoms were counted to a total of ~400 valves, where possible. This was reported as number of cells per unit area of substrate sampled (cells.cm⁻²), the size of each cell was also measured using Universal Serial Bus (USB) measuring software.

2.1.5 Statistical analysis

Principal Component Analysis (PCA) was used to decrease the complexity of the sample data and to analyse the physico-chemical variables of the water quality, the heavy metal concentrations, and the diatom diversity between various sites within different seasons and sampling dates (XLSTAT 2022). The diatom data were simplified using a presence/-absence representation method as biomass was not measured. Therefore, the PCA's containing species data, are rather an indication of species diversity than biomass. As presence absence data was used, PCA was deemed to be a suitable method. Other ordination methods such as CCA usually used for community composition analysis assume unimodal species distribution which does not always hold true given the extreme conditions present on mining sites.

Combination graphs and bar graphs were drawn up for each site using Microsoft Excel, to compare the different physico-chemical variables measured over a period of one year. Some of the bar graphs show standard deviation bars to examine the difference between winter and summer water quality data. This was only done for the samples collected in Sept-2022 and Oct-2022. DATAtab was used to run normal distribution tests, such as Kolmogorov-Smirnov, Kolmogorov-Smirnov (Lilliefors Corr.), Shapiro-Wilk, and Anderson-Darling, and draw up normal Quantile–Quantile (QQ) plots and normalized histogram plots to evaluate the data distribution.

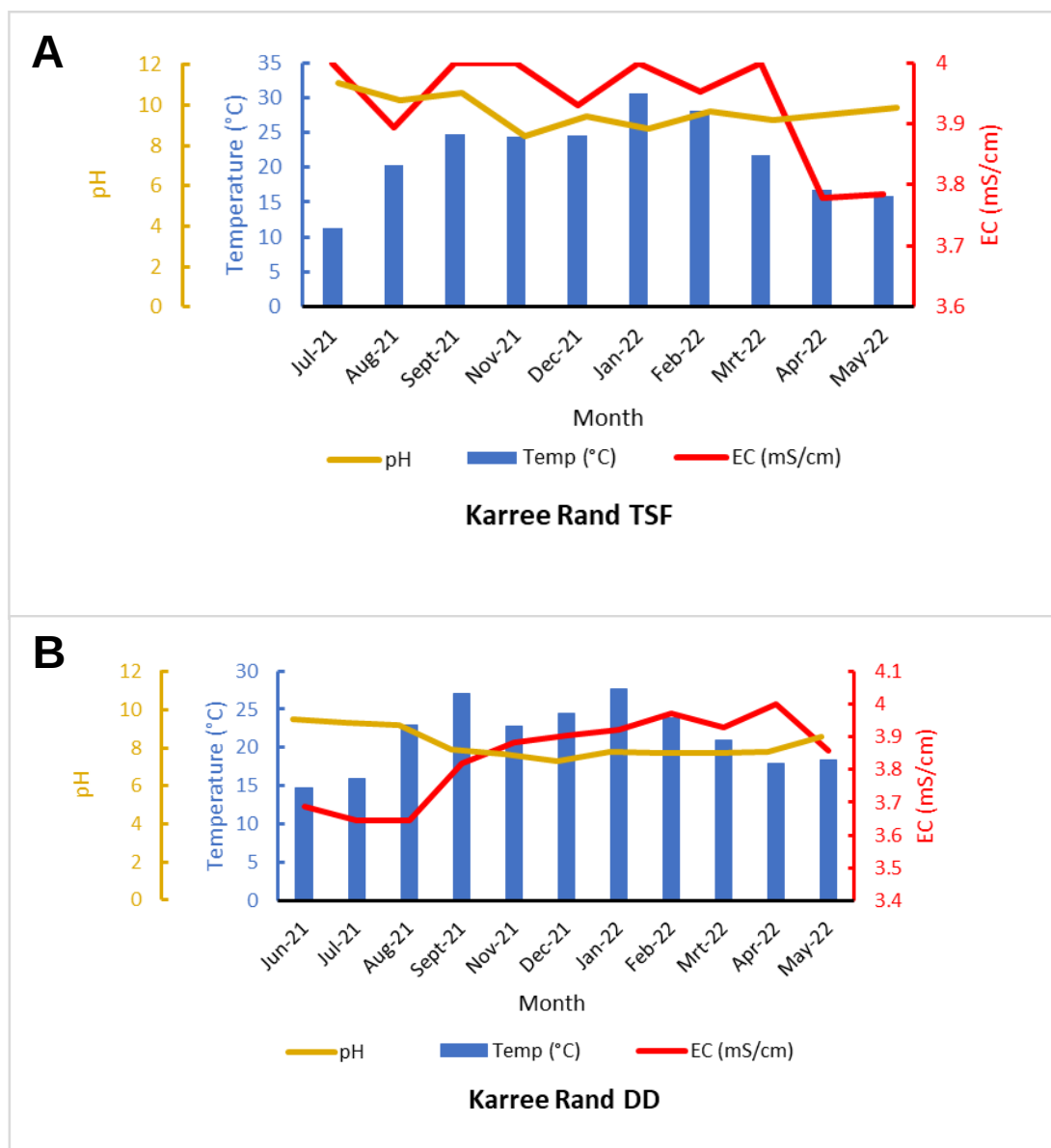
Chapter 3

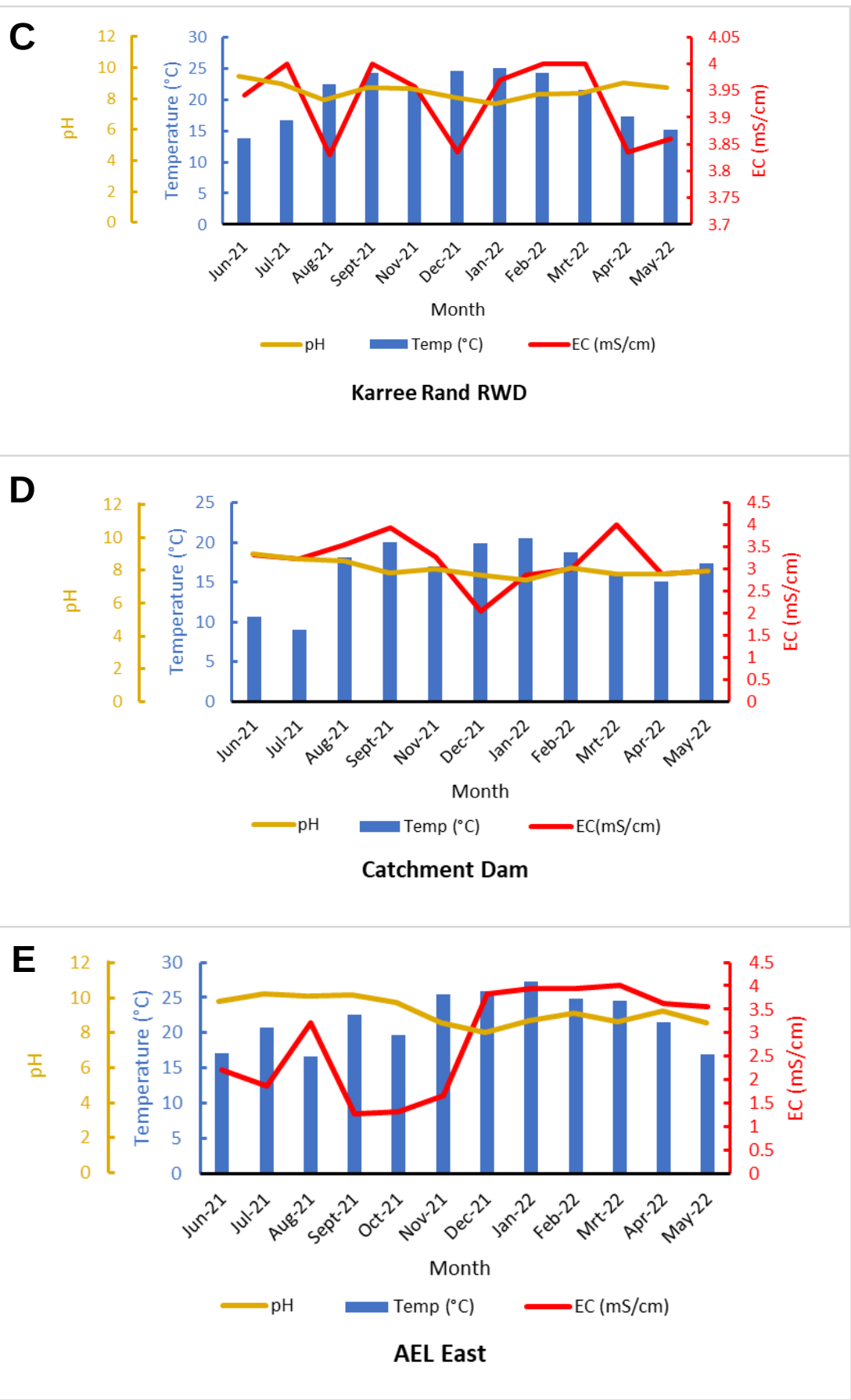
3.1. Results

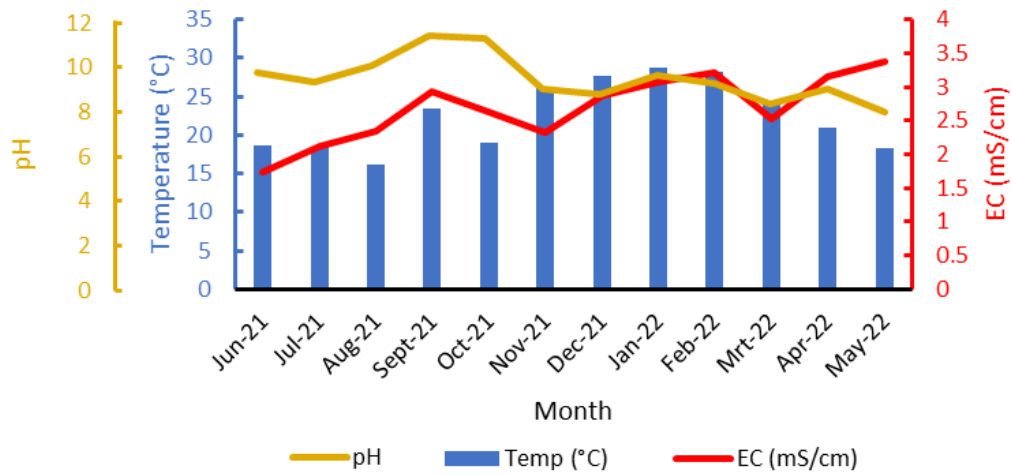
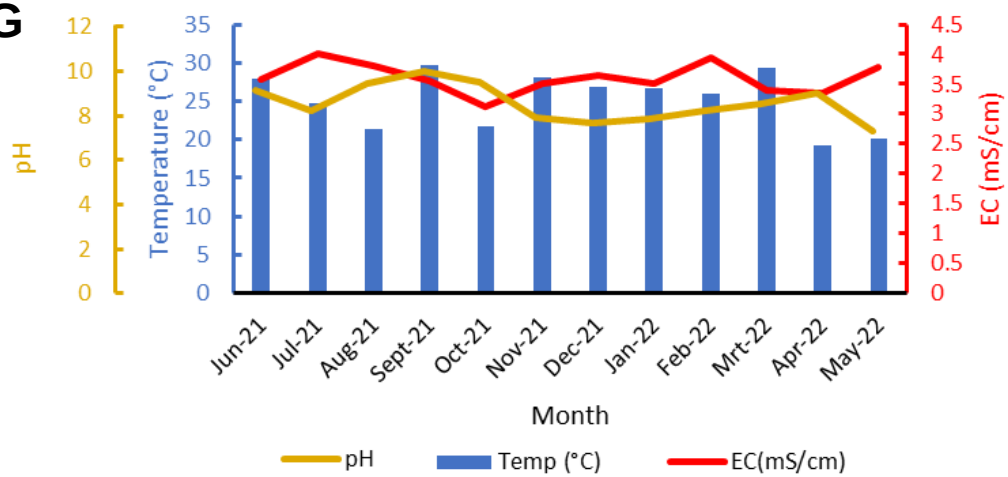
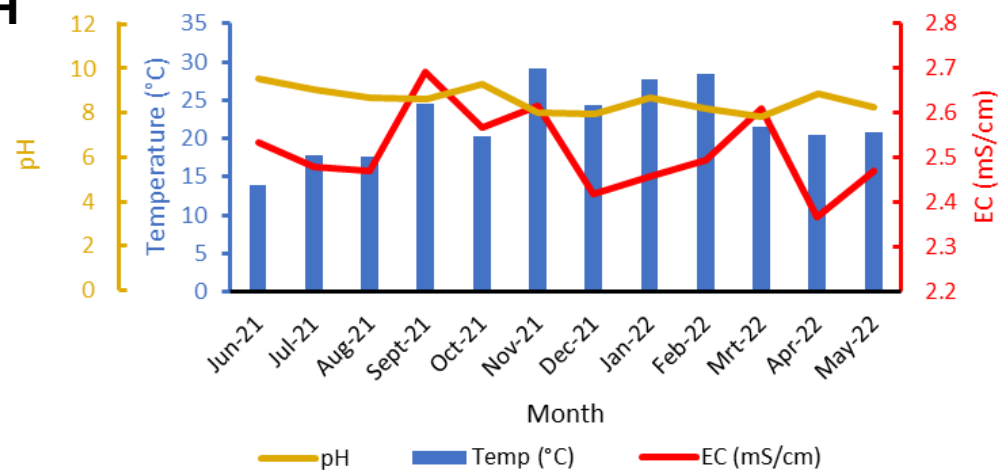
3.1.1. Physico-chemical parameters and elemental concentrations

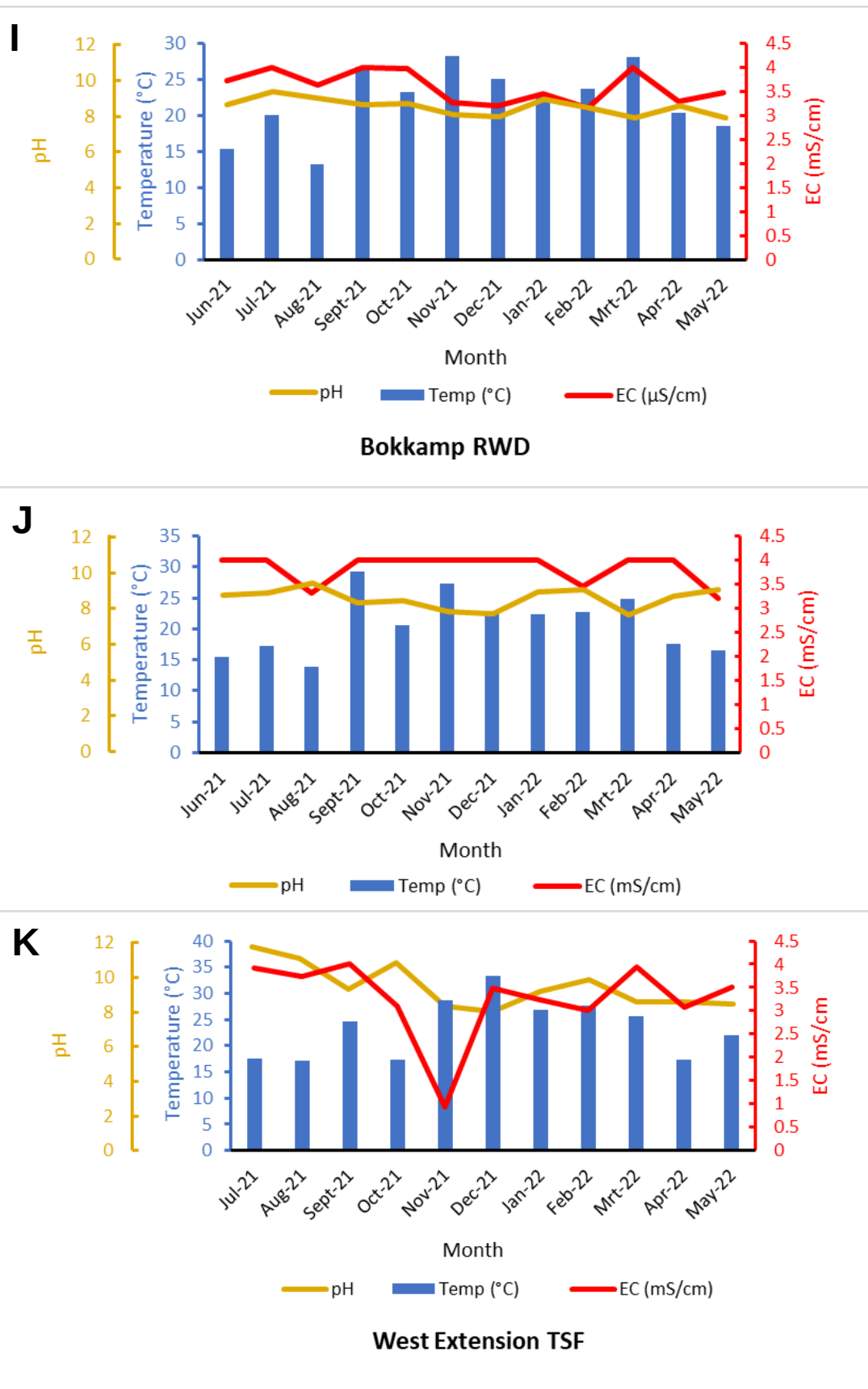
3.1.1.1 Standard analysis

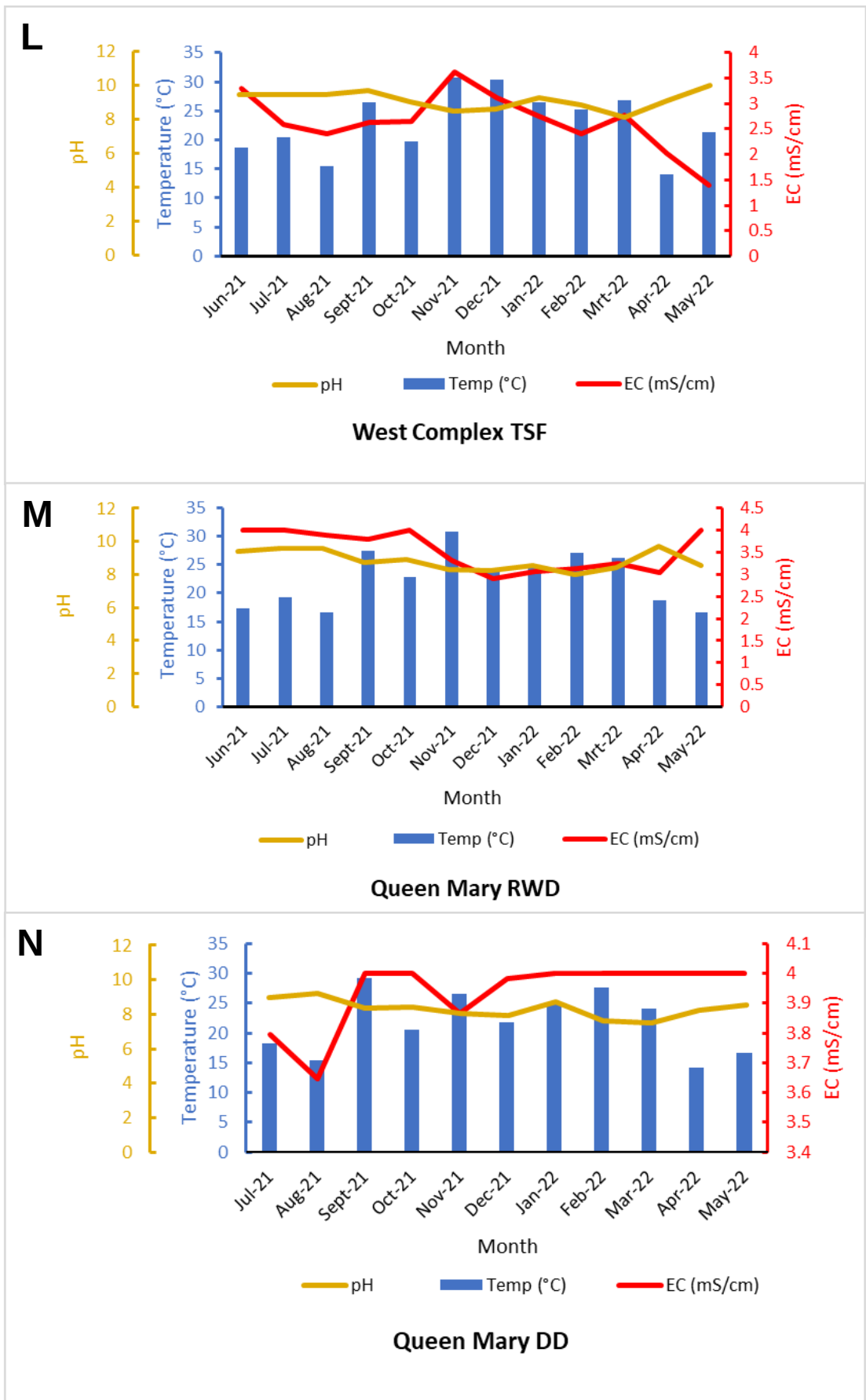
The graphs (Fig. 4) illustrate the different EC (electrical conductivity), pH levels and water temperatures of each site over a period of one year. In Figure 4 (A to S) the temperatures of all sites fluctuated over the year (Jun-21 to May-22), but this was due to seasonal changes although some sites showed a smaller change in temperature than others, such as AEL East, AEL DD, Bokkamp RWD, and Koekemoer Spruit (Fig. 4E, G, I and P).



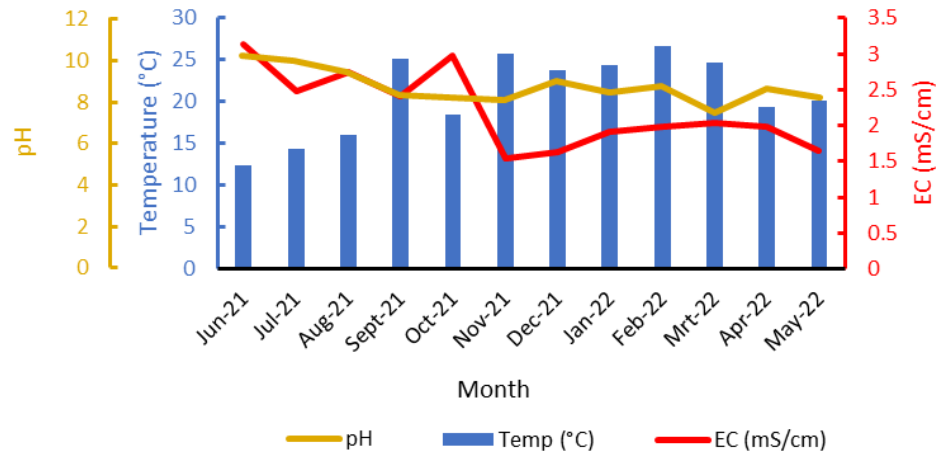


F**AEL South****G****AEL DD****H****Eye Dam**



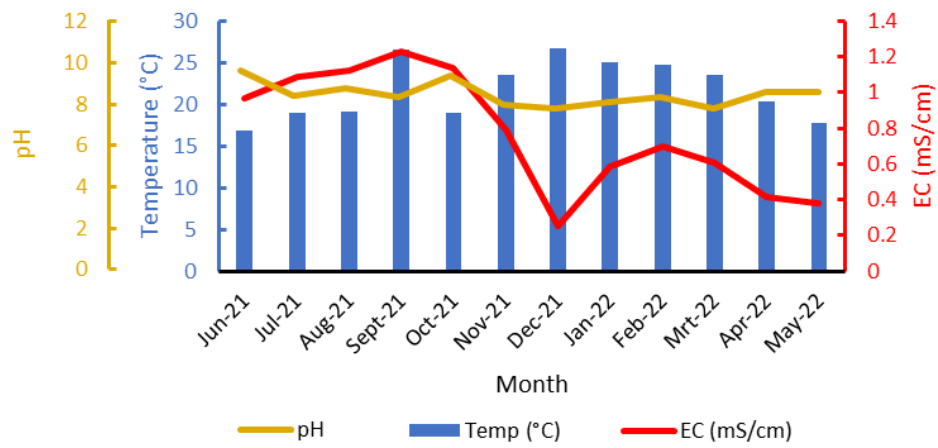


O



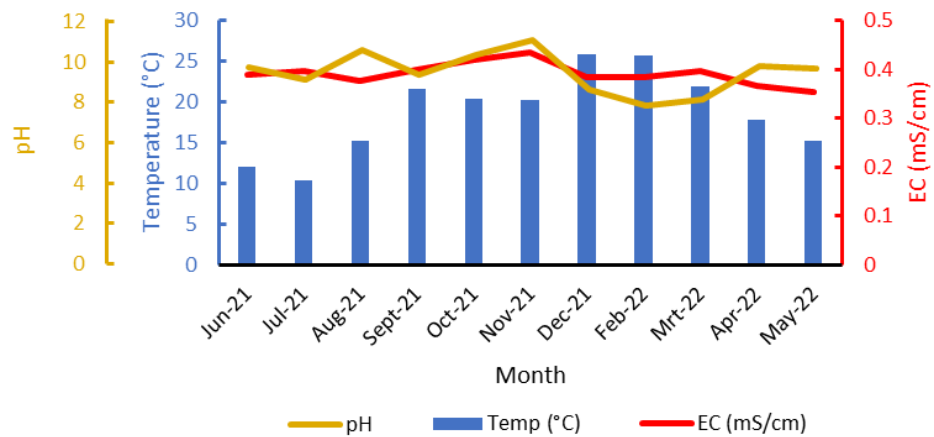
West Storm Water

P



Koekemoer Spruit

Q



Ref A

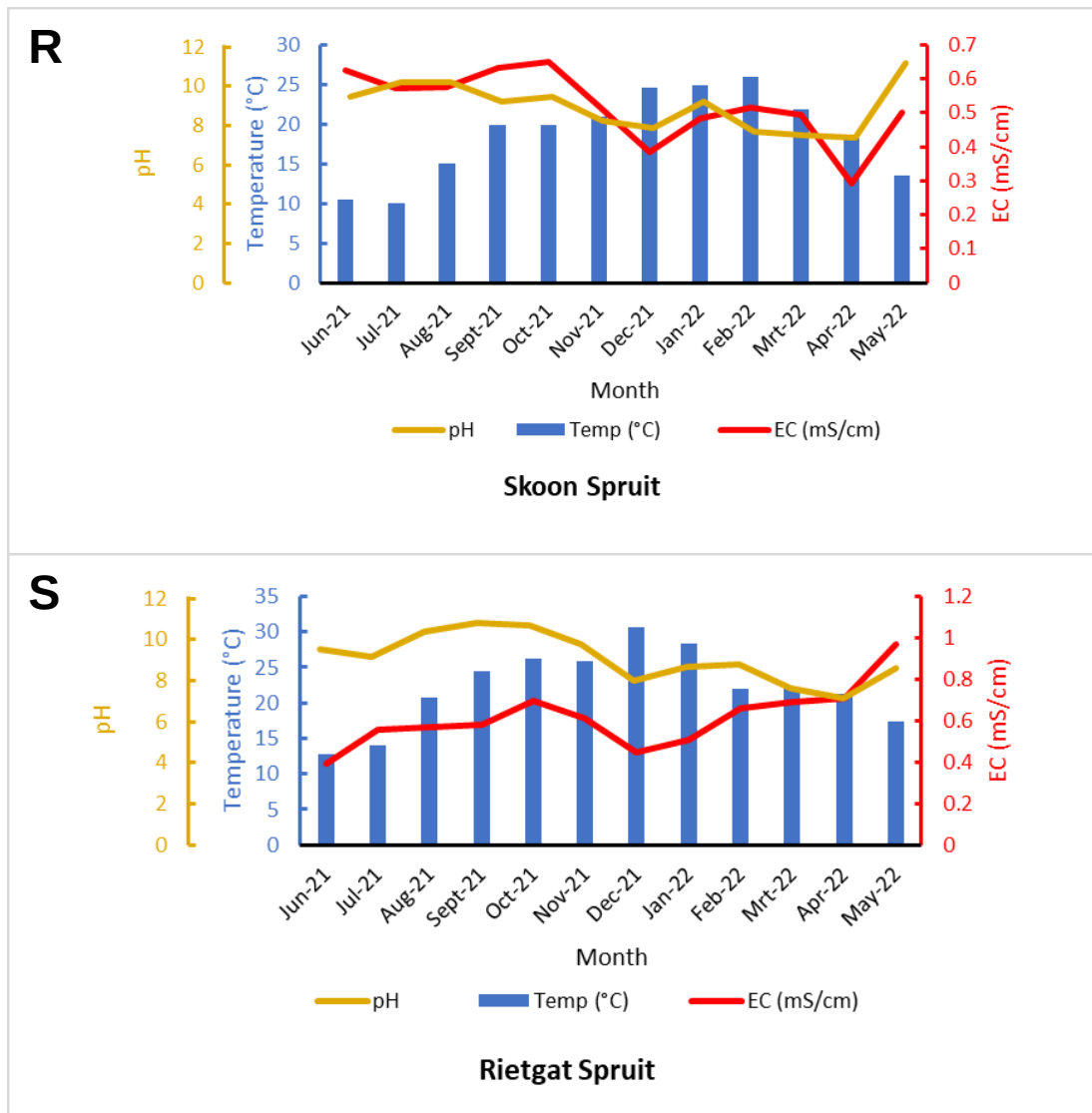
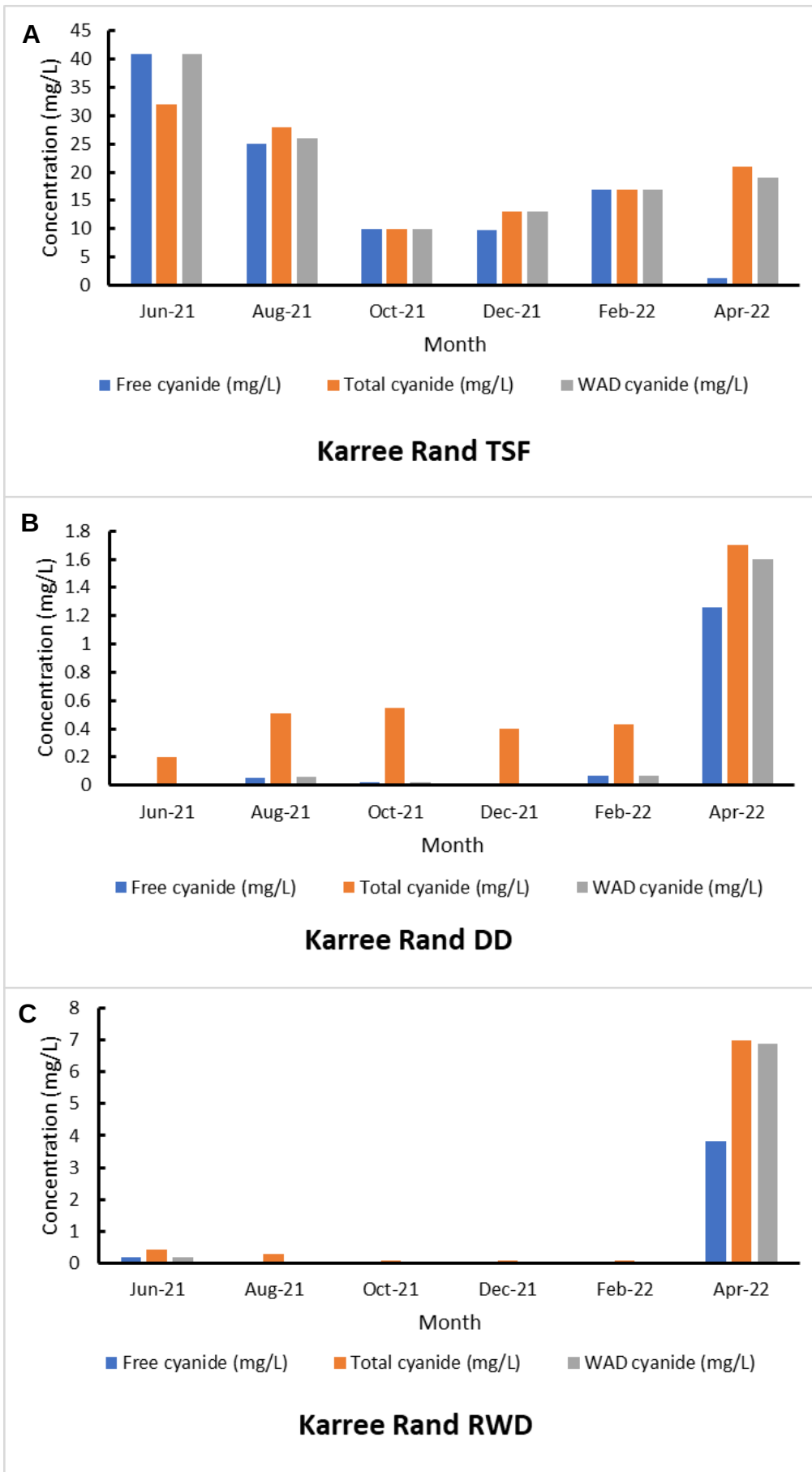
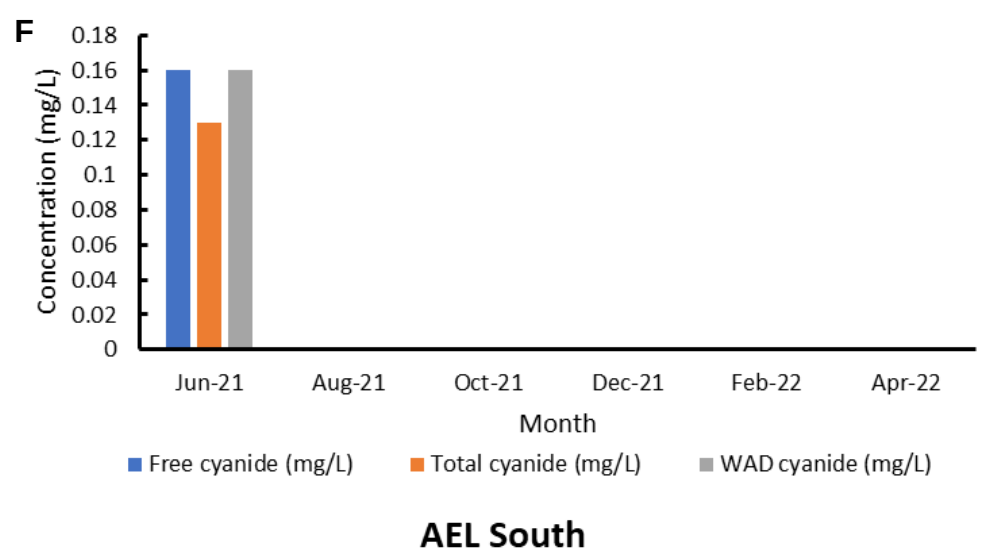
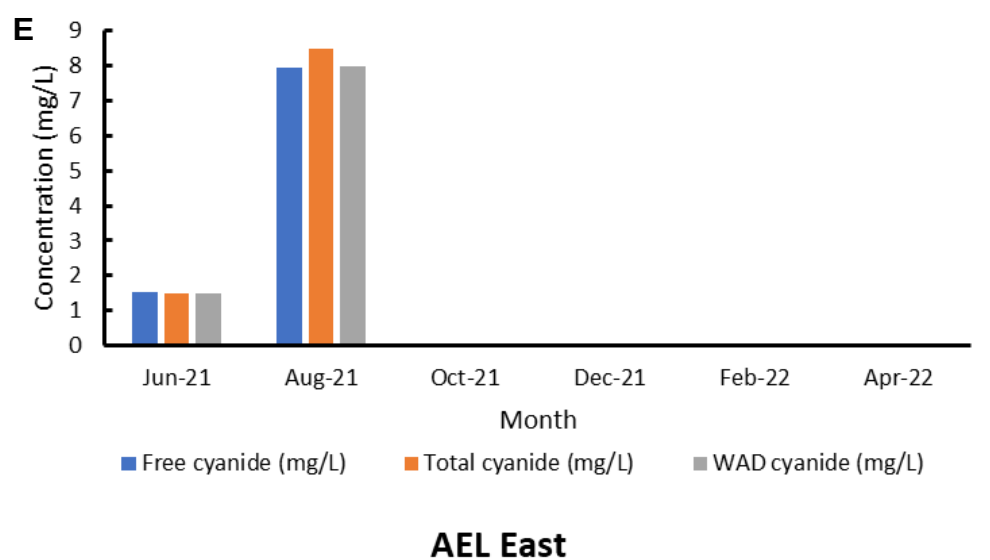
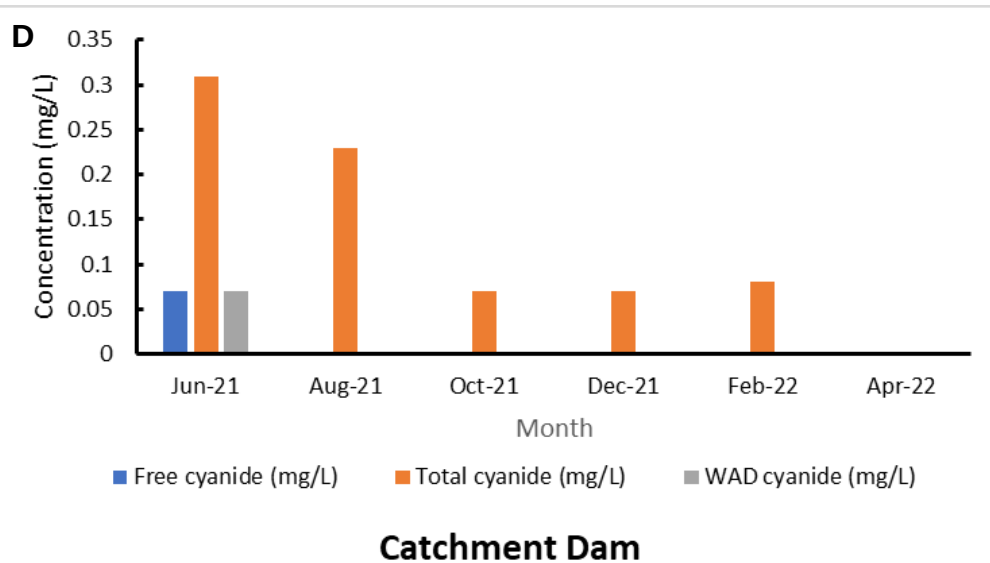


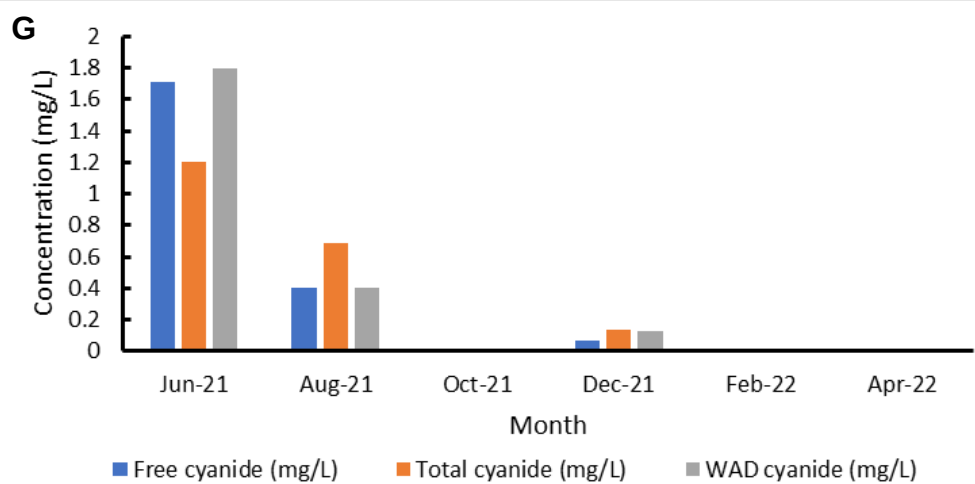
Figure 4: Graphs A to S indicate the comparison between the EC concentrations, pH values, and temperature in water between the different sites over a period of one year.

