

Evaluation of a diatom-based index for acid mine drainage impacted permanent pans in the Mpumalanga Highveld region

M Erasmus



orcid.org/0000-0003-3402-5030

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Supervisor: Prof JC Taylor

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25026917

PREFACE

“This is, I think, what holiness is:
the natural world, where every moment is full
of the passion to keep moving.
Inside every mind there's a hermit's cave full of light,
full of snow, full of concentration.
I've knelt there, and so have you,
hanging on to what you love,
to what is lovely.
-Mary Oliver, At the Lake”

Firstly, I want to thank my Heavenly Father for the wonderful world He created, for the opportunity to be a part of it and go on an adventure to see some of the most precious little jewels in nature. I have been blessed beyond my wildest dreams.

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ABSTRACT

The Mpumalanga Highveld region features a variety of wetlands, including permanent depressional wetlands such as grass pans, reed pans, open-water pans, and salt pans. The region also hosts the Highveld coal field, South Africa's second largest producing coal field. Increased mining and agriculture present the most serious threats to wetland ecosystems in the Mpumalanga Highveld. These wetlands are often used to store acid mine drainage (AMD) generated by coal mining activities. AMD contamination affects surface and ground water resources and agricultural lands in the vicinity, disrupting the growth and reproduction of aquatic and terrestrial organisms.

A water quality index based on diatoms was created in 2017 to determine the impacts of AMD on permanent pans in the region. Diatoms (Bacillariophyceae) are a ubiquitous group of algae with well-known ecological preferences and a history of successful use in biomonitoring of aquatic ecosystems. Being that natural variability among the pans may compromise the accuracy of diatom indices, indices sensitive to acid mine drainage (AMD) impacts were previously produced for a range of pan types. These indices were able to distinguish diatom communities present in non-impacted pans from communities in AMD impacted pans. However, indices accounting for natural variability perform better than those not accounting for natural variation and initially sampling was only done during the dry season (from May to August).

The aim of this study is to evaluate the performance of diatom indices created for different AMD-impacted pans in South Africa's Mpumalanga Highveld region. In order to assess the performance of the previously developed diatom index, it was tested on a validation dataset of diatoms sampled from pans impacted by AMD-disturbed and non-AMD disturbed pans (i.e. pans ranging from least disturbed by coal mining to highly disturbed). Epiphytic diatoms were sampled from reed, salt, and grass pans during both the wet and dry seasons. The AMD-multimetric index scores obtained for the pans were compared to scores of riverine indices as diatom-based indices that are frequently used in South Africa as several riverine indices have been recommended for biomonitoring in wetlands by reason that the majority of indices include cosmopolitan taxa with well-established correspondence to environmental parameters.

The present study found that factors that influenced the composition of diatom communities included the degree of AMD-disturbance, pan type and seasonal change. Similarity Percentage (SIMPER) analysis was used to determine the intra- and infra-group similarity among the samples resulting from three factors: disturbance level (AMD), pan type and season. Canonical Correspondence Analysis (CCA) was used to ascertain the relationship between diatom assemblages and measured environmental variables and Pearson's correlation coefficient was calculated to establish whether significant correlations exist between calculated index scores and water quality variables. For all indices tested, accuracy was determined as the percentage of correctly classified instances (CCI) Cohen's Kappa coefficient (K) was used as a measure of the agreement between indices in rating ecological condition.

Diatom assemblages showed strong correspondence with water quality variables which signify AMD-pollution. Higher AMD-MMIs scores were observed in non-impacted pans and low scores in impacted sites, which indicates proper correspondence with heavier coal mining impacts. The indicated conditions of reference pans were poor according to riverine indices, which were less successful in discriminating between reference sites and impacted sites. Riverine index scores correlated positively with indicators of AMD-disturbance. AMD-MMIs displayed strong negative correlations with AMD indicators and were more accurate in determining AMD-disturbance level than riverine indices. The index was explicitly developed for the indication of AMD and specifically for depressional wetlands and similar indices may be designed for the various other wetland types in the region.

Key terms: Ecological assessment; diatom index; acid mine drainage; depressional wetlands

OPSOMMING

Die Mpumalanga Hoëveld-streek bevat 'n verskeidenheid vleilande, insluitend permanente depressies soos graspanne, rietpanne, oopwaterpanne en soutpanne. Die streek huisves ook die Hoëveld-steenkoolveld, die tweede grootste produserende steenkoolveld in Suid-Afrika. In die Mpumalanga Hoëveld is toenemende mynbou en landbou die grootste bedreigings vir vleilandekosisteme. Hierdie vleilande word dikwels gebruik om suurmynafvoer (AMD) wat deur steenkoolmynaktiwiteite gegenereer word, te berg. AMD-besoedeling beïnvloed oppervlak- en grondwaterbronne asook landbougrond in die omgewing, wat die groei en voortplanting van akwatiese- en terrestriële organismes versteur.

'n Diatoomgebaseerde indeks is ontwikkel in 2017 om die impak van AMD op permanente panne in die streek te bepaal. Diatome (Bacillariophyceae) is 'n alomteenwoordige groep alge met bekende ekologiese voorkeure en is menigte male suksesvol toegepas in die biomonitoring van akwatiese ekosisteme. Aangesien die natuurlike wisselvalligheid tussen die panne die akkuraatheid van die diatoomindekse kan benadeel, is indekse ontwikkel vir 'n reeks verskillende tipes panne. Hierdie indekse kon onderskei tussen diatomgemeenskappe in panne wat nie geraak word nie en gemeenskappe in AMD-panne. Indekse wat rekening hou met natuurlike wisselvalligheid, presteer egter beter as dié wat nie natuurlike variasie in ag neem nie. Aanvanklik is monsterneming slegs gedurende die droë seisoen (van Mei tot Augustus) uitgevoer.

Die doel van die studie is om die vermoë van die diatom-indekse om AMD-versteuring in panne aan te dui, te evalueer. Ten einde die prestasie van die diatome-indeks wat deur Riato (2017) ontwikkel is, te beoordeel, is die indeks getoets op 'n valideringsdatastel wat saamgestel is uit diatome uit panne wat geraak word deur AMD en nie-AMD-versteurde panne (dws panne wat wissel van die minste versteur deur steenkoolmynbou tot hoogs versteurde panne). Epifitiese diatome is tydens die nat en droë seisoen uit riet-, sout- en graspanne versamel. Die AMD-multimetriese indekspunte wat vir die panne behaal is, is vergelyk met tellings van rivierindekse, aangesien diatome-gebaseerde indekse gereeld in Suid-Afrika gebruik word, en verskeie rivierindekse aanbeveel word vir biomonitoring in vleilande, omrede die meeste indekse kosmopolitiese taksa inkorporeer wat goed gevestigde ooreenkomste met omgewingsveranderlikes toon.

Faktore wat die samestelling van diatomgemeenskappe beïnvloed, sluit die mate van AMD-versteuring in, asook pannetipe en seisoenale verandering. Gelykheidspersentasie (SIMPER) -analise is gebruik om die intra- en infragroep-ooreenkoms tussen die monsters te bepaal wat voortspruit danksy drie faktore: versteuringsvlak (AMD), pannetipe en seisoen. Kanoniese korrespondensie-analise (CCA) is gebruik om die verband tussen diatoomgemeenskap samestellings en omgewingsveranderlikes vas te stel en Pearson se korrelasiekoëffisiënt is bereken om vas te stel of beduidende korrelasies tussen berekende indekspunte en watergehalte-veranderlikes bestaan. Vir alle indekse is die akkuraatheid bepaal as die persentasie korrek geklassifiseerde gevalle (CCI). Cohen se Kappa-koëffisiënt (K) is gebruik om die mate van ooreenkoms tussen indekse, wat ekologiese toestande evalueer, te bepaal.

Diatom-samestellings het sterk ooreenstemming getoon met die veranderlikes van watergehalte wat AMD-besoedeling aandui. Die multimetriese diatoomindeks het hoër tellings vir nie-versteurde panne gelewer en lae tellings vir versteurde panne wat ooreenstem met die toeneemende impak van steenkoolmynbou. Tellings wat deur die rivierindekse opgelewer is het positiewe korrelasies met AMD-aanwysers getoon. AMD-MMI's het sterk negatiewe korrelasies met AMD-aanwysers getoon en het meer akkurate bepalinge van AMD-versteuringsvlak gelewer as rivierindekse. Die indeks is ontwikkel om die voorkoms van AMD in spesifiek depressie vleilande uit te wys en soortgelyke indekse kan ontwerp word vir ander vleilandtypes in die streek.

Sleutelterm: Ekologiese assessering; diatom indeks; suurmynafvoer; depressie vleilande

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LIST OF ABBREVIATIONS

APDI - Artois-Picardie Diatom Index (Prygiel et al., 1996)
BDI - Biological Diatom Index (Lenoir & Coste, 1996)
CCA - Canonical Correspondence Analysis
CEC -Commission of Economical Community Index (Descy & Coste, 1991)
EC - Electrical Conductivity
EPI - Eutrophication/Pollution Index
FAI - Fish Assemblage Integrity Index
FRAI - Fish Response Assessment Index
FHI - Fish Health Index
GDI - Generic Diatom Index (Coste & Ayphassorho, 1991)
LHWP - Lesotho Highlands Water Project
LM - Light microscopy
LMI - Leclercq & Maquet's Index (Leclercq & Maquet, 1987)
MLD - Mpumalanga Lakes District
MMI - Multimetric index
SADI - South African Diatom Index for rivers and streams
SASS - South African Scoring System
SEM - Scanning Electron Microscopy
SHE - Schiefele and Schreiner's index (Schiefele & Schreiner, 1991)
SLA - Sládeček's index (Sládeček, 1986),
SPI - Specific Pollution sensitivity Index (Coste in CEMAGREF, 1982)
TDI - Trophic Diatom Index (Kelly & Whitton, 1995)
TDS - Total Dissolved Solids
WAT - Watanabe index (Watanabe et al., 1986; Watanabe, 1990)
WRC -Water Research Commission
%CCI - Percentage of Correctly Classified Instances
%PTV - Percentage of Pollution Tolerant Values

TABLE OF ABBREVIATIONS IN FIGURES

Acronym	Pan type	Season	Reference or Disturbed site
RWR	Reed	Winter	Reference
RWD	Reed	Winter	Disturbed
GWR	Grass	Winter	Reference
GWD	Grass	Winter	Disturbed
SWR	Salt	Winter	Reference
SWD	Salt	Winter	Disturbed
OWWD	Open water	Winter	Disturbed
RSR	Reed	Summer	Reference
RSD	Reed	Summer	Disturbed
GSR	Grass	Summer	Reference
GSD	Grass	Summer	Disturbed
SSR	Salt	Summer	Reference
SSD	Salt	Summer	Disturbed
OWSD	Open water	Summer	Disturbed

CHAPTER 1: INTRODUCTION AND BACKGROUND

Water is an essential and precious resource. Precipitation in South Africa is limited (less than 500 mm per annum) in comparison to many other countries. The provision of domestic water remains a challenge and many people in sub-Saharan Africa and Asia find water difficult to obtain (Dungumaro, 2007). Donnenfeld *et al.* (2018) mentions that more than 60% of the country's rivers are over-utilised while only a third of rivers are in good condition. Sources of potable drinking water include rivers, groundwater sources, wetlands and man-made dams, the levels of which have been declining during the past few years. As the population grows, the demand for water for agricultural, industrial and municipal use increases, placing more stress on South Africa's finite water resources. The cautious use and protection of aquatic ecosystems is imperative as water resource deterioration exacerbates the scarcity or limitation of water in South Africa.

1.1 Wetlands of South Africa

The South African National Water Act (NWA, 1998), provides the following definition for wetlands: "land which is transitional between terrestrial and aquatic systems where the water table is usually at or near the surface, or the land is periodically covered with shallow water, and which in normal circumstances supports or would support vegetation typically adapted to life in saturated soil". The Ramsar Convention on Wetlands defines wetlands as: "areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six metres". Cowan (1995) describes wetlands as regions of interaction between marine, aquatic and terrestrial organisms; ecosystems that support wide varieties of plants, invertebrates, fish, reptiles, birds and mammals. Wetland ecosystems differ from other aquatic ecosystems in that they are often not permanently inundated. Soils in these systems are typically water saturated and thus not classified as purely terrestrial. According to the Classification System for Wetlands and other Aquatic Ecosystems in South Africa, wetlands are considered a type of aquatic ecosystem due to the presence of water in the system, even if only periodically, which is the distinguishing feature between terrestrial and aquatic systems (Ollis *et al.*, 2013).

1.1.2 Wetland classification

The South African Highveld region features a considerable amount and variety of wetlands (Allan *et al.*, 1995). To distinguish between different types of wetlands, the South African

National Biodiversity Institute (SANBI) published the Classification System for Wetlands and other Aquatic Ecosystems in South Africa (Ollis *et al.*, 2013). The Classification System has a component focussing on inland systems, the tiered structure of which represents six categories used to classify ecosystems. These range from the type of system (inland, estuarine or marine) at the first level, followed by regional setting, landscape setting, hydrogeomorphic unit, hydrological regime and finally descriptors (e.g. pH, geology and vegetation type). Based on landform, hydrological features and hydrodynamics, ecosystems are classified as one of the following hydrogeomorphic units: river, floodplain wetland, channelled valley-bottom, unchannelled valley-bottom, depression, seep or wetland flat (Ollis *et al.*, 2013).

The classification system describes depressional wetlands as water filled concavities that decrease in depth from the centre outwards to the shallower edges. These depressions are often referred to as pans when possessing a flat base. An especially distinguishing feature of these wetlands are their closed off /isolated shape. Depressional wetlands are categorized as exorheic, endorheic or dammed, each with a sub-category based on having channelled inflow or not. Apart from some depressional wetlands where a river can be found flowing into or through them, most inland depressional wetlands are endorheic (Allan *et al.*, 1995).

The Mpumalanga Highveld region bears, among others, around 2600 depressional wetlands (Riato, 2017) which tend to be at most about three meters deep when fully inundated (although reed pans may be deeper). The naturally formed endorheic pans in the Mpumalanga Highveld are mostly circular or oval shaped. The pans are filled by means of direct precipitation, surface runoff or subsurface inflows (De Klerk *et al.*, 2016). Summers in the Mpumalanga Highveld region are warm and wet (>700 mm mean rainfall per annum) while winter months are cold and dry (Wessels *et al.*, 2007 and Riato *et al.*, 2014).

Feldspathic sandstones and shales from the Karoo Supergroup, within which intruding dolerite sills are also found, form the underlying geology (Tooth and McCarthy, 2007). The geology not only determines the types of soil formed in an area and the rate thereof, but also provides various anions and cations that contribute to the general slightly saline nature of the pans.

Though pans are widely spread throughout the Highveld, the area of Lake Chrissie known as the Mpumalanga Lakes District (MLD) has a particularly substantial number of large pans and inundation is more likely to be permanent in eastern pans as rainfall increases and evaporation rates decreased from west to east (Allan *et al.*, 1995).