The sites illustrated in Figures 4 (A-O) had relatively high EC values, whereas (P-S) which were located further from the mining sites (reference sites) had lower EC values. In Figure 4 (A-S) relatively high pH values occurred at all sites, ranging from 7.2 to 12 which specifies that both mining sites, as well as the reference sites can be distinguished as slightly alkaline to alkaline environments.

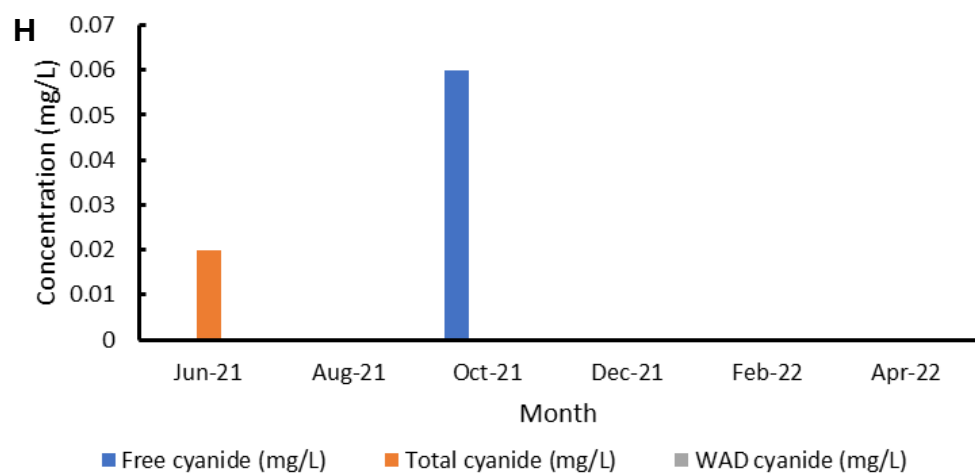
Figure 5 (A-P) indicates the different cyanide concentrations in the water at each site over a period of one year. This includes free cyanide, total cyanide, and weak acid dissociable (WAD) cyanide concentrations.



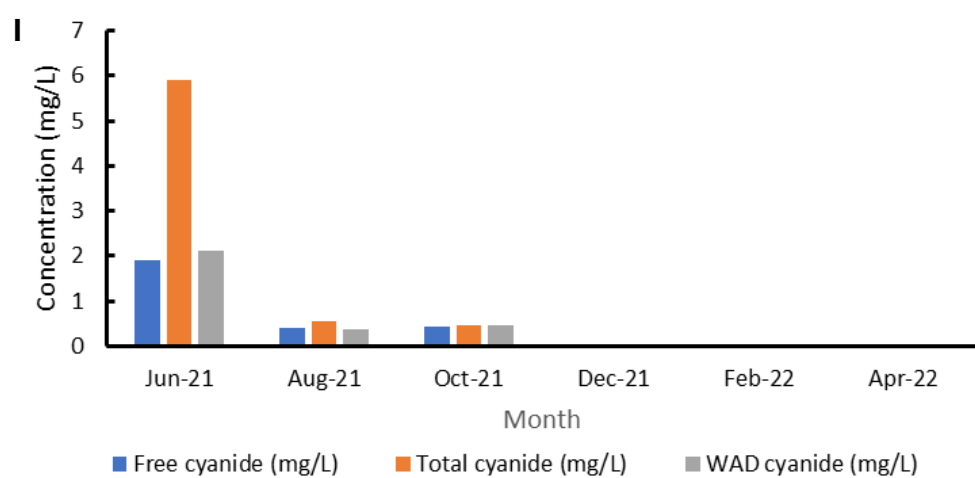




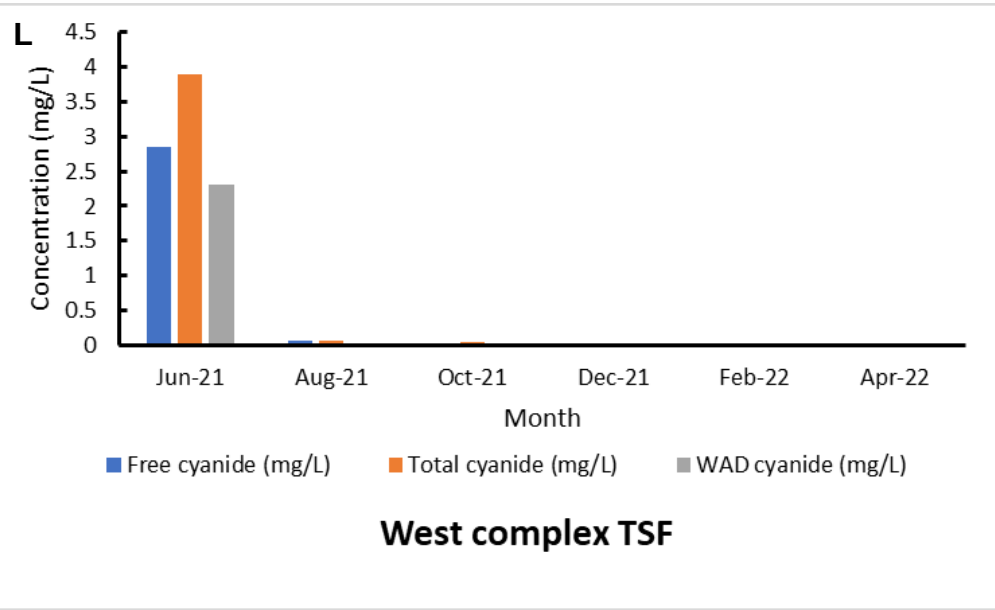
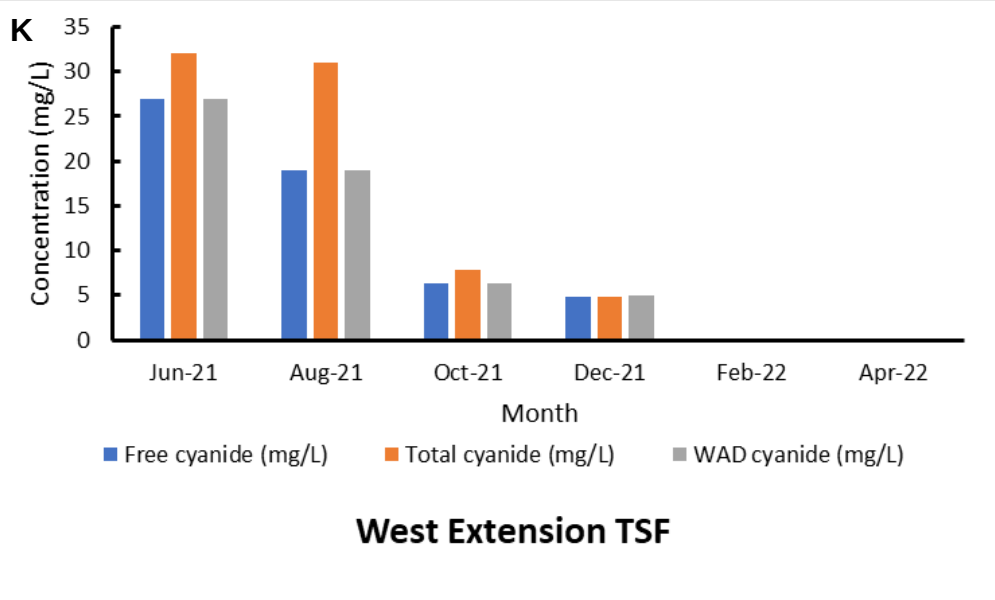
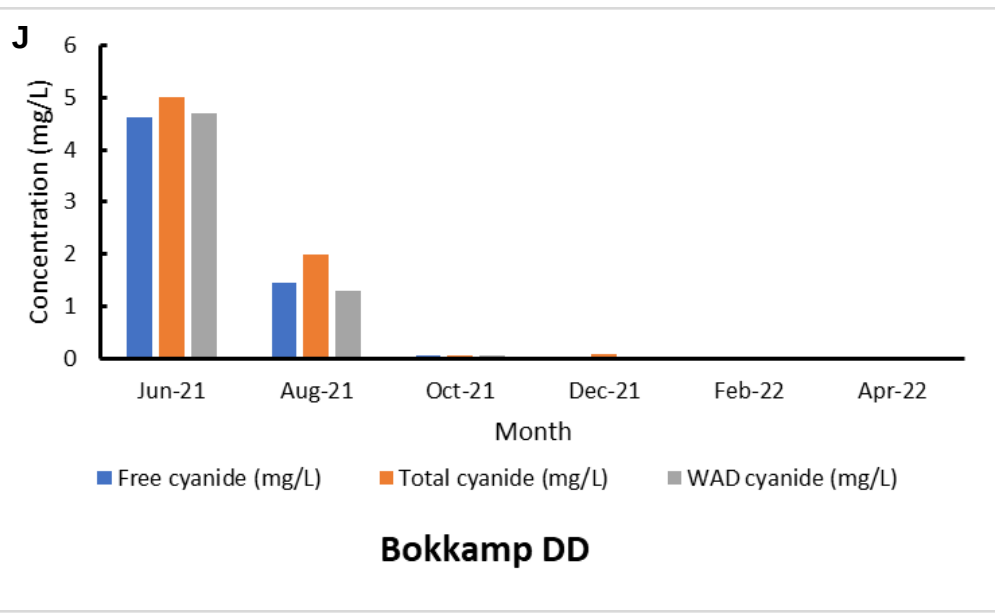
AEL DD

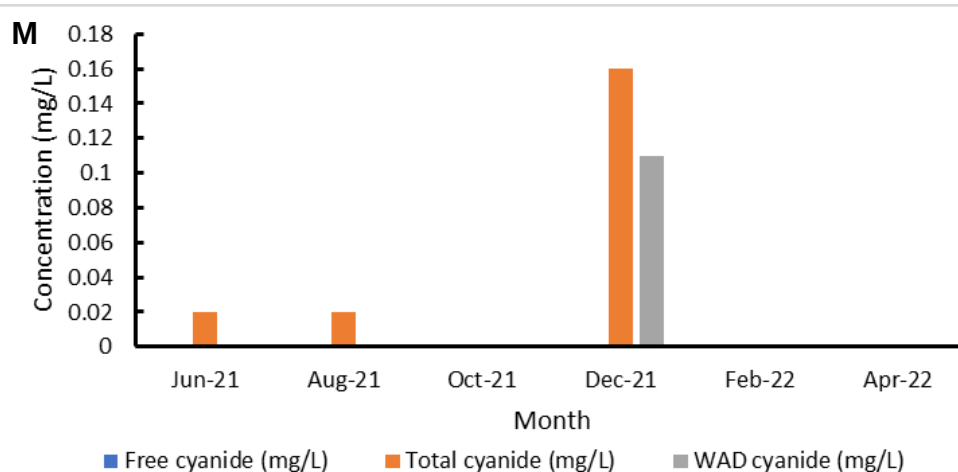


Eye Dam

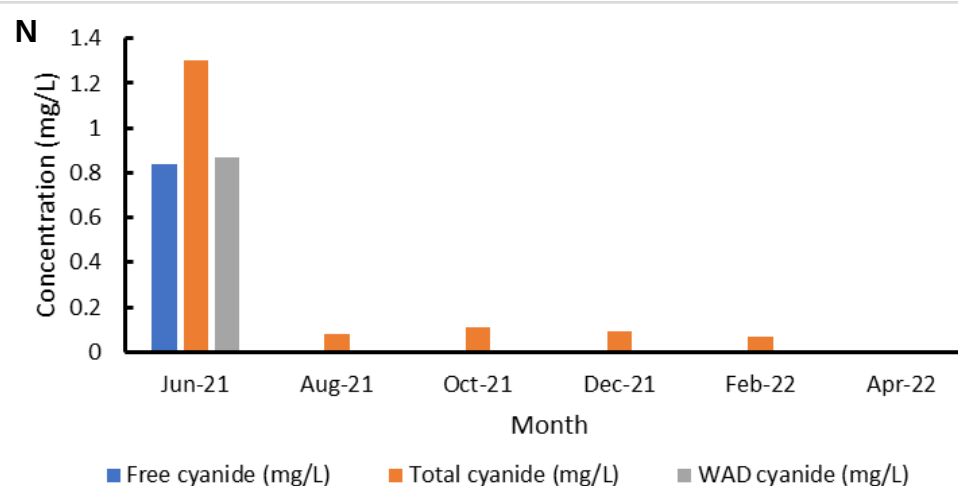


Bokkamp RWD

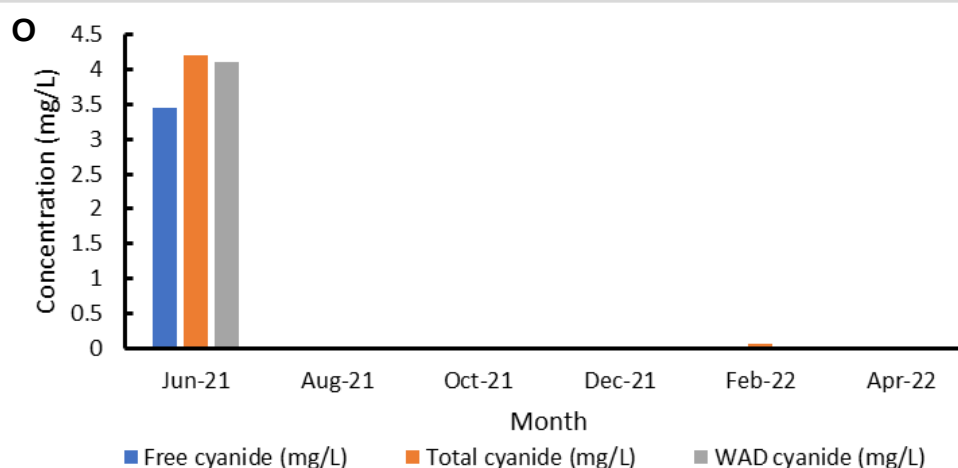




Queen Mary RWD



Queen Mary DD



West Storm Water

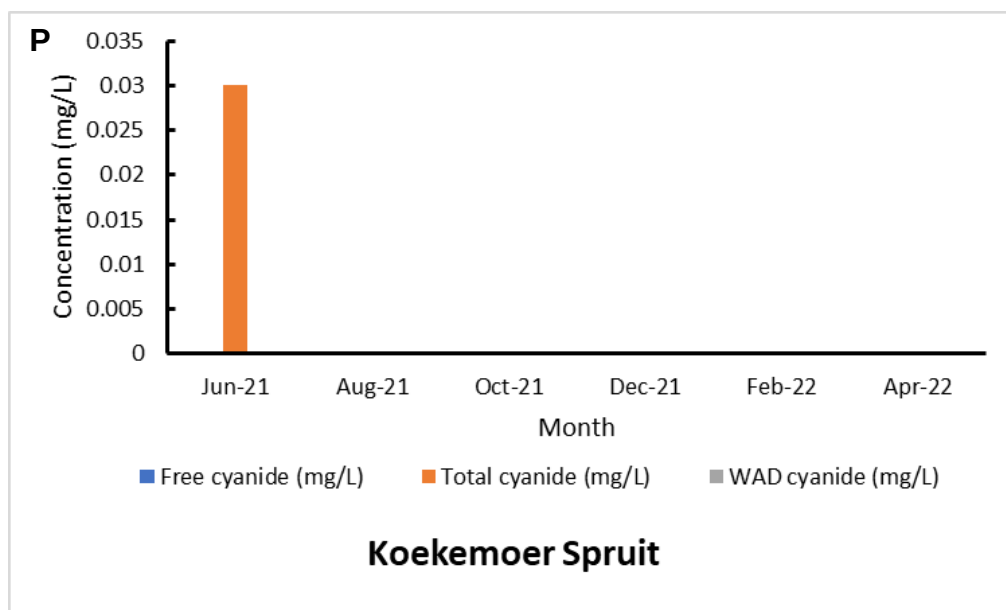


Figure 5: A comparison between the free, Weak Acid Dissociable (WAD), and total cyanide concentrations, between the different sites, over a period of one year.

As indicated in Figure 5, all the sites showed transient incidences of elevated cyanide concentrations. As specified by DWAF (1996c), the free cyanide concentrations were at acute levels (≥ 0.11 mg/L) at Karee Rand TSF (Fig. 5A) throughout the entire study, and West Extension TSF (Fig. 5K) from Jun-21 to Dec-21. Other sites also showed acute free cyanide concentrations, but only in some months; Karee Rand DD (Jun-21) (Fig. 5B), Karee Rand RWD (Jun-21 and Apr-2022) (Fig. 5C), Catchment Dam, AEL East, AEL DD, Bokkamp DD (Jun-21 to Aug-21) (Fig. 5D, E, G, J), AEL South, West complex TSF, Queen Mary DD, West Storm Water (Jun-21) (Fig. 5F, L, N, O), Bokkamp RWD (Jun-21, Aug-21, Oct-21) (Fig. 5J), and Queen Mary RWD (Dec-21) (Fig. 5 M). As seen in Table 3 and Figure 5P the reference sites had low cyanide concentrations (< 0.2 mg/L). But these sites may still contain chronic concentrations, since the chronic effect value for aquatic ecosystems is 0.004 mg/L (DWAF, 1996c).

Table 3: Cyanide concentrations in water of the three reference sites.

Ref A			
Date	Free Cyanide (mg/L)	Total Cyanide (mg/L)	WAD (mg/L)
Jun-21	< 0.02	< 0.02	< 0.02
Aug-21	< 0.02	< 0.02	< 0.02
Oct-21	< 0.02	< 0.02	< 0.02
Dec-21	-	-	-
Feb-22	< 0.02	< 0.02	< 0.02
Apr-22	-	-	-
Skoon Spruit			
Date	Free Cyanide (mg/L)	Total Cyanide (mg/L)	WAD (mg/L)
Jun-21	< 0.02	< 0.02	< 0.02
Aug-21	< 0.02	< 0.02	< 0.02
Oct-21	-	-	-
Dec-21	< 0.02	< 0.02	< 0.02
Feb-22	-	-	-
Apr-22	< 0.02	< 0.02	< 0.02
Rietgat Spruit			
Date	Free Cyanide (mg/L)	Total Cyanide (mg/L)	WAD (mg/L)
Jun-21	< 0.02	< 0.02	< 0.02
Aug-21	< 0.02	< 0.02	< 0.02
Oct-21	< 0.02	< 0.02	< 0.02
Dec-21	-	-	-
Feb-22	< 0.02	< 0.02	< 0.02
Apr-22	-	-	-

Figure 6 shows the mean cyanide values of each site over one year. This shows that the overall cyanide concentrations were higher at the tailing's storage facilities than at the reference sites, except West Complex TSF where deposition had stopped. Although this tailings storage facility and some other sites had lower concentrations of cyanide, concentrations were still chronic.

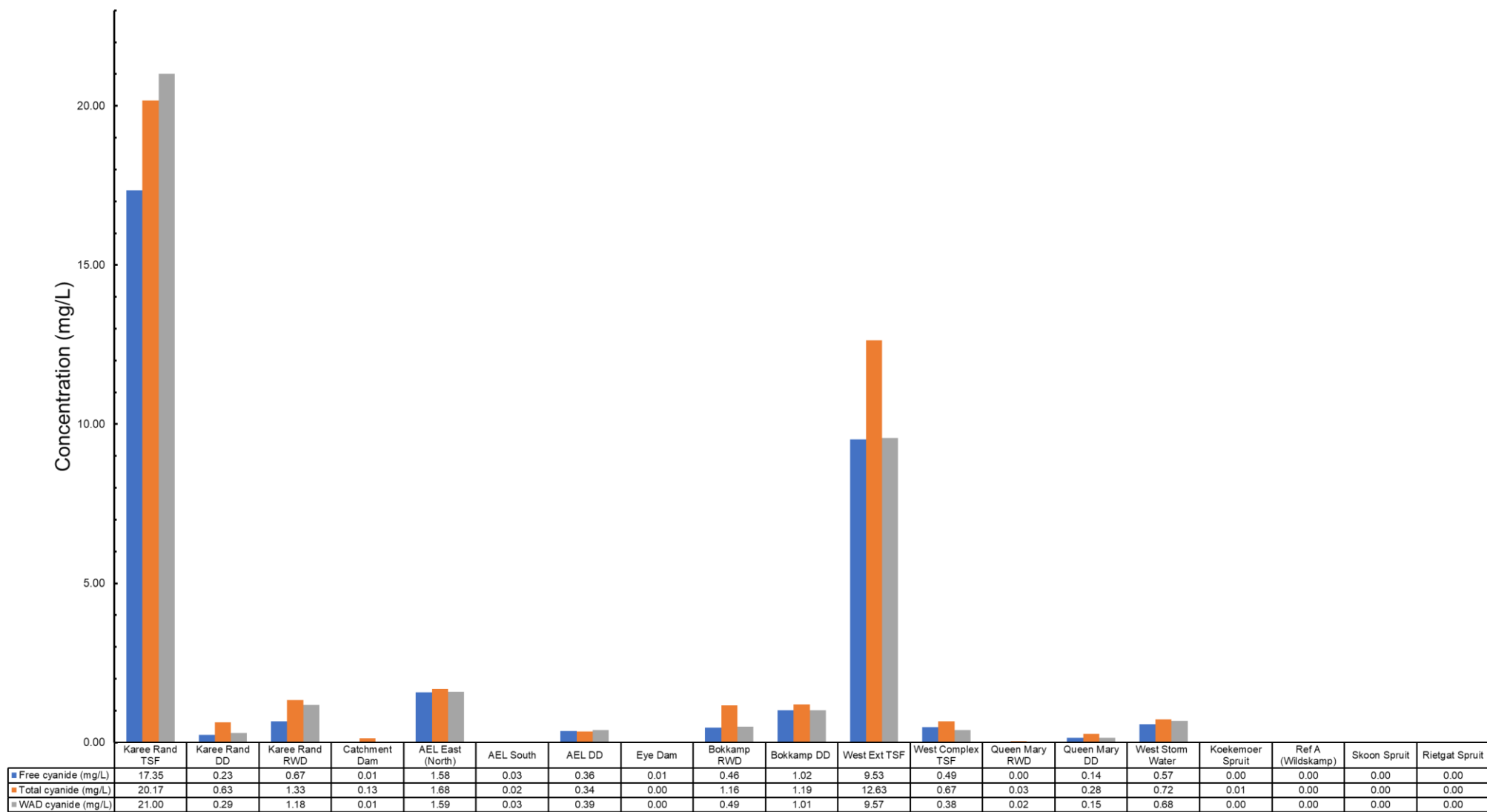


Figure 6: A comparison between the mean cyanide values of each site of one year.

The ordination biplot in Figure 7 (describing 75% of all variation) shows all similarities and dissimilarities in the water quality parameters among the various sites sampled in the North West province, from June 2021 to April 2022. The first axis (describing 42% of total variation) shows that as the distance increased from the TSF there was a decrease in total cyanide (recoverable cyanide) concentrations. The sites to the right of the Y axis (especially Karee Rand TSF and West extension TSF) had higher cyanide concentrations, due to cyanide being part of the gold extraction process (Rösner and Van Schalkwyk, 2000). Along the second axis (describing 33% of the total variation) the sites with a higher EC value were grouped at the top of the graph and the sites with a lower EC, but higher pH at the bottom.

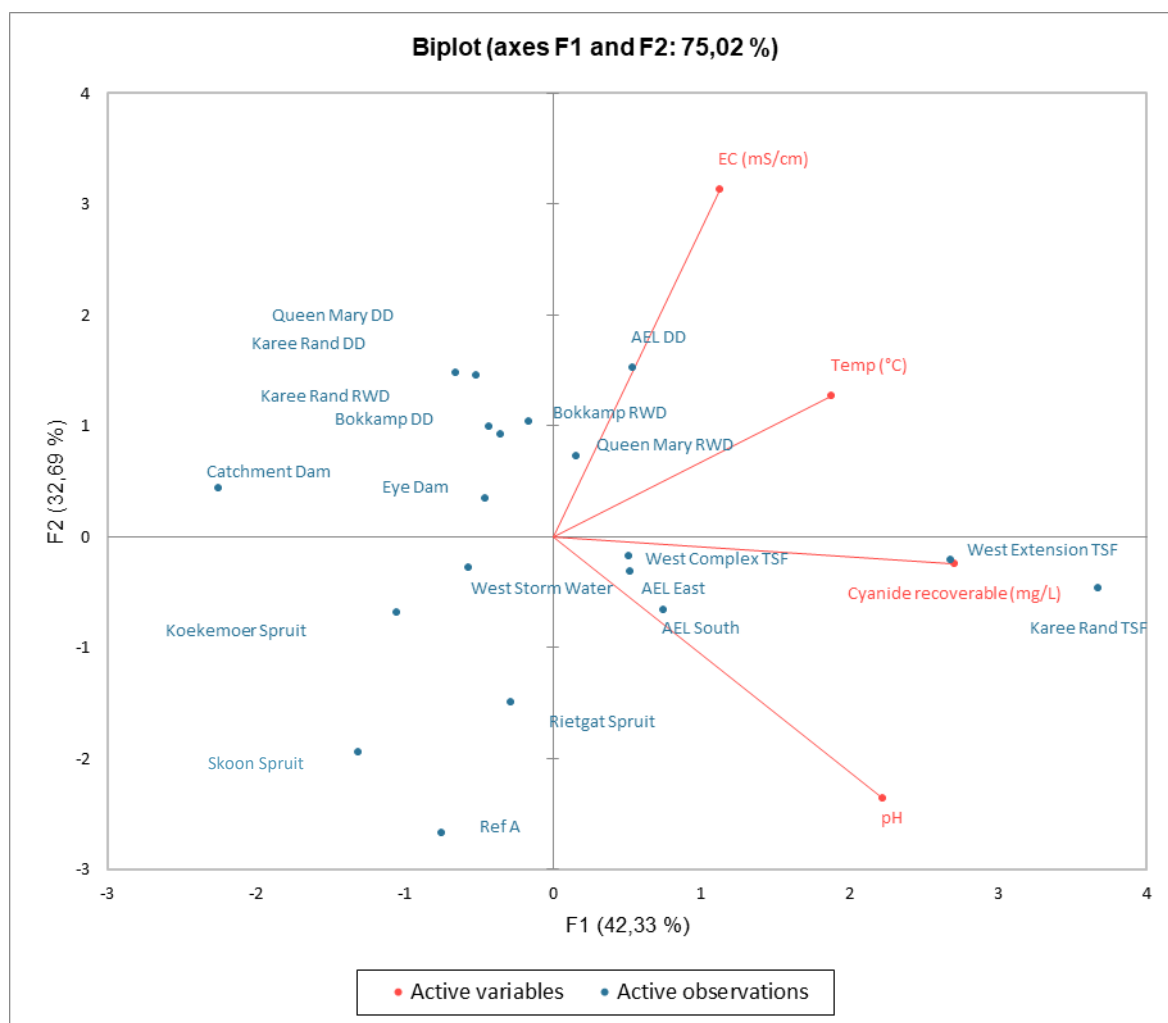
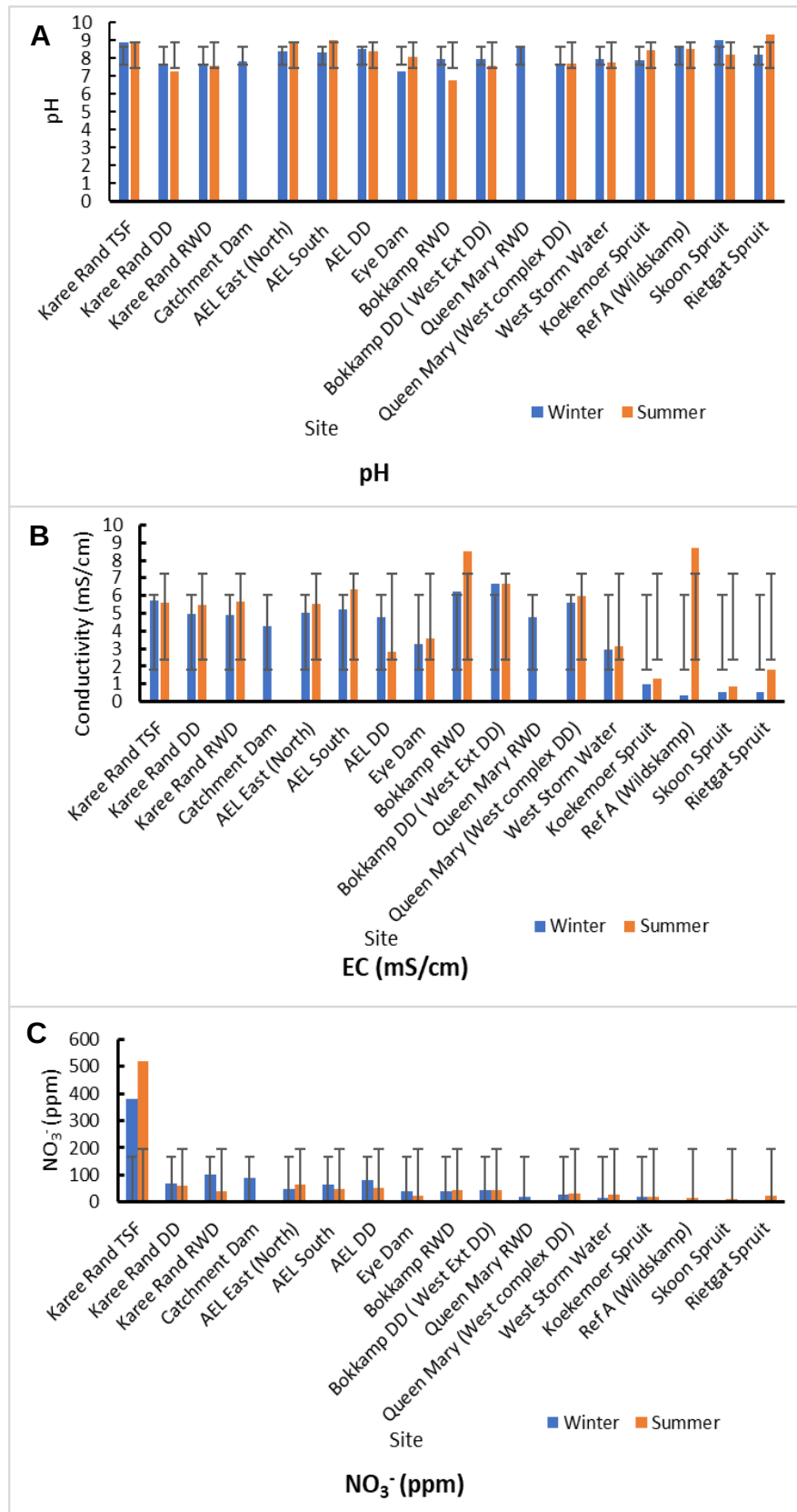


Figure 7: A PCA biplot of the similarities and dissimilarities between sites. The correlation biplot describes 75% of all variation, with 42% on the first axis and 33% on the second axis (Recoverable cyanide = Total cyanide)(Sampling dates: Jun-21, Aug-21, Oct-21, Dec-21, Feb-22, and Apr-22).

The bar graphs (Fig. 8) illustrate the different pH levels, EC, Nitrate (NO_3^-), dissolved oxygen (DO), and phosphate (PO_4^{3-}) concentrations, and temperatures in the water of the various sites in the winter and summer. From the normal distribution tests all data had a normal distribution pattern, except the nitrate and EC concentrations in the winter, due to outliers.

- The pH (Fig. 8A) stayed more or less the same, with no significant difference ($p>0.05$) between the sites or different seasons. The pH levels indicated slightly alkaline to alkaline conditions at all sites in both seasons.
- The EC concentration (Fig. 8B) also stayed mostly the same throughout the study with no significant differences ($p>0.05$) between the seasons at the various sites. There was a significant ($p<0.05$) difference between the sites in the winter, this could be due to the comparatively low concentrations found at the reference sites (Koekemoer Spruit, Rietgat Spruit, Ref A (Wildskamp) and Skoon Spruit). Summer had no significant differences, but this observation could be due to the large spike in EC concentrations at Ref A (Wildskamp) in this season.
- The NO_3^- concentrations (Fig. 8C) were relatively high at all sites, especially Karee Rand TSF, due to large amounts of explosives used in mining operations (Olenici *et al.*, 2017). The data indicated a significant difference ($p<0.05$) between the seasons for NO_3^- , as well as between the different sites. As specified by DWAF (1996c) all these sites sampled can be considered hypertrophic. Since the nitrate concentration alone was greater than the total inorganic nitrogen concentrations considered for hypertrophic conditions by the South Africa water quality guidelines for aquatic ecosystems, thus $>10 \text{ mg/L}$ (DWAF, 1996c).
- There was a relative decrease in the DO (Fig. 8D) from winter to summer, but the DO levels were still at a lethally low concentration (30-60%) in both seasons (DWAF, 1996c). There was no significant difference ($p>0.05$) in DO concentrations between the different sites in either season.
- The PO_4^{3-} concentrations (Fig. 8E) were relatively low in the winter and escalated in the summer, with hypertrophic conditions ($>0.25 \text{ mg/L}$) for both seasons, at all sites DWAF, 1996c. There was a significant difference ($p<0.05$) between the PO_4^{3-} concentrations at the various sites, as well as the different seasons.

- The temperatures (Fig. 8F) were similar for both the winter and summer seasons at Karee Rand TSF, whereas with the other sites the temperatures increased during the summer, which is normal for seasonal shifts.



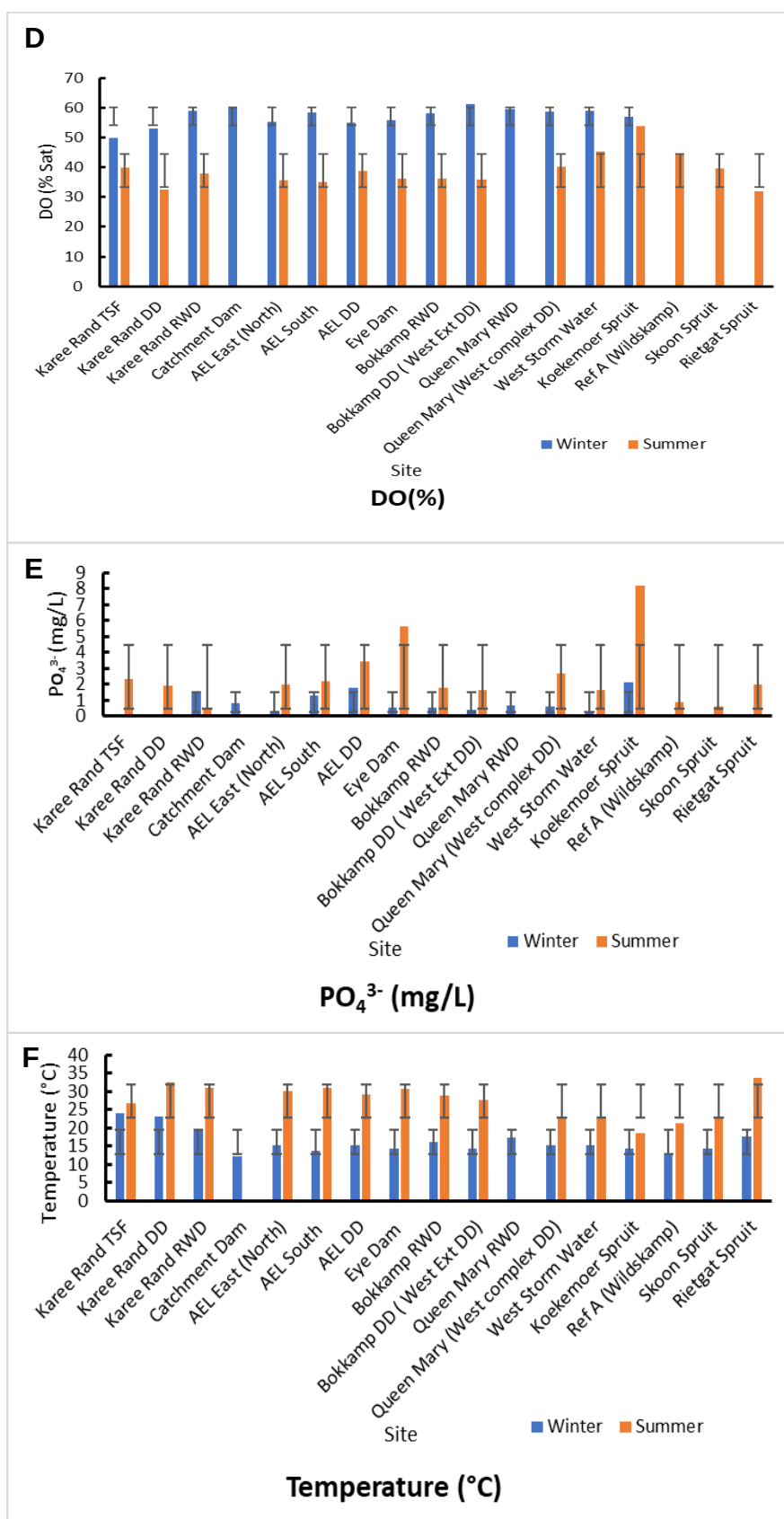


Figure 8: The column graphs (A-F) indicate the difference between pH, EC, NO_3^- , DO, and PO_4^{3-} concentrations and the temperature at the various sites between winter and summer. The bars indicate standard deviation.

3.1.1.2 Multivariate analyses

The correlation biplot in Figure 9 (accounting for 66% of all variation) illustrates similarities and dissimilarities in the water quality parameters among the various sites sampled in the winter. The closer the sample sites are to each other, the more they have in common. Variable vectors increase in the direction of the arrows and decreases away from the arrow vector. The closer the vectors ordinate towards each other in direction, the more that are co-variate. Perpendicular vectors have no association.

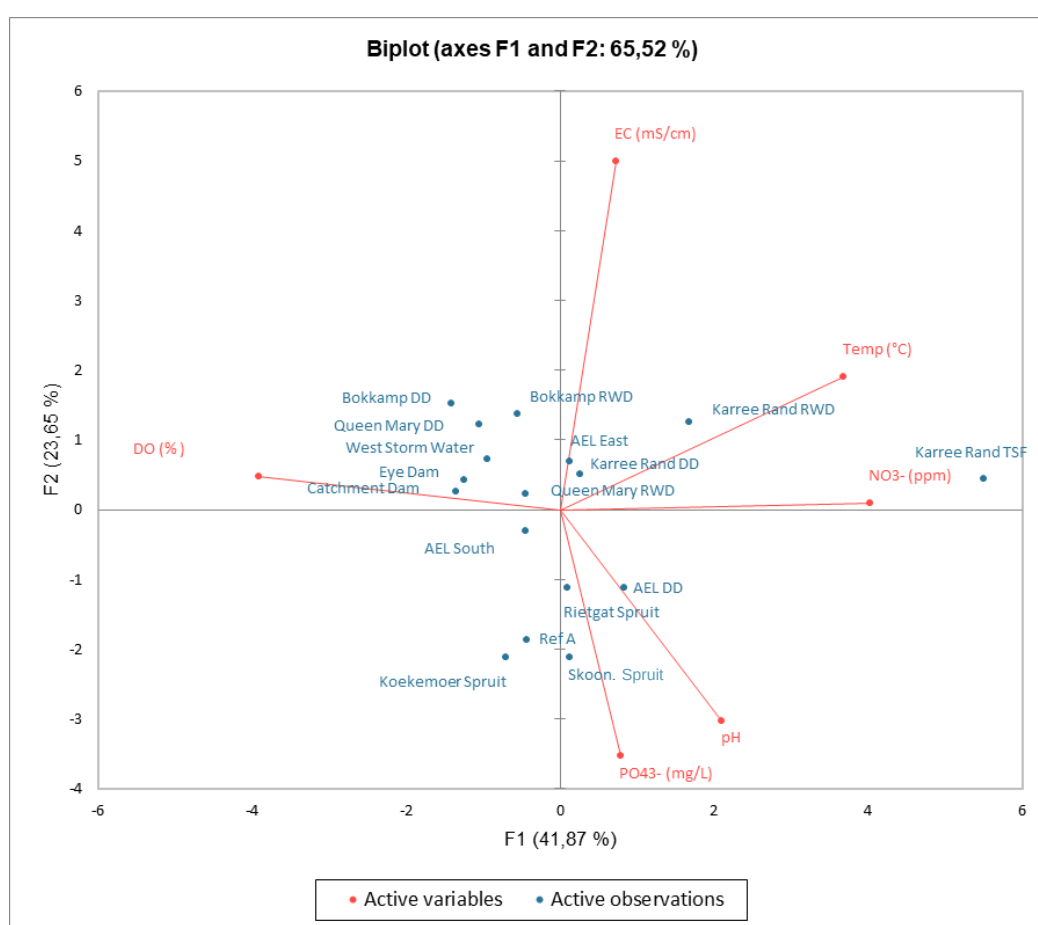


Figure 9: The PCA biplot showing the ordination of sites and water quality variables between the sites in the winter, the plots accounts for 66% of all measured variation, with 42% on the first axis and 24% on the second axis.

No diatoms were found at Karee Rand TSF throughout the whole study. Along the first axis (describing 42% of all variation) there was an increase in the NO_3^- concentration and temperature at the sites grouped at the right side of the graph, but with a decrease in DO concentrations. Whereas the sites grouped at the left side of the graph had higher DO concentration, but lower NO_3^- concentrations. Axis 1 therefore, showed a positive correlation with NO_3^- and temperature, with a strong negative correlation with

DO (%). On the second axis (describing 24% of all variation) the sites were grouped according to the PO_4^{3-} and pH variables, with a decrease in these values in the upper quadrants, while EC decreased to the lower half. The reference sites (Koekemoer Spruit, Ref A, Skoon Spruit, Rietgat Spruit) were grouped in the bottom quadrants, whereas the mining sites were grouped at the top, with higher EC concentrations, containing more dissolved solids, such as metals and dissolved salts.

The correlation biplot in Figure 10 (describing 62% of all variation) shows similarities and dissimilarities in water quality parameters among the various sites in summer. The variables associated with the first axis (describing 39% of all variation) are grouped according to their PO_4^{3-} and DO concentrations with a decrease to the left of the plot, Koekemoer Spruit had the highest PO_4^{3-} and DO concentrations. Along the second axis (describing 23% of all variation) the sites were grouped according to their NO_3^- and pH concentrations with a decrease in these concentrations toward the lower half of the plot, with Karee Rand TSF and Rietgat Spruit having the highest NO_3^- and pH concentrations. In comparison to the winter data in Figure 9, there was an entire shift in the relationship between water quality variables.

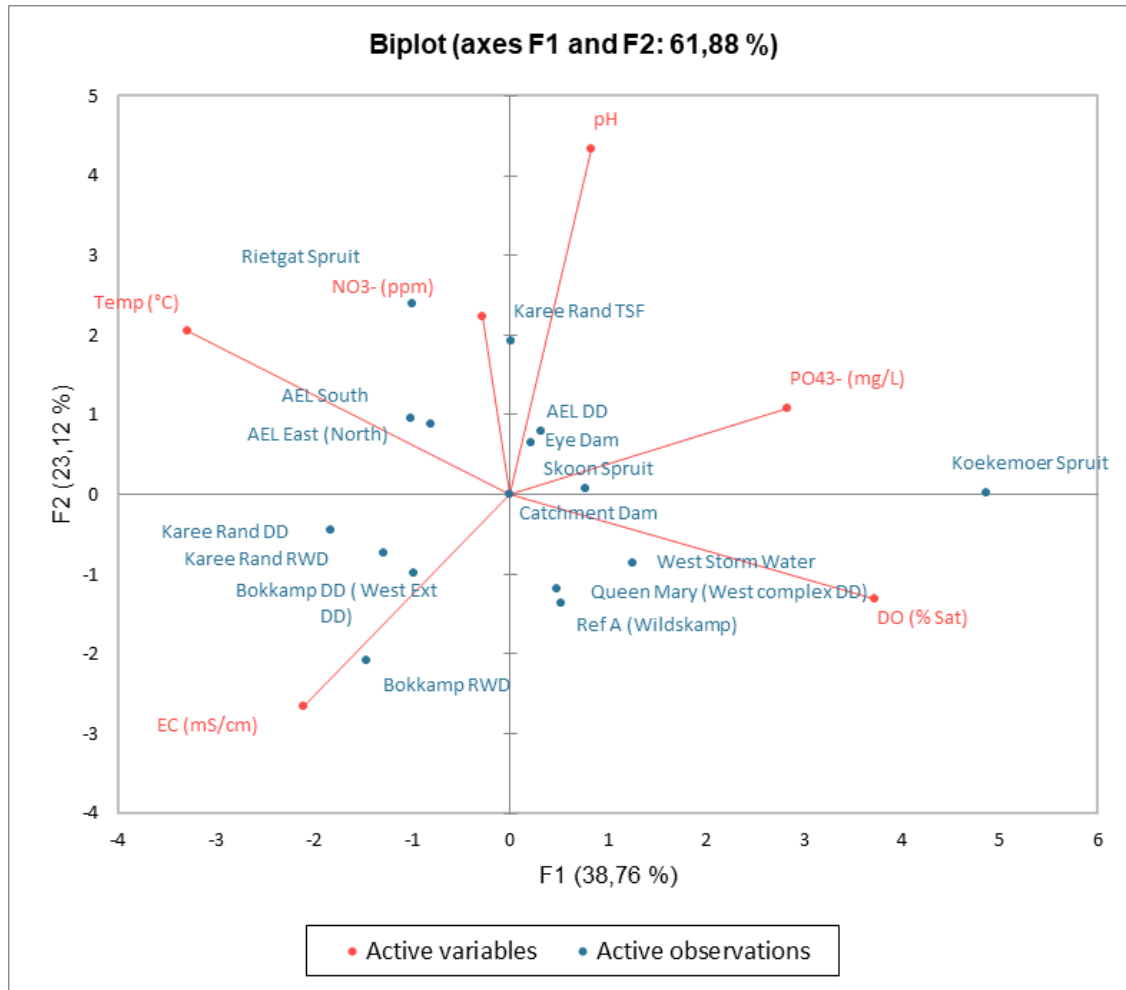


Figure 10: The PCA biplot of the differences between sites in the summer, showing 62% of all variation, with 39% on the first axis and 23% on the second axis.

Table 4 presents the elemental concentrations in sediments of the mining and reference sites. The elements with the highest concentrations included sodium (Na), magnesium (Mg), aluminium (Al), phosphorus (P), potassium (K), calcium (Ca), manganese (Mn) and iron (Fe), with values ranging from 59 mg/kg – 60000 mg/kg (Tables 4). The nickel (Ni), copper (Cu) and zinc (Zn) concentrations were especially high at AEL East, AEL South, Bokkamp RWD, and West Storm Water, which had concentrations of 3300 mg/kg – 14000 mg/kg.

When taking all elements into account, their concentrations were the highest at AEL East, AEL South, AEL DD, Bokkamp RWD and West Storm Water. In comparison with all the other sites, the concentrations of the elements were much lower at Karee Rand TSF, DD and RWD.

Table 4: Elemental concentrations (mg/kg DM) in the sediments at the different sampling sites (green indicates the minimum value and grey the maximum value for each element). (The number of minimum or maximum values found of an element per site is indicated in the last two rows). Locations and descriptions of the sites are provided in Figure 2 and Table 2 (Dates of sampling: June 2021, September 2021, January 2022, and May 2022).

Element	Site																		
	Karee Rand TSF	Karee Rand DD	Karee Rand RWD	Catchment Dam	AEL East	AEL South	AEL DD	Eye Dam	Bokkamp RWD	Bokkamp DD	West Extension TSF	West Complex TSF	Queen Mary RWD	Queen Mary DD	West Storm Water	Koekemoer Spruit	Ref A	Skoon Spruit	Rietgat Spruit
Be	0.34	0.23	0.26	0.96	1.4	0.92	1.6	1.1	0.98	0.67	0.58	0.74	0.7	0.3	0.52	1.6	0.86	0.95	1.6
B	2.6	1.4	1.2	8.4	7.4	39	6.2	8.9	5.7	3.9	3.3	4.5	5.3	1.5	3.8	12	7.9	4.8	14
Na	800	140	180	390	590	1200	690	750	1900	1300	480	480	1000	440	510	190	59	170	670
Mg	2400	2000	2200	3700	3000	3500	2900	2400	2800	2600	9000	3200	4500	6600	5300	380	2500	2500	3700
Al	4300	3200	3000	13000	11000	8600	9800	12000	9600	8000	7500	8900	7600	4300	7600	15000	11000	9900	17000
P	210	180	190	890	740	1400	700	1100	700	330	370	420	380	270	480	2200	700	850	1100
K	730	220	260	1800	1600	1400	1400	1800	2600	2200	1200	1900	1600	350	2100	1800	1300	870	1900
Ca	6200	2300	5100	6400	44000	60000	24000	32000	23000	5500	13000	6800	8500	12000	12000	5500	4600	6700	3200
Ti	58	74	60	170	150	120	95	250	65	59	150	86	98	190	130	250	180	230	280
V	7.3	7.5	6.3	42	19	21	16	37	19	15	17	19	16	13	21	47	28	37	50
Cr	36	26	29	72	53	46	66	76	58	47	52	57	54	40	56	79	62	100	140
Mn	610	320	420	710	7500	9900	2600	2400	24000	1800	600	1000	810	890	1100	590	220	470	440
Fe	16000	13000	15000	23000	15000	13000	16000	16000	16000	12000	14000	15000	13000	14000	16000	18000	13000	32000	18000
Co	44	32	38	61	220	170	73	190	140	27	17	26	31	24	180	26	8.2	9.3	10
Ni	70	50	65	94	1300	690	300	37	1800	74	63	75	93	57	690	60	19	31	42
Cu	36	18	42	150	7200	1400	310	23	10000	190	44	93	110	49	14000	34	11	16	21
Zn	100	67	92	120	1200	330	320	49	1100	98	74	100	79	47	630	79	21	17	62
As	87	51	61	150	210	150	200	9.1	260	75	29	81	49	56	150	7.6	1.9	2.6	20
Se	2.5	1.9	2.1	4.6	24	7.9	9.5	2.1	41	2.7	1.4	2.3	2.0	1.5	16	2.8	1.6	1.4	2.3
Rb	3.9	1.8	1.6	18	16	18	12	29	22	14	8	13	11	2.3	37	33	26	16	42
Sr	15.6	8.7	11	29	120	200	130	44	59	27	22	26	29	17	27	49	28	21	37

Mo	1.8	0.55	1.2	4.3	8.3	1.9	1.8	0.51	16	2.1	0.97	5.3	1.8	1.3	18	1	0.23	0.48	1.3
Pd	0.098	0.079	0.075	0.25	0.59	0.43	0.85	0.12	0.42	0.10	0.049	0.084	0.082	0.055	0.22	0.15	0.088	0.078	0.16
Ag	0.10	0.072	0.21	0.47	10	2.6	2.9	0.077	3.3	0.51	0.08	0.73	1.6	0.083	45	0.22	0.035	0.026	0.067
Cd	0.35	0.21	0.32	0.32	4.2	1.3	0.84	0.11	2.3	0.26	0.13	0.24	0.25	0.17	0.46	0.20	0.023	0.023	0.068
Sn	0.28	0.23	0.0022	0.59	0.58	0.42	1.5	0.36	0.96	0.41	1.3	0.91	1.2	1.5	0.4	1.2	0.45	0.36	0.82
Sb	2.2	0.82	1.3	2.1	4.3	2.3	1.2	0.23	8.6	0.99	0.80	1.2	1.8	0.93	6.5	0.75	0.096	0.18	0.24
Ba	48	28	31	130	120	210	220	570	230	140	51	150	78	30	73	1200	81	85	120
Pt	<LOQ	<LOQ	<LOQ	0.029	0.0057	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0140	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Au	0.061	<LOQ	0.80	0.095	0.43	1.0	1.1	0.80	0.16	0.035	0.08	0.16	0.60	0.060	12	0.12	<LOQ	<LOQ	<LOQ
Hg	0.31	0.12	0.17	0.51	2.8	0.77	2.4	0.10	0.67	0.34	1.7	0.58	2.0	0.27	0.55	1.3	0.055	<LOQ	0.014
Tl	0.029	0.0077	0.0075	0.12	0.12	0.14	0.10	0.24	0.20	0.070	0.057	0.070	0.059	0.0092	0.072	0.79	0.086	0.051	0.14
Pb	90	30	44	140	130	65	130	20	110	46	26	60	71	27	49	62	7.4	7.2	10
Th	22	16	18	29	23	13	30	4.8	23	11	6.5	12	9.5	6	12	5.6	3.4	3.1	5.1
U	65	63	59	78.0	120	82	120	11	190	23	13	22	20	17	39	12	0.5	0.47	1.0
Min	1	7	6	0	1	0	0	0	0	0	2	0	0	0	0	1	0	0	1
Max	0	0	0	2	4	3	4	0	8	2	0	0	0	1	4	4	9	7	6

3.1.2 Diatom community composition

Both planktonic and benthic diatoms were found at the various sites, presented in Table 5 organised according to order and family. Most of the taxa found were in the order Pennales, suborder Biraphideae, having a raphe on both valves.

Table 5: Classification of the diatoms found at the sites.

Order	Family	Description	Genus	Benthic Pelagic	Species richness
Centrales		Cylindrical/ filamentous taxa	<i>Aulacoseira</i>	P	3
			<i>Melosira</i>	B	1
		Single celled	<i>Cyclostephanos</i>	P	1
			<i>Cyclotella</i>	P	2
			<i>Discostella</i>	P	1
			<i>Stephanothrix</i>	P	1
			<i>Thalassiosira</i>	P	1
Pennales	Araphideae	Taxa with a pseudo raphe system	<i>Diatoma</i>	B	1
			<i>Fragilaria</i>	B	10
			<i>Pseudostaurosira</i>	B	1
			<i>Staurosira</i>	B	2
			<i>Staurosirella</i>	B	1
			<i>Tabularia</i>	B	1
	Monoraphideae	Taxa with a raphe on only one side of the valve	<i>Achnanthis</i>	B	4
			<i>Cocconeis</i>	B	4
			<i>Lemnicola</i>	B	1
			<i>Planothidium</i>	B	3
	Biraphideae	Taxa with raphe on both valves	<i>Amphora</i>	B	7
			<i>Anomoeneis</i>	B	1
			<i>Brachysira</i>	B	1
			<i>Caloneis</i>	B	2
			<i>Craticula</i>	B	6
			<i>Cymatopleura</i>	B/P	2
			<i>Cymbella</i>	B	4
			<i>Diadesmis</i>	B	1
			<i>Diploneis</i>	P	2
			<i>Encyonema</i>	B	2
			<i>Encyonopsis</i>	B	2
			<i>Entomoneis</i>	B	1
			<i>Eolimna</i>	B	4
			<i>Epithemia</i>	B	2
			<i>Fallacia</i>	B	1
			<i>Gomphonema</i>	B	14
			<i>Gyrosigma</i>	B/P	1

<i>Hantzschia</i>	P	1
<i>Hippodonta</i>	P	1
<i>Luticola</i>	P	2
<i>Mayamaea</i>	B	3
<i>Navicymbula</i>	B	1
<i>Navicula</i>	B	17
<i>Nitzschia</i>	B/P	28
<i>Pinnularia</i>	B	8
<i>Placoneis</i>	B	2
<i>Pleurosigma</i>	B/P	1
<i>Rhopalodia</i>	B/P	2
<i>Sellaphora</i>	B	2
<i>Stauroneis</i>	B/P	3
<i>Surirella</i>	B/P	2
<i>Tryblionella</i>	B/P	5

For the 19 sites, 6285 records of presence absence of diatom taxa were generated. In total, 49 genera and 169 taxa were identified in this study, with 98 tolerant species and 71 less tolerant species (Appendix C). The dominant taxa occurring in the sampled sites included *Achnantheidium* sp., *Amphora veneta*, *Brachysira neoexilis*, *Craticula buderi*, *Cyclotella meneghiniana*, *Eolimna subminuscula*, *Fragilaria ulna*, *Gomphonema parvulum*, *Navicula erifuga*, *Navicula riediana*, *Navicula veneta*, *Nitzschia amphibia*, *Nitzschia frustulum*, *Nitzschia nana*, *Nitzschia palea*, *Pinnularia* sp., and *Planothidium frequentissimum* (Appendix A).

The ordination biplot (describing 38% of diatom community variation) for similarities and dissimilarities of diatom communities among the various sites in the winter season is presented in Figure 11.

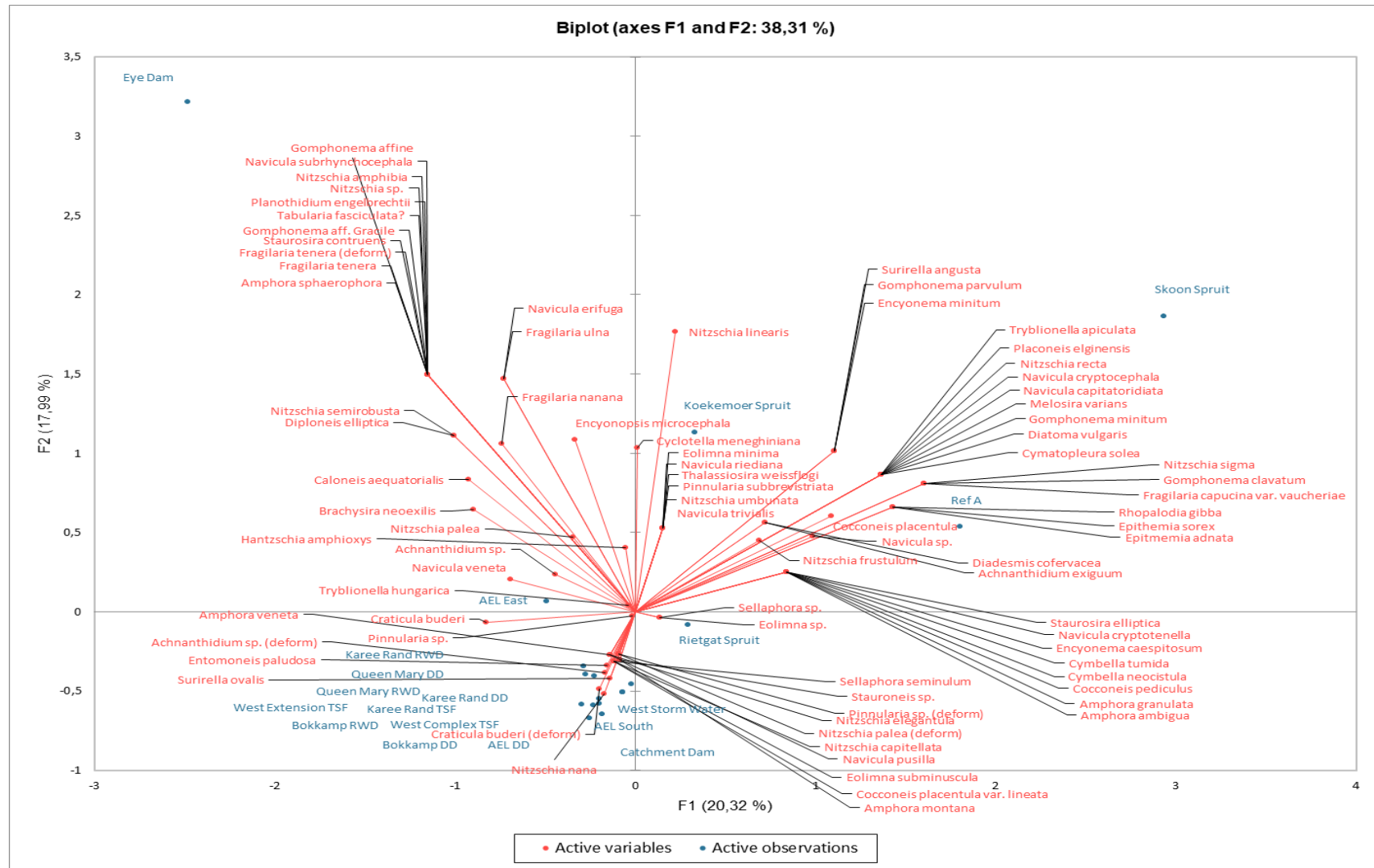


Figure 11: PCA showing the ordination of diatom taxa (presence or absence of diatom species or genus) among the sites in the winter. The two axes show 38% of all the variation, with 20% on the first axis and 8% and 18% on the second axis.

The first axis of Figure 11 (describing 20% of all variation) shows the grouping of the TSFs, as well as the sites near these facilities (such as the return water dams and drainage ditches) at the bottom left of the Y axis. These are sites with a low taxon richness, with *Craticula buderi* being dominant. The separation along the second axis (describing 18% of all variation) occurred as sites became more distant from the tailings storage facilities (TSFs), with an increase in taxon richness. All the mining sites had less taxa richness but had species resistant to industrial pollution, such as *Amphora montana*, *Amphora veneta*, *Craticula buderi*, *Entomoneis paludosa*, *Eolimna subminuscula*, *Nitzschia palea*, *Sellaphora seminulum*, and *Surirella ovalis*. Whereas Koekemoer Spruit, Skoon Spruit and Ref A grouped at the top right of the Y axis had a greater taxon richness, with less tolerant species, as well as tolerant species, such as *Cyclotella meneghiniana*, *Fragilaria capucina* var. *vaucheriae*, *Navicula cryptocephala*, *Nitzschia frustulum*, and *Nitzschia linearis*.

A biplot is presented in Figure 12 (describing 46% of all variation in the diatom community) showing similarities and dissimilarities between diatom communities of each site in summer. Along the first axis (describing 28% of all variation) (as was the case in Figure 11) the TSFs and associated sites are grouped together at the bottom left of the Y axis, with *Craticula buderi* deformed as one of the dominant species. The second axis (describes 18% of all variation), the sites with the highest taxon richness are grouped at the top left of the Y axis, but with a lower richness than within the winter. In comparison with Figure 11 the taxon richness decreased at both mining and reference sites from summer to winter. In comparison with winter (Fig. 11), there was a decrease in the taxon richness at all sites, including the reference sites located further away from the mining activities (Ref A, Koekemoer Spruit, Rietgat Spruit, Skoon Spruit). Figures 11 and 12 showed distinct separation of the reference sites from the mining sites.

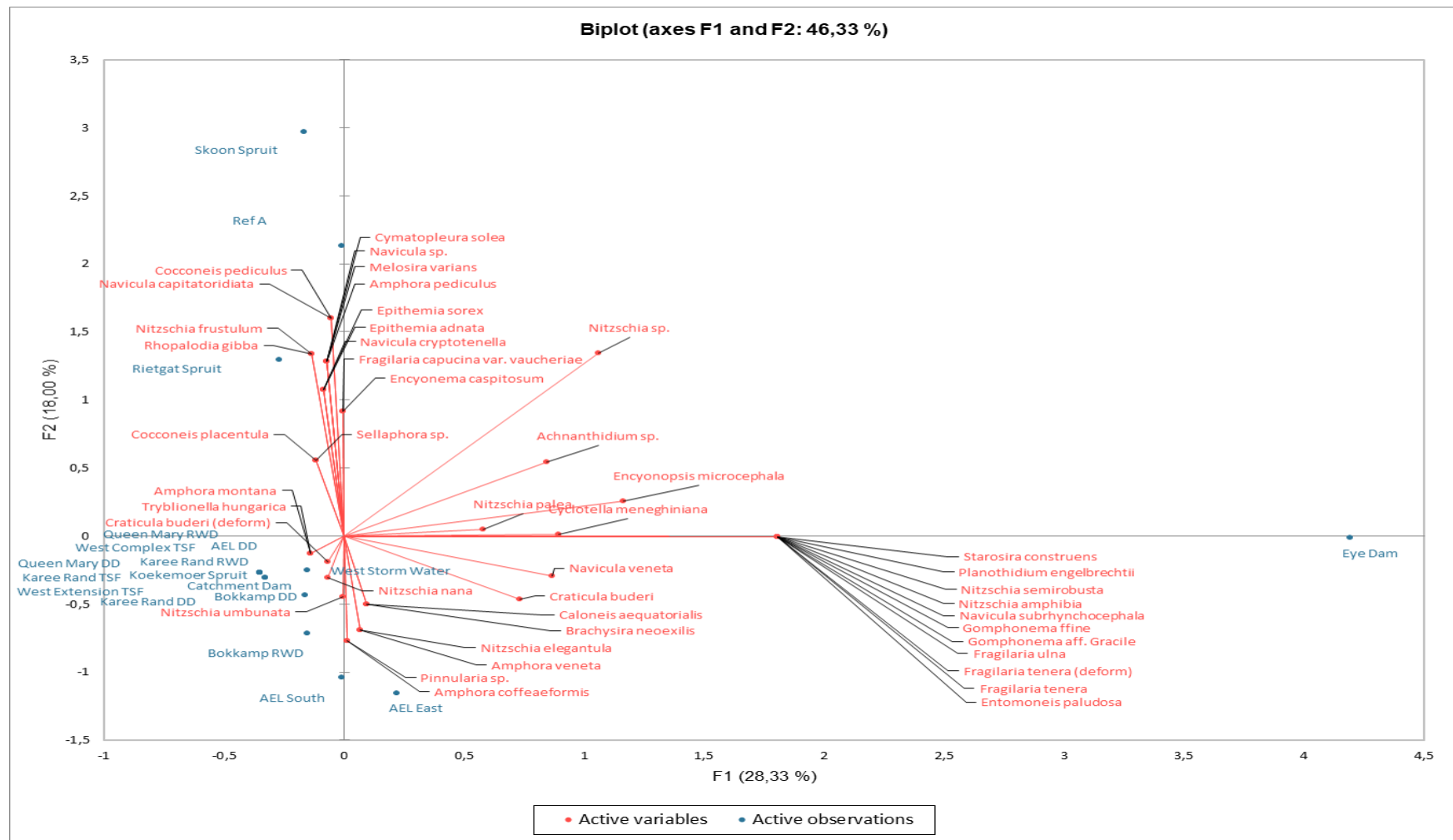


Figure 12: A PCA showing the difference in species diversity of the various sites in the summer, is showing 46% of all variation, with 28% on the first axis and 18% on the second axis.

A biplot ordination is presented in Figure 13 (describing 77% of all variation in the diatom community) showing similarities and dissimilarities between diatom communities with mean water quality variables from summer to winter. Along the first axis (describing 58% of all variation) the sites grouped according to their positive relationship to pH, DO and temperature. The sites having a higher pH, temperature and DO concentrations were grouped at the right side of the graph. Along the second axis (describing 18% of all variation) the NO_3^- and PO_4^{3-} concentrations show no association with each other.

The correlation biplot presented in Figure 14 (describing 75% of all diatom community variables) shows the structure of diatom communities in relation to electrical conductivity (EC), total cyanide (recoverable cyanide) concentrations, pH, and temperature from June 2021 to May 2022 (Fig. 14). Along the first axis (describing 42% of all variation), the sites are ordinated according to EC concentration. These sites consist of all the drainage ditches (Karee Rand DD, Queen Mary DD, AEL DD, Bokkamp DD) and return water dams (Karee Rand RWD, Bokkamp RWD, Queen Mary RWD) which had very high EC, especially AEL DD, which means that these sites had a very high dissolved salt content. As can be observed from Figure 14, these sites had a relatively low taxon richness, while those with lower EC had higher taxon richness.

Along the second axis (describing 33% of all variation), the sites are grouped according to total cyanide concentrations. The tailings storage facilities (West Extension TSF and Karee Rand TSF) were ordinated close together and associated with increasing cyanide concentrations and relatively low taxon richness (no diatoms were found at Karee Rand TSF throughout the whole study). With a decrease in cyanide concentrations taxon richness increased, especially Rietgat Spruit, Koekemoer Spruit, and Ref A (Wildskamp) (sites relatively far from the mining area, with an average distance of 31 km from the closest mining sites). Although deformities also occurred at the sites with lower cyanide concentrations, the number of deformities increased with an increase in cyanide concentrations. The total cyanide may have had an influence on the deformities, but deformities are difficult to ascribe to a single stressor with certainty, but what is clear is the impact of cyanide on the abundance and richness of the diatoms.

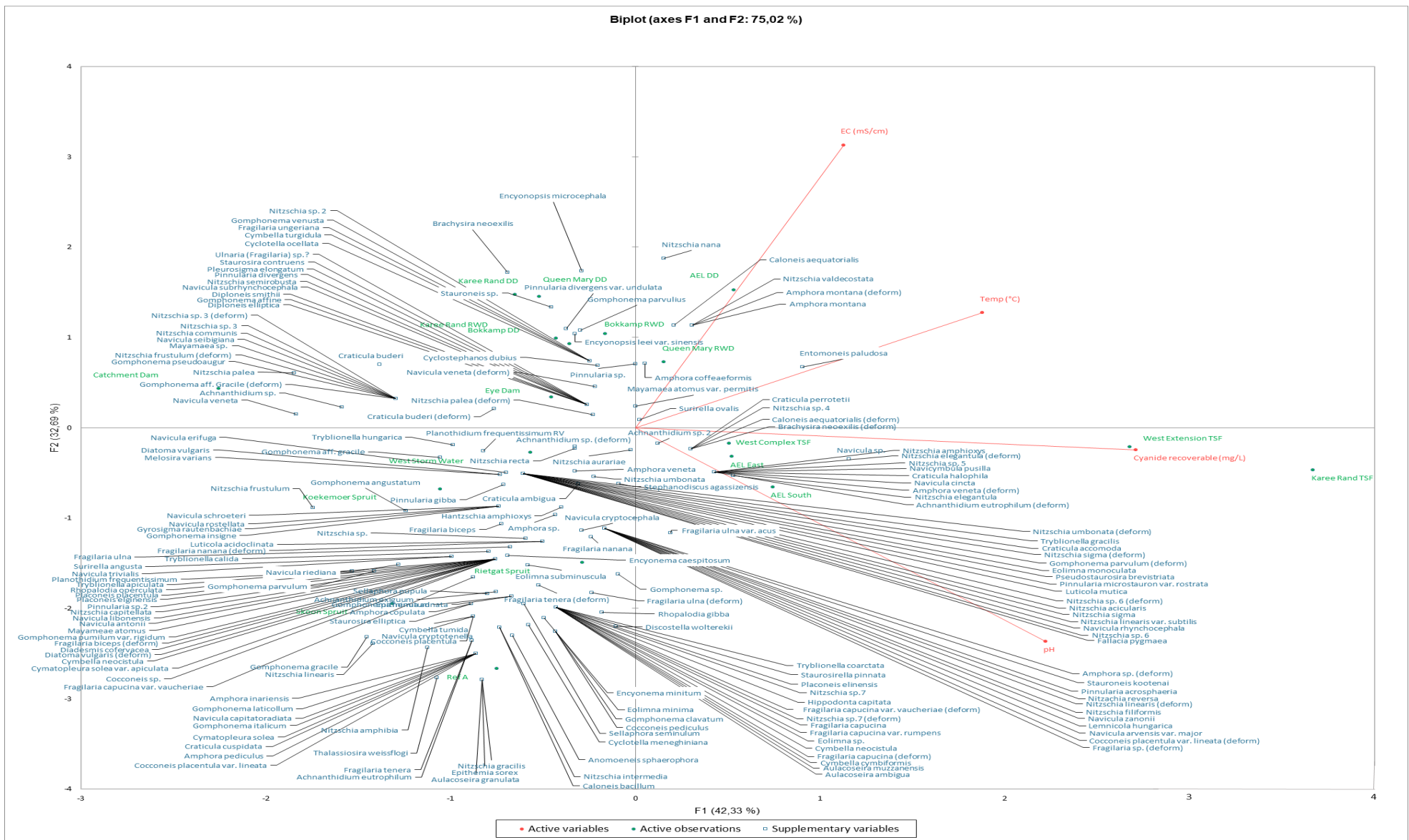


Figure 14: PCA plot illustrating the diatom community composition and water quality variables form an entire year (May- 2021 to May- 2022). The two axes account for 75% of all variation, with 42% on the first axis and 33% on the second axis (Recoverable cyanide = Total cyanide).