Different approaches to the classification of pans have been produced based on various features including geology, physical aspect and associated vegetation, however, De Klerk *et al.* (2016) recommend the vegetation-based classification developed by Allan (1987) as the more comprehensible and practical approach. Pans can be identified as reed pans, sedge pans, open water pans, grass pans or salt pans based on the type of vegetation present or the absence thereof. A summary of the characteristics of the different pan types is as follows:

- 1) Salt pans are saline open pans with acutely saline substrata (which may appear white when dry due to the precipitation of salts) and little to no vegetation. Salinity generally ranges from 5000 mg/l and upwards (Shaw and Thomas, 1989). The sedge, *Schoenoplectus triqueter*, is frequently found along the shoreline. Of the different pan types, salt pans are the most alkaline type with the highest overall pH (essentially around 9) and display considerably higher EC (electrical conductivity) values compared to other types of pans. Some recorded values range from less than 5 mScm⁻¹ to nearly 210 mScm⁻¹ (De Klerk *et al.*, 2016 and Riato *et al.*, 2017a). Higher concentrations of ammonium, phosphate, chloride and sulphates are also distinguishing features as sodium chloride, sodium and calcium sulphates and sodium carbonate salts are especially common (Seaman *et al.*, 1991).
- 2) An open water surface, substrata of bare rock or shallow soils and lack of vegetation characterise open-water pans. Vegetation, if present, is limited to the edges and may include grasses and sedges for example *Cynodon dactylon*, *Scirpus*, *Cyperus* and *Juncus* species (Allan *et al.*, 1995 and De Klerk *et al.*, 2016). They contain fresh to slightly saline, alkaline water (pH also around 9 but generally less than that of salt pans). Open pans exhibit lower conductivity (e.g. between 1 mScm⁻¹ and 10 mScm⁻¹) than salt pans but higher values than reed and grass pans, as is the case for nutrient and salt concentrations.
- 3) Sedge pans typically consist of black, clay-rich soil and a diverse range of emergent or submerged vegetation in and around the pan. Species such as *Schoenoplectus corymbosus* and *Eleocharis palustris* commonly occur. The fresh to slightly saline water with alkaline pH levels is comparable to those of open pans. EC values and ion concentrations also resemble those of open pans. De Klerk *et al.* (2016) maintains that open-water pans and sedge pans should be classified as a single type- open pans- based on their similar water quality parameters and the difference in vegetation resulting from different substrata.

- 4) Reed pans are characterised by a cluster of emergent reeds in the centre, typically *Phragmites* sp., surrounded by a belt of open water that stretches to the edges of the pan. Reed pans often exceed other types of pans in depth, are typically saline and have a relatively neutral pH (De Klerk *et al.*, 2012 and De Klerk *et al.*, 2016). Except for grass pans, reed pans have lower EC values than other types of pans. Values between 0.2 mScm⁻¹ and 2 mScm⁻¹ were recorded by De Klerk *et al.* (2016). De Klerk and Wepener (2013) documented EC values from reed pans ranging from 819 mScm⁻¹ to 1 614 mScm⁻¹ and Riato *et al.* (2017a) values between 0.84 mScm⁻¹ and 2.8 mScm⁻¹. In contrast to other pans, lower concentrations of nutrients can be measured in reed pans due to the influence of groundwater inflow and vegetation.
- 5) Grass pans are characterised by grasses and sedges (e.g. *Schoenoplectus* sp.) extending from the edges through to the centre of the pan. These pans are often small and non-perennial. Like reed and open pans, grass pans have fresh to slightly saline water but differ in that they are more acidic and generally demonstrate pH levels of around six (Hoffman, 2012 and De Klerk *et al.*, 2016). Grass pan EC values and nutrient concentrations are likewise the lowest of the different pan types. This might be due to the vegetation within the pans and seasonal dilution. Values as low as 0.16 mScm⁻¹ were observed by Hoffman (2012) ranging to 0.25 mScm⁻¹.

1.1.3 The value of wetlands

Humans benefit greatly from wetlands both directly and indirectly (Collins, 2005). As well as being sources of fresh water, depressional wetlands support floral, faunal and microbial diversity. Anaerobic microbes contribute to the detoxifying capabilities of wetlands. In addition to water purification, flood attenuation, nutrient and carbon storage, and erosion control represent some of their ecologically important functions (Collins, 2005; Gala and Young, 2015). These wetlands often serve as cultural heritage sites, fisheries or valuable land in terms of cultivation. Socioeconomic benefits include support of local farming (e.g. cattle), aesthetic and recreational value, as well as ecotourism. Despite the many benefits derived from wetlands, according to Driver *et al.* (2011) 65% of South Africa's wetland ecosystems are threatened and only 11% are protected.

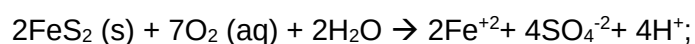
Regrettably, wetlands are threatened with degradation and loss due to urban development and dam construction that cause changes to the natural flow regime, eventually leading to more channelled flow and a decrease in water saturation (Collins, 2005 and Driver *et al.*, 2011). This is accompanied by the removal of wetland vegetation and consequently soil

disruption and erosion. Wetland ecosystems are disturbed by pollution and invasive plants which consequently threatens native species and biodiversity. In addition, mining activities which are known to have severe ecological impacts on terrestrial as well as aquatic habitats, are prevalent in the Mpumalanga Highveld region and likewise jeopardize wetlands in the region (Sieben *et al.*, 2017).

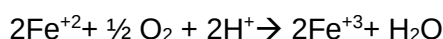
1.1.3.1 Coal mining and acid mine drainage

1.1.3.1.1 The significance of coal in South Africa and formation of acid mine drainage

According to Munnik *et al.* (2010) South Africa exports an estimated 28% of its produced coal and is the world's fourth largest coal exporting country. In South Africa, coal is used mainly in electricity generation, fuel production and industrial and household activities. Coal accounts for approximately 72% of the country's energy needs, as it is used to generate 90% of the country's electricity and 30% of liquid fuel (Ratshomo and Nembahe, 2016). The Highveld coal field in Mpumalanga is South Africa's second largest coal producing field, the mining and distribution of which, contributes significantly to the local economy (Fosso-Kankeu *et al.*, 2016). However, the coal seam contains the mineral pyrite (FeS_2) which is exposed to atmospheric oxygen or water rich in dissolved oxygen by mining activities (Ochieng *et al.*, 2010). Acid mine drainage (AMD) is known to originate from the oxidation of sulphur bearing minerals such as pyrite (the reaction occurs as follows:



followed by the further oxidation of ferrous iron to ferric iron:



(Akcil and Koldas, 2006; Pinetown *et al.*, 2007). Smucker and Vis (2009) list high conductivity, low pH and high sulphate (SO_4^{2-}) concentration as significant evidence of AMD contamination. Acid mine drainage contamination affects freshwater sources and agricultural lands in the vicinity, disrupting the growth and reproduction of aquatic and terrestrial organisms. Increased mining and agriculture present the most serious threats to wetland ecosystems in the Mpumalanga Highveld (Riato, 2017). Many of the pans are subjected to the impacts of coal mining by being used directly to store AMD.

1.1.3.1.2 The effects of acid mine drainage

The dangers posed to wetland and other ecosystems by AMD include increased concentrations of heavy metals and often significant drops in pH. Certain metals have been known to occur as unavailable hydrated hydroxides above neutral pH conditions. These metals increase in availability and toxicity as pH levels decrease and consequently, they form highly toxic ions. Metals such as, aluminium, cadmium, cobalt, copper, lead, manganese, mercury, nickel, silver and zinc accumulate in water bodies and soils, where they are known to have harmful effects associated with low pH conditions (Dallas and Day, 2004). These metals pose a threat to human health insofar as they accumulate in agricultural products or are ingested directly from contaminated water. Furthermore, the presence of AMD in wetlands and other water bodies leads to contamination of potable water supplies, accumulation in and contamination of the food chain, depletion of aquatic organisms and the degradation of natural ecosystems (Ochieng *et al.*, 2010). The acidification caused by acid mine drainage, is signified by elevated concentration of hydrogen ions that are known to disrupt many organisms' mechanisms of ionic regulation (Coetzee, 1995). Therefore, AMD not only possess considerable risks to human health and ecosystem health overall, but also presents a threat to wetland recreational activities and tourism. This leaves communities who are socioeconomically dependant on wetlands especially vulnerable.