The samples collected further from the tailings storage facilities (Ref A, Rietgat Spruit, Skoon Spruit) had a relatively large species richness, with up to 25 species per sample (cyanide concentrations < 0.02 mg/L) (Fig. 5 & Appendix A).

The samples collected relatively near the tailing storage facilities, had a wide-ranging species richness, with 1 to 19 species per sample (cyanide concentrations < 0.02 to 8.5 mg/L). For example, AEL East (North) had 10 species present in August 2021, even with the cyanide concentrations of 8.5 mg/L, and Karee Rand RWD had 7 species present in April 2022, with cyanide concentrations of 7 mg/L (Fig. 5 & Appendix A). West Extension and West Complex tailings storage facilities were characterised by a much lower number of species, from 1 to 5 species per sample (cyanide concentrations < 0.02 mg/L), although diatoms were absent at West Extension and West Complex tailings storage facilities until cyanide concentrations dropped to below 0.02 mg/L. On the other hand, Karee Rand tailings storage facility, diatoms were completely absent throughout the study (cyanide concentrations 21 to 32 mg/L) (Fig. 5 & Appendix A).

The correlation biplot (describing 55% of all diatom community variation and heavy metal concentrations) for similarities and dissimilarities of the diatom communities and the mean elemental concentrations in the sediments from June 2021 to May 2022 is presented in Figure 15. Along the first axis (describing 34% of all variation), the sites grouped in conformity with their relationship with cobalt (Co), manganese (Mn), radium (Rd), selenium (Se), nickel (Ni), cadmium (Cd), zinc (Zn), copper (Cu), lead (Pb), antimony (Sb), arsenic (As), molybdenum (Mo), and uranium (U). The sites having the highest concentrations of these elements ordinated to the right, which included AEL East, AEL South, AEL DD, West Storm Water, and Bokkamp RWD. As observed from the biplot, the species having deformities increased with an increase in these elements, which consisted of *Achnantheidium* sp., *Craticula buderi*, *Fragilaria nanana*, *Fragilaria ulna*, *Gomphonema* aff. *gracile*, *Nitzschia palea* and *Nitzschia frustulum*. It can also be observed that there was an increase in the species richness with a decrease in the metal concentrations. Along the second axis (describing 21% of all variation), the sites ordinated together in conformity to their association with vanadium (V), chromium (Cr), barium (Ba), aluminium (Al), phosphorus (P) and rubidium (Rb).

Despite the response correlations, there were no associations found between the environmental concentrations, water quality, and deformations.

Species abundance ranges were ascribed a scale (Table 6 & 7). The majority of the samples had very low abundances, with diatoms being completely absent from some. At Karee Rand TSF there was an overall absence of diatoms, whereas at the West Extension TSF and West Complex TSF the diatoms increased from being absent to an abundance of >10 -100 (Mar-22 to May-22) at West Complex TSF and $>100 - 200$ cells.cm⁻² (Mar-22 to May-22) at West Extension TSF in autumn. The drainage ditches and return water dams (Karee Rand DD, Karee Rand RWD, Bokkamp RWD, Bokkamp DD (West Ext DD, Queen Mary RWD, and Queen Mary DD (West Complex DD)) had low abundances, with a mean of $>10 - 100$ cells.cm⁻², but both Karee Rand DD and Bokkamp DD (West Extension DD) had a large spike in diatom biomass in summer, with a range of $>200 - 300$ and $>300 - 400$ cells.cm⁻² respectively.

The Catchment dam and AEL East (North) had a relatively large biomass range in winter and spring ($>300 - 400$ & >400 cells.cm⁻²) but this decreased drastically in summer and autumn ($>100 - 200$ cells.cm⁻²). AEL South and Eye Dam had a relatively large increase in their biomass from spring to autumn with all samples containing $>200 - 300$ or more cells.cm⁻². AEL DD had low abundances throughout the year (>10 cells.cm⁻²), except for winter where there were at least $>100 - 200$ cells.cm⁻². As for the sites further from the mining activities (Koekemoer Spruit, Skoon Spruit, and Rietgat Spruit) they had the greatest abundance in spring with $>200 - 300$ or more cells, but with a mean of $>100 - 200$ cells.cm⁻² throughout the rest of the year. As for Ref A (Wildskamp) also located further away from the mining activities it had a relatively high abundance throughout the year with a mean abundance of $>300 - 400$ cells.cm⁻² throughout the study period.

Throughout the study, Eye dam and Ref A (Wildskamp) had the highest diatom abundances with a mean of $>300 - 400$ cells.cm⁻², and Karee Rand TSF the lowest, with a complete absence of diatoms.

Table 6: Diatom abundance range of each site for each season.

Site	Season					Scale	Mean diatom abundance range (cells.cm ⁻²)
	Winter (Jun-21 - Aug-21)	Spring (Sept-21 - Nov-21)	Summer (Dec-21 - Feb-22)	Autumn (Mar-22 - May-22)			
Karee Rand TSF	0	0	0	0	0		No Cells
Karee Rand DD	1	1	4	1	1		>0 - 10
Karee Rand RWD	1	0	0	1	2		>10 - 100
Catchment Dam	5	6	2	3	3		>100 - 200
AEL East (North)	5	5	2	3	4		>200 - 300
AEL South	2	4	4	6	5		>300 - 400
AEL DD	3	1	1	1	6		>400
Eye Dam	3	5	5	5			
Bokkamp RWD	1	2	2	5			
Bokkamp DD (West Ext DD)	1	0	6	4			
West Extention TSF	0	0	0	3			
West Complex TSF	0	0	1	2			
Queen Mary RWD	2	2	0	4			
Queen Mary (West complex DD)	0	3	1	0			
West Storm Water	1	2	6	6			
Koekemoer Spruit	2	4	3	2			
Ref A (Wildskamp)	5	4	5	4			
Skoon Spruit	1	4	3	3			
Rietgat Spruit	2	5	4	3			

Table 7: The mean diatom abundance range of one year for each site.

Site	Mean abundance of the entire year (Jun-21 - May-22)	Scale	Mean diatom abundance range (cells.cm ⁻²)
Karee Rand TSF	0	0	No Cells
Karee Rand DD	2	1	>0 - 10
Karee Rand RWD	1	2	>10 - 100
Catchment Dam	4	3	>100 - 200
AEL East (North)	4	4	>200 - 300
AEL South	4	5	>300 - 400
AEL DD	2	6	>400
Eye Dam	5		
Bokkamp RWD	3		
Bokkamp DD (West Ext DD)	3		
West Extension TSF	1		
West Complex TSF	1		
Queen Mary RWD	2		
Queen Mary (West complex DD)	1		
West Storm Water	4		
Koekemoer Spruit	3		
Ref A (Wildskamp)	5		
Skoon Spruit	3		
Rietgat Spruit	4		

3.1.3. Diatom deformations

The response of diatoms to chemical contamination is normally hard to assess, because they have the same reaction to acidification (Olenici *et al.*, 2017). As seen in Figures 4 and 8, the pH was relatively alkaline at all sites with a mean of 8.9, indicating that the diatoms mainly responded to the elemental and water quality variables, apart from pH condition.

The species that showed deformities in their cell structure related to elevated elemental concentrations, included *Achnanthydium eutrophilum*, *Achnanthydium* sp., *Amphora* sp., *Amphora montana*, *Amphora veneta*, *Brachysira neoexilis*, *Caloneis aequatorialis*, *Cocconeis placentula* var. *lineata*, *Craticula buderi*, *Diatoma vulgare*, *Fragilaria biceps*, *Fragilaria capucina*, *Fragilaria capucina* var. *vaucheriae*, *Fragilaria nanana*, *Fragilaria tenera*, *Fragilaria ulna*, *Gomphonema* aff. *gracile*, *Gomphonema parvullum*, *Navicula veneta*, *Nitzschia elegantula*, *Nitzschia frustulum*, *Nitzschia linearis*, *Nitzschia palea*, *Nitzschia sigma*, *Nitzschia* sp. 7, and *Nitzschia umbonata*, of which *Achnanthydium eutrophilum*, *Achnanthydium* sp., *Craticula buderi*, *Fragilaria nanana*, *Fragilaria tenera* and *Nitzschia palea* had the most deformed cells.

Table 8 illustrates the types of deformities that occurred in the different cells and species, these are grouped into Type 1 (deformed valve outline), Type 2 (alterations in the striation pattern, costae, and septae), Type 3 (raphe canal system alterations (twisted, disordered, extended fibulae), and Type 4 (mixed type (valve shows more than one deformity)).

There were irregular striation patterns found in *Craticula buderi*, *Fragilaria* sp. and *Diatoma vulgare*. *Achnanthydium eutrophilum*, *Achnanthydium* sp., *Amphora* sp., *Amphora montana*, *Amphora veneta*, *Brachysira neoexilis*, *Caloneis aequatorialis*, *Cocconeis placentula* var. *lineata*, *Craticula buderi*, *Diatoma vulgare*, *Fragilaria biceps*, *Fragilaria capucina*, *Fragilaria capucina* var. *vaucheriae*, *Fragilaria nanana*, *Fragilaria tenera*, *Fragilaria ulna*, *Gomphonema* aff. *gracile*, *Gomphonema parvullum*, *Navicula veneta*, *Nitzschia linearis*, *Nitzschia sigma*, *Nitzschia* sp.7 and *Nitzschia umbonata*, showed an abnormal outline of the valve, as well as slightly bent valves. *Nitzschia elegantula*, *Nitzschia frustulum* and *Nitzschia palea* had an abnormal orientation of the marginal raphe system, orientated toward the centre of the valve.

Table 8: Types of deformities that occurred at the various sites during the study (All photos in table by Talitha Brits).

Types	Description	Examples		
Type 1	Deformed valve outline			
		<i>Craticula buderi</i> in Catchment Dam	<i>Craticula buderi</i> in West Ext TSF	<i>Epithemia adnata</i> in Rietgat Spruit
Type 2	Alterations in the striation pattern, costae, and septae			
		<i>Diatoma vulgaris</i> in Skoon Spruit		
Type 4	Raphe canal system alterations (twisted, disorder, extended fibulae)			
		<i>Nitzschia palea</i> in AEL East (North)	<i>Nitzschia elegantula</i> in AEL South	<i>Nitzschia frustulum</i> in Catchment Dam
Type 5	Mixed type (one valve shows more than one deformity)			
		<i>Fragilaria sp.</i> in Eye Dam	<i>Craticula buderi</i> in AEL South	<i>Nitzschia sp.7</i> in Catchment Dam

The primary deformities include an abnormal valve outline and alterations in the raphe system, whereas secondary deformation includes modifications in the stria organisation and fibulae distribution (Table 8).

Chapter 4

4.1. Discussion

4.1.1. *Physico-chemical parameters in water*

The condition of aquatic environments can be inferred by measuring the water quality variables of that environment (Kock *et al.*, 2019). Measurements may include cyanide (CN^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), electrical conductivity (EC), temperature, and pH.

4.1.1.1 *Electrical conductivity*

Electrical conductivity (EC) is the water's ability to conduct an electrical current and is an estimation of the total dissolved salts or ions (Pal *et al.*, 2015). EC has a high dependency on the temperature of the water (Pal *et al.*, 2015). The high EC values that were obtained in the study (Fig. 4 & Fig. 8 B) are due to the occurrence of ions, metals or dissolved salts such as iron (Fe^{3+}), sulphate (SO_4^{3-}), phosphorus (P), and nitrate (NO_3^-) within the mine wastewater (Acheampong *et al.*, 2013). The EC of the TSFs; Karee Rand TSF, West Extension TSF, and West Complex TSF (Fig. 4A, K, L) was most probably high due to an increase in dissolved concentration as a result of high evaporation rates (Acheampong *et al.*, 2013), as well as leaching of salts from the base minerals. One of the reference sites, Ref A (Wildskamp) also had a large spike in summer (Figure 8 B), this could be due to intensive game farming and agriculture observed, runoff generated during rainfall events, but likely a combination of both.

Electrical conductivity is therefore considered to play a role that will stress biological systems in water in most of the mining sites, but not at the reference sites.

4.1.1.2 *pH*

The pH of surface waters within South Africa normally ranges from 4 to 11, with the well buffered waters ranging between 6 and 8 (DWAF, 1996c). The pH of the samples collected in the present study ranged between 6.79 and 11.74 (Fig. 4 & Fig. 8A), which puts them in the water quality range of South Africa's water quality guidelines for

aquatic ecosystems, although any pH above or at 11 can be considered extremely caustic. This also means that the sites ranged from weakly acidic to alkaline environments. The relatively high pH values can most likely be due to liming, which is added to the acid slime before it is disposed onto the tailings dam (Rösner and Van Schalkwyk, 2000). Even though the pH of the effluent was very high (highly alkaline), the TSFs (Fig. 4A, K, L), return water dams (Fig. 4C, I, M) and drainage ditches (Fig. 4B, J, N) waste water, as well as other sites (Fig. 4D-I, O-S) pH's progressively decreased over time, as a result of the neutralisation of the alkaline environment due to rainwater and probably also through the uptake of carbon dioxide (Acheampong *et al.*, 2013).

The production of NO_3^- and SO_4^{2-} through the biodegradation of cyanide and thiocyanate may also have played a part in the slight decrease within pH values observed in the TSFs (Fig. 4A, K, L) (Acheampong *et al.*, 2013). Although the pH levels ranged between weakly acidic and alkaline in the present study, these environmental conditions may also have an influence on aquatic organisms. If there is a change in the H^+ (hydrogen) and OH^- (hydroxide) ion concentrations, it could influence the ionic balance of the organism's body fluids. Slight changes in the pH are considered as non-lethal but can still impede growth rates and reduce fertility due to the physiological stress placed on the organism (Day and Dallas, 2011).

Since pH varies little and largely stays between norms according to season, it is not expected to have a direct impact on biological systems in the water. However, as will be seen below, it may affect the toxicity of other elements that occurred at high concentrations at some of the mining and reference sites.

4.1.1.3 Nitrate

Nitrate (NO_3^-) is a highly oxidised form of nitrogen that is commonly found in water and wastewater and causes algal blooming, resulting in eutrophication in the surface water (Acheampong *et al.*, 2013). The NO_3^- concentrations measured during the present study for all sites (Fig. 8C) indicated hypertrophic conditions, when these values are compared to South Africa's water quality guidelines for aquatic ecosystems (DWA, 1996c). It must be kept in mind that these regulations are formulated for riverine systems. The nitrate concentrations also exceed the target water quality range (0-6

mg/L) for domestic use (DWAF, 1996a), which ranged at the sites from 11-520 mg/L (Fig. 8C). The inorganic nitrogen concentrations within South Africa in unimpacted, aerobic surface waters are usually below 0.5 mg N/L but can be 10 mg N/L in highly enriched waters (DWAF, 1996c). The high NO_3^- concentrations found in the present study may be due to the use of explosives in the mining industry (Olenici *et al.*, 2017) and the mineral salts deriving from rocks and soils during the mining process (DWAF, 1996c).

NO_3^- is therefore likely to stress biological systems in the aquatic environment of this study where it exceeds norms in both reference and mining sites.

4.1.1.4 Temperature

The water temperature plays an important role in the rate at which chemical reactions occur (DWAF, 1996c; Bhateria and Jain, 2016), consequently also the metabolic rates of the organisms, thus affecting the distribution patterns of organisms (DWAF, 1996c). The temperatures of the sampled sites ranged from 9-33 °C (Fig. 4 & Fig. 8F), which is in the general range of inland water, in compliance with South Africa's water quality guidelines for aquatic ecosystems (DWAF, 1996c). The temperatures are minimum and maximum values for one year, thus accounting for all season changes, and therefore are not an immediate temperature change, but a temperature change over time.

Since temperature plays a role in chemical reaction rate, it can have an influence on the toxicity of some of the chemical parameters, elements, and the oxygen holding capacity of water (DWAF, 1996c; Acheampong *et al.*, 2013). But temperature alone is suspected to have no impact on the biological systems in the water of both reference and mining sites.

4.1.1.5 Dissolved oxygen

Adequate dissolved oxygen (DO) concentrations are crucial for the persistence and functioning of aquatic organisms, and can therefore be a useful measure in assessing the overall condition of the aquatic environment (DWAF, 1996c). The DO concentrations ranged from 50% to 61% in the winter and from 32% to 53% in the

summer. These low DO concentrations can have serious effects on aquatic organisms (DWAF, 1996c). In accordance with the South African water quality guidelines for aquatic ecosystems (1996c), if the DO concentration drops below 100%, the DO is exhausted from the theoretical equilibrium concentration. The low DO values obtained in this study for the tailings dam wastewater as well as the supplementary sites could be due to the insufficient aeration (Fig. 8D). In addition, the low oxygen-holding capacity of the wastewater is most likely due to the high temperatures and the (bio) chemical oxidation of the sulphide ore minerals (DWAF, 1996c; Acheampong *et al.*, 2013). Temperature also has an effect on the DO concentrations, causing a decrease in the DO with an increase in temperature (Bhateria and Jain, 2016), which also accounts for the drastic change in DO concentrations between winter and summer (Fig. 8D).

Therefore, the very low DO concentrations found at both reference and mining sites, especially in the summer, is likely to cause stress to the biological systems in the aquatic environment.

4.1.1.6 Phosphate

Phosphate (PO_4^{3-}) is one of the most important nutrients for plants and animals within the food web. As phosphorus is generally limited in most fresh water sources, even a slight change in these concentrations might have a large effect on the aquatic environment, including accelerated plant and algal growth, as well as oxygen depletion as plants die off (Bhateria and Jain, 2016). The PO_4^{3-} concentrations were very high at all sites, ranging from 0.4 to 8.2 mg/L (Fig. 8E), indicating hypertrophic water conditions (>0.25 mg/L) (DWAF, 1996c), except for AEL East and West Storm Water, which showed eutrophic conditions (0.025-0.25 mg/L) in the winter, with PO_4^{3-} concentrations of 0.2 mg/L. The high phosphorus concentrations at the sites can be attributed to the high PO_4^{3-} concentrations within the mine wastewater (Acheampong *et al.*, 2013). Another natural occurring event that could have played an additional role in the increase in the phosphate concentration, was the high rainfall that occurred throughout the year, carrying nutrients overland and into water sources (DWAF, 1996c).

When Figure 9 (summer data) and Figure 8 (winter data) were compared, there was an entire shift in the relationship between water quality variables, which is expected, because with warmer temperatures there is an increase in the chemical reaction rate (DWAF, 1996c; Bhateria and Jain, 2016).

The high water quality variable concentrations at the Bokkamp Dam are expected, due to the dam being a licenced pollution control dam, which receives wastewater from the TSFs and recycles it back to the metallurgical process (Lusilao-Makiese *et al.*, 2014).

PO_4^{3-} is a very important nutrient; its high concentrations at the sites in this study will most likely affect the biological systems in the water of some sites. One of the reference sites (Koekemoer Spruit) is expected to be affected the most, since it had the highest PO_4^{3-} concentration, but this occurrence can be attributed to the rural settlement and grazing cattle next to this stream, not the mining activities.

4.1.1.7 Cyanide

The cyanide contamination is due to its utilisation in the mineral (gold) extraction process. The toxicity of the cyanide is dependent upon temperature, pH, DO concentrations, and salinity, as well as other ions (DWAF, 1996c; Kuyucak and Akcil, 2013). The decrease in cyanide concentration in the wastewater of the TSFs in Figure 5 (A, K, and L) could be a result of a natural degradation, such as volatilisation, complexation, or biodegradation. Naturally existing microbial activity causes the transformation of cyanide to NH_3 (ammonia) (Acheampong *et al.*, 2013; Kuyucak and Akcil, 2013), which is then oxidised to nitrate (Kuyucak and Akcil, 2013), but this process is limited to a maximum cyanide concentration of 200 mg/L, which means that this process will occur at the sites, since all the cyanide concentrations were <200 mg/L (Kuyucak and Akcil, 2013).

Cyanide reacts with sulphide minerals, as well as fractionally oxidises sulphur intermediates to produce thiocyanate (Acheampong *et al.*, 2013). The thiocyanate is considered less toxic and more stable than cyanide. The natural conversion of cyanide to NH_3 mostly depends upon the environmental conditions and may not produce an effluent that complies to the discharge requirements (Acheampong *et al.*, 2013). In the case West Complex TSF (Fig. 5L) the lower concentrations might also be due to the

termination of waste deposition, especially the sudden drop in concentrations at West Complex TSF. Considering Karee Rand TSF (Fig. 5A), the continuous discharge of tailings into the dam, may slow or hamper the rate at which the natural cyanide conversion occurs (Acheampong *et al.*, 2013).

Looking at the other sites sampled surrounding the tailings storage facilities (TSFs), although the cyanide concentrations were not in the preferred water quality range, the TSFs showed adequate in removing more than 90% of the free cyanide in the wastewater. This is likely due to the dissociation of cyanide through sunlight which produces free cyanide that is released into the atmosphere (Johnson, 2015). This shows that the TSFs are effective settlers removing >90% of all cyanide concentrations. As a result of the continuous loading of the tailings storage facilities with fresh process effluent, the biodegradation of the cyanide may be low. Nevertheless, the dam is capable of containing the toxic effluent and preventing severe environmental contamination of the surrounding area with toxic metals and cyanide. As mentioned in the results section (Figure 5 P and Table 3) the cyanide concentrations of the reference sites were low (< 0.2 mg/L). However, these sites may still contain chronic concentrations, since the chronic effect value for aquatic ecosystems is 0.004 mg/L (DWAF, 1996c), especially Koekemoer Spruit. These sites are located relatively far upstream; thus, the possibility of acute concentrations may be due to runoff from the mining sites, or groundwater contamination.

The cyanide code has a 50 mg/L WAD cyanide concentration limit for the protection of wildlife for open waters (International Cyanide Management Institute, 2021). Even though the WAD cyanide from the TSFs were high, they still complied with this code being less than 50 mg/L.

The toxicity of cyanide can increase due to low DO concentrations (DWAF, 1996c), as found at the study sites (Fig. 7D). Which means that although the cyanide concentrations complied with the cyanide code, it might still have an effect on the biological systems in the water. The reference sites may be affected to a much lesser extent, with slight to no drastic effects.

Summary: Of all the physico-chemical and cyanide parameters discussed above, cyanide, DO, NO_3^- and PO_4^{3-} will most likely have the largest negative effects,

individually and in combination, on biological systems in the water of some of the mining and reference sites.

4.1.2. Elemental parameters in sediments

Chalcophile trace elements such as Cd, Cu, Ag, Zn, Hg, Pb, Se, Tl, and Mo are geologically connected to sulphide minerals that include pyrite (Aucamp and Van Schalkwyk, 2003). Chalcophile elements are elements with a strong affinity for sulphur (Chalcophile, 2024). Following gold extraction from the pyrite minerals, these trace elements persist in the tailings (Aucamp and Van Schalkwyk, 2003). Cobalt, Cr, Cu, and Zn concentrations within the gold mine tailings are normally higher than the threshold concentrations of the soils. Therefore, gold mine tailings are considered as one of the main sources of soil pollution associated with mining areas (Rösner and Van Schalkwyk, 2000). These trace elements' concentrations increased with a decrease in pH as was expected. The metal contamination of the water released to the environment would be due to the processing method and the type of gold-bearing ore processed (Acheampong *et al.*, 2013).

4.1.2.1 Calcium

Calcium is an important nutrient for all living organisms, especially those with calcium carbonate skeletal elements. Calcium and magnesium (Mg) are the main constituents of water hardness. The harder the water, the higher the Ca concentration. A known characteristic of Ca is to mitigate the toxicity of some toxic metals (DWAF, 1996a). All the sites had high Ca concentrations (2300 mg/kg – 60000 mg/kg) (Table 4) in their sediments, which is expected due to the wastewater undergoing a liming process, before the acid slime is added to the tailings dam (Rösner and Van Schalkwyk, 2000).

The high Ca concentrations in sediment found at the sites is not expected to have a direct effect on aquatic biology. However, it may decrease the toxicity of some elements as will be listed below.

4.1.2.2 *Iron*

When the availability of iron (Fe) is low, it can cause a decrease in the photosynthetic productivity of plants (DWAF, 1996c). However, this was not the case in this study since the Fe concentrations ranged between 12000 mg/kg and 32000 mg/kg (Table 4). Although Fe is an essential micro-nutrient, it can also be toxic (DWAF, 1996c, Zaid *et al.*, 2020). Fe is considered toxic to plants at concentrations >500 mg/kg (Zaid *et al.*, 2020). This toxicity is dependent on its ferrous or ferric state, but still has limited toxicity (DWAF, 1996c).

Therefore, the high Fe concentrations found in the sediments of all the study sites, especially the reference site, Skoon Spruit, may be considered toxic and can cause stress and affect the biological systems that are exposed.

4.1.2.3 *Manganese*

Manganese (Mn) is comparable to Fe due to its chemical reaction toward air and water and is often found associated with Fe. The concentration of Mn in the water depends on the DO concentrations, pH levels, and organic matter. Although Mn is an essential nutrient, it is toxic at high concentrations (DWAF, 1996c) just like Fe. The Mn concentrations were relatively high in the sediments at all sites, ranging from 220 mg/kg to 24000 mg/kg (Table 4).

Although the high Mn concentrations in sediments can be toxic to the aquatic mining environments, the reference sites will be affected to a lesser extent.

4.1.2.4 *Zinc*

The toxicity of zinc (Zn) is dependent on numerous interactions in the aquatic environment. The hardness of the water has a major effect on the toxicity, with a decrease in the toxicity coupled with an increase in the water hardness. Although copper can increase the toxicity of Zn in soft water, it cannot in hard water. Zinc toxicity can also increase with a decrease in DO concentrations and an increase in cyanide concentrations (DWAF, 1996c). The Zn concentrations ranged from 17 mg/kg to 1200 mg/kg (Table 4). The Canadian interim sediment quality guidelines for Zn are 123

mg/kg and the probable effect concentrations are 315 mg/kg (Canadian Environmental Quality Guidelines, 1999g). In compliance with these guidelines, most sites had concentrations lower than the interim sediment quality guidelines, except for AEL DD (320 mg/kg), AEL South (330 mg/kg), West Storm Water (630 mg/kg), Bokkamp RWD (1100 mg/kg), and AEL East (1200 mg/kg).

As mentioned earlier in this section, all sites had high Ca concentrations, which can then decrease the toxicity of the Zn. But with the low DO concentrations and relatively high cyanide concentrations at the sites, the Zn can still have a large negative impact on the plant life. Thus, Zn can still be considered toxic to the biological systems in the water of some mining sites, as well as the other sites, depending on species, physico-chemical parameters and bioavailability of the Zn (Canadian Environmental Quality Guidelines, 1999g).

4.1.2.5 *Molybdenum*

Molybdenum concentrations in unpolluted soils normally range between 0.10 mg/kg to 40 mg/kg (DWAF, 1996b). There are no quality guidelines for Mo in sediments, therefore soil guidelines were used. This could be used because most of the dams sampled can dry out. The concentrations found in the sediments at the sites ranged from 0.23 mg/kg to 18 mg/kg (Table 4), which still falls in the natural occurring range. The availability of Mo to plants tends to increase with an increase in pH. This element is considered essential, but only in low concentrations, although plants can take up a relatively large amount, without any negative effects (DWAF, 1996b).

Since the Mo concentrations in sediments of this study are within natural range with relatively low concentrations, this element is not expected to have a direct effect on aquatic biological systems. However, considering the high pH levels found at the sites, Mo might have a slight toxic effect at some reference and mining sites.

4.1.2.6 *Rubidium*

Rubidium (Rb) is associated with potassium (K) and cesium (Cs) minerals in soils (Campbell *et al.*, 2005). These two elements are alkali metals (group IA on the periodic table) and share similar chemical characteristics. Both elements also have the same

number of valence electrons, which is one. It may be an important nutrient for humans as well as other organisms, including plants (Campbell *et al.*, 2005). The measured Rb concentrations ranged between 1.6 mg/kg and 42 mg/kg at the different sites (Table 4). Rb uptake by plants can increase with increasing pH (Kosla *et al.*, 2002), but the uptake can be governed in environments with high K concentrations (Kosla *et al.*, 2002), which means that this element might have only been absorbed minimally. There is no Canadian sediment quality guideline for Rb.

Therefore, since the Rb concentrations were relatively low at most sites, and the K concentrations very high at all sites, the chances of Rb having an effect on the biological systems are considered very low.

4.1.2.7 Selenium

Selenium (Se) is considered an essential nutrient for animals but not plants (DWAF, 1996c). There is no Canadian sediment quality guideline for Se, but this element normally occurs in the range of 0.01 to 2 mg/kg in unpolluted soils (Van Hoewyk, 2013). The sediments at most sites had concentrations more or less in this range, except AEL South (7.9 mg/kg), AEL DD (9.5 mg/kg), West Storm Water (16 mg/kg), AEL East (24 mg/kg), Bokkamp RWD (41 mg/kg), which were much higher (Table 4). This element can also decrease the toxicity of mercury (Hg), thallium (Th), silver (Ag), and cadmium (Cd) (DWAF, 1996c).

Thus, this element might only have a negative effect on the biological system of some of the sites, but not the reference sites.

4.1.2.8 Sodium

Some halophytes may need sodium (Na) for growth (Mäser *et al.*, 2002). However, in general, this element is considered toxic to most plants at high concentrations (Mäser *et al.*, 2002; Nieves-Cordones *et al.*, 2016), which can cause osmotic stress, hampering of essential enzyme activity, and competition with K (Mäser *et al.*, 2002). There is no Canadian sediment quality guideline for Na, but as seen in Table 4 the Na concentrations were relatively high at most sites (ranging from 59 mg/kg to 1900

mg/kg) which could have an influence on the abundance as well as the type of vegetation and algal species found at the various sites.

Therefore, the Na concentrations could have a restrictive effect on biological systems in the water of both reference and mining sites.

4.1.2.9 Copper

Copper (Cu) is also an essential mineral for plant growth and development (Yruela, 2005) which is regularly found as an impurity in numerous mineral ores (DWAF, 1996c). Although considered an essential element, it could also cause disorders in plant growth as well as development, when insufficient or in excess (Yruela, 2005). The Canadian interim sediment quality guideline for Cu is 35.7 mg/kg and the probable effect concentration is 197 mg/kg (Canadian Environmental Quality Guidelines, 1999d). The Cu concentrations in sediments of the present study were relatively low, most sites having concentrations from 11 mg/kg to 93 mg/kg, except Queen Mary RWD (110 mg/kg), Catchment Dam (150 mg/kg), Bokkamp DD (190 mg/kg), AEL South (1400 mg/kg), AEL East (7200 mg/kg), and West Storm Water (14000 mg/kg) (Table 4). This means that only six sites (Ref A (11 mg/kg), Skoon Spruit (16 mg/kg), Karee Rand DD (18 mg/kg), Rietgat Spruit (21 mg/kg), Eye Dam (23 mg/kg), and Koekemoer Spruit (34 mg/kg) had concentrations below the guideline levels.

Copper concentrations >100 mg/kg are toxic to various plants but are considered deficient when below or equal to 5 mg/kg (Zaid *et al.*, 2020). The toxicity of this element is also highly dependent on the water quality of the aquatic environment. The toxicity of this element decreases with an increase in pH and the presence of Zn, sulphate, Ca or magnesium (Mg). Copper concentrations may increase with a decrease in DO and pH, and the presence of some other elements (DWAF, 1996c).

Although the sites with the high Cu concentrations had high pH levels (Fig. 4 & 8A) as well as Ca and Mg concentrations (Table 4), the DO concentrations were very low at all sites (Fig. 8D), which means that the Cu could still have had a serious effect on the plant life, even though these other elements were present. Even at the sites with the lower Cu concentrations, it can still have a serious effect on plant life, because of very low DO concentrations. Thus, the Cu concentrations are considered toxic to biological

systems in the water at most mining sites. Although some of the other sites, including the reference sites, consisted of low concentrations, the Cu might still have an effect on the aquatic environment. Copper, therefore, stands out as an important toxic element in this study.

4.1.2.10 Arsenic

The arsenic (As) concentration could be high at mining sites as a consequence of its use in ore extraction processes (DWAF, 1996c, Hasanuzzaman *et al.*, 2015). Arsenic is a non-vital and toxic element to plants, especially in high concentrations, inhibiting plant growth. In the case of very high concentrations, it can even cause plant death due to the interference with metabolic processes (Hasanuzzaman *et al.*, 2015).

Some of the sites had very high As concentrations, including Catchment Dam (150 mg/kg), West Storm Water (150 mg/kg), AEL South (150 mg/kg), AEL DD (200 mg/kg), AEL East (210 mg/kg), and Bokkamp RWD (260 mg/kg). The TSFs also had relatively high As concentrations, Karee Rand TSF (87 mg/kg), and West Complex TSF (81 mg/kg), which can partially be attributed to water evaporation leading to concentration of the element. West extension TSF on the other hand had relatively low As concentrations (29 mg/kg). The interim sediment quality guidelines for As are 5.9 mg/kg and the probable effect concentrations are 17 mg/kg (Canadian Environmental Quality Guidelines, 1999a). This indicates that most sites had concentrations higher than the probable effect value, except Ref A (1.9 mg/kg), Skoon Spruit (2.6 mg/kg), Koekemoer Spruit (7.6 mg/kg), and Eye Dam 9.1 mg/kg (Table 4).

Moreover, the arsenic bioavailability is also associated with the pH level of the wastewater (Acheampong *et al.*, 2013). At low pH, the As will be adsorbed onto ferric hydroxide particles, which settles to the sediment. But the pH of these TSFs were not low enough for this process to occur (Fig. 4A, K, L & Fig 8A). That being so, the amount of soluble arsenic in liquid phase in the TSFs remained consistently high.

Temperature also plays an important role in the toxicity of As, with the toxicity increasing with an increase in temperature. This means that As would have a larger impact on organisms in the environment in the summer compared with winter (DWAF, 1996c).

Therefore, As is expected to have negative effects on the biological systems at most mining sites and two of the reference sites, and less to no effect on the aquatic life at the reference sites located further from the mining activities (Ref A, Skoon Spruit) due to their low As concentrations. Arsenic therefore, should be considered a major concern in this region.

4.1.2.11 Aluminium

Aluminium (Al) concentrations were high at the study sites, ranging from 3000 mg/kg to 17000 mg/kg (Table 4), which is most probably due to the mining activities which allow for the solubilization of Al. This element is normally found as aluminosilicate minerals, which are regarded as not soluble enough to take part in bio-chemical reactions (DWAF, 1996c). The solubility of Al is highly dependent on the pH levels (DWAF, 1996c; Roy *et al.*, 1988). In the natural pH range, this element is considered insoluble, but becomes soluble in a highly alkaline environment (with pH > 8) or in the presence of complexing ligands (DWAF, 1996c; Delhaize and Ryan, 1995). Although this element is soluble in these conditions, it still occurs as biologically inaccessible hydroxide complexes.

It has been shown that aluminium can influence both the cycling and availability of elements such as phosphorus, organic carbon, and specific trace metals (DWAF, 1996c), such as Cu, Zn, Ca, Mg, Mn, K, Pb and Fe, which in turn can affect plant growth (Roy *et al.*, 1988). However, given that Al toxicity is largely attributed to acidic conditions, it is normally assumed that Al^{3+} is the leading phytotoxic species (Delhaize and Ryan, 1995). There is no Canadian sediment quality guideline for Al.

Therefore, although the sites had very high Al concentrations, they most likely occurred in the form of $\text{Al}(\text{OH})^{2+}$ or $\text{Al}(\text{OH})_2^+$, which is not as toxic as Al^{3+} , which has a lesser effect on plant growth (Delhaize and Ryan, 1995). This implies that this element would only have a slight effect on the biological systems in the water of both mining and reference sites.

4.1.2.12 Cadmium

Cadmium (Cd) is a very toxic element which can impede the growth and development of plants (Nazar *et al.*, 2012; Zulfiqar *et al.*, 2022). The solubility of Cd is pH dependent (DWAF, 1996c; Nazar *et al.*, 2012). A decrease in solubility follows an increase in pH above 9 (DWAF, 1996c). Cd itself is considered insoluble in water, but numerous of its organic, as well as inorganic salts are extremely soluble. The presence of other metals can have an influence on the toxicity of Cd. In the presence of both Cd and mercury (Hg), it can cause a decrease in both elements' toxicity. However, if Cd and copper (Cu) occur together, it can cause a drastic increase in the toxicity of both the elements. Zinc on the other hand can increase the toxicity of Cd to certain aquatic plants (DWAF, 1996c) as Zn increases the uptake of Cd (Nazar *et al.*, 2012). Selenium has a shielding effect against Cd toxicity for both aquatic plants and animals (Nazar *et al.*, 2012; DWAF, 1996c). This is attributed to the characteristic of Se being able to form a complex with metals and the binding of low molecular-mass proteins (Nazar *et al.*, 2012).

The cyanide concentrations in an aquatic environment can also influence the toxicity of Cd. The bioaccumulation and intake of Cd within an aquatic environment is highly dependent on water quality conditions. The uptake by algae and bacteria increases with increase in temperature and decreased pH levels (DWAF, 1996c). It has also been shown that Cd can have a negative influence on the uptake and assimilation of essential nutrients, causing nutrient deficiency (Zulfiqar *et al.*, 2022).