The impacts of AMD on these wetlands are well understood and it is vital that they be accurately quantified and mitigated as much as possible. Monitoring ecological conditions and anthropogenic impacts in aquatic ecosystems can be difficult. As mentioned by Taylor *et. al.* (2007c), chemical analyses give accurate representations of water quality in ecosystems, however, measurements are only representative of the water's characteristics at that specific point in time and in that specific location. These types of analyses are too expensive to be carried out frequently and repeatedly, creating a need for monitoring methods that are more repeatable and cost effective.

1.1.3.2 Biological assessment in South Africa

Living organisms can be studied over time in order to determine changes in a given environment. The term biomonitoring (from biological monitoring) refers to the use of representative organisms to assess the state of the environment and water quality (Rand, Wells and McCarty, 1995). In the interest of conserving South Africa's wetlands, the ecological condition can be evaluated in terms of functional, physical and biotic variables (Kotze *et al.*, 2012). Biological assessment offers a means of quantifying the degree to which wetlands have

been altered from their natural state. For instance, disturbance can be measured as relative change in species composition and abundance using various organisms as indicative of change. Communities of diatoms, macrophytes, macroinvertebrates and fish are well known aquatic bioindicators, especially in rivers (Kleynhans, 1999; Dickens and Graham, 2002; Taylor *et al.*, 2007c; Dallas *et al.*, 2010). The South African Scoring System (SASS) Version 5 uses macroinvertebrate communities to assess water quality and is designed specifically for rivers. Similarly, fish indices such as the Fish Assemblage Integrity Index (FAII) and the Fish Response Assessment Index (FRAI) use fish assemblages and responses to describe the ecological state of rivers.

Allan *et al.* (1995) mention that temporary pans are known to host a wide variety of invertebrates (referring especially to species with opportunistic lifestyles that are capable of surviving desiccation or reach reproductive maturity rapidly during periods of inundation). Most studies of macroinvertebrates in pans occur in temporary pans, as permanent pans are comparatively much less abundant. De Klerk and Wepener (2013) were able to determine that changes in water quality (due to seasonal variability and anthropogenic impacts) could be demonstrated by changes in the macroinvertebrate assemblages of permanent reed pans in the Mpumalanga Highveld. While most pans in the Mpumalanga Highveld do not harbour any fish, smallmouth bass (*Micropterus dolomieu*) and carp have been introduced to some of the larger permanent pans in the Lake Chrissie area (Allan *et al.*, 1995). Being absent in most pans and introduced in only a few, fish do not offer a dependable approach to biomonitoring in Highveld pans.

1.1.3.2.1 Diatom-based biomonitoring in South Africa

Diatoms are widely used as bio-indicators due to their general abundance and sensitivity towards changes in the environment (Hattikudur *et al.* 2015 and Lobo *et al.*, 2016). Diatoms are present in most aquatic habitats and have been used as bio-indicators the world over, often comparing well with more conventional methods, and making them broadly applicable. Lobo *et al.* (2016) mention that, for many diatom species, the ecological preferences concerning factors such as pH, electrical conductivity (EC), the concentrations of organic matter and trophic state or nutrient requirements, among others, have been determined. Though South Africa has established methods based on fish and macroinvertebrate bioindicators, certain diatoms are substantially more tolerant of the highly acidic conditions produced by acid mine drainage than other organisms (Battarbee *et al.*, 2004; De Nicola and Stapleton, 2002). Diatoms reproduce rapidly and maintain a shorter generation time (around two weeks) than macroinvertebrates and fish (Harding *et al.*, 2005) and are sensitive to physio-

chemical changes in water. This means that diatom assemblages can change quickly according to deviations from natural conditions. Wu (1999) states that diatoms were especially used to detect organic pollution. Subsequently they were then used to indicate salinity and eutrophication, and later aspects such as acidification to water quality in general. Besides being indicative of organic pollution, diatoms can also indicate heavy metal pollution. High concentrations of heavy metals such as chromium (Cr), cadmium (Cd), zinc (Zn), strontium (Sr) and copper (Cu) cause unusual morphology and abnormalities in ornamentations on frustules (deformation) (Tapia, 2008).

Benthic diatoms generally represent specific environmental conditions more accurately than planktonic specimens, which are passively carried by along streams and currents. Sampling epiphytic and epilithic diatoms is simpler compared to sampling macroinvertebrates or fish (Taylor *et al.* 2007a; Dallas, 1997; Kleyhans, 1999).

In 2004, Taylor tested several European indices in the Vaal catchment and found significant correlations between the ambient water quality and the scores of the Biological Diatom Index as well as the Specific Pollution sensitivity Index (SPI). Following these, Leclercq and Maquet's Index (LMI) and the Generic Diatom Index (GDI) also displayed good capability in representing general water quality. The Eutrophication/Pollution Index/ EPI was not recommended for general water quality monitoring but proved useful for determining eutrophication levels. In terms of organic pollution, Sládeček's index (SLA) was found to correlate the best.

Taylor (2006) tested a variety of indices on the same data obtained from various sources in the Crocodile-Marico Catchment. Indices of which the scope of application was tested are the Generic Diatom Index (GDI), the Specific Pollution sensitivity Index (SPI), the Biological Diatom Index (BDI), the Artois-Picardie Diatom Index (APDI), Sládeček's index (SLA), the Eutrophication/Pollution Index (EPI), Rott's Index (ROT), Leclercq and Maquet's Index or (LMI), the Commission of Economical Community Index (CEC), Schiefele and Schreiner's index (SHE), the Trophic Diatom Index (TDI) and the Watanabe index (WAT). Due to the similar environmental preferences of widespread diatom species, various European indices are applicable to South African freshwater systems to an extent. The presence of endemic species elicits the need for customisation of these indices to be truly representative of South Africa's ecological condition (Taylor *et al.*, 2007c). Diatom indices used in South Africa include the South African Diatom Index (SADI) for rivers and streams, based especially on the Specific Pollution sensitivity Index (SPI) (Harding and Taylor, 2011) and the Lange-Bertalot method was proven to be applicable to South African conditions when adjusted (Schoeman, 1979 and Harding *et al.*, 2005).

Taylor *et al.* 2007d points out that most of the aforementioned indices are calculated using the formula of Zelinka and Marvan (1961). Taxa most compatible with the prevailing water quality will be the most abundant (Taylor, 2004). Thus, indices provide scores that reflect biotic integrity or pollution levels by using a weighted average equation in which each taxon in a sample is assigned a sensitivity value (s-value) and an indicator value (v-value) then weighted by the abundance of that taxon. The sensitivity value expresses a species' preference for water of a specific quality and the indicator value denotes how strongly a taxon is associated with water of a specific quality. Higher indicator values indicate a stronger association and increases the influence of a taxon.

The software package OMNIDIA (Lecointe *et al.*, 1993) calculates and presents the scores of 18 indices up to a score of 20 with the exception of the TDI (up to 100; Taylor, 2004) and the percentage of Pollution Tolerant Values (%PTV). According to the index scores obtained, aquatic environments fall into one of five water quality classes: high quality (17-20), good quality (15-17), moderate quality (12-15), poor quality (9-12) and bad quality (9 or less) (Matlala *et al.*, 2011).

In 2011 Matlala *et al.* recommended the use of certain diatom indices in wetlands until such time as sufficient information on South African wetlands could be collected to facilitate the development of a customised diatom-based index for wetlands. Three indices proved particularly reliable because they incorporate the majority of diatoms found in the studied wetlands. These were the Generic Diatom Index (GDI), Specific Pollution sensitivity Index (SPI) as well as the Trophic Diatom Index (TDI). A subsequent study tested the application of the Generic Diatom Index (GDI), Specific Pollution sensitivity Index (SPI), the Biological Diatom Index (BDI) and the percentage of Pollution Tolerant Values (%PTV) in wetlands from South Africa's Western Cape (Olivier, 2016). The Specific Pollution sensitivity Index (SPI) and the Biological Diatom Index correlated most significantly with water quality variables. The substrata from which diatoms were sampled was found to effect SPI scores (up to two quality classes). The study also produced essential information on the diversity of diatoms in wetlands in the Western Cape Province and their ecological requirements.

Not only have diatom-based indices been developed for eutrophication and organic pollution detection, but efforts have been made towards the use of diatoms for monitoring pollution associated with coal mining (especially acid mine drainage). In the Western Allegheny Plateau of south eastern Ohio, a species-level Bray–Curtis (BC) similarity to reference index as well as a genus-level diatom model affinity (DMA) index was produced for the quantification of agricultural and acid mine drainage (AMD) impacts on streams (Smucker and Vis, 2009). The study not only compared diatom assemblages at sites varying in levels of disturbance, but macroinvertebrate and fish assemblages as well, reporting definite impact indication by all the

assemblages. Fish were deemed the least useful as they were not present in all the sites. Negative physical effects (e.g. deformities) were still noticed contrary to high species richness and index of biotic integrity scores suggesting the recovery of (NaOH) treated sites. Metrics regarding macroinvertebrate assemblages reflected impairment at AMD impacted sites but remained comparable between non-impacted and treated sites. Diatom metrics and indices very clearly reflected agricultural and mining impacts as well as noticeably discriminating between non-impacted and treated sites. Diatom indices proved more sensitive to AMD impacts and illustrated ecological conditions more finely than fish or macroinvertebrates.

In a study published in 2010, Zalack *et al.* developed and tested a multi-metric AMD-diatom index of biotic integrity (AMD-DIBI) to indicate AMD impacts in streams. The correlations between various AMD-DIBI metrics and water chemistry variables- NO₃, PO₄, SO₄, Fe, Mn, alkalinity, pH, conductivity and dissolved oxygen- were determined and the effectiveness of the index in differentiating AMD disturbance levels, was compared to that of an established multi-metric macroinvertebrate community index (ICI). The AMD-DIBI includes the following metrics:

- Richness
- % Sensitive species
- % Tolerant species
- Number of Reference species
- % Acidophilic species
- % Eutraphentic species
- Chlorophyll a
- % similarity to reference sites
- % *Cymbella* species

Metrics and the AMD-DIBI responded well to variations in water quality and gave results similar to those of the multi-metric macroinvertebrate community index (ICI).

Though previously some studies were done on the macroinvertebrate and zooplankton communities of Highveld pans, little information existed on diatoms in these pans (Riato *et al.*, 2014). After determining that much was known about South African pans in terms of physical and chemical attributes, a description of the zooplankton and epiphytic diatom communities of the pans in this region was provided by Riato *et al.* in 2014. The study provided indispensable information on the zooplankton and diatoms of both ephemeral and permanently inundated pans, from which 140 species of epiphytic diatoms were identified. It was found that temporary pans were occupied by species such as *Nitzschia acidoclinata* Lange-Bertalot, *Gomphonema*

gracile Ehrenberg and *Eunotia bilunaris* (Ehrenberg) Schaarschmidt- indicative of acidic water with low oxygen and nutrient levels. In contrast, permanent pans hosted species representative of more saline conditions e.g., *Nitzschia frustulum* (Kützing) Grunow, *Amphora veneta* Kützing and *Planothidium engelbrechtii* (Cholnoky) Round & Bukhityarova and mostly excluded species from the genera *Pinnularia* and *Eunotia* which are typical of acidic conditions.

The genus *Nitzschia* was represented by the most taxa and contained the rarest species, followed in numbers of taxa by *Navicula*, *Gomphonema* and *Eunotia*. Species encountered across more than half of all sites included *Gomphonema parvulum* Kützing, *Nitzschia frustulum* (Kützing) Grunow, *Nitzschia palea* (Kützing) W Smith, *Gomphonema gracile* Ehrenberg, *Eunotia bilunaris*, *Nitzschia acidoclinata* and *Nitzschia palea* var. *debilis* (Kützing) Grunow.

On account of coal mines in the Mpumalanga Highveld using many of these permanently inundated pans for the storage of AMD, Riato *et al.* (2017a) developed a diatom-based multimetric index for acid mine drainage impacted permanent pans. The multimetric indices (MMI) based on epiphytic diatoms were specialised for different wetland types. The use of multiple metrics provides a collective indication of ecological condition due to the integration of a variety of taxonomic and functional groups -each with varying responses to different stressors. Metrics were selected based on possessing a broad range, high separation power and there being a low correlation among metrics. The metrics used in the indices represent different components of the diatom assemblages' structure and function; and fall into three categories (to exhibit a gradient of human disturbance): similarity to reference sites, functional groups, and taxonomic composition. Natural variation was accounted for by classifying wetland types based on diatom typologies.

1.1.3.2.2 Diatoms as representative of ecological conditions

Diatoms (Bacillariophyta) are a morphologically diverse group of microscopic algae characterized by unique siliceous cell walls known as frustules (Mann, 2002) and a golden-brown appearance resulting from photosynthetic xanthophyll pigments (Burliga and Kociolek, 2016). Some characteristics of diatoms that make them outstanding bioindicators include their impressive diversity and occurrence in most aquatic habitats. Diatoms occur in moist terrestrial habitats as well. Taxa are identifiable by the shape, size, symmetry and ornamentation of frustule and species have discernible tolerance ranges for physical and chemical water quality parameters (Telford *et al.*, 2002). For example, different species of *Nitzschia* are associated with different types of contamination such as organic pollution, eutrophic conditions, salinity and heavy metals (Trobajo *et al.*, 2013). The life span of approximately two weeks enables

species composition to change more quickly according to disturbances, reflecting ecological conditions over time (and with less delay).

Diatom frustules can remain preserved long after the cells have died due to silica (SiO_2) making frustules very hard and resistant. The siliceous nature of the frustules is conducive to the fossilisation of diatoms and fossils dating back to the lower Cretaceous period have been found (Telford *et al.*, 2002). Diatoms therefore, are not only excellent indicators of present changes in ecological conditions but demonstrate environmental changes over enormous timescales (Wachnicka *et al.*, 2010). Siemińska (2015) reported that diatoms have occurred from as early as the Paleoproterozoic Era (2,500 to 1,600 million years ago). Finally, identification keys and diatom-based water quality indices are noted to be broadly applicable geographically, as many diatom taxa are presumed cosmopolitan in their occurrence (Telford *et al.*, 2002 and Harding *et al.*, 2005).

Based on the shape and symmetry of the frustule, diatoms are categorized into two elementary groups: centric (radially symmetrical) and pennate (bilaterally symmetrical) (D'Ippolito *et al.*, 2015). Generally, centric diatoms are adapted to living suspended in the water column as phytoplankton, (triangular, square, and stellate forms also occur especially in marine phytoplankton). Pennate taxa are more commonly abundant in surface-associated biofilms (Taylor *et al.*, 2007b). Although essentially periphytic, pennate diatoms may often be resuspended in the water column and consequently sink beyond the photic zone. Some pennate species avoid sinking by forming extended colonies (Sabater, 2009).

The ecological condition of aquatic habitats can be deduced from the growth forms predominant in the diatom community as well the ecological guild represented by the majority of species. Growth form, according to Hudon and Legendre (1987), refers to organisms' adaptations for a specific mode of life (adaptations usually advantageous in various habitats and competition for available resources). Three features collectively define the growth form of benthic diatoms: form (occurring either as solitary cells or in colonies), posture (prostrate or erect) and mobility.