The interim sediment quality guidelines for Cd are 0.6 mg/kg and the probable effect concentrations are 3.5 mg/kg (Canadian Environmental Quality Guidelines, 1999c). Most of the sites had a Cd concentration of ≤ 0.6 mg/kg, except AEL DD (0.84 mg/kg), AEL South (1.3 mg/kg), Bokkamp RWD (2.3 mg/kg), and AEL East (4.2 mg/kg) (Table 4).

This suggests that the Cd concentrations in sediments at the sites pose little threat to the environment, although it could still have a slight effect at the sites with the higher concentrations.

4.1.2.13 *Lead*

Lead (Pb) is a hazardous element to many forms of life (DWAF, 1996c). This element is neither essential nor has it any role in the metabolic process, but rather hampers photosynthesis, nutrition, and enzyme activity (Hadi and Aziz, 2015). This element is toxic and rather accessible to aquatic organisms (DWAF, 1996c). The bioavailability of Pb is mainly dependent on pH (Hadi and Aziz, 2015), increasing with a decrease in pH (DWAF, 1996c). Due to the pH, Pb is considered more toxic in soft water than in hard water. Although an increase in water hardness can decrease the toxicity of Pb, the decrease in DO on the other hand can increase the toxicity of the Pb (DWAF, 1996c). The threshold of Pb concentrations in soils for plants is 2 mg/kg and for agricultural soils 50 – 300 mg/kg. If these limits are exceeded, it could have adverse effects on plant growth (Zulfiqar *et al.*, 2019).

The interim sediment quality guidelines for Pb are 35 mg/kg and the probable effect concentrations are 91.3 mg/kg (Canadian Environmental Quality Guidelines, 1999e). Only three of the sites had concentrations below the guidelines level, Skoon Spruit (7.2), Ref A (7.4 mg/kg), and Rietgat Spruit (10 mg/kg), and four sites had concentrations above the probable effect value, Bokkamp RWD (110 mg/kg), AEL DD (130 mg/kg), AEL East (130 mg/kg), and Catchment Dam (140 mg/kg).

At the sampling sites in the present study, all Pb concentrations in sediments were in the range of 7.2 mg/kg to 140 mg/kg (Table 4) suggesting that the Pb concentrations would potentially not have an adverse effect on the natural environment although this depends on DO concentrations (Fig. 8D). The sites which will be affected the most is the ones containing Pb concentrations ≥ 91.3 mg/kg.

Lead, therefore, should be considered a major concern for the aquatic systems of the mining sites.

4.1.2.14 *Chromium*

Chromium (Cr) is a serious pollutant within the natural environment when in excess due to its wide range of uses and consequent releases (Kumar *et al.*, 2016). The water hardness (Ca concentrations) has a significant effect on the toxicity of Cr, causing a decrease in toxicity, with an increase in pH (DWAF, 1996c). But the valence state in

which it occurs, can also have an effect on the toxicity of Cr, with Cr(VI) being much more toxic than Cr(II) (Kumar *et al.*, 2016).

The Cr concentrations of the various sites ranged between 26 mg/kg and 140 mg/kg (Table 4). The interim sediment quality guidelines for Cr are 37.3 mg/kg and the probable effect concentrations are 90 mg/kg (Canadian Environmental Quality Guidelines, 1999c). Most sites had concentrations below the probable effect concentrations, except two of the reference sites, Skoon Spruit (100 mg/kg) and Rietgat Spruit (140 mg/kg).

Although sediments at most sites had concentrations higher than the quality guideline levels, it is hard to evaluate the toxicity as the valence state was not measured. However, it may affect plant growth and living organisms occurring at some sites. Therefore, Cr should be added to the list of elements that are of environmental concern.

4.1.2.15 Mercury

Mercury (Hg) is toxic to aquatic organisms and this element has the potential to accumulate in the aquatic food web (DWAF, 1996c). The concentration of the Hg absorbed by plants differs from that found in soils or sediments, which depends on several factors such as cation-exchange capacity, soil or sediment pH, aeration and plant species. The toxicity of Hg can be reduced by high pH levels or the presence of high concentrations of lime or salts. Mercury binds with sulphur or nitrogen ligands, which enters the ionic channels, competing with other toxic metals such as Cd, or essential metals such as Zn, Cu, and Fe (Azevedo and Rodriguez, 2012) in animals, but this theory has yet to be tested on plants.

The interim sediment quality guidelines for Hg are 0.17 mg/kg and the probable effect concentrations are 0.486 mg/kg (Canadian Environmental Quality Guidelines, 1999f). The sediments of all sites had Hg concentrations higher than the probable effects value, especially West extension TSF (1.7 mg/kg), Queen Mary RWD (2.0 mg/kg), AEL DD (2.4 mg/kg), and AEL East (2.8 mg/kg), except for Skoon Spruit (<LOQ), Rietgat Spruit (0.014 mg/kg), Ref A (0.055 mg/kg), Eye Dam (0.10 mg/kg), Karee Rand

DD (0.12 mg/kg), and Karee Rand RWD (0.17 mg/kg), which had concentrations lower or equal to the interim sediment quality guidelines (Table 4).

Therefore, the Hg concentrations at most sites are expected to have toxic effects on the biological systems of the mining sites. The reference sites will be the least affected, except for Koekemoer Spruit. This is expected due to Koekemoer Spruit being closer to the mining activities than the other reference sites. Although amalgamation of gold with mercury is not used by the mines to extract gold from the ores, illegal mining may contribute to pollution as mercury is known to be used here (Personal observations and conversations, Talitha Brits and Ian Luyt). Mercury plays a substantial role in the gold extraction process of illegal mining. Illegal miners (zamma zamas) are supplied with this metal by former mine workers. The Hg is then irresponsibly disposed of, which can be washed away by rain, causing contamination of surrounding areas, and increasing the risk of trace metals in crops planted near such areas (Munakamwe, 2017).

Mercury, therefore, should be considered as an element of critical concern for the aquatic systems of the mining area, as it is also likely that associated terrestrial systems may be affected.

4.1.2.16 Vanadium

Metallic vanadium (V) does not occur naturally in the environment, but V salts can be easily taken up by living organisms and are usually unable to strongly adsorb onto soil particles (DWAF, 1996a; DWAF, 1996b). Vanadium absorption is dependent on the pH levels, with a decrease with an increase in pH, and the mobility of V in soils and water is dependent on the Fe and Al hydroxides. Vanadium is a vital element, especially in aquatic ecosystems, where it is utilised by algae, but in very low concentrations. Normally the concentrations found in plants are similar to those found in the soils (Roychoudhury, 2020). There is no Canadian sediment quality guideline for V, but the natural occurring V concentrations normally range between 10 and 220 mg/kg in soils (Imtiaz *et al.*, 2015). The V concentrations found in the sediments of the study sites were very low, ranging from 6.3 mg/kg to 50 mg/kg (Table 4), whereas another study showed that V concentrations in mining sites can range between 738 and 3505 mg/kg (Imtiaz *et al.*, 2015). The natural occurring concentrations in

unpolluted soils were used, because there are no sediment quality guidelines for this element.

Therefore, since the V concentrations of all sites were relatively low and fell into the natural occurring ranges found in soils, it most likely had no effect on the biological systems in the water of the sites.

4.1.2.17 *Beryllium*

As with most other trace elements, beryllium (Be) is strongly adsorbed by soils and sediments (DWAF, 1996b, Bolan *et al.*, 2023). Beryllium can be found in numerous silicate and oxide minerals. The natural occurring silicate will limit the available Be in the solution (DWAF, 1996b). This element is insoluble in the pH range of 5 to 8, but soluble at levels higher than 8 (Bolan *et al.*, 2023). Beryllium is not considered an essential nutrient and can be toxic to both plants and animals at very low concentrations (DWAF, 1996b), which can have substantial effects on the biochemical and physiological process in plants. There is no Canadian sediment quality guideline for Be, but this element is considered toxic to plants at concentrations > 1 mg/kg (Shah *et al.*, 2016), which would mean that Be was only found in toxic concentrations at Eye Dam (1.1 mg/kg), AEL East (1.4 mg/kg), AEL DD (1.6 mg/kg), Koekemoer Spruit (1.6 mg/kg), and Rietgat Spruit (1.6 mg/kg) (Table 4).

Therefore, this element may only have little effect on the biological systems in the water of a few sites, and less to no effect on the aquatic environments of the other sampling sites, including the reference sites.

4.1.2.18 *Cobalt*

Cobalt (Co) is an essential metal in numerous biological enzymes and the majority living organisms, as well as higher plants, but can in large concentrations cause irreversible damages to plants (Lwalaba *et al.*, 2017). Concentration of Co in a solution is highly dependent on pH, with its solubility decreasing with an increase in pH (DWAF, 1996b).

There is no Canadian sediment quality guideline for Co. The concentrations occurring naturally in soils are normally between 8 to 25 mg/kg (Lwalaba *et al.*, 2017). The reference sites had very low Co concentrations in their sediments; Ref A (8.2 mg/kg), Skoon Spruit (9.3 mg/kg), and Rietgat Spruit (10 mg/kg), except Koekemoer Spruit (26 mg/kg), which was just above natural soil concentrations, whereas the other sites had concentrations ranging from 24 to 220 mg/kg (Table 4). The limit of Co found to be tolerable in soils by plants is 0.20 – 0.50 mg/kg, but some plants can even resist concentrations of 4000 – 10000 mg/kg (Mahey *et al.*, 2020).

Thus, even though all sites' Co concentrations could be considered toxic to most plant life, it is relatively species dependent. Cobalt therefore, might affect only certain species or organisms occurring in the water of some sites, but this determination is qualified.

4.1.2.19 Nickel

Nickel (Ni) mostly occurs as a sulphide ore (DWAF, 1996b). Even though Ni is largely water insoluble, it is still considered toxic at high concentrations (Biswas and Choudhury, 2021). The solubility of Ni is dependent on pH, with the solubility decreasing, with an increase in pH (DWAF, 1996c). There is no Canadian sediment quality guideline for Ni. Ni is not considered as an important nutrient, but it has been proven that this element may improve the growth of some plants, when in small concentrations (DWAF, 1996b), these concentrations range between 0.05 – 10 mg/kg (Bhalerao *et al.*, 2015). Ni can cause Fe deficiency in plants, by hampering the absorption or binding in the roots (Yusuf *et al.*, 2011). Ni occurs naturally in soils with concentrations of less than 100 mg/kg but can reach concentrations up to 26000 mg/kg in polluted soils (Yusuf *et al.*, 2011). Most of the sites had concentrations in their sediments lower than those occurring naturally in the environment, except AEL DD (300 mg/kg), AEL South (690 mg/kg), West Storm Water (690 mg/kg), AEL East (1300 mg/kg), and Bokkamp RWD (1800 mg/kg) (Table 4), which were very high.

When considering the tolerance range for plants (0.05 – 10 mg/kg), all sites contained toxic concentrations of Ni. Thus, Ni will have an effect and cause stress to the biological systems in the water of some reference and mining sites and should be considered an element of concern.

4.1.2.20 Antimony

Antimony (Sb) concentrations can be very high in areas polluted by anthropogenic activities. Elevated Sb concentrations are usually associated with high arsenic concentrations within sulphide ores. Sb is also used as an indicator of gold, due to its chalcophylic nature (Filella *et al.*, 2002). Antimony is considered a chalcophile mineral that occurs with sulphur and some toxic metals, such as Cu, Pb, and Ag (silver) (Anderson, 2012). Its toxicity is closely related to that of As (Pintor *et al.*, 2020). There is no Canadian sediment quality guideline for Sb, but this element is normally found in concentrations lower than 1 mg/kg in unpolluted areas but can increase to 15 mg/kg at sites polluted by anthropogenic activities (Tschan *et al.*, 2009). The Sb concentrations in the sediments of the sites were very low, of which half had concentrations lower than 1 mg/kg and the other from 1.2 to 8.6 mg/kg (Table 4). Even if soils contain very high concentrations such as 105 mg/kg the plant will only absorb a very small amount, but he also showed that the amount absorbed increases with the amount Sb in the soils (Tschan *et al.*, 2009). Soil values were used due to Sb not having specific Canadian sediment guideline values.

Considering the sampling sites, the Sb concentrations is expected to have almost no effect on the biological systems in the water of the mining sites, and no effect on the aquatic environment in the reference sites.

4.1.2.21 Uranium

Uranium (U) is an important environmental pollutant, normally originating from anthropogenic activities (Chen *et al.*, 2021), and is a relatively reactive element (DWAF, 1996c). There is no Canadian sediment quality guideline for U, but all soils contain a U concentration between 0.7 to 9 mg/kg (DWAF, 1996b), and causes a considerable amount of damage to plants, animals and microbes, however the toxic effects on plants are species specific, and also dependent on U concentrations and environmental conditions (Chen *et al.*, 2021). A study has shown that plants only contain a mean of approximately 10^{-7} U g/g, due to this element's low solubility in water (Chen *et al.*, 2021). The U concentrations in the sediments of the three reference sites

(Ref A, Skoon Spruit, and Rietgat Spruit) ranged from 0.5 – 1.0 mg/kg, whereas the rest of the sites ranged between 11 and 190 mg/kg (Table 4). All sites had carcinogenic concentrations (>0.05 mg/kg) for humans (Chen *et al.*, 2021). The no-effect limit for terrestrial plants is 250 mg/kg, whereas that for freshwater plants is substantially lower (Sheppard *et al.*, 2005).

This means that the U concentrations at the sites most likely had little to no effect on the terrestrial plants, but most likely had a drastic effect on the freshwater plant and algal species occurring in these biological systems in the water. Uranium, therefore, should be considered as an element of significant concern.

4.1.2.22 Barium

Barium (Ba) is not an important plant nutrient but has a wide occurrence in the natural environment (Verbruggen *et al.*, 2020), at comparably high concentrations (Suwa *et al.*, 2008). The toxicity and uptake of Ba is still not fully understood. The Ba concentration can be decreased when in the presence of sulphate and carbonate (Verbruggen *et al.*, 2020). There is no Canadian sediment quality guideline for Ba, but natural concentrations in the subsoil normally ranges between 13 – 20500 mg/kg and in the topsoil between 30 and 1870 mg/kg, but not all Ba can be readily absorbed by living organisms (Suwa *et al.*, 2008). Although the toxicity of this element is poorly understood, all sites' concentrations ranged from 28 mg/kg to 12004 mg/kg (Table 4). Natural occurring ranges in soils were used, since there is no sediment quality value for this element. These values can be used because most of the dams sampled can dry up.

This indicates that the concentrations found in the sediments of the sites are at least in the natural occurring range, and that it would supposedly have little to no serious effect on the environment in this range.

4.1.2.23 Platinum

Platinum (Pt) can be released into the natural environment through anthropogenic activities. The release of this element can be enhanced by low pH levels, high chlorine concentrations and organic matter. The uptake by organisms increases with an

increase in pH (Fortin *et al.*, 2011), but with plants it is also considered species dependent (Gawrońska *et al.*, 2018). The toxicity of this element is dependent on the exposure time, concentration and water hardness. The toxicity decreases with an increase in water hardness (Fortin *et al.*, 2011). There is no Canadian sediment quality guideline for Pt, but this element normally occurs in unpolluted soils at concentrations of 0,00014 mg/kg and in agricultural soils at concentrations of 0,0011 mg/kg (Gawrońska *et al.*, 2018). The Pt concentrations were below limits of quantification for most sites in the present study, except Catchment Dam (0.029 mg/kg), AEL East (0.0057 mg/kg), and Queen Mary RWD (0.014 mg/kg) (Table 4). Natural occurring ranges and agricultural ranges were used, since this element does not have any sediment quality guideline values.

Therefore, this element will only have a slight to no effect on the biological systems in the water of the sites sampled.

4.1.2.24 Strontium

Strontium (Sr) is a toxic compound (Gupta and Walther, 2018), which is mainly found in the natural environment as a mineral ore, such as celestite and strontianite (Mikhailovskaya and Pozolotina, 2020). Strontium is not an important nutrient but can be easily taken up by plants due to its chemistry which is similar to Ca (Mikhailovskaya and Pozolotina, 2020; Gupta and Walther, 2018), which makes it easy for Sr to enter the food web (Gupta and Walther, 2018). There is no Canadian sediment quality guideline for Sr, but the mean Sr concentration found in soils is 0.035% but can increase to up to 0.2% in some areas. Strontium concentrations of 600mg/kg are considered normal in soil (Mikhailovskaya and Pozolotina, 2020). There is a lack of research on the toxicity and tolerances of Sr to plants, but one report showed that 30 mg/kg can be toxic to plants (Sasmaz and Sasmaz, 2017). Measured values in the present study indicated that seven sites (Eye Dam (44 mg/kg), Koekemoer Spruit (49 mg/kg), Bokkamp RWD (59 mg/kg), AEL East (120 mg/kg), AEL DD (130 mg/kg) and AEL South (200 mg/kg)) had Sr concentrations that are considered toxic to plants (Table 4).

Therefore, this element might be toxic and cause stress to the biological systems in the water of most sites, even the reference sites. Even with the tolerance level of plants found by the one report.

4.1.2.25 *Summary of the elements*

As mentioned in the results, when considering all elements, the sites with the highest concentrations were AEL East, AEL South, AEL DD, Bokkamp RWD and West Storm Water (Table 4), which means that these sites had the highest levels of contamination. One interesting occurrence was that the concentrations of the elements were much lower at Karee Rand TSF, DD, and RWD, which was not expected since this is a TSF, drainage ditch and return water dam. Of all the elements discussed above the As, Cr, Cu, Fe, Hg, Mn, Na, Pb, Ni, Se, Sr, and U and Zn will most likely have the largest toxic effects on biological systems in the water of some of the sites, either individually or in combinations.

4.1.2.26 *Other observations*

Although most variables were measured using laboratory techniques, there were also some visual observations. Some reddish precipitates were observed on the sediment of Queen Mary and Karee Rand drainage ditches, as well as in Karee Rand return water dam. These precipitates are caused by the upward capillary movement of salt-rich liquids, resulting from tailings material. In cases of water deficiency, the salt-rich solutions evaporate, which causes the salt precipitates. These deposits are normally highly soluble and rich in trace metal elements (Simate and Ndlovu, 2014, Aucamp and Van Schalkwyk, 2003). The Skoon Spruit also received spillages (pre- 1940's) from the TSFs, as well as industries, graveyards, and artisanal mining activities (Lusilao-Makiese *et al.*, 2014) which would account for the large amounts of micro-copper and gold (visual observation on the slides) found on the sample slides. Although the water quality variables were measured over a period of one year, it is advisable to also use aquatic communities to evaluate the condition of the water sources, since they are exposed to a number of environmental changes over time, and they can react directly to any chemical or physical changes within their environment (Morin *et al.*, 2008; Kock *et al.*, 2019).

4.1.3. Diatom community composition

Diatoms are constantly exposed to their aquatic environment, therefore making them good indicators of water quality, thus a combination of water quality variables and diatom community data can be a good representation of the health of a specific water body. Taxon diversity and richness were used as a measure of alterations in the community structure by differentiating between the impacted and reference sites. Changes in the diatom community along the pollution gradient were observed, in regard to their abundance, dominance and similarity, with a notable decrease in the diversity and richness between the impacted and reference sites.

The Klerksdorp-Orkney-Stilfontein-Hartebeesfontein (KOSH) region had 169 diatom taxa in 49 genera. The diatom component of the study area was dominated by biraphids 131 (77%). The remaining components comprised 12 monoraphids (7%) and 16 araphids (9.4%), with centrics being the least in abundance, with 10 species, 4 (2.3%) of which were cylindrical/ filamentous taxa and 6 (3.5%) single celled taxa. The species rich genera were *Nitzschia* (28), followed by *Navicula* (17), *Gomphonema* (14), and *Fragilaria* (9) (Appendix C).

The species with the greatest abundances included *Achnantheidium* sp., *Amphora veneta*, *Brachysira neoexilis*, *Craticula buderi*, *Cyclotella meneghiniana*, *Eolimna subminuscula*, *Fragilaria ulna*, *Gomphonema parvulum*, *Navicula erifuga*, *Navicula riediana*, *Navicula veneta*, *Nitzschia amphibia*, *Nitzschia frustulum*, *Nitzschia nana*, *Nitzschia palea*, *Pinnularia* sp., and *Planothidium frequentissimum* (Appendix A), of which 11 of these species are pollution tolerant (Appendix C), which can occur in critically polluted sites (Taylor *et al.*, 2007b).

The high nitrate (NO_3^-), phosphate (PO_4^{3-}) and low dissolved oxygen (DO) concentrations, suggest that all sites experience hypertrophic conditions, which means systems are highly productive in terms of biomass, usually with low levels of species diversity (DWAF, 1996c). How much of this PO_4^{3-} is bio-available is unknown. Biraphid diatoms mostly occur in environments where there are steep physical and chemical gradients (Gooden, 2002) which could be the reason for the high diversity of biraphid genera found at the sampling sites in the present study. The species that occurred at the sites, are mostly characteristic of eutrophic or oligotrophic conditions (Taylor *et al.*, 2007b), although most sites were found to be hypertrophic.

Diversity was used as an indicator of alterations in the community structure when comparing reference and impacted sites. The diatom community showed an obvious decrease in diversity between the reference and impacted sites (Fig.11-15). The succession of diatom communities can be driven by metal contamination, causing an increase in more tolerant species, which could explain the large number of tolerant species found at the sites (Appendix C). Moreover, it can also cause a decrease in the species diversity and richness (da Silva *et al.*, 2009) (Appendix A, B & Fig. 11-15).

Diatom growth is also sensitive to changes in the phosphate concentrations (Katiyar *et al.*, 2010), due to its important role in the diatom community composition of assemblages (Leira *et al.*, 2009). Diatoms have an optimum cell growth rate at a phosphate concentration of 0.15 mg/L (Katiyar *et al.*, 2010), which decreases with extreme phosphate concentrations, which can be explained by their competitive abilities or physiological tolerances (Leira *et al.*, 2009). Both summer and winter seasons, had high phosphate concentrations with accelerated phosphate peaks at some sites in the summer (Fig. 8E), which can also have an influence on the diatom diversity, because the species richness declines towards extreme conditions, due to competitive abilities, as well as physiological tolerances (Leira *et al.*, 2009).

Although relatively low diatom abundances were expected, due to the stressful environmental conditions, relatively high abundances were found at some sites, during some seasons (Table 6 & 7). One reason for the higher than expected abundances could be due to the alkaline environment, since diatoms are better able to survive in alkaline, saline and Ca rich environments as compared to other groups of algae (Orlekowsky *et al.*, 2013). Normally, mine wastewater has a low pH, which increases the solubility of elements including aluminium and decreases the bioavailability of essential nutrients, such as phosphorus and potassium (Orlekowsky *et al.*, 2013).

Although Al is not a vital nutrient for plant growth, it is absorbed into the diatom frustules, which may be a result of the detoxification process. Even small amounts of Al can have adverse effects on algae, causing growth inhibition and mortalities. This leads to the elimination of species in a habitat having high Al concentrations within the waters. Aluminium can also impact the bioavailability of phosphate, which is a crucial element for diatom growth (Köhler *et al.*, 2017). As observed in the present study all sites were characterised by very high Al concentrations, which could have played a

role in the very small amount of dead cells (1 - 3 cells.cm⁻²) found at some sites (when live, unprocessed samples were examined), as well as changes in species composition.

Arsenic can also cause a decrease in both diversity and abundance in diatoms, especially the more sensitive species (Chen *et al.*, 2015), this could be seen at the sites characterised by having high As concentrations. Vanadium can improve the growth rate of algae, but at low concentrations. It has been shown that diatom communities favour relatively high Mn concentrations (Patrick, 1978). Zinc can also cause a decrease in photosynthetic activity (DWAF, 1996c), potentially resulting in a decrease in the diatom biomass.

The three species that occurred the most and were the most abundant at the mining sites, were *Achnantheidium* sp., *Craticula buderi*, and *Nitzschia palea*.

Numerous of studies have shown *Nitzschia palea* to be tolerant of pollution and this taxon is often used as a bioindicators for organic pollution and heavy metal contamination (Rimet *et al.*, 2014; Delgado *et al.*, 2012; Salomoni *et al.*, 2006). As with *Nitzschia palea*, *Craticula buderi* is also considered a good indicator of mining effluent, as well as polluted water with elevated electrolyte content (Taylor *et al.*, 2007b). These taxa also occur in calcareous waters (Taylor *et al.*, 2007b), typical of mine effluent, due to liming to neutralise the acid mine drainage, before deposition (Rösner and Van Schalkwyk, 2000).

The *Achnantheidium* sp. found at the sites, have a resemblance to *Achnantheidium minitissimum*, this species had a high abundance at some of the sites with a high metal concentration, *Achnantheidium minitissimum* is considered as a relatively sensitive taxon when compared with *Craticula buderi* or *Nitzschia palea* for instance, not tolerating more than a moderate level of pollution (Taylor *et al.*, 2007b). Some other studies also showed *Achnantheidium minitissimum* occurring in highly metal contaminated environments (da Silva *et al.*, 2009; Gold *et al.*, 2003; Biswas and Choudhury, 2021) and can be considered as an indicator of metal contamination (Biswas and Choudhury, 2021). Gold *et al.* (2003) hypothesised that this resistance may be attributed to the way these adnate types with a short mucilaginous stalk live beneath other periphytic taxa. The periphytic layer (biofilm) protects them through the

production of extracellular polymeric substances, thus minimising metal bioavailability and toxicity.

Small species like *Achnantheidium minutissimum* that are tightly attached to a substrate are more able to endure metal stress, since they are encapsulated in an organic matrix which functions as a barrier against metal toxicity. In comparison, the capability of filamentous and motile diatoms to move beyond this barrier would most likely make them more sensitive to toxicants (Morin *et al.*, 2008). *Achnantheidium minutissimum* is also considered to be quite tolerant to metal contamination as well as high arsenic (As) concentrations (Chen *et al.*, 2015), and is also found in waters containing a low nutrient availability (Leira *et al.*, 2009) which in the present study could be a factor considering the high aluminium concentrations at some sites, such as AEL East, Catchment Dam, Koekemoer Spruit, Ref A, Rietgat Spruit, and Eye Dam.

The majority of *Navicula* and *Nitzschia* species thrive in nutrient-rich environments (Delgado *et al.*, 2012), which illustrates that the diatom community characterisation of the highly impacted sites is comprised of nutrient tolerant taxa such as *Craticula buderi*, *Cyclotella meneghiniana*, *Gomphonema parvulum*, *Navicula erifuga*, *Navicula veneta*, *Nitzschia amphibia*, *Nitzschia palea*, and *Planothidium frequentissimum*. These species occurred in samples with phosphate (PO_4^{3-}) concentrations up to 8.2 mg/L, nitrate (NO_3^-) concentrations up to 100mg/L, and EC concentrations up to 8.5 mS/cm, hence they are indicators of high levels of nutrient pollution, as well as high electrical conductivity concentrations.

Species of the genus *Cyclotella* is considered metal sensitive (Morin *et al.*, 2008), which makes the appearance of *Cyclotella meneghiniana* and *Cyclotella ocellata* at AEL South and Eye Dam in the summer and autumn remarkable, but this occurrence was most likely related to high nutrient availability during this period (NO_3^- ranging between 23 – 65 ppm and P between 0.5 – 5.6 mg/L), which may have allowed these species to grow.

Exposure of diatoms to metals can cause failure of metabolic, and reproductive processes, as well as elevated mortality rates. The community size can be reduced in three ways: (1) decrease in cell quantity, (2) an increase in small-sized species, and a (3) size reduction in the occurrence of certain species (Morin *et al.*, 2012).

The tolerance of diatoms as well as other micro-algae occurring in metal polluted sites, has already been tested through laboratory toxicity tests of their photosynthetic activity (Gold *et al.*, 2003).

The presence of multiple toxic elements exceeding guideline values (As, Cr, Cu, Fe, Hg, Mn, Na, Pb, Ni, Se, Sr, and U and Zn) most likely created an ecological competitive elimination scenario, where the competition among metal-tolerant species and numerous other species, merged with additional environmental conditions, contributing to the final community structures found in the present study. Despite some of the taxa recorded in the present study were also found in unpolluted sites, their mutual presence and co-dominance might be viewed as a sign of metal contamination (Morin *et al.*, 2008).

Although there were changes in the diatom diversity and abundance due to the ecological state of their habitat, there were also morphological changes in their cell structure that occurred due to these conditions.

4.1.4. Diatom deformations

Diatoms not only react to their environment on community level or diversity changes, but also on individual level, through alterations in their frustule morphology (da Silva *et al.*, 2009). These cell wall characteristics (stria structure, cell outline, etc) are normally considered to be stable and are used for the identification of diatom species. Any changes to these characteristics of the cell wall indicate that the metabolic pathway of silica was disrupted in the newly formed cell at some stage. These alterations in the cell structure can be caused by many pollutants including some chemicals commonly associated with mining operations (Sienkiewicz, *et al.*, 2023).

Metals normally exist at low concentrations in the natural environment. The essential metals (iron (Fe), zinc (Zn), and copper (Cu)) are absorbed by diatoms to help with the fabrication of membranes and cell walls, as well as cell growth, photosynthetic and respirational processes. The most favourable concentrations of crucial metals are usually very narrow. Low Fe concentrations can restrict algal growth. In contrast, the nonessential toxic metals (e.g. mercury (Hg), cadmium (Cd), lead (Pb)) can have notable effects even at low concentrations, due to the damage caused to metabolic

pathways used for the uptake of essential elements and metals (Falasco *et al.*, 2009; Sienkiewicz, *et al.*, 2023).

The impact of toxic metals on the environment is highly dependent on the presence of distinct metal species. Therefore, if a metal or metalloid is available in a more favourable chemical form for absorption, it is likely that biological damage, such as morphological changes will increase, due to undissolved metals entrapped by Fe or Mn hydroxides cloaking the cell wall (Luís *et al.*, 2016). The occurrence of abnormal and deformed individuals is considered a basis for the assessment of metal pollution. Metals have a high affinity for silicic acids sulfhydryl groups, which causes a decrease in silicone uptake due to these binders (Cd, Cu, Hg, Ag), causing ineffective silica deposition (Luís *et al.*, 2011). In comparison with metals, high phosphate concentrations have also been shown to cause deformities in diatoms (Falasco *et al.*, 2021).

Although cell deformities were observed, they were much less than anticipated, taking in consideration their environmental conditions. Most of the cell abnormalities showed asymmetrical, abnormal, and bent frustules (Table 8), which is most likely due to the occurrence of Cd, Cu, and Zn (Falasco *et al.*, 2009; Sienkiewicz, *et al.*, 2023). These alterations in cell structure can be visible only few days after contamination occurred (Sienkiewicz *et al.*, 2023).

According to Falasco *et al.* (2009) and Olenci *et al.* (2017), Cu and Zn contamination mainly affects the outline of the valve, leaving the shape and pattern of the ornamentations unaltered. Cell size reduction is associated with generally high metal concentrations, whereas cell deformities are associated with Cu (Olenici *et al.*, 2017). It has also been shown that Cu and Zn toxicity can cause oxidative stress in diatoms which could result in an increase in cell surface and roughness (Marella *et al.*, 2020). Gold is a very toxic element and can be accumulated by pennate diatoms within their frustules (Biswas and Choudhury, 2021).

Diatoms can accumulate large amounts of metals from the aquatic environment which can interfere with their biochemical and cellular processes when in excess. Metal exposure can lead to the malfunction of cell processes, such as primary productivity, respiration, nutrient and oligoelement variability (Morin *et al.*, 2012). The metabolism of phosphate is also affected by metal accumulation (Morin *et al.*, 2012). The sizes of

the diatoms in the present study were measured, to evaluate any size reductions due to metal stress, but no size reduction results were obtained. Size reduction of the cells is most probably a protective measure against heavy metal accumulation, because metal absorption is associated with the surface area exposed to the metal nanoparticles (Morin *et al.*, 2012). In general diatoms reproduce vegetatively using McDonald Pfitzer's diminutive law (Morin *et al.*, 2012; Biswas and Choudhury, 2021). As the vegetative reproduction is proceeding and the cell size progressively declining, within the metal contaminated areas, the diatoms face a threat in their vegetative reproduction, as a result of the cells reaching their limiting size at a faster rate, which limits the diatom communities in the metal impacted areas (Pandey and Bergey, 2016; Morin *et al.*, 2012; Biswas and Choudhury, 2021).

With the stressed forms reaching vital size long before the metal resistant forms, they are more easily removed and replaced by more tolerant species (Luís *et al.*, 2011).

Diatom valves having deformities are most likely disadvantaged in their natural habitat. The ability of abnormal valves to thrive in their environment can be associated with the type of teratology. For example, raphe displacement can cause a decrease in their reproductive capacity, particularly in their natural habitat, where adhesion ability of the cells is crucial. In other cases, deformations in the stria, as well as mixed deformities are likely to enable the cells to survive and reproduce in such drastic environments (Falasco *et al.*, 2021).

The most challenging deformities to observe were those involving changes in the valve surface, in particular the small species. This can be attributed to the restricted resolution of the photomicroscope that was used in the present study but may also be an indication that smaller species are more tolerant to morphological changes (Morin *et al.*, 2012).

Although toxic compounds such as cyanide, polycyclic hydrocarbons (PAH), and pesticides can also cause teratological forms (Falasco *et al.*, 2009), those observed were most likely due to the As, Cu, Hg, Ni, and Zn concentrations, and to a lesser extent the chromium Cr, and Pb concentrations as shown in Chapter 3.

Diatoms require essential metals and ions such as potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), manganese ($Mn(IV)$), zinc (Zn^{2+}), iron ($Fe(II)$), copper ($Cu(I)$), nickel (Ni^{2+}), and molybdenum (Mo^{2+}), for growth and establishment. Whereas

nonessential metals such as cobalt (Co^{2+}), sodium (Na^+), cadmium (Cd^{2+}) and mercury (Hg^{2+}) have no specific physiological role within diatoms. Nevertheless, both essential and nonessential metals can cause morphological changes and size reduction and other morphological changes if in excess (Morin *et al.*, 2012).

Chapter 5

5.1 Conclusions and recommendations

This chapter will conclude the study by outlining the fundamental aims and research questions, along with the benefit and contribution thereof. Limitations that were encountered during the study will be presented, as well as opportunities for future research.

The current research aimed to assess the effect of gold mine tailings on the diatom community composition and abundance, and in the process establish the condition of the water bodies related to gold mining activities, by using the diatoms as bio-indicators. The results indicate that diatoms are wide-ranging and also frequently the main primary producers within a certain aquatic environment. Numerous environmental variables have an impact on the diatom community structure, as well as causing morphological abnormalities (deformities) in different diatom species at the cellular level. Therefore, both physical and chemical data is needed to determine the major causes for the diatoms' reactions towards their environment.

In this study the main variables affecting the biological systems of the sites are the physico-chemical parameters in the water which includes cyanide, dissolved oxygen (DO), NO_3^- and PO_4^{3-} and elements in the sediments such as As, Cr, Cu, Fe, Hg, Mn, Na, Pb, Ni, Se, Sr, and U and Zn. These variables can either have an individual or combined effect on the biological systems. Whereas the main causes of the diatom deformations found is the toxic concentrations of As, Cu, Hg, Ni, and Zn found in the sediments of the sites, with cyanide having a small effect in the water. These observations showed that diatoms are a suitable biological indicator for the observation of metal pollution, supplying us with relevant ecological data on particular sensitivity or tolerances at a community level, as well as an individual level.

As for the condition of these mine associated waterbodies, these sites contained much lower metal concentrations than found in some other studies, and the tailings storage facilities allow for the removal of >90% of all cyanide. Nevertheless, these waterbodies are still considered toxic to the natural environment, as seen in the morphological changes in the diatoms, as well as the changes in their abundance and species diversity (or complete absence at very high cyanide concentrations).

There were some limitations that occurred during the study. Phosphate measurements, using a field photometer, could not be collected at some sites, due to the high calcium and ferrous iron concentrations. Some phosphate measurements were still made during some of the months at these sites, when conditions allowed for these measurements. Although the use of the phosphate photometer was very convenient in the field, in the case of mine related sites, it would be better to use laboratory-based techniques in the future to obtain the phosphate concentrations. Another challenging aspect was the identification of deformities in the smaller diatom species, due to the restricted resolution of the photomicroscope that was used, but in general, most deformed cells could be identified.

Another problem was with the comparison of sediment data with terrestrial soil standards for some elements. This was deemed to be the most accurate way in which to conduct such a comparison as comparing classifying sediment based on water quality standards would be highly inaccurate. Unfortunately, there are no available sediment quality guidelines for sediments in South Africa.

Diatoms as bio-indicators can provide us with a substantial amount of information about the environment they inhabit, which can be very important in quality water studies for the purposes of resource management. There are not many studies done on diatoms in mine or industrial waste waters, especially in South Africa. This study brought about a better understanding about the effect that these conditions have on the diatoms at both community and individual level, since their reactions are mainly dependent on their environmental conditions, simply extrapolating studies from other parts of the world would not provide sufficient information for monitoring efforts as mining sites are highly unique environments, differing from operation to operation. Although these findings of this study are generally compatible with other diatom related research at mining sites in other countries, there was a difference in elemental concentrations in the sediments of the sites and in the diatom community composition.

Although the research questions for this project were addressed, new questions were raised for future research. The occurrence of *Cyclotella meneghiniana* at some of these highly metal polluted sites is of interest, since it is generally considered to be very sensitive to metal pollution. This species may be more metal tolerant than we expect, or its occurrence might have been due to the excess amount of nutrients.