Adnate taxa are either biraphid or monoraphid and live attached to a substrate by adhering firmly to the substrate surface with their valve face. Adnate forms advance and proliferate on substrate surfaces in a manner that is parallel to the surface, seldom forming colonies. Diatom species from genera such as *Amphora*, *Cocconeis*, *Epithemia* and *Rhopalodia* exemplify adnate taxa (Totti *et al.*, 2009). What is more, diatom taxa can be categorized in terms of ecological guild as low-profile, high-profile or motile. Rimet and Bouchez (2012b) define an ecological guild as a group of taxa of corresponding taxonomic or functional group that utilize

the same resources within the same environment (albeit by different adaptations). Passy (2007) explains the low-profile guild as comprised of species that are short in stature and/or slow moving as well as solitary centric species. The low-profile guild encompasses adnate, prostrate and minor erect forms of which species from the genera *Achnanthes*, *Achnanthidium*, *Amphora*, *Cocconeis* and *Cymbella* are representative. Low-profile taxa have proven suitably adapted to tolerate low nutrient and light conditions and physical disturbance associated with fast flowing water conditions common in river headwaters (Passy, 2007; Rimet *et al.*, 2009; Tang *et al.*, 2012).

Species in the high-profile guild thrive under conditions in which light and nutrients are abundant and current velocity reduced. Members are erect, tall in stature and prone to forming colonies (specifically, colonies that are filamentous, branched, chainlike or tubular as well as colonial centric species) which make them vulnerable to physical disturbance in fast moving water. This includes species from genera such as *Diademesmis*, *Diatoma*, *Encyonema*, *Eunotia*, *Fragilaria*, *Gomphonema*, *Gomphoneis*, *Melosira* and *Ulnaria*.

Erect forms typically grow in a vertical direction from the substrate, attached to the surface by means of mucilaginous stalks, as in the case of *Grammatophora*, or by pads as do *Cyclophora* and *Synedra* (Rimet *et al.*, 2009). Erect taxa from the genera *Achnanthes*, *Achnanthidium*, *Fragilaria*, *Gomphonema* and *Ulnaria* which attach either by stalks or pads are also referred to as pedunculate. Some erect forms form rosette colonies that are fan-shaped (e.g. *Ulnaria acus*) whereas others including *Licmophora*, *Gomphonema*, and *Rhoicosphenia* form arborescent (treelike) colonies (Hudon and Legendre, 1987). Linear chains are formed by taxa such as *Fragilaria*, *Melosira*, *Biddulphia* and *Rhabdonema*. Species like *Eunotia bilunaris*, *Eunotia minor* and *Eunotia pectinalis* (Riati, 2017) and *Fragilaria capucina* var. *vaucheriae* form ribbon colonies, where diatoms are connected to each other by means of interlocking spines or bonded at the valve face by a mucous layer (Rimet and Bouchez, 2012b). Also in the high-profile category, are tube-dwelling colonies which are composed of motile species living in mucilaginous tubes (Totti *et al.*, 2009). Tube-living taxa move freely within the stationary tube of mucous. Tube-forming taxa include species of *Frustulia* and *Encyonema* (e.g. *Encyonema mesianum* (Cholnoky) D.G. Mann and *Encyonema minutum* (Hilse) D.G. Mann) (Rimet and Bouchez, 2012b). Fast moving diatom species like those belonging to genera such as *Navicula*, *Nitzschia*, *Sellaphora*, and *Surirella* comprise the motile guild. This guild excludes species that are mobile but slow moving (Passy, 2007). Species from the motile guild exhibit tolerance for polluted and eutrophic environments but are vulnerable to physical disturbance.

Rimet and Bouchez (2012b) contend that solitary centric species from the genera *Cyclotella* and *Stephanodiscus* for example, are not sturdy enough in their attachment to be considered low-profile while *Aulacoseira* and *Skeletonema* were also withdrawn from the high-profile guild and placed in a fourth guild, the planktonic guild. This guild contains taxa adjusted to lentic environments (e.g. *Cyclotella meneghiniana* Kützing, *Fragilaria tenera* (WM Smith) Lange-Bertalot, *Ulnaria acus*, *Nitzschia acicularis*).

According to Hudon and Legendre (1987), the structure of diatom communities is subject to change based on environmental changes, an occurrence facilitated by their ability to colonise new substrates. Pioneer species are those that colonise bare substrates faster than others and usually substrates are initially colonised by adnate and motile diatoms (Totti *et al.*, 2009). Pioneer species form the base of the diatom biofilm, upon which erect forms are established as colonisation progresses. Erect forms tend to be dominant in climax communities (Majewska *et al.*, 2013).

The relationship between diatom species and physico-chemical properties in water has been widely determined and well documented. Changes in the distribution of diatoms along environmental gradients have repeatedly proven to be evident (Pan and Stevenson, 1996; Wachnicka *et al.*, 2010; Nodine and Gaiser, 2013). Rimet and Bouchez (2012b) point out that a great number of diatom-based indices are based on species ecological preferences because higher taxonomic resolution (species level) is considered more discriminative and accurate in its representation of conditions than higher classifications. However, some difficulties may be faced in the use of species in indices, especially when applied on a large geographical scale. The immense diversity of diatoms, constant discovery of new species and taxonomic intricacies makes application at species level difficult and time-consuming where taxonomic knowledge or tools for identification is limited (Wu, 1999 and Rimet and Bouchez, 2012a).

The use of lower taxonomic resolution (specifically genus level) may be adequate where very precise taxonomic resolution is unnecessary, as diatom genera are also correlated with physico-chemical parameters (Smucker and Vis, 2009). Diatom growth forms and ecological guilds also change predictably along nutrient and disturbance gradients as well as in response to organic pollution (Passy, 2007; Rimet and Bouchez, 2012a; Rimet and Bouchez, 2011), and can be used to observe changes in water quality when the use of species would be impractical.

1.2 Problem statement

Multimetric indices use multiple metrics to better discern between non-impacted and impacted sites and provide an extensive determination of the ecological condition. Diatom indices are designed to determine the extent to which a given pan is impacted by AMD. In order to assess the performance of the diatom index developed by Riato (2017), the index must be tested on a validation dataset consisting of a range of AMD-disturbed pan sites and non-AMD disturbed pans. The application of the indices using a validation dataset will provide an unbiased assessment of the indices' accuracy in discriminating between heavily impacted sites and those least disturbed.

Additionally, sampling for the initial study was only done once during the dry season (from May to August). Sampling during two seasons (the wet as well as dry season) would allow for identifying natural variation in the diatom assemblages associated with seasonal changes and enable the use of the indices throughout the year.

1.3 Research aim and objectives

1.3.1 Research aim

The aim of this study is to evaluate the performance of, and determine deficiencies in diatom indices created for different AMD-disturbed pans in the Mpumalanga Highveld region. Potential further refinements can be made for the purpose of producing a practical means of assessing the ecological condition of these pans. In other words, developing an effective assessment and management strategy.

1.3.2 Research objectives

It is necessary to determine whether the diatom-based multimetric indices can be used to accurately distinguish between sites highly impacted by coal mining activities, sites moderately impacted, and sites not impacted. By using a validation dataset that incorporates both the dry and wet season, the performance of the diatom indices developed for salt pans, reed pans, open water/ grass pans and all different pan types combined can be evaluated unbiasedly.

Firstly, the differences in diatom communities of different types of pans must be determined as well as differences in physical and chemical variables. As mentioned previously, riverine diatom indices such as the GDI, SPI and the TDI explain the acronyms display applicability in wetlands on account of the incorporated species occurring in wetlands as well. In the event of acid mine drainage pollution however, a diatom-based multimetric index designed to indicate acid mine drainage would be more appropriate. Even more so when including diatom species

associated with pans. The ability of diatom based MMIs to distinguish between impacted sites and non-impacted sites are to be compared to that of other widely used diatom indices as well as their applicability to different types of pans. The influence of seasonal change on index scores will also be compared.

Assessment of the effectiveness of diatom based MMIs compared to established indices may contribute to the development of an index and a user-friendly guide for the ecological assessment of the pans in the Mpumalanga Highveld.

CHAPTER 2: MATERIALS AND METHODS

2.1 Study area:

The study area extends across the Mpumalanga Highveld Region, from the area of Ogies to the Mpumalanga Lakes District (MLD) around Lake Chrissie. As mentioned, the region is characterised by warm, wet summers and cold, dry winters. The region's extensive number of endorheic pans are either ephemeral or perennial (Riato *et al.*, 2014). Direct precipitation, surface runoff or subsurface inflows provide the means by which the pans are filled, and reed pans are more likely to be permanently inundated as they are deeper than other types of pans (De Klerk *et al.*, 2016). Mpumalanga's prevalent coal mining and agricultural activities threaten the health of these wetlands and their unique diversity (Ferreira *et al.*, 2012 and Riato *et al.*, 2014). In the interest of evaluating the diatom based multimetric index for acid mine drainage impacted pans, sampling took place during the rainy season (December to February) and again during the dry season (May to August) from permanently inundated pans. Samples were taken from a range of reference pans where no mining occurs in the catchment, which represents non-impacted depressional wetlands as well as AMD-impacted depressional wetlands (Riato *et al.*, 2017a). Reference sites should not be disturbed by agricultural activities or surrounded by overgrazed pastures.

2.1.1 Site identification and selection

Sites were selected using Google Earth Pro and time-sequenced satellite imagery and information provided by experts and local landowners. After locating as many coal mines as possible in the study area, depressional wetlands in the vicinity were located, and it was determined from time-sequenced satellite imagery and local landowners whether they are permanently inundated. More than 30 potential sites were identified, of which at least 10 sites fall into one of 3 categories of disturbance: 1) Highly AMD impacted, 2) Moderately AMD impacted and 3) Least impacted (these serve as reference sites). Sites nearest to coal mines were categorized as highly disturbed by mining activities. Sites not directly adjacent to mines but still in close proximity were considered moderately impacted. Reference sites (least impacted by mining activities) were selected based on a lack of mining within the catchment and are located in the Lake Chrissie area. A total of 28 pan sites were sampled between 9 May and 20 August 2019, each from which one diatom sample and one water sample was collected. Sites were classified according to level of mine disturbance, ranging from least disturbed (14 sites), moderately disturbed (9 sites) and highly disturbed (5 sites). Sampling was repeated in the summer from 15 January to 7 February 2020. Two sites, S21 (moderately

impacted) and S9 (reference site) could not be resampled during the summer. Figure 1 displays the distribution of reference sites, moderately disturbed sites and highly disturbed sites across the Mpumalanga Highveld region. Table 1 shows the pan type, level of disturbance and coordinates of each site.

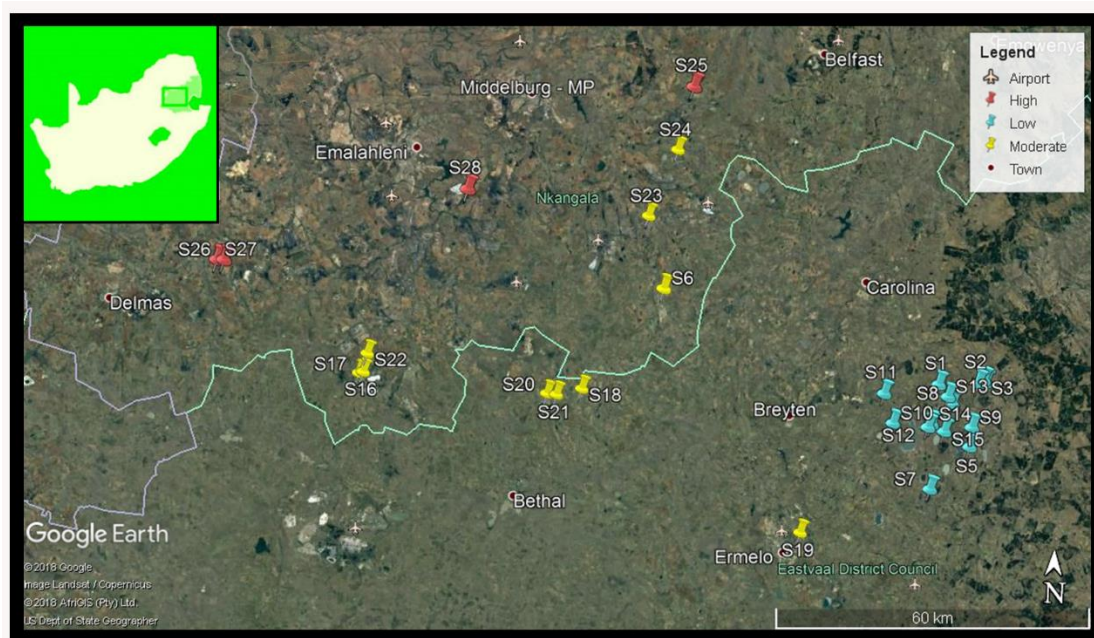


Figure 1: Distribution of pan sites across the Mpumalanga Highveld region.

Table 1: Depressional wetlands sampled in winter 2019 and summer 2020

Site	Coordinates	Pan type	Disturbance level	Winter Date collected	Summer Date collected
S1	26°15'26.20"S 30°15'29.61"E	Reed	Low	03/06/2019	17/01/2020
S2	26°15'5.87"S 30°20'1.28"E	Reed	Low	02/06/2019	17/01/2020
S3	26°14'46.44"S	Reed	Low	02/06/2019	17/01/2020

	30°20'36.37"E				
S4	26°14'59.59"S 30°20'49.19"E	Reed	Low	02/06/2019	17/01/2020
S5	26°21'39"S 30°19'23"E	Grass	Low	03/06/2019	18/01/2020
S6	26°06'59"S 29°43'40"E	Salt	Medium	23/07/2019	20/01/2020
S7	26°26'10"S 30°15'04"E	Salt	Low	04/06/2019	16/01/2020
S8	26°17'24"S 30°17'08"E	Grass	Low	03/06/2019	16/01/2020
S9	26°19'39.60"S 30°19'32.23"E	Salt	Low	04/06/2019	-
S10	26°19'36.62"S 30°10'27.29"E	Salt	Low	04/06/2019	16/01/2020
S11	26°16'37.99"S 30° 9'17.00"E	Grass	Low	02/06/2019	16/01/2020
S12	26°19'51.05"S 30°14'30.09"E	Grass	Low	04/06/2019	16/01/2020
S13	26°16'38.11"S 30°16'38.08"E	Grass	Low	02/06/2019	17/01/2020
S14	26°19'30.68"S	Grass	Low	04/06/2019	16/01/2020

	30°14'52.80"E				
S15	26°20'12.04"S 30°16'23.16"E	Grass	Low	03/06/2019	18/01/2020
S16	26°16'41.47"S 29° 9'58.70"E	Grass	Medium	25/07/2019	06/02/2020
S17	26°16'50.63"S 29° 9'29.33"E	Salt	Medium	25/07/2019	06/02/2020
S18	26°17'25.96"S 29°34'55.10"E	Salt	Medium	23/07/2019	20/01/2020
S19	26°31'05.0"S 30°00'33.0"E	Grass	Medium	01/06/2019	15/01/2020
S20	26°18'5.01"S 29°30'58.85"E	Salt	Medium	23/07/2019	05/02/2020
S21	26°18'8.46"S 29°32'3.74"E	Salt	Medium	23/07/2019	-
S22	26° 14' 47.69"S 29° 10' 21.79"E	Salt	Medium	25/07/2019	06/02/2020
S23	25°59'31.53"S 29°41'36.64"E	Salt	Medium	08/07/2019	05/02/2020
S24	25°52'38,61"S 29°44'35,04"E	Grass	High	23/07/2019	20/01/2020

S25	25°46'16.49"S 29°46'3.39"E	Open water	High	09/05/2019	20/01/2020
S26	26° 5'59.27"S 28°53'32.99"E	Salt	High	22/07/2019	07/02/2020
S27	26° 5'59.63"S 28°52'44.22"E	Reed	High	22/07/2019	07/02/2020
S28	25° 57'45"S 29°20'56,80"E	Reed	High	20/08/2019	06/02/2020

2.2 Field procedures

2.2.1 Equipment necessary for wetland diatom sampling

- Waders or gumboots
- Knife or pruning shears for cutting of macrophyte stems
- Toothbrushes for scrubbing diatoms from solid substrata
- Ziplock bags for removing diatom from macrophyte stems
- Sample bottles for the storage of water and diatom samples
- Marking pen
- Appropriate measuring equipment for measuring water quality variables in situ.