Another important factor to investigate is if size reduction is worth using as one of the main indications of severe metal contamination, since it was not clearly visible in this study and with the very limited number of cells present in some samples were very difficult to quantify. The last possible factor that could be looked at into the future, is if resistant diatom species can successfully develop in water containing relatively high cyanide concentrations, if there is enough organic matter present.

This study showed that metal contamination as well as other environmental variables can have adverse effects on the diatom communities, on both individual and community level. Although some challenges were encountered throughout the study, very useful data were obtained, an important foundation for future research.

Chapter 6

6.1 References

(2004). 'Periphyton'. *Australian Oxford Dictionary*. Second edition. Oxford University Press. Available at:

https://www.oxfordreference.com/view/10.1093/acref/9780195517965.001.0001/m-en_au-msdict-00001-0041050 (Accessed: 4 July 2023).

(2006). 'Phototroph', in Cammack, R., Atwood, T., Campbell, P., Parish, H., Smith, A., Vella, F., and Stirling, J. (Eds.) *Oxford Dictionary of Biochemistry and Molecular Biology*. Second edition. Oxford University Press. Available at:

<https://www.oxfordreference.com/view/10.1093/acref/9780198529170.001.0001/acref-9780198529170-e-15523> (Accessed: 4 July 2023).

(2019a). 'Eutrophic', in Hine, R. (Ed.) *A Dictionary of Biology*. Eighth edition. Oxford University Press. Available at:

<https://www.oxfordreference.com/view/10.1093/acref/9780198821489.001.0001/acref-9780198821489> (Accessed: 4 July 2023).

(2014). 'Oligoelement'. *Cambridge Dictionary*. Cambridge University Press and Assessment. Available at: <https://dictionary.cambridge.org/us/dictionary/french-english/oligo-element> (Accessed: 18 March 2024).

(2019b). 'Oligotrophic', in Hine, R. (Ed.) *A Dictionary of Biology*. Eighth edition. Oxford University Press. Available at:

<https://www.oxfordreference.com/view/10.1093/acref/9780198821489.001.0001/acref-9780198821489-e-3074> (Accessed: 4 July 2023).

(2019c). 'Unicellular', in Hine, R. (Ed.), *A Dictionary of Biology*. Eighth edition. Oxford University Press. Available from:

<https://www.oxfordreference.com/view/10.1093/acref/9780198821489.001.0001/acref-9780198821489-e-4590> (Accessed: 4 July 2023).

(2020). 'Eukaryotic organism', in Gorse, C., Johnston, D., and Pritchard, M. (Eds.) *A Dictionary of Construction, Surveying and Civil Engineering*. Second edition. Oxford University Press. Available at:

<https://www.oxfordreference.com/view/10.1093/acref/9780198832485.001.0001/acref-9780198832485-e-2225> (Accessed: 4 July 2023).

- Acheampong, M.A., Adiyiah, J. and Ansa, E.D.O. (2013). Physico-chemical characteristics of a gold mining tailings dam wastewater. *Journal of Environmental Science and Engineering*, A2(8A), p.469.
- Ali, S.H. (2006). Gold mining and the golden rule: a challenge for producers and consumers in developing countries. *Journal of Cleaner Production*, 14(3-4), pp.455-462.
- Allaby, M. (2015). 'Diatom'. *A Dictionary of Ecology*. Fifth edition. Oxford University Press. Available at:
<https://www.oxfordreference.com/view/10.1093/acref/9780191793158.001.0001/acref-9780191793158> (Accessed: 4 July 2023).
- Allaby, M. (2020a). 'Electrical conductivity'. *A Dictionary of Geology and Earth Sciences*. Fifth edition. Oxford University Press. Available at:
<https://www.oxfordreference.com/view/10.1093/acref/9780198839033.001.0001/acref-9780198839033-e-2644> (Accessed: 4 July 2023).
- Allaby, M. (2020b). 'Eutrophication'. *A Dictionary of Zoology*. Fifth edition. Oxford University Press. Available at:
<https://www.oxfordreference.com/view/10.1093/acref/9780198845089.001.0001/acref-9780198845089-e-3135> (Accessed: 4 July 2023).
- Allaby, M. (2020). 'Chalcophile'. *A Dictionary of Geology and Earth Sciences*. Oxford University Press. Available at:
<https://www.oxfordreference.com/view/10.1093/acref/9780198839033.001.0001/acref-9780198839033-e-1384> (Accessed: 17 March 2024).
- Aucamp, P. and Van Schalkwyk, A. (2003). Trace element pollution of soils by abandoned gold mine tailings, near Potchefstroom, South Africa. *Bulletin of Engineering Geology and the Environment*, 62, pp.123-134.
- Anderson, C.G. (2012). The metallurgy of antimony. *Geochemistry*, 72, pp.3-8.
- Azevedo, R. and Rodriguez, E. (2012). Phytotoxicity of mercury in plants: a review. *Journal of Botany*, 2012, pp.1 – 6.

- Bakatula, E.N. and Tutu, H. (2016). Characterization and speciation modelling of cyanide in effluent from an active slimes dam. *South African Journal of Chemistry*, 69, pp.140-147.
- Belle, G., Fossey, A., Esterhuizen, L. and Moodley, R. (2021). Contamination of groundwater by potential harmful elements from gold mine tailings and the implications to human health: A case study in Welkom and Virginia, Free State Province, South Africa. *Groundwater for Sustainable Development*, 12, p.100507.
- Bellinger, E.G. and Sigeo, D.C. (2015). *Freshwater algae: identification, enumeration and use as bioindicators*. John Wiley and Sons.
- Bhalerao, S.A., Sharma, A.S. and Poojari, A.C. (2015). Toxicity of nickel in plants. *International Journal of Pure and Applied Bioscience*, 3(2), pp.345-355.
- Bhateria, R. and Jain, D. (2016). Water quality assessment of lake water: a review. *Sustainable Water Resources Management*, 2, pp.161-173.
- Biswas, R.K. and Choudhury, A.K. (2021). Diatoms: Miniscule biological entities with immense importance in synthesis of targeted novel bioparticles and biomonitoring. *Journal of biosciences*, 46(4), p.102.
- Bolan, S., Wijesekara, H., Tanveer, M., Boschi, V., Padhye, L.P., Wijesooriya, M., Wang, L., Jasemizad, T., Wang, C., Zhang, T. and Rinklebe, J. (2023). Beryllium contamination and its risk management in terrestrial and aquatic environmental settings. *Environmental Pollution*, 320, p.121077.
- Bozarth, A., Maier, U.G. and Zauner, S. (2009). Diatoms in biotechnology: modern tools and applications. *Applied microbiology and biotechnology*, 82, pp.195-201.
- Campbell, L.M., Fisk, A.T., Wang, X., Köck, G. and Muir, D.C. (2005). Evidence for biomagnification of rubidium in freshwater and marine food webs. *Canadian Journal of Fisheries and Aquatic Sciences*, 62(5), pp.1161-1167.
- Canadian Environmental Quality Guidelines (1999a). *Canadian Sediment Quality Guidelines for the Protection of Aquatic Life: Arsenic*. Canadian Council of Ministers of the Environment. Available at: <https://ccme.ca/en/res/arsenic-canadian-sediment-quality-guidelines-for-the-protection-of-aquatic-life-en.pdf> (Date Accessed: 17 March 2024).

Canadian Environmental Quality Guidelines (1999b). *Canadian Sediment Quality Guidelines for the Protection of Aquatic Life: Cadmium*. Canadian Council of Ministers of the Environment. Available at: <https://ccme.ca/en/res/cadmium-canadian-sediment-quality-guidelines-for-the-protection-of-aquatic-life-en.pdf> (Date Accessed: 17 March 2024).

Canadian Environmental Quality Guidelines (1999c). *Canadian Sediment Quality Guidelines for the Protection of Aquatic Life: Chromium*. Canadian Council of Ministers of the Environment. Available at: <https://ccme.ca/en/res/chromium-canadian-sediment-quality-guidelines-for-the-protection-of-aquatic-life-en.pdf> (Date Accessed: 17 March 2024).

Canadian Environmental Quality Guidelines (1999d). *Canadian Sediment Quality Guidelines for the Protection of Aquatic Life: Copper*. Canadian Council of Ministers of the Environment. Available at: <https://ccme.ca/en/res/copper-canadian-sediment-quality-guidelines-for-the-protection-of-aquatic-life-en.pdf> (Date Accessed: 17 March 2024).

Canadian Environmental Quality Guidelines (1999e). *Canadian Sediment Quality Guidelines for the Protection of Aquatic Life: Lead*. Canadian Council of Ministers of the Environment. Available at: <https://ccme.ca/en/res/lead-canadian-sediment-quality-guidelines-for-the-protection-of-aquatic-life-en.pdf> (Date Accessed: 17 March 2024).

Canadian Environmental Quality Guidelines (1999f). *Canadian Sediment Quality Guidelines for the Protection of Aquatic Life: Mercury*. Canadian Council of Ministers of the Environment. Available at: <https://ccme.ca/en/res/mercury-canadian-sediment-quality-guidelines-for-the-protection-of-aquatic-life-en.pdf> (Date Accessed: 17 March 2024).

Canadian Environmental Quality Guidelines (1999g). *Canadian Sediment Quality Guidelines for the Protection of Aquatic Life: Zinc*. Canadian Council of Ministers of the Environment. Available at: <https://ccme.ca/en/res/zinc-canadian-sediment-quality-guidelines-for-the-protection-of-aquatic-life-en.pdf> (Date Accessed: 17 March 2024).

- Chen, G., Shi, H., Tao, J., Chen, L., Liu, Y., Lei, G., Liu, X. and Smol, J.P. (2015). Industrial arsenic contamination causes catastrophic changes in freshwater ecosystems. *Scientific reports*, 5(1), p.17419.
- Chen, L., Liu, J., Zhang, W., Zhou, J., Luo, D. and Li, Z. (2021). Uranium (U) source, speciation, uptake, toxicity and bioremediation strategies in soil-plant system: a review. *Journal of Hazardous Materials*, 413, p.125319.
- da Silva, E.F., Almeida, S.F., Nunes, M.L., Luís, A.T., Borg, F., Hedlund, M., de Sá, C.M., Patinha, C. and Teixeira, P. (2009). Heavy metal pollution downstream the abandoned Coval da Mó mine (Portugal) and associated effects on epilithic diatom communities. *Science of the total environment*, 407(21), pp.5620-5636.
- Dash, R.R., Gaur, A. and Balomajumder, C. (2009). Cyanide in industrial wastewaters and its removal: a review on biotreatment. *Journal of hazardous materials*, 163(1), pp.1-11.
- Day, J. and Dallas, H. (2011). 'Understanding the basics of water quality', in Quentin Grafton, R. and Hussey, K. (Eds.) *Water resources planning and management*, pp. 68 - 89.
- Delgado, C., Pardo, I. and García, L. (2012). Diatom communities as indicators of ecological status in Mediterranean temporary streams (Balearic Islands, Spain). *Ecological indicators*, 15(1), pp.131-139.
- Delhaize, E. and Ryan, P.R. (1995). Aluminum toxicity and tolerance in plants. *Plant physiology*, 107(2), p.315.
- Dixit, S.S., Smol, J.P., Kingston, J.C. and Charles, D.F. (1992). Diatoms: powerful indicators of environmental change. *Environmental science and technology*, 26(1), pp.22-33.
- Dudumashe, N. and Thomas, A. (2020). Assessment of land surface temperature and drought indices for the Klerksdorp-Orkney-Stilfontein-Hartebeesfontein (KOSH) region. In *Forest ecosystems under climate change: biological productivity and remote monitoring*, 6, pp. 46-68.

Durand, J.F. (2012). The impact of gold mining on the Witwatersrand on the rivers and karst system of Gauteng and North West Province, South Africa. *Journal of African Earth Sciences*, 68, pp.24-43.

DWAF (1993a). *South African water quality guidelines: Volume 1-Domestic use*. Department of Water Affairs and Forestry.

DWAF (1993b). *South African water quality guidelines: Volume 4-Agricultural use: irrigation*. Department of Water Affairs and Forestry.

DWAF (1993c). *South African water quality guidelines: Volume 7-Aquatic ecosystems*. Department of Water Affairs and Forestry.

Eisler, R. and Wiemeyer, S.N. (2004). Cyanide hazards to plants and animals from gold mining and related water issues. *Reviews of environmental contamination and toxicology*, 183, pp.21-54.

Element, C.A.S. (2007). Method 3051A microwave assisted acid digestion of sediments, sludges, soils, and oils. *Z. Für Analytical Chemistry*, 111, pp.362-366.

Falasco, E., Bona, F., Badino, G., Hoffmann, L. and Ector, L. (2009). Diatom teratological forms and environmental alterations: a review. *Hydrobiologia*, 623, pp.1-35.

Falasco, E., Ector, L., Wetzel, C.E., Badino, G. and Bona, F. (2021). Looking back, looking forward: a review of the new literature on diatom teratological forms (2010–2020). *Hydrobiologia*, 848, pp.1675-1753.

Filella, M., Belzile, N. and Chen, Y.W. (2002). Antimony in the environment: a review focused on natural waters: I. Occurrence. *Earth-science reviews*, 57(1-2), pp.125-176.

Fortin, C., Wang, F. and Pitre, D. (2011). *Critical Review of Platinum Group Elements (Pd, Pt, Rh) in Aquatic Ecosystem*. Available at: <https://espace.inrs.ca/id/eprint/879/1/R001269.pdf> (Accessed: 11 August 2023).

Fuelling, L.J. 2013. *Interactive effects of AMD and grazing on Periphyton productivity, biomass, and Diatom diversity* (Doctoral dissertation, Ohio University).

- Gawrońska, H., Przybysz, A., Szalacha, E., Pawlak, K., Brama, K., Miszczak, A., Stankiewicz-Kosyl, M. and Gawroński, S.W. (2018). Platinum uptake, distribution and toxicity in *Arabidopsis thaliana* L. plants. *Ecotoxicology and Environmental Safety*, 147, pp.982-989.
- Giannakoula, A., Moustakas, M., Mylona, P., Papadakis, I. and Yupsanis, T. (2008). Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. *Journal of plant physiology*, 165(4), pp.385-396.
- Gold, C., Feurtet-Mazel, A., Coste, M. and Boudou, A. (2003). Impacts of Cd and Zn on the development of periphytic diatom communities in artificial streams located along a river pollution gradient. *Archives of environmental contamination and toxicology*, 44, pp.0189-0197.
- Gooden, W.B.S. (2002). *Periphyton responses to surfactants: Community structure and mat architecture*. Bowling Green State University.
- Gupta, D.K. and Walther, C. Eds. (2018). *Behaviour of Strontium in Plants and the Environment*. Cham: Springer International Publishing.
- Hadi, F. and Aziz, T. (2015). A mini review on lead (Pb) toxicity in plants. *Journal of Biology and Life Science*, 6(2), pp.91-101.
- Hallock, P. (1997). Reefs and reef limestones in Earth history. *Life and death of coral reefs*, pp.13-42.
- Hasanuzzaman, M., Nahar, K. and Fujita, M. (2015). Arsenic toxicity in plants and possible remediation. *Soil remediation and plants: Prospects and challenges*, pp.433-501.
- Imtiaz, M., Rizwan, M.S., Xiong, S., Li, H., Ashraf, M., Shahzad, S.M., Shahzad, M., Rizwan, M. and Tu, S. (2015). Vanadium, recent advancements and research prospects: A review. *Environment International*, 80, pp.79-88.
- International Cyanide Management Institute (2016). Guidance for the mining operations verification protocol. Available at: <https://cyanidecode.org/wp-content/uploads/2021/06/15-Mining-Guidance-JUNE-2021.pdf> (Accessed: 4 February 2024).

Ireland, R. (2020). 'Amalgamation', in Yeung, C. (Ed.), *A Dictionary of Dentistry*. Second edition. Oxford University Press. Available at: <https://www.oxfordreference.com/view/10.1093/acref/9780191828621.001.0001/acref-9780191828621-e-176> (Accessed: 4 July 2023).

Johnson, C.A. (2015). The fate of cyanide in leach wastes at gold mines: An environmental perspective. *Applied geochemistry*, 57, pp.194-205.

Kamunda, C., Mathuthu, M. and Madhuku, M. (2016). An assessment of radiological hazards from gold mine tailings in the province of Gauteng in South Africa. *International Journal of environmental research and public health*, 13(1), p.138.

Katiyar, D., Lall, A.M. and Singh, B. (2010). Effect of phosphate on growth of diatoms. *Indian Journal of Scientific Research*, 1(2), pp.103-106.

Kock, A., Taylor, J.C. and Malherbe, W. (2019). Diatom community structure and relationship with water quality in Lake Sibaya, KwaZulu-Natal, South Africa. *South African journal of botany*, 123, pp.161-169.

Köhler, L., Machill, S., Werner, A., Selzer, C., Kaskel, S. and Brunner, E. (2017). Are diatoms "green" aluminosilicate synthesis microreactors for future catalyst production? *Molecules*, 22(12), p.2232.

Kosla, T., Skibniewska, E., Debski, B. and Urbanska-Slomka, G. (2002). Rubidium in the trophic chain soil-plants-animals. *Trace elements and electrolytes*, 19(4), pp.171-176.

Kroth, P. (2007). Molecular biology and the biotechnological potential of diatoms. *Transgenic microalgae as green cell factories*, 616, pp.23-33.

Kumar, R., Saha, S., Dhaka, S., Kurade, M.B., Kang, C.U., Baek, S.H. and Jeon, B.H. (2017). Remediation of cyanide-contaminated environments through microbes and plants: a review of current knowledge and future perspectives. *Geosystem Engineering*, 20(1), pp.28-40.

Kumar, V., Suryakant, P., Kumar, S. and Kumar, N. (2016). Effect of chromium toxicity on plants: A review. *Agriways*, 4(1), pp.107-120.

Kuyucak, N. and Akcil, A. (2013). Cyanide and removal options from effluents in gold mining and metallurgical processes. *Minerals Engineering*, 50, pp.13-29.

Laitos, J.G. (2012). Cyanide, mining, and the environment. *Pace Environmental Law Review*, 30. Available at:

<https://digitalcommons.pace.edu/cgi/viewcontent.cgi?article=1728&context=pelr>
(Accessed: 4 February 2024).

Leira, M., Chen, G., Dalton, C., Irvine, K. and Taylor, D. (2009). Patterns in freshwater diatom taxonomic distinctness along an eutrophication gradient. *Freshwater Biology*, 54(1), pp.1-14.

Luís, A.T., Durães, N., de Almeida, S.F.P. and da Silva, E.F. (2016). Integrating geochemical (surface waters, stream sediments) and biological (diatoms) approaches to assess AMD environmental impact in a pyritic mining area: Aljustrel (Alentejo, Portugal). *Journal of environmental sciences*, 42, pp.215-226.

Luís, A.T., Teixeira, P., Almeida, S.F.P., Matos, J.X. and da Silva, E.F. (2011). Environmental impact of mining activities in the Lousal area (Portugal): chemical and diatom characterization of metal-contaminated stream sediments and surface water of Corona stream. *Science of the Total Environment*, 409(20), pp.4312-4325.

Lusilao-Makiese, J.G., Tessier, E., Amouroux, D., Tutu, H., Chimuka, L., Weiersbye, I. and Cukrowska, E.M. (2014). Seasonal distribution and speciation of mercury in a gold mining area, north-west province, South Africa. *Toxicological and Environmental Chemistry*, 96(3), pp.387-402.

Lwalaba, J.L.W., Zvobgo, G., Fu, L., Zhang, X., Mwamba, T.M., Muhammad, N., Mundende, R.P.M. and Zhang, G. (2017). Alleviating effects of calcium on cobalt toxicity in two barley genotypes differing in cobalt tolerance. *Ecotoxicology and Environmental Safety*, 139, pp.488-495.

Mahey, S., Kumar, R., Sharma, M., Kumar, V. and Bhardwaj, R. (2020). A critical review on toxicity of cobalt and its bioremediation strategies. *SN Applied Sciences*, 2, pp.1-12.

Marella, T.K., Saxena, A. and Tiwari, A. (2020). Diatom mediated heavy metal remediation: A review. *Bioresource Technology*, 305, p.123068.

Mäser, P., Gierth, M. and Schroeder, J.I. (2002). Molecular mechanisms of potassium and sodium uptake in plants. In *Progress in Plant Nutrition: Plenary Lectures of the XIV International Plant Nutrition Colloquium: Food security and sustainability of agro-ecosystems through basic and applied research* (pp. 43-54). Springer Netherlands.

Mayhew, S. (2023). 'Benthic'. *A Dictionary of Geography*. Sixth edition. Oxford University Press. Available at: <https://www.oxfordreference.com/view/10.1093/acref/9780192896391.001.0001/acref-9780192896391-e-315> (Accessed: 3 July 2023).

Mikhailovskaya, L.N. and Pozolotina, V.N. (2020). Spatial distribution of 90 Sr from different sources in soils of the Ural region, Russia. *Strontium Contamination in the Environment*, 88, pp.141-158.

Millaleo, R., Reyes-Díaz, M., Ivanov, A.G., Mora, M.L. and Alberdi, M.

(2010). Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *Journal of soil science and plant nutrition*, 10(4), pp.470-481.

Mishra, M., Arukha, A.P., Bashir, T., Yadav, D. and Prasad, G.B.K.S. (2017). All new faces of diatoms: potential source of nanomaterials and beyond. *Frontiers in Microbiology*, 8, p.1239.

Molino, P.J. and Wetherbee, R. (2008). The biology of biofouling diatoms and their role in the development of microbial slimes. *Biofouling*, 24(5), pp.365-379.

Morin, S., Cordonier, A., Lavoie, I., Arini, A., Blanco, S., Duong, T.T., Tornés, E., Bonet, B., Corcoll, N., Faggiano, L. and Laviale, M. (2012). Consistency in diatom response to metal-contaminated environments. *Emerging and priority pollutants in rivers: bringing science into river management plans*, 19, pp.117-146.

Munakamwe, J. (2017). Zamazama–Livelihood strategies, mobilisation and resistance in Johannesburg, South Africa. *Mining Africa: Law, environment, society and politics in historical and multidisciplinary perspectives*, pp.155-185.

- Nazar, R., Iqbal, N., Masood, A., Khan, M.I.R., Syeed, S. and Khan, N.A. (2012). Cadmium toxicity in plants and role of mineral nutrients in its alleviation. *American Journal of Plant Sciences*, 3(10), pp. 1476-1489.
- Morin, S., Duong, T.T., Dabrin, A., Coynel, A., Herlory, O., Baudrimont, M., Delmas, F., Durrieu, G., Schäfer, J., Winterton, P. and Blanc, G. (2008). Long-term survey of heavy-metal pollution, biofilm contamination and diatom community structure in the Riou Mort watershed, South-West France. *Environmental Pollution*, 151(3), pp.532-542.
- Nel, E. and Binns, T. (2002). Decline and response in South Africa's Free State goldfields: local economic development in Matjhabeng. *International Development Planning Review*, 24(3), pp.249-270.
- Nieves-Cordones, M., Al Shiblawi, F.R. and Sentenac, H. (2016). Roles and transport of sodium and potassium in plants. *The alkali metal ions: Their role for life*, 16, pp.291-324.
- Olenici, A., Blanco, S., Borrego-Ramos, M., Momeu, L. and Baciú, C. (2017). Exploring the effects of acid mine drainage on diatom teratology using geometric morphometry. *Ecotoxicology*, 26, pp.1018-1030.
- Orlekowsky, T., Venter, A., Van Wyk, F. and Levanets, A. (2013). Cyanobacteria and algae of gold mine tailings in the Northwest Province of South Africa. *Nova Hedwigia*, 97(3-4), pp.281-294.
- Pal, M., Samal, N.R., Roy, P.K. and Roy, M.B. (2015). Electrical conductivity of lake water as environmental monitoring—A case study of Rudrasagar Lake. *Journal of Environmental Science, Toxicology and Food Technology*, 9(3), pp.66-71.
- Pandey, L.K. and Bergey, E.A. (2016). Exploring the status of motility, lipid bodies, deformities and size reduction in periphytic diatom community from chronically metal (Cu, Zn) polluted waterbodies as a biomonitoring tool. *Science of the Total Environment*, 550, pp.372-381.
- Park, C., & Allaby, M. (2017a). 'Acidification'. *A Dictionary of Environment and Conservation*. Third edition. Oxford University Press. Available at:

<https://www.oxfordreference.com/view/10.1093/acref/9780191826320.001.0001/acref-9780191826320-e-57> (Accessed: 4 July 2023).

Park, C., & Allaby, M. (2017b). 'Acid mine drainage'. *A Dictionary of Environment and Conservation*. Third edition. Oxford University Press. Available at: <https://www.oxfordreference.com/view/10.1093/acref/9780191826320.001.0001/acref-9780191826320-e-47> (Accessed: 4 July 2023).

Park, C. and Allaby, M. (2017a). 'Hypertrophic'. *A Dictionary of Environment and Conservation*. Third edition. Oxford University Press. Available at: <https://www.oxfordreference.com/view/10.1093/acref/9780191826320.001.0001/acref-9780191826320-e-3888> (Accessed: 3 July 2023).

Park, C. and Allaby, M. (2017b). 'Phytoremediation'. *A Dictionary of Environment and Conservation*. Third edition. Oxford University Press. Available at: <https://www.oxfordreference.com/view/10.1093/acref/9780191826320.001.0001/acref-9780191826320-e-6094> (Accessed: 4 July 2023).

Park, C., and Allaby, M. (2017c). 'Phytotoxic'. *A Dictionary of Environment and Conservation*. Third edition. Oxford University Press. Available at: <https://www.oxfordreference.com/view/10.1093/acref/9780191826320.001.0001/acref-9780191826320-e-6095> (Accessed: 3 July 2023).

Park, C., and Allaby, M. (2017d). 'Saturated zone'. *A Dictionary of Environment and Conservation*. Third edition. Oxford University Press. Available at: <https://www.oxfordreference.com/view/10.1093/acref/9780191826320.001.0001/acref-9780191826320-e-7132> (Accessed: 3 July 2023).

Park, C., and Allaby, M. (2017e). 'Wetland'. *A Dictionary of Environment and Conservation*. Third edition. Oxford University Press. Available at: <https://www.oxfordreference.com/view/10.1093/acref/9780191826320.001.0001/acref-9780191826320-e-8906> (Accessed: 4 July 2023).

Park, J., Lee, H., Depuydt, S., Han, T. and Pandey, L.K. (2020). Assessment of five live-cell characteristics in periphytic diatoms as a measure of copper stress. *Journal of Hazardous Materials*, 400, p.123113.

- Patrick, R. (1978). Effects of Trace Metals in the Aquatic Ecosystem: The diatom community, base of the aquatic food chain, undergoes significant changes in the presence of trace metals and other alterations in water chemistry. *American scientist*, 66(2), pp.185-191.
- Petersen, W., Willer, E. and Willamowski, C. (1997). Remobilization of trace elements from polluted anoxic sediments after resuspension in oxic water. *Water, Air, and Soil Pollution*, 99, pp.515-522.
- Pintor, A.M., Vieira, B.R., Boaventura, R.A. and Botelho, C.M. (2020). Removal of antimony from water by iron-coated cork granulates. *Separation and Purification Technology*, 233, p.116020.
- Rimet, F., Trobajo, R., Mann, D.G., Kermarrec, L., Franc, A., Domaizon, I. and Bouchez, A. (2014). When is sampling complete? The effects of geographical range and marker choice on perceived diversity in *Nitzschia palea* (Bacillariophyta). *Protist*, 165(3), pp.245-259.
- Rösner, T. and Van Schalkwyk, A. (2000). The environmental impact of gold mine tailings footprints in the Johannesburg region, South Africa. *Bulletin of Engineering Geology and the Environment*, 59, pp.137-148.
- Round, F.E., Crawford, R.M. and Mann, D.G. (1990). *Diatoms: biology and morphology of the genera*. Cambridge university press.
- Roy, A.K., Sharma, A. and Talukder, G. (1988). Some aspects of aluminum toxicity in plants. *The botanical review*, 54, pp.145-178.
- Roychoudhury, A. (2020). Vanadium uptake and toxicity in plants. *SF Journal of Agricultural and Crop Management*, 1(2), 1010.
- Saaltink, R.M., Dekker, S.C., Eppinga, M.B., Griffioen, J. and Wassen, M.J. (2017). Plant-specific effects of iron-toxicity in wetlands. *Plant and Soil*, 416, pp.83-96.
- Salomoni, S.E., Rocha, O., Callegaro, V.L. and Lobo, E.A. (2006). Epilithic diatoms as indicators of water quality in the Gravataí River, Rio Grande do Sul, Brazil. *Hydrobiologia*, 559, pp.233-246.

Sasmaz, M. and Sasmaz, A. (2017). The accumulation of strontium by native plants grown on Gumuskoy mining soils. *Journal of Geochemical Exploration*, 181, pp.236-242.

Shah, A.N., Tanveer, M., Hussain, S. and Yang, G. (2016). Beryllium in the environment: Whether fatal for plant growth? *Reviews in Environmental Science and Biotechnology*, 15, pp.549-561.

Sheppard, S.C., Sheppard, M.I., Gallerand, M.O. and Sanipelli, B. (2005). Derivation of ecotoxicity thresholds for uranium. *Journal of Environmental Radioactivity*, 79(1), pp.55-83.

Simate, G.S. and Ndlovu, S. (2014). Acid mine drainage: Challenges and opportunities. *Journal of environmental chemical engineering*, 2(3), pp.1785-1803.

Singh, M. and Parikh, P. (2020). Freshwater diatoms as bio-indicators in urban wetlands of Central Gujarat, India. *Indian Journal of Ecology*, 47(1), pp.7-11.

Sienkiewicz, E., Gąsiorowski, M., Sekudewicz, I., Kowalewska, U. and Matoušková, Š. (2023). Responses of diatom composition and teratological forms to environmental pollution in a post-mining lake (SW Poland). *Environmental Science and Pollution Research*, 30(51), pp.110623-110638.

Sivarajah, B., Korosi, J.B., Blais, J.M. and Smol, J.P. (2019). Multiple environmental variables influence diatom assemblages across an arsenic gradient in 33 subarctic lakes near abandoned gold mines. *Hydrobiologia*, 841, pp.133-151.

Srivastava, P., Verma, J., Grover, S. and Sardar, A. (2016). On the importance of diatoms as ecological indicators in river ecosystems: a review. *Indian Journal of Plant Sciences*, 5(1), pp.70-86.

Suwa, R., Jayachandran, K., Nguyen, N.T., Boulenouar, A., Fujita, K. and Saneoka, H. (2008). Barium toxicity effects in soybean plants. *Archives of Environmental Contamination and Toxicology*, 55, pp.397-403.

Taylor, J. C. and Cocquyt, C. (2016). *Diatoms from the Congo and Zambezi Basins – Methodologies and identification of the genera*, Vol. 16, Abc Taxa, The Belgian development cooperation, Belgium.

Taylor, J.C., Harding, W.R. and Archibald, C.G.M. (2007a). A methods manual for the collection, preparation and analysis of diatom samples. *Version, 1*, p.60.

Taylor, J.C., de la Rey, P.A. and van Rensburg, L. (2005). Recommendations for the collection, preparation and enumeration of diatoms from riverine habitats for water quality monitoring in South Africa. *African Journal of Aquatic Science*, 30(1), pp.65-75.

Taylor, J.C., Harding, W.R. and Archibald, C.G.M. (2007b). *An illustrated guide to some common diatom species from South Africa* (pp. 1-225). Pretoria: Water Research Commission.

Thomas, A. (2020). Mapping of land use/land cover of Klerksdorp–Orkney–Stilfontein–Hartebeestfontein (KOSH) region for the years 2020 and 2019 using sentinel-2 data. In *Forest ecosystems under climate change: biological productivity and remote monitoring*, 6, pp. 6-28.

Tschan, M., Robinson, B.H. and Schulin, R. (2009). Antimony in the soil–plant system—a review. *Environmental chemistry*, 6(2), pp.106-115.

Van Hoewyk, D. (2013). A tale of two toxicities: malformed selenoproteins and oxidative stress both contribute to selenium stress in plants. *Annals of botany*, 112(6), pp.965-972.

Verbruggen, E.M.J., Smit, C.E. and Van Vlaardingen, P.L.A. (2020). *Environmental quality standards for barium in surface water: Proposal for an update according to the methodology of the Water Framework Directive*. Available at: <https://rivm.openrepository.com/bitstream/handle/10029/623740/2020-0024.pdf?sequence=5> (Accessed: 13 Augustus 2023).

Wang, Y., Cai, J., Jiang, Y., Jiang, X. and Zhang, D. (2013). Preparation of biosilica structures from frustules of diatoms and their applications: current state and perspectives. *Applied microbiology and biotechnology*, 97, pp.453-460.

Wassie, T.A. (2008). *Comparative study of macro invertebrates and diatoms as bioindicators of river water quality in Addis Ababa* (Doctoral dissertation, MSc thesis, Addis Ababa University, Addis Ababa, Ethiopia).

Yruela, I. (2005). Copper in plants. *Brazilian Journal of Plant Physiology*, 17, pp.145-156.

Yusuf, M., Fariduddin, Q., Hayat, S. and Ahmad, A. (2011). Nickel: an overview of uptake, essentiality and toxicity in plants. *Bulletin of environmental contamination and toxicology*, 86, pp.1-17.

Zaid, A., Ahmad, B., Jaleel, H., Wani, S.H. and Hasanuzzaman, M. (2020). A critical review on iron toxicity and tolerance in plants: role of exogenous phytoprotectants. *Plant micronutrients: Deficiency and toxicity management*, pp.83-99.

Zulfiqar, U., Ayub, A., Hussain, S., Waraich, E.A., El-Esawi, M.A., Ishfaq, M., Ahmad, M., Ali, N. and Maqsood, M.F. (2022). Cadmium toxicity in plants: Recent progress on morpho-physiological effects and remediation strategies. *Journal of soil science and plant nutrition*, 22, pp.1-58.

Zulfiqar, U., Farooq, M., Hussain, S., Maqsood, M., Hussain, M., Ishfaq, M., Ahmad, M. and Anjum, M.Z. (2019). Lead toxicity in plants: Impacts and remediation. *Journal of environmental management*, 250, p.109557.

Appendix A

Diatom species present at sites from June 2021 to May 2022

Group 1 - Karee Rand TSF complex

Karee Rand TSF												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
	x	x	x	x	x	x	x	x	x	x	x	x
Species	0	0	0	0	0	0	0	0	0	0	0	0
Species containing deformities	0	0	0	0	0	0	0	0	0	0	0	0
Number of deformed cells	0	0	0	0	0	0	0	0	0	0	0	0

Present	✓
Absent	x
If cells were enough to count they are indicated by a number	

Karee Rand RWD												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnantheidium sp.</i>	x	x	x	x	x	✓	x	x	x	x	✓	x
<i>Caloneis aequatorialis</i>	x	x	✓	x	x	x	x	x	x	x	✓	x
<i>Cocconeis placentula</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Craticula buderi</i>	✓	x	✓	x	x	x	x	x	x	x	x	x
<i>Cyclotella ocellata</i>	x	x	x	x	x	✓	x	x	x	x	x	x
<i>Cymbella tumida</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Cymbella turgidula</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Diatoma vulgaris</i>	✓	x	x	x	x	x	x	x	x	x	✓	x
<i>Encyonopsis leei</i> var. <i>sinensis</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Fragilaria capucina</i> var. <i>vaucheriae</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Fragilaria ungeriana</i>	x	x	x	x	x	x	x	x	x	x	✓	x

Present	✓
Absent	x
If cells were enough to count they are indicated by a number	

<i>Gomphonema parvulum</i>	✓	x	x	x	x	x	x	x	x	x	✓	x
<i>Gomphonema venusta</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Melosira varians</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Navicula cryptotenella</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Navicula veneta</i>	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Nitzschia frustulum</i>	x	x	x	x	x	✓	x	x	x	x	x	x
<i>Nitzschia palea</i>	✓	x	✓	x	x	x	x	x	✓	x	✓	x
<i>Nitzschia</i> sp. 2	x	✓	x	x	x	x	x	x	x	x	x	x
<i>Stauroneis</i> sp.?	x	x	x	x	x	x	x	x	x	x	✓	x
Species	5	1	3	0	0	3	0	0	1	0	15	0
Species containing deformities	0	0	0	0	0	0	0	0	0	0	0	0
Number of deformed cells	0	0	0	0	0	0	0	0	0	0	0	0

Karee Rand DD												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnanthidium sp.</i>	x	x	✓	x	x	x	✓	x	20	x	x	x
<i>Amphora coffeaeformis</i>	x	x	x	x	x	x	x	✓	x	x	x	x
<i>Amphora veneta</i>	x	x	✓	x	x	x	x	x	x	x	x	x
<i>Brachysira neoexilis</i>	x	x	✓	x	x	x	✓	x	x	✓	x	x
<i>Caloneis aequatorialis</i>	x	x	✓	x	x	x	x	x	x	x	x	x
<i>Craticula buderi</i>	x	x	✓	x	x	✓	x	✓	64	x	x	✓
<i>Encyonema caespitosum</i>	x	x	x	x	x	x	✓	x	x	x	x	x
<i>Encyonopsis microcephala</i>	x	x	✓	x	x	x	✓	x	x	x	x	x
<i>Eolimna subminuscula</i>	x	x	x	x	x	x	x	x	x	✓	x	x
<i>Fragilaria ulna</i>	x	x	x	x	x	x	✓	x	x	x	x	x
<i>Gomphonema angustatum</i>	x	x	x	x	x	x	x	x	4	x	x	x

Present	✓
Absent	x
If cells were enough to count they are indicated by a number	

<i>Gomphonema parvulum</i>	x	x	x	x	x	x	x	x	87	x	x	x
<i>Navicula erifuga</i>	x	x	x	x	x	x	✓	x	x	x	x	x
<i>Navicula veneta</i>	x	x	✓	x	x	x	x	✓	15	x	x	x
<i>Nitzschia frustulum</i>	x	x	x	x	x	x	x	x	205	x	x	x
<i>Nitzschia palea</i>	x	x	x	x	x	x	✓	x	3	x	x	x
<i>Nitzschia</i> sp.	x	x	✓	x	✓	x	x	x	x	x	x	x
<i>Pinnularia divergens</i> var. <i>undulata</i>	x	x	✓	x	x	x	x	x	x	x	x	x
<i>Pinnularia</i> sp.	x	x	x	x	x	x	x	✓	x	x	x	x
<i>Planothidium frequentissimum</i>	x	x	x	x	x	x	x	x	3	x	x	x
<i>Planothidium frequentissimum</i> RV	x	x	x	x	x	x	x	x	3	x	x	x
<i>Stauroneis</i> sp.	x	x	x	x	x	x	x	x	x	x	x	✓
Species	0	0	9	0	1	1	7	4	9	2	0	2
Species containing deformities	0	0	0	0	0	0	0	0	0	0	0	0

Number of deformed cells	0	0	0	0	0	0	0	0	0	0	0	0
--------------------------	---	---	---	---	---	---	---	---	---	---	---	---

Catchment Dam												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnanthidium</i> sp.	3	20	✕	2	8	109	✕	✕	3	✕	✕	✓
<i>Brachysira neoexilis</i>	✕	✕	✕	✕	✕	✕	✕	✕	2	✕	✕	✕
<i>Craticula buderi</i>	49	127	✓	18	46	99	✕	✕	✕	✓	✓	✓
<i>Craticula buderi</i> (deform)	2	1	✕	2	✕	1	✕	✕	✕	✕	✕	✕
<i>Gomphonema</i> aff. <i>Gracile</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓
<i>Gomphonema</i> aff. <i>Gracile</i> (deform)	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓
<i>Gomphonema angustatum</i>	✕	✕	✕	36	✕	✕	✕	✕	✕	✕	✓	✕
<i>Gomphonema gracile</i>	50	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Gomphonema parvulum</i>	259	224	✓	291	302	133	✕	✕	✕	✓	✓	✓

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

<i>Gomphonema pseudoaugur</i>	x	x	x	x	x	x	x	x	x	✓	x	x
<i>Mayamaea</i> sp.	x	x	x	x	x	x	x	x	x	x	x	✓
<i>Navicula riediana</i>	1	x	x	x	x	x	x	x	x	x	x	x
<i>Navicula seibigiana</i>	x	x	✓	x	x	x	x	x	x	✓	x	x
<i>Navicula veneta</i>	14	17	✓	11	6	8	x	x	x	✓	✓	✓
<i>Nitzschia communis</i>	x	x	x	8	x	x	x	x	x	x	x	x
<i>Nitzschia frustulum</i>	x	x	✓	4	7	x	x	x	x	✓	✓	✓
<i>Nitzschia frustulum</i> (deform)	x	x	x	2	x	x	x	x	x	x	✓	x
<i>Nitzschia linearis</i>	1	x	✓	6	45	24	x	x	x	x	x	✓
<i>Nitzschia nana</i>	x	x	x	2	14	2	x	x	x	x	x	x
<i>Nitzschia palea</i>	20	11	x	18	x	14	x	x	388	x	x	x
<i>Nitzschia palea</i> (deform)	2	x	x	x	x	x	x	x	9	x	x	x
<i>Nitzschia</i> sp.3	x	x	x	x	x	7	x	x	x	x	x	x
<i>Nitzschia</i> sp. 3 (deform)	x	x	x	x	x	1	x	x	x	x	x	x
<i>Pinnularia gibba</i>	x	x	x	x	x	2	x	x	x	x	x	x

<i>Planothidium frequentissimum</i>	2	x	✓	1	x	x	x	x	x	✓	x	x
<i>Tryblionella hungarica</i>	x	x	✓	1	3	x	x	x	x	x	✓	x
Species	8	5	8	12	8	10	0	0	3	7	6	8
Species containing deformities	2	1	0	0	0	1	0	0	1	0	1	1
Number of deformed cells	4	1	0	0	0	1	0	0	9	0	1	1

Group 2 – West Complex TSF complex

West Complex TSF												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Cyclotella meneghiniana</i>	x	x	x	x	x	x	x	x	x	x	✓	✓
<i>Gomphonema parvulum</i>	x	x	x	x	x	x	x	x	✓	x	x	x
<i>Navicula erifuga</i>	x	x	x	x	x	x	x	x	✓	x	x	x
<i>Nitzschia aurariae</i>	x	x	x	x	x	x	x	✓	✓	x	x	x
<i>Nitzschia palea</i>	x	x	x	x	x	✓	x	✓	✓	x	✓	✓
<i>Surirella ovalis</i>	x	x	x	x	x	x	x	x	x	x	✓	✓
<i>Tryblionella hungarica</i>	x	x	x	x	x	x	x	x	x	x	x	✓
Species	0	0	0	0	0	1	0	2	4	0	3	4
Species containing deformities	0	0	0	0	0	0	0	0	0	0	0	0
Number of deformed cells	0	0	0	0	0	0	0	0	0	0	0	0

Present	✓
Absent	x
If cells were enough to count they are indicated by a number	

Queen Mary RWD												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnantheidium</i> sp.	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕	✕
<i>Amphora</i> sp.	✕	✓	✕	✓	✕	✕	✕	✕	✕	✕	✕	✕
<i>Craticula buderi</i>	✕	✓	✕	✕	✕	✕	✕	✕	✕	✓	✓	382
<i>Navicula erifuga</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	1
<i>Navicula veneta</i>	✕	✕	✓	✕	✕	✓	✕	✕	✕	✕	✓	4
<i>Nitzschia nana</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕
<i>Nitzschia palea</i>	✓	✓	✓	✓	✓	✓	✕	✓	✕	✓	✓	16
Species	1	3	2	2	1	2	0	1	0	3	4	4
Species containing deformities	0	0	0	0	0	0	0	0	0	0	0	0
Number of deformed cells	0	0	0	0	0	0	0	0	0	0	0	0

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

Queen Mary (West complex DD)												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnantheidium</i> sp.	x	x	x	x	x	x	✓	x	x	x	x	x
<i>Brachysira neoexilis</i>	x	x	x	x	✓	x	x	x	x	x	x	x
<i>Craticula buderi</i>	x	x	x	x	✓	✓	x	x	x	x	x	x
<i>Entomoneis paludosa</i>	x	x	x	✓	x	x	x	x	x	x	x	x
<i>Gomphonema parvulus</i>	x	x	x	x	✓	x	x	x	x	x	x	x
<i>Navicula veneta</i>	x	x	x	x	x	✓	x	x	x	x	x	x
<i>Nitzschia nana</i>	x	x	x	✓	✓	✓	x	x	x	x	x	x
<i>Nitzschia palea</i>	x	x	x	✓	x	✓	x	x	x	x	x	x
Species	0	0	0	3	4	4	1	0	0	0	0	0
Species containing deformities	0	0	0	0	0	0	0	0	0	0	0	0
Number of deformed cells	0	0	0	0	0	0	0	0	0	0	0	0

Present	✓
Absent	x
If cells were enough to count they are indicated by a number	

West Storm Water												
	Jun- 21	Jul- 21	Aug- 21	Sept- 21	Oct- 21	Nov- 21	Dec- 21	Jan- 22	Feb- 22	Mar- 22	Apr- 22	May- 22
<i>Achnantheidium</i> sp.	✕	✕	✕	✕	✕	✕	396	396	397	391	372	394
<i>Achnantheidium</i> sp. (deform)	✕	✕	✕	✕	✕	✕	✕	4	3	7	8	✕
<i>Craticula buderi</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	1	✕
<i>Nitzschia aurariae</i>	✕	✕	✕	✕	✕	✕	4	✕	✕	✕	✕	✕
<i>Nitzschia frustulum</i>	✕	✕	✓	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Nitzschia palea</i>	✓	✓	✕	✕	✓	✓	1	3	3	2	21	9
<i>Nitzschia palea</i> (deform)	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	1	✕
Species	1	1	1	0	1	1	3	2	2	2	3	2
Species containing deformities	0	0	0	0	0	0	0	1	1	1	2	0
Number of deformed cells	0	0	0	0	0	0	0	4	3	7	9	0

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

Group 3 – West Extension TSF complex

West Ext TSF												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Entomoneis paludosa</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓	3
<i>Navicula</i> sp.	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	13
<i>Nitzschia nana</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	3
<i>Nitzschia palea</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓	374
<i>Nitzschia palea</i> (deform)	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓	10
Species	0	0	0	0	0	0	0	0	0	0	2	4
Species containing deformities	0	0	0	0	0	0	0	0	0	0	1	1
Number of deformed cells	0	0	0	0	0	0	0	0	0	0	3	10

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

Bokkamp RWD												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnanthidium</i> sp.	✓	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Amphora coffeaeformis</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	1	✕
<i>Craticula buderi</i>	✕	✕	✕	✕	✕	✕	✕	✕	✓	✓	335	329
<i>Craticula buderi</i> (deform)	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	6	✕
<i>Cyclotella meneghiniana</i>	✕	✕	✕	✕	✓	✓	✕	✕	✕	✕	1	✕
<i>Nitzschia nana</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕	4
<i>Nitzschia palea</i>	✓	✓	✕	✓	✓	✓	✓	✕	✓	✓	52	58
<i>Nitzschia palea</i> (deform)	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	4	8
<i>Pinnularia</i> sp.	✕	✕	✕	✕	✕	✕	✓	✕	✓	✓	2	3
Species	2	1	0	1	2	2	2	0	3	4	5	4
Species containing deformities	0	0	0	0	0	0	0	0	0	0	2	1

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

Number of deformed cells	0	0	0	0	0	0	0	0	0	0	10	8
---------------------------------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	-----------	----------

Bokkamp DD (West Ext DD)												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Craticula buderi</i>	✕	✕	✕	✕	✕	✕	✕	✕	49	✓	34	✓
<i>Craticula buderi</i> (deform)	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	1	✕
<i>Cyclostephanos dubius</i>	✕	✕	✓	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Encyonopsis leei</i> var. <i>sinensis</i>	✕	✕	✕	✕	✕	✕	✕	1	✕	✕	✕	✕
<i>Encyonopsis microcephala</i>	✕	✕	✕	✕	✕	✕	✕	2	✕	✕	✕	✕
<i>Navicula erifuga</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	1	✕
<i>Navicula veneta</i>	✕	✕	✕	✕	✕	✕	✓	✕	✕	✕	3	✕
<i>Nitzschia nana</i>	✕	✕	✕	✕	✕	✕	✓	24	224	✓	1	✓
<i>Nitzschia palea</i>	✕	✕	✓	✕	✕	✕	✓	363	133	✓	345	✓
<i>Nitzschia palea</i> (deform)	✕	✕	✕	✕	✕	✕	✕	14	✕	✕	17	✕
Species	0	0	2	0	0	0	3	4	3	3	6	3
Species containing deformities	0	0	0	0	0	0	0	1	0	0	1	0

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

Number of deformed cells	0	0	0	0	0	0	0	14	0	0	18	0
---------------------------------	----------	----------	----------	----------	----------	----------	----------	-----------	----------	----------	-----------	----------

Group 4 – East Complex

AEL South												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnantheidium eutrophilum</i> (deform)	✕	✕	✕	✕	5	✕	✕	✕	✕	✕	✕	✕
<i>Achnantheidium</i> sp.	✕	✕	✓	✕	95	✕	✕	54	23	169	297	145
<i>Achnantheidium</i> sp. 2	✕	✕	✕	✕	✕	68	✕	✕	✕	✕	✕	✕
<i>Amphora coffeaeformis</i>	✕	✕	✕	✕	✕	✕	✕	✕	14	4	✕	✕
<i>Amphora veneta</i>	✕	✕	✓	✕	122	240	✕	32	52	3	4	7
<i>Amphora veneta</i> (deform)	✕	✕	✕	✕	6	✕	✕	✕	✕	✕	✕	✕
<i>Caloneis aequatorialis</i>	✕	✕	✕	✕	6	1	✕	✕	10	✕	✕	✕
<i>Craticula buderi</i>	✕	✕	✓	✕	15	14	✕	27	59	99	51	81
<i>Craticula buderi</i> (deform)	✕	✕	✕	✕	✕	✕	✕	✕	✕	12	✕	✕
<i>Craticula halophila</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	20	✕
<i>Cyclotella meneghiniana</i>	✕	✕	✕	✕	12	48	✕	26	6	7	3	3

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

<i>Discostella woltereckii</i>	x	x	x	x	x	x	x	x	1	x	x	x
<i>Eolimna subminuscula</i>	x	x	x	x	x	x	x	x	x	1	x	x
<i>Fragilaria ulna</i>	x	x	x	x	x	x	x	2	x	1	x	x
<i>Fragilaria ulna</i> (deform)	x	x	x	x	x	x	x	5	x	x	x	x
<i>Fragilaria unlna</i> var. <i>acus</i>	x	x	x	x	x	x	x	x	x	x	2	x
<i>Gomphonema parvulum</i>	x	x	x	x	x	1	x	x	x	x	x	x
<i>Navicula cincta</i>	x	x	x	x	20	x	x	x	x	x	x	x
<i>Navicula erifuga</i>	x	x	x	x	x	x	x	x	x	x	x	15
<i>Navicula</i> sp.	x	x	x	x	x	x	x	x	x	1	x	x
<i>Navicula veneta</i>	x	x	x	x	x	2	x	x	x	x	x	4
<i>Navicymbula pusilla</i>	x	x	x	x	x	x	x	6	68	x	11	x
<i>Nitzschia amphioxys</i>	x	x	x	x	1	x	x	x	x	x	x	x
<i>Nitzschia elegantula</i>	x	x	✓	x	113	24	x	200	94	91	13	140
<i>Nitzschia elegantula</i> (deform)	x	x	x	x	x	x	x	x	x	x	x	4
<i>Nitzschia palea</i>	x	x	x	x	7	9	x	46	74	16	1	1

<i>Nitzschia palea</i> (deform)	x	x	x	x	1	x	x	8	x	x	x	2
<i>Nitzschia</i> sp. 5	x	x	x	x	x	1	x	x	x	x	x	x
<i>Pinnularia</i> sp.	x	x	x	x	x	x	x	x	x	x	x	1
<i>Sellaphora seminulum</i>	x	x	x	x	x	1	x	x	x	x	x	x
Species	0	0	4	0	9	11	0	8	10	10	9	9
Species containing deformities	0	0	0	0	3	0	0	2	0	1	0	1
Number of deformed cells	0	0	0	0	12	0	0	13	0	12	0	2

AEL East (North)												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnanthidium</i> sp.	✕	1	✓	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Amphora veneta</i>	✕	✕	✕	✕	4	✕	✕	✕	✕	✕	✕	✕
<i>Anomoeneis sphaerophora</i>	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕	✕	✕
<i>Brachysira neoexilis</i>	✓	175	✓	✓	143	170	✕	✓	✕	✕	10	✓
<i>Brachysira neoexilis</i> (deform)	✕	4	✕	✕	10	✕	✕	✕	✕	✕	✕	✕
<i>Caloneis aequatorialis</i>	✕	4	✓	✕	21	6	✕	✓	✕	✕	1	✕
<i>Caloneis aequatorialis</i> (deform)	✕	✕	✕	✕	1	✕	✕	✕	✕	✕	✕	✕
<i>Craticula buderi</i>	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕	✕	✕
<i>Craticula perrotetii</i>	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕	✕	✕
<i>Cyclotella meneghiniana</i>	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕	✕	✕
<i>Encyonema caespitosum</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Encyonema minitum</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

<i>Fragilaria biceps</i>	x	x	x	x	x	x	x	x	✓	x	x	x
<i>Fragilaria nanana</i>	x	x	x	x	x	x	x	x	x	x	x	x
<i>Gomphonema parvulum</i>	x	x	✓	x	x	x	x	x	✓	x	x	x
<i>Gomphonema</i> sp.	x	x	✓	x	x	x	x	x	x	x	x	x
<i>Hantzschia amphioxys</i>	x	x	x	x	x	x	x	x	✓	x	x	x
<i>Mayamaea atomus</i> var. <i>permitis</i>	x	x	✓	x	x	x	x	x	x	x	x	x
<i>Navicula cryptocephala</i>	x	x	x	x	x	x	x	x	✓	x	x	x
<i>Navicula riediana</i>	✓	x	x	x	x	x	x	x	✓	x	x	x
<i>Nitzschia linearis</i> var. <i>subtilis</i>	x	x	x	x	x	x	x	x	✓	x	x	x
<i>Nitzschia linearis</i>	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Nitzschia elegantula</i>	x	x	✓	x	x	x	x	x	x	x	x	x
<i>Nitzschia frustulum</i>	x	x		✓	x	x	x	x	x	x	x	x
<i>Nitzschia nana</i>	x	x	✓	x	1	x	x	✓	x	x	x	x
<i>Nitzschia palea</i>	✓	174	✓	x	157	161	x	✓	✓	x	378	✓

<i>Nitzschia palea</i> (deform)	x	4	x	x	50	22	x	x	x	x	12	x
<i>Nitzschia umbonata</i>	x	7	x	x	x	x	x	x	x	x	x	x
<i>Nitzschia</i> sp. 4	x	x	x	x	x	44	x	x	x	x	x	x
<i>Pinnularia gibba</i>	x	x	x	x	x	x	x	x	✓	x	x	x
<i>Pinnularia</i> sp.	✓	22	✓	✓	14	x	x	✓	x	x	2	x
<i>Rhopalodia gibba</i>	x	x	x	x	x	x	x	x	✓	x	x	x
Species	5	6	10	3	6	4	0	5	13	0	4	2
Species containing deformities	0	2	0	0	3	1	0	0	0	0	1	0
Number of deformed cells	0	8	0	0	61	22	0	0	0	0	12	0

AEL DD												
	Jun- 21	Jul- 21	Aug- 21	Sept- 21	Oct- 21	Nov- 21	Dec- 21	Jan- 22	Feb- 22	Mar- 22	Apr- 22	May- 22
<i>Achnanthidium</i> sp.	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕
<i>Amphora coffeaeformis</i>	✕	✕	✕	✕	✓	✕	✕	✕	✕	✕	✕	✕
<i>Amphora montana</i>	✕	166	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕
<i>Amphora montana</i> (deform)	✕	5	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Amphora</i> sp.	✕	✕	✓	✕	✕	✕	✕	✕	✕	✓	✕	✕
<i>Brachysira neoexilis</i>	✕	1	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Caloneis aequatorialis</i>	✕	✕	✕	✕	✕	✓	✕	✕	✕	✕	✕	✕
<i>Encyonopsis microcephala</i>	✕	✕	✓	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Mayamaea atomus</i> var. <i>permitis</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕
<i>Navicula</i> sp.	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕	✕
<i>Navicula veneta</i>	✕	201	✓	✕	✓	✕	✕	✕	✕	✕	✓	✕
<i>Navicula veneta</i> (deform)	✕	1	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Nitzschia frustulum</i>	✕	19	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

<i>Nitzschia nana</i>	x	x	x	x	x	x	✓	x	x	x	✓	x
<i>Nitzschia palea</i>	x	10	✓	x	x	x	✓	x	✓	✓	✓	x
<i>Nitzschia recta</i>	x	x	x	x	✓	✓	x	x	x	x	x	x
<i>Nitzschia valdecostata</i>	x	x	x	x	✓	x	x	x	x	x	x	x
<i>Stephanodiscus agassizensis</i>	x	x	x	x	✓	x	x	x	x	x	x	x
Species	0	5	4	0	5	2	2	0	1	3	6	0
Species containing deformities	0	2	0	0	0	0	0	0	0	0	0	0
Number of deformed cells	0	6	0	0	0	0	0	0	0	0	0	0

Eye Dam												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnanthidium eutrophilum</i>	✕	✕	✕	✕	✕	40	✕	✕	✕	✕	✕	✕
<i>Achnanthidium</i> sp.	✓	✕	✓	✕	35	✕	✕	✕	✕	✕	✕	61
<i>Achnanthidium</i> sp. 2	✕	✕	✕	✕	55	✕	30	✓	31	✕	✕	✕
<i>Amphora copulata</i>	✓	✓	✓	✕	✕	1	✕	✕	✕	✕	✕	✕
<i>Amphora veneta</i>	✕	✕	✕	✕	✕	✕	1	✕	1	✕	✕	✕
<i>Anomoeneis sphaerophora</i>	✕	✕	✕	✕	✕	2	✕	✕	✕	1	✕	✕
<i>Craticula ambigua</i>	✕	✕	✕	✕	✕	✕	2	✕	✕	2	✕	✕
<i>Craticula buder</i>	✕	✓	✓	✕	✕	83	9	✓	172	✕	✓	61
<i>Craticula buder</i> (deform)	✕	✕	✕	✕	✕	✕	✕	✓	✕	✕	✕	✕
<i>Cyclotella meneghiniana</i>	✓	✓	✓	✓	72	18	13	✓	✕	✕	✓	16
<i>Diploneis elliptica</i>	✕	✕	✕	✕	✕	1	1	✕	✕	✕	✕	✕
<i>Diploneis smithii</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	2
<i>Encyonopsis microcephala</i>	✓	✓	✓	✕	33	152	13	✓	118	182	✓	144
<i>Fragilaria biceps</i>	✕	✕	✕	✕	49	✕	✕	✕	✕	✕	✕	✕
<i>Fragilaria nanana</i>	✕	✕	✕	✕	✕	✕	57	✓	31	✕	✓	✕

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

<i>Fragilaria nanana</i> (deform)	x	x	x	x	x	x	18	x	x	x	x	x
<i>Fragilaria tenera</i>	✓	x	x	x	x	x	x	x	x	51	x	39
<i>Fragilaria tenera</i> (deform)	x	x	x	x	x	x	x	x	x	3	x	x
<i>Fragilaria ulna</i>	x	x	x	x	x	31	122	✓	31	17	✓	10
<i>Fragilaria ulna</i> (deform)	x	x	x	x	x	x	x	✓	x	x	x	x
<i>Gomphonema aff. Gracile</i>	x	x	x	x	1	3	x	x	x	x	x	x
<i>Gomphonema affine</i>	x	x	x	x	x	x	8	x	x	x	x	3
<i>Gomphonema insigne</i>	x	x	✓	x	x	x	x	x	x	1	x	x
<i>Gomphonema parvulum</i>	x	x	x	x	x	x	x	x	2	x	x	x
<i>Gyrosigma rautenbachiae</i>	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Hantzschia amphioxys</i>	x	x	x	x	x	x	1	✓	x	1	x	x
<i>Luticola acidoclinata</i>	x	x	x	x	x	x	x	x	x	1	x	x
<i>Navicula</i> <i>subrhynchocephala</i>	x	x	x	x	27	x	x	✓	x	2	✓	1
<i>Navicula erifuga</i>	x	x	✓	x	x	x	4	x	x	1	✓	x
<i>Navicula rostellata</i>	x	x	x	x	x	5	18	x	x	x	x	x
<i>Navicula schroeteri</i>	x	x	x	x	3	x	x	x	x	x	x	x
<i>Navicula veneta</i>	✓	x	x	x	x	26	47	x	x	x	x	1
<i>Nitzschia amphibia</i>	x	x	x	x	20	14	34	x	x	x	x	x

<i>Nitzschia frustulum</i>	x	✓	x	x	x	x	x	✓	3	x	✓	1
<i>Nitzschia palea</i>	x	x	✓	x	x	x	10	✓	12	16	✓	x
<i>Nitzschia palea</i> (deform)	x	x	x	x	x	1	x	x	x	x	x	x
<i>Nitzschia semirobusta</i>	✓	x	✓	x	29	18	x	x	7	21	✓	61
<i>Pinnularia divergens</i>	x	✓	✓	✓	1	x	x	x	x	x	x	x
<i>Pinnularia</i> sp.	x	x	x	x	x	x	5	x	x	x	x	x
<i>Pleurosigma elongatum</i>	x	x	x	x	x	x	x	✓	x	x	x	x
<i>Sellaphora pupula</i>	x	x	✓	x	x	x	x	x	x	x	x	x
<i>Staurosira contruens</i>	✓	✓	✓	x	66	6	8	✓	3	x	✓	5
<i>Staurosira elliptica</i>	x	x	x	x	x	x	3	x	x	x	x	x
<i>Surirella ovalis</i>	x	✓	✓	x	x	x	x	x	x	x	x	x
<i>Tryblionella calida</i>	x	x	x	x	x	x	x	x	x	x	x	x
<i>Ulnaria (Fragilaria) sp.?</i>	x	x	x	x	x	x	x	x	x	x	✓	x
Species	9	8	12	2	12	14	18	12	11	11	12	13
Species containing deformities	0	0	0	0	0	1	1	2	0	1	0	0
Number of deformed cells	0	0	0	0	0	1	18	4	0	3	0	0

Group 5 – Reference sites

Koekemoer SpruitKoekemoer Spruit												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnanthidium exiguum</i>	x	x	✓	x	x	x	x	x	x	x	✓	x
<i>Amphora veneta</i>	x	x	x	x	3	x	x	x	x	x	x	x
<i>Caloneis bacillum</i>	x	✓	x	x	x	x	x	x	x	x	✓	x
<i>Craticula accomoda</i>	x	✓	✓	x	x	x	x	x	x	x	x	x
<i>Cyclotella meneghiniana</i>	x	x	x	x	x	x	✓	x	✓	✓	✓	✓
<i>Encyonema minutum</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Eolimna monoculata</i>	x	x	x	x	11	x	x	x	x	x	x	x
<i>Eolimna subminuscula</i>	x	✓	✓	x	x	69	x	✓	✓	✓	✓	x
<i>Fallacia pygmaea</i>	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Fragilaria biceps</i>	x	x	x	x	x	x	x	x	x	x	x	✓
<i>Fragilaria ulna</i>	x	x	x	x	x	x	✓	x	x	x	x	✓
<i>Gomphonema parvulum</i>	✓	✓	✓	x	122	240	✓	✓	✓	x	✓	x

Present	✓
Absent	x
If cells were enough to count they are indicated by a number	

<i>Gomphonema parvulum</i> (deform)	x	x	x	x	4	6	x	x	x	x	x	x
<i>Hantzschia amphioxys</i>	x	x	x	x	x	x	✓	x	x	x	x	x
<i>Luticola mutica</i>	x	x	x	x	1	x	x	x	x	x	x	x
<i>Mayamaea atomus</i> var. <i>permitis</i>	x	✓	x	x	x	x	x	x	x	x	✓	x
<i>Navicula cryptocephala</i>	x	x	x	x	x	x	x	x	x	✓	x	x
<i>Navicula erifuga</i>	x	x	x	x	x	x	✓	x	x	x	x	x
<i>Navicula rhynchocephala</i>	x	x	x	x	x	x	x	x	x	x	x	✓
<i>Navicula riediana</i>	x	✓	x	x	x	x	x	x	x	x	✓	✓
<i>Navicula trivialis</i>	✓	x	x	x	x	x	x	x	x	x	✓	x
<i>Navicula veneta</i>	x	x	✓	x	112	80	x	x	x	x	✓	x
<i>Navicula veneta</i> (deform)	x	x	x	x	2	x	x	x	x	x	x	x
<i>Nitzschia acicularis</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Nitzschia amphibia</i>	x	x	x	x	x	11	x	x	x	x	✓	x
<i>Nitzschia frustulum</i>	✓	x	✓	x	x	x	x	x	x	x	✓	x
<i>Nitzschia intermedia</i>	x	x	x	x	x	x	x	x	x	x	x	✓

<i>Nitzschia linearis</i>	x	x	x	x	x	x	x	x	x	x	x	✓
<i>Nitzschia linearis</i> var. <i>subtilis</i>	x	x	x	x	x	x	✓	x	x	x	x	x
<i>Nitzschia palea</i>	x	x	x	x	x	x	✓	x	✓	✓	x	✓
<i>Nitzschia sigma</i>	x	x	x	x	2	x	x	x	x	x	x	x
<i>Nitzschia sigma</i> (deform)	x	x	x	x	1	x	x	x	x	x	x	x
<i>Nitzschia</i> sp. 6	✓	✓	x	x	47	x	x	x	x	x	x	x
<i>Nitzschia</i> sp. 6 (deform)	x	x	x	x	13	x	x	x	x	x	x	x
<i>Nitzschia umbonata</i>	✓	✓	x	x	71	x	x	x	x	x	x	x
<i>Nitzschia umbonata</i> (deform)	x	x	x	x	9	x	x	x	x	x	x	x
<i>Pinnularia microstauron</i> var. <i>rostrata</i>	x	x	x	x	x	x	x	x	x	✓	x	x
<i>Pseudostaurosira</i> <i>brevistriata</i>	x	x	x	x	x	x	✓	x	x	x	x	x
<i>Sellaphora pupula</i>	✓	x	✓	x	x	x	x	x	✓	x	✓	x
<i>Sellaphora seminulum</i>	x	x	x	x	x	x	x	✓	x	✓	x	✓
<i>Surirella angusta</i>	x	x	x	x	2	x	x	x	x	x	x	x

<i>Thalassiosira weissflogi</i>	x	x	x	x	x	x	✓	x	x	x	x	✓
<i>Tryblionella calida</i>	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Tryblionella gracilis</i>	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Tryblionella hungarica</i>	x	x	✓	x	x	x	x	x	x	x	x	x
Species	9	8	8	0	9	4	9	3	5	6	14	10
Species containing deformities	0	0	0	0	5	1	0	0	0	0	0	0
Number of deformed cells	0	0	0	0	29	6	0	0	0	0	0	0

Ref A (Wildskamp)												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnanthidium eutrophilum</i>	✕	✕	✓	✕	25	✕	✕	✕	✕	✕	✕	✓
<i>Achnanthidium exiguum</i>	✕	✕	✓	✕	✕	2	✓	✕	4	✕	✕	✓
<i>Achnanthidium</i> sp.	23	43	✕	✕	✕	63	✕	✕	10	✓	✕	✕
<i>Amphora copulata</i>	1	2	✓	✕	✕	✕	✓	✓	3	✕	✕	✕
<i>Amphora inariensis</i>	✕	5	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Amphora pediculus</i>	✕	✕	✕	✕	9	✕	✕	✕	✕	✕	✕	✕
<i>Amphora</i> sp.	✕	✕	✓	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Anomoeneis sphaerophora</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕	✕
<i>Aulacoseira ambigua</i>	✕	✕	✕	✕	✕	✕	✕	✓	✕	✓	✓	✓
<i>Aulacoseira granulata</i>	1	✕	✕	✕	✕	✕	✓	✓	59	✓	✓	✕
<i>Aulacoseira muzzanensis</i>	✕	✕	✕	✕	✕	✕	✕	✕	9	✕	✕	✕
<i>Caloneis bacillum</i>	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕
<i>Cocconeis pediculus</i>	✕	✕	✓	✕	✕	✕	✕	✓	✕	✕	✕	✕

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

<i>Cocconeis placentula</i>	2	1	x	x	x	x	x	x	3	x	x	x
<i>Cocconeis placentula</i> var. <i>lineata</i>	x	x	x	x	20	3	✓	x	x	✓	x	x
<i>Craticula buderi</i>	x	x	x	x	x	x	x	x	3	x	x	x
<i>Craticula cuspidata</i>	x	x	x	x	x	x	x	x	x	x	x	✓
<i>Cymatopleura solea</i>	x	x	✓	x	x	x	x	x	x	x	x	x
<i>Cyclotella meneghiniana</i>	x	x	x	x	x	x	x	x	x	✓	✓	✓
<i>Cymbella cymbiformis</i>	x	x	x	x	4	x	x	x	x	x	x	x
<i>Cymbella neocistula</i>	x	4	x	x	x	2	x	x	x	x	x	✓
<i>Cymbella tumida</i>	x	3	x	x	x	x	✓	x	11	✓	x	✓
<i>Discostella wolterekii</i>	x	x	x	x	x	x	x	x	10	✓	✓	x
<i>Encyonema caespitosum</i>	7	9	x	x	209	160	✓	x	8	✓	✓	✓
<i>Encyonema minitum</i>	9	2	x	x	x	x	x	x	x	x	x	x
<i>Eolimna minima</i>	x	x	x	x	x	x	✓	x	x	x	x	x
<i>Eolimna</i> sp.	x	17	x	x	x	x	x	x	x	x	x	x
<i>Eolimna subminuscula</i>	x	x	x	x	x	x	x	x	x	x	✓	x

<i>Epithemia sorex</i>	8	3	✓	✕	20	✕	✓	✓	58	✓	✕	✕
<i>Fragilaria capucina</i>	✕	✕	✕	✕	74	✕	✕	✕	✕	✕	✕	✕
<i>Fragilaria capucina</i> (deform)	✕	✕	✕	✕	5	✕	✕	✕	✕	✕	✕	✕
<i>Fragilaria capucina</i> var. <i>rumpens</i>	71	44	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Fragilaria capucina</i> var. <i>vaucheriae</i>	154	117	✓	✕	✕	✕	✓	✕	44	✓	✕	✓
<i>Fragilaria capucina</i> var. <i>vaucheriae</i> (deform)	✕	2	✕	✕	✕	✕	✕	✕	4	✕	✕	✕
<i>Fragilaria nanana</i>	✕	✕	✕	✕	✕	✕	✕	✕	19	✓	✕	✕
<i>Fragilaria nanana</i> (deform)	✕	✕	✕	✕	✕	✕	✕	✕	27	✕	✕	✕
<i>Fragilaria tenera</i>	2	✕	✕	✕	✕	✕	✓	✕	✕	✕	✕	✓
<i>Fragilaria ulna</i>	✕	✕	✕	✕	✕	16	✕	✕	✕	✕	✕	✓
<i>Fragilaria ulna</i> (deform)	✕	✕	✕	✕	✕	3	✕	✕	✕	✕	✕	✕
<i>Gomphonema angustatum</i>	✕	✕	✕	✕	✕	✕	✓	✕	✕	✕	✕	✕
<i>Gomphonema clavatum</i>	✕	1	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Gomphonema gracile</i>	✕	2	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕

<i>Gomphonema italicum</i>	3	4	x	x	x	x	x	x	x	x	x	x
<i>Gomphonema laticollum</i>	2	x	x	x	x	x	x	x	x	x	x	x
<i>Gomphonema parvulum</i>	x	x	x	x	x	x	x	x	3	✓	x	x
<i>Gomphonema</i> sp.	x	x	x	x	1	x	x	x	x	x	x	x
<i>Hippodonta capitata</i>	x	x	x	x	x	1	x	x	x	x	x	x
<i>Luticola acidoclinata</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Navicula capitatoradiata</i>	11	3	x	x	27	17	✓	x	30	✓	x	x
<i>Navicula cryptotenella</i>	x	x	x	x	x	x	x	x	57	✓	x	✓
<i>Navicula veneta</i>	x	1	x	x	x	x	x	x	x	x	x	x
<i>Nitzschia amphibia</i>	x	x	x	x	4	2	✓	x	5	x	x	x
<i>Nitzschia frustulum</i>	19	45	x	x	x	x	x	x	x	✓	x	x
<i>Nitzschia gracilis</i>	x	x	x	x	x	x	x	x	x	x	x	✓
<i>Nitzschia intermedia</i>	x	x	x	x	x	x	x	x	x	✓	x	x
<i>Nitzschia linearis</i>	x	x	x	x	x	x	x	x	x	x	x	✓
<i>Nitzschia palea</i>	x	x	x	x	x	x	✓	x	37	x	x	✓

<i>Nitzschia</i> sp.	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Nitzschia</i> sp.7	x	x	x	x	x	35	x	x	x	x	x	x
<i>Nitzschia</i> sp.7 (deform)	x	x	x	x	x	3	x	x	x	x	x	x
<i>Placoneis elinensis</i>	x	x	x	x	x	x	x	x	3	x	x	x
<i>Planothidium frequentissimum</i>	x	x	x	x	2	x	x	x	x	x	x	x
<i>Rhopalodia gibba</i>	x	x	x	x	x	x	x	x	x	x	x	✓
<i>Sellaphora pupula</i>	x	x	x	x	x	x	x	✓	x	x	✓	x
<i>Staurosira elliptica</i>	88	93	✓	x	19	x	✓	✓	x	x	x	x
<i>Staurosirella pinnata</i>	x	x	x	x	x	x	x	✓	x	x	x	x
<i>Stephanodiscus agassizensis</i>	x	x	x	x	x	x	x	x	x	✓	x	x
<i>Thalassiosira weissflogi</i>	x	x	x	x	x	x	x	✓	x	x	x	x
<i>Tryblionella calida</i>	x	x	x	x	x	x	x	x	2	x	x	x
<i>Tryblionella coarctata</i>	x	x	✓	x	x	x	x	x	x	x	x	x
Species	15	19	10	0	11	10	15	9	19	18	10	16

Species containing deformities	0	1	0	0	1	2	0	0	2	0	0	0
Number of deformed cells	0	2	0	0	5	6	0	0	31	0	0	0

Skoon Spruit												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnanthidium eutrophilum</i>	x	x	x	x	x	x	x	x	x	x	✓	✓
<i>Achnanthidium</i> sp.	✓	✓	x	x	x	x	✓	x	x	x	x	x
<i>Amphora copulata</i>	✓	x	x	✓	x	✓	x	x	x	x	x	x
<i>Amphora inariensis</i>	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Amphora pediculus</i>	x	x	x	x	x	x	x	x	✓	✓	x	x
<i>Amphora</i> sp.	x	x	x	✓	x	x	x	x	x	x	x	x
<i>Anomoeneis sphaerophora</i>	x	x	x	✓	x	x	x	x	x	x	x	x
<i>Aulacoseira granulata</i>	x	x	x	✓	x	x	x	x	x	x	x	x
<i>Cocconeis placentula</i>	✓	✓	x	x	x	x	x	x	x	x	x	✓
<i>Cocconeis placentula</i> var. <i>lineata</i>	x	x	x	x	27	✓	✓	✓	✓	✓	✓	x
<i>Cocconeis</i> sp.	x	x	x	✓	x	x	x	x	x	x	x	x
<i>Craticula cuspidata</i>	x	x	x	x	2	x	x	x	x	x	x	x

Present	✓
Absent	x
If cells were enough to count they are indicated by a number	

<i>Cyclotella meneghiniana</i>	✓	✓	✗	✗	14	✗	✓	✓	✓	✓	✓	✓
<i>Cymatopleura solea</i>	✗	✗	✗	✗	6	✗	✗	✗	✗	✗	✗	✗
<i>Cymatopleura solea</i> var. <i>apiculata</i>	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓	✗
<i>Cymbella neocistula</i>	✗	✗	✗	✓	✗	✗	✗	✗	✗	✗	✗	✗
<i>Cymbella tumida</i>	✗	✗	✗	✗	1	✓	✗	✓	✓	✗	✗	✗
<i>Diadmesmis cofervacea</i>	✗	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
<i>Diatoma vulgare</i>	✓	✓	✗	✗	7	✗	✗	✗	✗	✗	✗	✗
<i>Diatoma vulgare</i> (deform)	✗	✗	✗	✗	✗	✗	✗	✓	✗	✗	✗	✗
<i>Encyonema caespitosum</i>	✗	✗	✗	✓	✗	✗	✗	✓	✗	✗	✗	✗
<i>Epithemia adnata</i>	✗	✓	✗	✗	1	✗	✗	✗	✗	✗	✗	✗
<i>Epithemia sorex</i>	✗	✓	✗	✓	168	✓	✓	✗	✗	✗	✗	✗
<i>Fragilaria biceps</i>	✗	✓	✗	✗	✗	✗	✓	✓	✗	✗	✗	✗
<i>Fragilaria biceps</i> (deform)	✗	✓	✗	✗	29	✗	✓	✗	✗	✗	✗	✗
<i>Fragilaria capucina</i> var. <i>vaucheriae</i>	✗	✗	✗	✓	✗	✗	✗	✗	✗	✗	✗	✗

<i>Fragilaria tenera</i>	x	✓	x	x	x	x	x	x	x	x	x	x
<i>Gomphonema gracile</i>	x	x	x	x	x	x	x	✓	x	x	x	x
<i>Gomphonema insigne</i>	x	x	x	x	4	x	x	x	x	x	x	x
<i>Gomphonema italicum</i>	x	x	x	x	16	✓	x	x	x	x	x	x
<i>Gomphonema laticollum</i>	x	x	x	x	2	x	x	x	x	x	x	x
<i>Gomphonema minutum</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Gomphonema parvulum</i>	x	x	x	x	x	x	x	✓	x	✓	✓	✓
<i>Gomphonema pumilum</i> <i>var. rigidum</i>	x	x	x	x	x	x	x	x	x	x	x	✓
<i>Gyrosigma</i> <i>rautenbachiae</i>	x	✓	x	x	x	x	✓	x	x	x	x	x
<i>Mayameae atomus</i>	x	x	x	x	x	x	x	x	x	x	x	✓
<i>Melosira varians</i>	x	✓	x	x	x	x	x	x	x	✓	x	✓
<i>Navicula antonii</i>	x	x	x	x	x	x	x	x	✓	x	x	✓
<i>Navicula capitatoradiata</i>	✓	✓	x	x	x	x	x	x	x	x	x	x
<i>Navicula cryptotenella</i>	x	x	x	x	28	x	x	x	x	✓	x	x
<i>Navicula erifuga</i>	x	x	x	x	x	x	x	x	x	x	x	✓

<i>Navicula libonensis</i>	x	✓	x	x	x	x	x	x	x	x	✓	x
<i>Navicula riediana</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Navicula rostellata</i>	x	x	x	x	x	x	x	x	x	x	x	✓
<i>Navicula schroeteri</i>	x	x	x	x	x	x	x	x	✓	✓	x	x
<i>Navicula trivialis</i>	x	✓	x	x	x	✓	x	x	x	✓	x	x
<i>Navicula veneta</i>	x	✓	x	x	x	x	x	x	x	x	✓	✓
<i>Nitzschia amphibia</i>	x	x	x	✓	34	x	x	x	x	✓	x	x
<i>Nitzschia capitellata</i>	x	✓	x	x	x	x	x	x	x	x	x	x
<i>Nitzschia frustulum</i>	✓	✓	x	x	x	✓	x	x	x	x	✓	✓
<i>Nitzschia gracilis</i>	x	x	x	x	x	x	x	x	x	✓	x	✓
<i>Nitzschia linearis</i>	x	x	x	x	x	x	x	x	x	x	x	✓
<i>Nitzschia palea</i>	x	x	x	x	x	x	x	x	x	✓	✓	x
<i>Nitzschia recta</i>	x	x	x	x	x	x	x	x	✓	x	x	x
<i>Pinnularia sp.2</i>	x	x	x	x	x	x	x	x	x	✓	x	x
<i>Placoneis elginensis</i>	✓	✓	x	x	x	x	x	x	x	x	x	x

<i>Placoneis placentula</i>	✓	✓	✕	✕	20	✓	✓	✕	✕	✕	✕	✕
<i>Planothidium frequentissimum</i>	✓	✓	✕	✕	41	✓	✕	✕	✕	✕	✕	✓
<i>Planothidium frequentissimum</i> (raphed)	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓
<i>Rhopalodia operculata</i>	✕	✓	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕
<i>Sellaphora seminulum</i>	✕	✕	✕	✕	✕	✕	✕	✕	✓	✕	✕	✕
<i>Staurosira elliptica</i>	✕	✓	✕	✓	✕	✕	✕	✕	✕	✕	✕	✕
<i>Surirella angusta</i>	✕	✓	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓
<i>Thalassiosira weissflogi</i>	✕	✕	✕	✕	✕	✕	✕	✕	✓	✓	✕	✕
<i>Tryblionella apiculata</i>	✕	✓	✕	✕	✕	✕	✕	✕	✕	✕	✕	✕
Species	11	24	0	11	16	9	7	7	9	13	11	17
Species containing deformities	0	1	0	0	0	0	1	1	0	0	0	0
Number of deformed cells	0	4	0	0	0	0	2	3	0	0	0	0

Rietgat Spruit												
	Jun-21	Jul-21	Aug-21	Sept-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
<i>Achnanthydium eutrophilum</i>	x	x	x	x	x	x	x	x	x	✓	x	x
<i>Achnanthydium</i> sp.	✓	✓	✓	x	116	28	x	x	x	x	x	✓
<i>Amphora coffeaeformis</i>	x	x	x	x	x	x	x	✓	x	x	x	x
<i>Amphora</i> sp. (deform)	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Amphora veneta</i>	✓	✓	x	x	32	5	x	x	x	x	x	x
<i>Anomoeneis sphaerophora</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Aulacoseira granulata</i>	x	x	x	x	x	x	6	x	x	x	x	✓
<i>Caloneis bacillum</i>	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Cocconeis pediculus</i>	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Cocconeis placentula</i>	x	✓	x	x	x	x	x	x	x	x	x	x
<i>Cocconeis placentula</i> var. <i>lineata</i>	x	x	x	x	13	7	x	x	x	✓	x	x

Present	✓
Absent	x
If cells were enough to count they are indicated by a number	

<i>Cocconeis placentula</i> var. <i>lineata</i> (deform)	x	x	x	x	1	x	x	x	x	x	x	x
<i>Craticula ambigua</i>	x	x	x	x	x	x	1	x	x	x	x	x
<i>Craticula buderi</i>	x	x	x	x	7	x	22	✓	✓	✓	✓	x
<i>Craticula buderi</i> (deform)	x	x	x	x	4	x	x	x	x	x	x	x
<i>Cyclotella meneghiniana</i>	x	✓	x	x	x	3	x	x	x	x	✓	x
<i>Cymbella tumida</i>	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Discostella woltereckii</i>	x	x	x	x	4	x	x	x	x	x	x	x
<i>Encyonema minitum</i>	x	✓	x	x	13	x	x	x	x	x	x	✓
<i>Eolimna minima</i>	x	x	x	x	x	x	x	x	x	✓	x	x
<i>Eolimna subminuscula</i>	x	x	x	x	x	x	x	✓	x	x	✓	x
<i>Epithemia adnata</i>	x	✓	x	x	23	26	x	x	x	x	x	x
<i>Epithemia sorex</i>	x	✓	x	✓	118	295	x	x	x	x	x	✓
<i>Fragilaria</i> sp. (deform)	x	x	x	x	4	x	x	x	x	x	x	x
<i>Fragilaria tenera</i>	x	✓	x	x	x	x	x	x	x	x	x	x
<i>Fragilaria tenera</i> (deform)	x	x	x	x	x	1	x	x	x	x	x	x

<i>Fragilaria ulna</i>	x	x	x	x	x	x	1	x	x	x	x	x
<i>Fragilaria ulna</i> (deform)	x	x	x	x	x	x	2	x	x	x	x	x
<i>Fragilaria ulna</i> var. <i>acus</i>	x	x	x	x	x	x	x	x	x	✓	✓	x
<i>Gomphonema</i> aff. <i>gracile</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Gomphonema</i> <i>angustatum</i>	x	✓	x	x	x	x	x	x	x	✓	✓	x
<i>Gomphonema clavatum</i>	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Gomphonema gracile</i>	x	✓	x	x	x	x	x	x	x	x	x	x
<i>Gomphonema minutum</i>	x	x	x	x	x	x	x	x	✓	x	x	x
<i>Gomphonema parvulum</i>	✓	✓	x	x	6	19	x	x	✓	✓	x	x
<i>Hantzschia amphioxys</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Lemnicola hungarica</i>	✓	x	x	x	x	x	x	x	x	x	x	x
<i>Navicula arvensis</i> var. <i>major</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Navicula cryptocephala</i>	x	x	x	x	x	x	13	x	x	x	x	x
<i>Navicula cryptotenella</i>	x	x	x	x	x	x	x	x	x	✓	✓	✓
<i>Navicula erifuga</i>	x	x	x	x	x	x	x	x	x	x	✓	x

<i>Navicula riediana</i>	x	x	x	x	x	7	11	x	✓	✓	✓	x
<i>Navicula</i> sp.	x	✓	x	x	x	x	x	x	x	x	x	x
<i>Navicula veneta</i>	x	x	x	x	x	x	x	x	✓	x	✓	x
<i>Navicula zanonii</i>	x	✓	x	x	11	x	x	x	x	x	x	x
<i>Nitzachia reversa</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Nitzschia amphibia</i>	x	✓	x	x	48	13	x	x	x	x	x	x
<i>Nitzschia filiformis</i>	x	✓	x	x	x	x	x	x	x	x	x	x
<i>Nitzschia frustulum</i>	✓	✓	x	✓	x	x	x	x	x	x	✓	✓
<i>Nitzschia gracilis</i>	x	x	x	x	x	x	x	x	x	✓	x	x
<i>Nitzschia intermedia</i>	x	x	x	x	x	x	11	x	x	x	x	x
<i>Nitzschia linearis</i>	x	x	x	x	x	x	8	x	x	x	x	x
<i>Nitzschia linearis</i> (deform)	x	x	x	x	x	x	1	x	x	x	x	x
<i>Nitzschia palea</i>	x	x	x	x	x	x	266	✓	✓	✓	✓	x
<i>Nitzschia palea</i> (deform)	x	x	x	x	x	x	26	x	x	x	x	x
<i>Nitzschia</i> sp.	x	✓	x	x	x	x	x	x	x	x	x	x

<i>Pinnularia acrosphaeria</i>	x	x	x	x	x	x	x	x	x	x	✓	x
<i>Pinnularia gibba</i>	x	x	x	x	x	x	38	x	✓	x	x	x
<i>Planothidium frequentissimum</i>	x	x	x	x	1	x	x	x	x	x	x	x
<i>Rhopalodia gibba</i>	x	✓	x	x	x	x	x	x	x	x	x	x
<i>Sellaphora pupula</i>	✓	x	x	x	x	2	1	x	x	x	✓	x
<i>Sellaphora seminulum</i>	✓	✓	x	x	x	x	1	x	x	x	✓	x
<i>Stauroneis kootenai</i>	x	x	x	x	x	x	1	x	x	x	x	x
<i>Thalassiosira weissflogi</i>	x	x	x	x	x	x	x	x	x	x	✓	x
Species	11	19	1	2	12	10	13	4	7	11	20	6
Species containing deformities	1	0	0	0	3	1	3	0	0	0	0	0
Number of deformed cells	1	0	0	0	9	1	29	0	0	0	0	0

Appendix B

Diatom species present at sites in winter (2 - 4 August 2022) and summer (25 – 26 October 2022)

Group 1 - Karee Rand TSF complex

Karee Rand TSF		
	Aug-22	Oct-22
	✕	✕
Species	0	0
Species containing deformities	0	0
Number of deformed cells	0	0

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

Karee Rand RWD		
	Aug-22	Oct-22
<i>Brachysira neoexilis</i>	✓	✕
<i>Caloneis aequatorialis</i>	✓	✕
<i>Craticula buderi</i>	✓	✕
<i>Navicula veneta</i>	✓	✕
<i>Nitzschia palea</i>	✓	✕
Species	5	0
Species containing deformities	0	0
Number of deformed cells	0	0

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

Karee Rand DD		
	Aug-22	Oct-22
<i>Craticula buderi</i>	✓	✕
<i>Navicula</i> sp.	✓	✕
<i>Nitzschia palea</i>	✓	✕
Species	3	0
Species containing deformities	0	0
Number of deformed cells	0	0

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

Catchment Dam		
	Aug-22	Oct-22
<i>Achnantheidium</i> sp.	✓	
<i>Cocconeis placentula</i> var. <i>lineata</i>	✓	
<i>Craticula buderi</i>	✓	
<i>Eolimna submiscula</i>	✓	
<i>Navicula veneta</i>	✓	
<i>Nitzschia capitellata</i>	✓	
<i>Sellaphora seminulum</i>	✓	
Species	7	
Species containing deformities	0	
Number of deformed cells	0	

Present	✓
Absent	✕
No Sample	
If cells were enough to count they are indicated by a number	

Group 2 – West Complex TSF complex

West complex TSF		
	Aug-22	Oct-22
Species		
Species containing deformities		
Number of deformed cells		

Present	✓
Absent	✕
No Sample	
If cells were enough to count they are indicated by a number	

Queen Mary RWD		
	Aug-22	Oct-22
<i>Achnantheidium</i> sp.	✓	
<i>Craticula buderi</i>	✓	
<i>Cyclotella meneghiniana</i>	✓	
<i>Entomoneis paludosa</i>	✓	
<i>Navicula veneta</i>	✓	
<i>Nitzschia palea</i>	✓	
<i>Tryblionella hungarica</i>	✓	
Species	7	
Species containing deformities	0	
Number of deformed cells	0	

Present	✓
Absent	✕
No Sample	
If cells were enough to count they are indicated by a number	

Queen Mary DD		
	Aug-22	Oct-22
<i>Achnantheidium</i> sp.	✓	✕
<i>Brachysira neoexilis</i>	✓	✕
<i>Craticula buderi</i>	✓	✕
<i>Navicula veneta</i>	✓	✕
<i>Nitzschia palea</i>	✓	✕
Species	5	0
Species containing deformities	0	0
Number of deformed cells	0	0

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

West Storm Water		
	Aug-22	Oct-22
<i>Achnantheidium</i> sp.	333	57
<i>Achnantheidium</i> sp. (deform)	28	✕
<i>Craticula buderi</i>	29	336
<i>Craticula buderi</i> (deform)	1	✕
<i>Nitzschia palea</i>	12	9
Species	3	3
Species containing deformities	2	0
Number of deformed cells	29	0

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

Group 3 – West Extension TSF complex

West Ext TSF		
	Aug-22	Oct-22
Species		
Species containing deformities		
Number of deformed cells		

Present	✓
Absent	✗
No Sample	
If cells were enough to count they are indicated by a number	

Bokkamp RWD		
	Aug-22	Oct-22
<i>Amphora coffeaeformis</i>	✗	1
<i>Cocconeis placentula</i>	✗	1
<i>Craticula buder</i>	267	388
<i>Craticula buder</i> (deform)	47	7
<i>Cyclotella meneghiniana</i>	2	✗
<i>Nitzschia nana</i>	1	1
<i>Nitzschia palea</i>	50	2
<i>Nitzschia palea</i> (deform)	32	✗
<i>Pinnularia</i> sp.	2	3
<i>Pinnularia</i> sp. (deform)	1	✗
Species	5	6
Species containing deformities	3	1
Number of deformed cells	80	7

Present	✓
Absent	✗
If cells were enough to count they are indicated by a number	

Bokkamp DD		
	Aug-22	Oct-22
<i>Craticula buder</i>	✓	358
<i>Craticula buder</i> (deform)	✗	3
<i>Entomoneis paludosa</i>	✓	✗
<i>Navicula veneta</i>	✓	7
<i>Nitzschia nana</i>	✓	✗
<i>Nitzschia palea</i>	✓	36
<i>Surirella ovalis</i>	✓	✗
<i>Tryblionella hungarica</i>	✓	✗
Species	7	3
Species containing deformities	0	1
Number of deformed cells	0	3

Present	✓
Absent	✗
If cells were enough to count they are indicated by a number	

Group 4 – East Complex

AEL South		
	Aug-22	Oct-22
<i>Achnanthidium</i> sp.	117	59
<i>Achnanthidium</i> sp. (deform)	8	✗
<i>Amphora coffeaeformis</i>	5	22
<i>Amphora veneta</i>	4	21
<i>Craticula buder</i>	203	23
<i>Craticula buder</i> (deform)	7	✗
<i>Cyclotella meneghiniana</i>	4	21

Present	✓
Absent	✗
If cells were enough to count they are indicated by a number	

<i>Fragilaria ulna</i> var. <i>acus</i>	✕	2
<i>Navicula veneta</i>	12	2
<i>Navicymbula pusilla</i>	3	✕
<i>Nitzschia palea</i>	4	✕
<i>Nitzschia elegantula</i>	36	249
<i>Nitzschia umbunata</i>	✕	1
<i>Pinnularia</i> sp.	✕	1
Species	9	10
Species containing deformities	2	0
Number of deformed cells	15	0

AEL East (North)		
	Aug-22	Oct-22
<i>Achnantheidium</i> sp.	✓	✓
<i>Amphora veneta</i>	✕	✓
<i>Amphora coffeaeformis</i>	✕	✓
<i>Brachysira neoexilis</i>	✓	✓
<i>Caloneis aequatorialis</i>	✓	✓
<i>Craticula buderii</i>	✕	✓
<i>Cyclotella meneghiniana</i>	✕	✓
<i>Diploneis elliptica</i>	✓	✕
<i>Encyonopsis microcephala</i>	✓	✓
<i>Hantzschia amphioxys</i>	✓	✕

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

<i>Navicula veneta</i>	✕	✓
<i>Nitzschia elegantula</i>	✕	✓
<i>Nitzschia palea</i>	✓	✓
<i>Nitzschia semirobusta</i>	✓	✕
<i>Pinnularia</i> sp.	✕	✓
Species	8	12
Species containing deformities	0	0
Number of deformed cells	0	0

AEL DD		
	Aug-22	Oct-22
<i>Amphora montana</i>	✓	✓
<i>Navicula veneta</i>	✓	✕
<i>Nitzschia nana</i>	✓	✕
<i>Nitzschia palea</i>	✓	✓
<i>Pinnularia</i> sp.	✓	✓
<i>Stauroneis</i> sp.	✓	✕
<i>Surirella ovalis</i>	✓	✕
Species	7	3
Species containing deformities	0	0
Number of deformed cells	0	0

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

Eye Dam		
	Aug-22	Oct-22
<i>Achnantheidium</i> sp.	57	53
<i>Anomoeneis sphaerophora</i>	1	✕
<i>Brachysira neoexilis</i>	1	✕
<i>Caloneis aequatorialis</i>	1	✕
<i>Craticula buderi</i>	78	18
<i>Cyclotella meneghiniana</i>	15	1
<i>Diploneis elliptica</i>	1	✕
<i>Encyonopsis microcephala</i>	109	147
<i>Entomoneis paludosa</i>	✕	2
<i>Fragilaria nanana</i>	1	✕
<i>Fragilaria tenera</i>	6	24
<i>Fragilaria tenera</i> (deform)	1	2
<i>Fragilaria ulna</i>	✕	15
<i>Gomphonema</i> aff. <i>Gracile</i>	1	✕
<i>Gomphonema affine</i>	1	✕
<i>Navicula erifuga</i>	5	✕
<i>Navicula subrhynchocephala</i>	✕	5
<i>Navicula veneta</i>	8	4
<i>Nitzschia amphibia</i>	3	✕
<i>Nitzschia linearis</i>	1	✕
<i>Nitzschia palea</i>	3	2
<i>Nitzschia semirobusta</i>	104	127

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

<i>Nitzschia</i> sp.	✕	2
<i>Planothidium engelbrechtii</i>	✕	1
<i>Staurosira construens</i>	4	1
<i>Tabularia fasciculata?</i>	1	✕
Species	20	14
Species containing deformities	1	1
Number of deformed cells	1	2

Group 5 – Reference sites

Koekemoer SpruitKoekemoer Spruit		
	Aug-22	Oct-22
<i>Achnantheidium exiguum</i>	✓	✗
<i>Craticula buderi</i>	✓	✗
<i>Cyclotella meneghiniana</i>	✓	✗
<i>Diadesmis confervacea</i>	✓	✗
<i>Encyonema minutum</i>	✓	✗
<i>Eolimna minima</i>	✓	✗
<i>Fragilaria ulna</i>	✓	✗
<i>Gomphonema parvulum</i>	✓	✗
<i>Hantzschia amphioxys</i>	✓	✗
<i>Navicula erifuga</i>	✓	✗
<i>Navicula riediana</i>	✓	✗
<i>Navicula trivialis</i>	✓	✗
<i>Navicula veneta</i>	✓	✗
<i>Nitzschia frustulum</i>	✓	✗
<i>Nitzschia linearis</i>	✓	✗
<i>Nitzschia palea</i>	✓	✓
<i>Nitzschia umbunata</i>	✓	✗
<i>Pinnularia</i> sp.	✓	✗
<i>Pinnularia subbrevistriata</i>	✓	✗
<i>Surirella angusta</i>	✓	✗

Present	✓
Absent	✗
If cells were enough to count they are indicated by a number	

<i>Thalassiosira weissflogi</i>	✓	✗
<i>Tryblionella hungarica</i>	✓	✓
Species	22	2
Species containing deformities	0	0
Number of deformed cells	0	0

Ref A (Wildskamp)		
	Aug-22	Oct-22
<i>Achnantheidium exiguum</i>	✓	✗
<i>Achnantheidium</i> sp.	✓	✓
<i>Aulacoseira ambigua</i>	✓	✗
<i>Aulacoseira granulata</i>	✓	✗
<i>Cocconeis pediculus</i>	✓	✓
<i>Cymbella neocistula</i>	✓	✗
<i>Cymbella tumida</i>	✓	✗
<i>Diadesmis confervacea</i>	✓	✗
<i>Encyonema caespitosum</i>	✓	✓
<i>Encyonopsis microcephala</i>	✓	✓
<i>Epithemia adnata</i>	✓	✓
<i>Epithemia sores</i>	✓	✓
<i>Fragilaria capucina</i> var. <i>vaucheriae</i>	✓	✓
<i>Gomphonema clavatum</i>	✓	✗
<i>Navicula capitatoradiata</i>	✗	✓
<i>Navicula cryptotenella</i>	✓	✓
<i>Nitzschia frustulum</i>	✓	✗
<i>Nitzschia palea</i>	✗	✓
<i>Nitzschia sigma</i>	✓	✗
<i>Nitzschia</i> sp.	✗	✓
<i>Rhopalodia gibba</i>	✓	✗
<i>Staurosira elliptica</i>	✓	✗

Present	✓
Absent	✗
If cells were enough to count they are indicated by a number	

Species	19	11
Species containing deformities	0	0
Number of deformed cells	0	0

Skoon Spruit		
	Aug-22	Oct-22
<i>Achnantheidium</i> sp.	✕	✓
<i>Amphora pediculus</i>	✕	✓
<i>Cocconeis pediculus</i>	✕	✓
<i>Cocconeis placentula</i>	✓	✕
<i>Cyclotella meneghiniana</i>	✓	✓
<i>Cymatopleura solea</i>	✓	✓
<i>Diatoma vulgaris</i>	✓	✕
<i>Encyonema minitum</i>	✓	✕
<i>Epithemia adnata</i>	✓	✕
<i>Epithemia sores</i>	✓	✕
<i>Fragilaria capucina</i> var. <i>vaucheriae</i>	✓	✕
<i>Gomphonema minitum</i>	✓	✕
<i>Gomphonema parvulum</i>	✓	✕
<i>Gomphonema clavatum</i>	✓	✕
<i>Melosira varians</i>	✓	✓
<i>Navicula capitatoridiat</i>	✓	✓
<i>Navicula cryptocephala</i>	✓	✕
<i>Navicula</i> sp.	✓	✓

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

<i>Nitzschia frustulum</i>	✕	✓
<i>Nitzschia linearis</i>	✓	✕
<i>Nitzschia palea</i>	✓	✕
<i>Nitzschia recta</i>	✓	✕
<i>Nitzschia sigma</i>	✓	✕
<i>Nitzschia</i> sp.	✕	✓
<i>Placoneis elginensis</i>	✓	✕
<i>Rhopalodia gibba</i>	✓	✓
<i>Surirella angusta</i>	✓	✕
<i>Tryblionella apiculata</i>	✓	✕
Species	23	11
Species containing deformities	0	0
Number of deformed cells	0	0

Rietgat Spruit		
	Aug-22	Oct-22
<i>Achnanthidium</i> sp.	✓	✕
<i>Cocconeis placentula</i>	✓	✓
<i>Craticula buderi</i>	✓	✓
<i>Eolimna</i> sp.	✓	✕
<i>Epithemia adnata</i>	✓	✓
<i>Epithemia sorex</i>	✓	✓
<i>Fragilaria nanana</i>	✓	✕
<i>Navicula cryptotenella</i>	✕	✓

Present	✓
Absent	✕
If cells were enough to count they are indicated by a number	

<i>Navicula veneta</i>	✓	✓
<i>Nitzschia frustulum</i>	✓	✓
<i>Nitzschia palea</i>	✕	✓
<i>Rhopalodia gibba</i>	✓	✓
<i>Sellaphora</i> sp.	✕	✓
Species	10	10
Species containing deformities	0	0
Number of deformed cells	0	0

Appendix C

Species list of the tolerant and less tolerant species found at the sites

Less tolerant diatom taxa	Tolerant diatom taxa
<i>Achnantheidium eutrophilum</i> (Lange-Bertalot) Lange-Bertalot	<i>Achnantheidium exiguum</i> (Grunow) Czarnecki
<i>Achnantheidium</i> sp.	<i>Amphora coffeaeformis</i> (Agardh) Kützing
<i>Achnantheidium</i> sp. 2	<i>Amphora copulata</i> (Kützing) Schoeman & Archibald
<i>Aulacoseira ambigua</i> (Grunow) Simonsen	<i>Amphora inariensis</i> Krammer
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	<i>Amphora montana</i> Krasske
<i>Aulacoseira muzzanensis</i> (Meister) Krammer	<i>Amphora pediculus</i> (Kützing) Grunow
<i>Brachysira neoexilis</i> (Grunow) DG Mann	<i>Amphora</i> sp.
<i>Caloneis aequatorialis</i> Hustedt	<i>Amphora veneta</i> Kützing
<i>Cocconeis placentula</i> Ehrenberg	<i>Anomoeneis sphaerophora</i> (Ehrenberg) Pfister
<i>Cocconeis placentula</i> var. <i>lineata</i> (Ehrenberg) Van Heurck	<i>Caloneis bacillum</i> (Grunow) Cleve
<i>Cocconeis</i> sp.	<i>Cocconeis pediculus</i> Ehrenberg
<i>Cyclotella ocellata</i> Pantocsek	<i>Craticula accomoda</i> (Hustedt) DG Mann
<i>Cymbella cymbiformis</i> Agardh	<i>Craticula ambigua</i> (Ehrenberg) DG Mann
<i>Cymbella neocistula</i> Krammer	<i>Craticula buderii</i> (Hustedt)
<i>Cymbella tumida</i> (Brébisson) Van Heurck	<i>Craticula cuspidata</i> (Kützing) DG Mann
<i>Cymbella turgidula</i> Grunow	<i>Craticula halophila</i> (Grunow) DG Mann
<i>Diatoma vulgare</i> Bory	<i>Craticula perrotetii</i> Grunow
<i>Diploneis elliptica</i> (Kützing) Cleve	<i>Cyclostephanos dubius</i> (Fricke) Round
<i>Encyonema minutum</i> (Hilse) DG Mann	<i>Cyclotella meneghiniana</i> Kützing
<i>Encyonopsis leei</i> var. <i>sinensis</i> Metzeltin & Krammer	<i>Cymatopleura solea</i> (Brébisson) W Smith
<i>Encyonopsis microcephala</i> (Grunow) Krammer	<i>Cymatopleura solea</i> var. <i>apiculata</i> (W Smith) Ralfs
<i>Epithemia adnata</i> (Kützing) Brébisson	<i>Diadesmis cofervacea</i> (Kützing) DG Mann
<i>Epithemia sorex</i> Kützing	<i>Diploneis smithii</i> (Brébisson) Cleve
<i>Fragilaria capucina</i> Desmazières	<i>Discostella woltereckii</i> (Hustedt) Houk & Klee
<i>Fragilaria capucina</i> var. <i>rumpens</i> (Kützing) Lange-Bertalot	<i>Encyonema caespitosum</i> Kützing
<i>Fragilaria capucina</i> var. <i>vaucheriae</i> (Kützing) Lange-Bertalot	<i>Entomoneis paludosa</i> (W Smith) Reimer
<i>Fragilaria nanana</i> Lange-Bertalot	<i>Eolimna minima</i> (Grunow) Lange-Bertalot
<i>Fragilaria tenera</i> (W Smith) Lange-Bertalot	<i>Eolimna monoculata</i> (Hustedt) DG Mann
<i>Fragilaria ulna</i> (Nitzsch) Lange-Bertalot	<i>Eolimna</i> sp.
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing) Lange-Bertalot	<i>Eolimna subminuscule</i> (Manguin) Lange-Bertalot
<i>Fragilaria ungeriana</i> Grunow	<i>Fallacia pygmaea</i> (Kützing) Sickel & Mann

<i>Gomphonema aff. gracile</i>	<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot
<i>Gomphonema affine</i> Kützing	<i>Gomphonema angustatum</i> (Kützing) Rabenhorst
<i>Gomphonema insigne</i> Gregory	<i>Gomphonema clavatum</i> Ehrenberg
<i>Gomphonema italicum</i> Kützing	<i>Gomphonema gracile</i> Ehrenberg sensu stricto
<i>Gomphonema laticollum</i> Reichardt	<i>Gomphonema parvulus</i> Lange-Bertalot & Reichardt
<i>Gomphonema pumilum</i> var. <i>rigidum</i> Reichardt & Lange-Bertalot	<i>Gomphonema parvulum</i> (Kützing) Kützing sensu stricto
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	<i>Gomphonema pseudoaugur</i> Krammer
<i>Hippodonta capitata</i> (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	<i>Gomphonema</i> sp.
<i>Lemnicola hungarica</i> (Grunow) Round & Basson	<i>Gomphonema venusta</i> Passy, Kociolek & Lowe
<i>Navicula cryptotenella</i> Lange-Bertalot	<i>Gyrosigma rautenbachiae</i> Cholnoky
<i>Navicula riediana</i> Lange-Bertalot & Rumrich	<i>Luticola acidoclinata</i> Lange-Bertalot
<i>Navicula seibigiana</i> Lange-Bertalot	<i>Luticola mutica</i> (Kützing) DG Mann
<i>Navicula subrhynchocephala</i> Hustedt	<i>Mayamaea atomus</i> var. <i>permitis</i> (Hustedt) Lange-Bertalot
<i>Navicula zanonii</i> Hustedt	<i>Mayamaea</i> sp.
<i>Nitzschia amphioxys</i> (Ehrenberg) W Smith	<i>Mayameae atomus</i> (Kützing) Lange-Bertalot
<i>Nitzschia elegantula</i> Grunow	<i>Melosira varians</i> Agardh
<i>Nitzschia linearis</i> (Agardh) W Smith	<i>Navicula antonii</i> Lange-Bertalot
<i>Nitzschia linearis</i> var. <i>subtilis</i> Grunow	<i>Navicula arvensis</i> var. <i>major</i> Lange-Bertalot
<i>Nitzschia nana</i> Grunow	<i>Navicula capitatoradiata</i> Germain
<i>Nitzschia recta</i> Hantzsch	<i>Navicula cincta</i> (Ehrenberg) Ralfs
<i>Pinnularia acrosphaeria</i> W Smith	<i>Navicula erifuga</i> (OF Müller) Bory
<i>Pinnularia gibba</i> Ehrenberg	<i>Navicula libonensis</i> Schoeman
<i>Pinnularia microstauron</i> var. <i>rostrata</i> Krammer	<i>Navicula rhynchocephala</i> Kützing
<i>Pinnularia</i> sp.	<i>Navicula rostellata</i> Kützing
<i>Pinnularia</i> sp.2	<i>Navicula schroeteri</i> Meister
<i>Placoneis elginensis</i> (Gregory) EJ Cox	<i>Navicula</i> sp.
<i>Placoneis placentula</i> (Ehrenberg) Heinzerling	<i>Navicula trivialis</i> Lange-Bertalot
<i>Pleurosigma elongatum</i> W Smith	<i>Navicula veneta</i> Kützing
<i>Pseudostaurosira brevistriata</i> (Grunow in van Heurk) Williams & Round	<i>Navicymbula pusilla</i> (Grunow) Krammer
<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	<i>Nitzachia reversa</i> W Smith
<i>Stauroneis kootenai</i> Bahls	<i>Nitzschia acicularis</i> (Kützing) W Smith
<i>Stauroneis</i> sp.	<i>Nitzschia amphibia</i> Grunow
<i>Stauroneis</i> sp.?	<i>Nitzschia aurariae</i> Cholnoky
<i>Staurosira contruens</i> Ehrenberg	<i>Nitzschia capitellata</i> Hustedt
<i>Staurosira elliptica</i> (Schumann) Williams & Round	<i>Nitzschia communis</i> Rabenhorst

<i>Staurosirella pinnata</i> (Ehrenberg) Williams & Round	<i>Nitzschia filiformis</i> (W Smith) Van Heurk
<i>Stephanodiscus agassizensis</i> Håkansson & Kling	<i>Nitzschia frustulum</i> (Kützing) Grunow
<i>Tryblionella coarctata</i> (Grunow) DG Mann	<i>Nitzschia gracilis</i> Hantzsch
<i>Ulnaria (Fragilaria) sp.?</i>	<i>Nitzschia intermedia</i> Hantzsch
	<i>Nitzschia palea</i> (Kützing) W Smith
	<i>Nitzschia semirobusta</i> Lange-Bertalot
	<i>Nitzschia sigma</i> (Kützing) W Smith
	<i>Nitzschia sp.</i>
	<i>Nitzschia sp. 2</i>
	<i>Nitzschia sp. 3</i>
	<i>Nitzschia sp. 4</i>
	<i>Nitzschia sp. 5</i>
	<i>Nitzschia sp. 6</i>
	<i>Nitzschia sp. 7</i>
	<i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot
	<i>Nitzschia valdecostata</i> Lange-Bertalot & Simonsen
	<i>Pinnularia divergens</i> W Smith
	<i>Pinnularia divergens</i> var. <i>undulata</i> (Péragallo & Heribaud)
	<i>Planothidium frequentissimum</i> (Lange-Bertalot) Round & Bukhityarova
	<i>Planothidium frequentissimum</i> (raphed) (Lange-Bertalot) Round & Bukhityarova
	<i>Rhopalodia operculata</i> (Agardh) Hakanasson
	<i>Sellaphora pupula</i> (Kützing) Mereschowsky sensu lato
	<i>Sellaphora seminulum</i> (Grunow) DG Mann
	<i>Surirella angusta</i> Kützing
	<i>Surirella ovalis</i> Brébisson
	<i>Thalassiosira weissflogi</i> (Grunow) Fryxell & Hasle
	<i>Tryblionella apiculata</i> Gregory
	<i>Tryblionella calida</i> (Grunow) DG Mann
	<i>Tryblionella gracilis</i> W Smith
	<i>Tryblionella hungarica</i> (Grunow) DG Mann