2.2.2 Diatom sampling

Epiphytic diatoms were sampled during the wet and dry season from each site according to standard procedures (Taylor *et al.*, 2007b). Diatoms were sampled from the submerged stems and leaves of different macrophytes by removing a stem and its branches, placing the stem in a clear plastic bag with 50ml of water then squeezing, rubbing and shaking the bag vigorously. The water containing the dislodged diatoms is then poured into a bottle. At least five replicates were sampled per site. Stems were up to 50 cm deep from the water surface. In the event that macrophytes are absent from the pan a clean toothbrush was used to scrub diatoms off boulders, cobbles, pebbles and other submerged solid substrata then depositing the sample into a sample bottle. Macrophytes and other substrata were sampled as close to centre of the pan as possible and the edges avoided in order to obtain samples that best represented conditions within the pan.

2.2.3 Physico-chemical variables

Physical and physico-chemical variables measured in situ include temperature, pH, conductivity, nitrates (NO_3^-), calcium (Ca^{2+}), sodium (Na^+) and potassium (K^+) using a Horiba LAQUAtwin Kit. Water samples- at least 500 ml- were collected from each pan and kept on ice until being assessed in the laboratory.

Water quality variables determined in the laboratory include orthophosphates (PO_4^{3-}) and total phosphorus (TP) using a HACH DR 3900 spectrophotometer, alkalinity as CaCO_3 (mg/L; SALM.5.0 Potentiometric titration), ammonia (NH_3 in mg/L; SALM.6.0 Flow Injection Colorimetry), chloride (Cl^- in mg/L; SALM.1.0 Flow Injection Colorimetry), magnesium (Mg^{2+} in mg/L; MALS6.5-A ICP OES Detection), sulphates (SO_4^{2-} in mg/L; MALS6.5-A ICP OES

Detection), total dissolved solids (TDS in mg/L; SALM.26 Gravimetric Measurement), hardness as CaCO_3 (mg/L), cations and anions (meq/L) and Mg hardness as CaCO_3 (mg/L).

2.3 Laboratory procedures

2.3.1 Equipment necessary for cleaning of diatom samples

- Personal protective equipment (Acid resistant gloves, lab coat, goggles)
- Fume cabinet
- Beakers
- Test tubes
- Dropper
- Hot Plate for heating diatom material
- Vortex mixer
- Centrifuge
- Potassium permanganate (KMnO_4)
- Hydrochloric acid
- Hydrogen peroxide
- Distilled water

2.3.2 Cleaning techniques: hot HCl and KMnO_4 method

Once collected, the diatom samples were left for 24 hours and given time to settle out of suspension. Afterwards the supernatant was decanted. Between five and ten millilitres of the remaining sample transferred to correspondingly marked glass tubes. To oxidize the organic material in the sample, ten millilitres of saturated potassium permanganate (KMnO_4) solution was added to each sample, mixed then left for another 24 hours. Depending on the amount of organic material, between five and five and ten millilitres of concentrated hydrochloric acid (HCl, 32%) were added. Tubes containing the diatoms material, potassium permanganate and hydrochloric acid were placed in a hot bath and heated at 90°C for 1 to 2 hours. The solution gradually became clearer as oxidation occurs until finally reaching a slightly yellowish hue. A few drops of hydrogen peroxide were added which created foaming until oxidation was complete. When the addition of hydrogen peroxide no longer produced foaming, the samples were left to cool. The cooled samples were vigorously swirled in order to resuspend the diatoms and transferred to centrifuge tubes to be rinsed. Samples were centrifuged at 2500 rpm for ten minutes, after which the supernatant was decanted and distilled water added to resuspend the diatom material. This procedure was repeated four times. Cleaned samples were then stored in appropriately labelled glass bottles.

2.3.3 Equipment necessary for preparation of diatom slides

- Distilled water
- Pipette
- Microscope slides
- Coverslips
- Diatom mountant such as Pleurax

2.3.4 Diatom slide preparation for light microscopy

Using a pipette, the concentrated and cleaned diatom material is diluted with distilled water and placed onto a clean coverslip (18 mm diameter), covering the entire surface. The coverslips were left in a fume cabinet for 24 hours and allowed to air-dry. Dried coverslips were mounted onto glass slides using Pleurax.

2.3.5 Diatom enumeration and identification

Diatoms were identified and counted by means of light microscopy using a Nikon 80i light microscope equipped with a Nikon DS-Fi1 5MP digital camera. In each slide, a minimum of 400 valves were counted following arbitrarily chosen transects and identified to the lowest possible taxonomic level using available literature (DeNicola, 2000; Verb and Vis, 2000; Sonneman *et al.*, 2000; Taylor *et al.*, 2007a; Riato *et al.*, 2014).

2.4 Diatom indices and calculations

As mentioned, four indices were created to determine the level of impact caused by AMD in different types of pans (Riato *et al.*, 2017a). The first index, MMI1, employs metrics calculated from all reference sites for comparison to impacted sites. This includes every reference site from all pan types. Metrics from the indices for reed pans (MMI2) and salt pans (MMI3) are calculated using only reed and salt reference or disturbed pans, respectively. Due to the similar water quality conditions of grass and open water pans, a single index-MMI4- was created that incorporates both types and utilizes reference sites of both pan types. After the enumeration and identification of the wetland diatoms, metrics and subsequent index scores were calculated.

2.5 Metric calculation

The relative abundance of each species in every sample was calculated and the dominant species identified. For each index, the taxa characteristic of reference sites within that index were identified as well as taxa characteristic of disturbed sites. The indices are composed of various metrics from three metric categories and described in Table 2 (Riato *et al.*, 2017a).

Table 2: Description of metrics within metric three metric categories: 1) similarity to reference sites, 2) functional group and 3) taxonomic composition and the response of metric values to increased levels of AMD-disturbance (increase or decrease) as described by Riato *et al.* (2017a). Diatom functional groups have previously been discussed in chapter one.

Metric category	Description	Metric value response to elevated AMD-disturbance level
Similarity to reference sites		
% Reference taxa	Based on the relative abundances of taxa from reference sites, taxa most characteristic of reference sites are identified. Out of the total number of taxa in a sample, the percentage of taxa identified as reference taxa: $= \frac{n \text{ reference taxa}}{\text{total } n \text{ taxa}} \times 100$	Decrease
% Tolerant taxa	Based on the relative abundances of taxa from AMD-disturbed sites, taxa most characteristic of impacted sites are identified. Out of the total number of taxa in a sample, the percentage of taxa identified as tolerant taxa: $= \frac{n \text{ tolerant taxa}}{\text{total } n \text{ taxa}} \times 100$	Increase
% Similarity to reference sites	Bray-Curtis similarity in taxa composition to all reference sites	Decrease
% Reference taxa found in reference sites that occurred in impaired sites	Relative abundance of taxa found in reference sites that also occurred in impacted sites: $= \frac{n \text{ taxa found in reference sites as well as impacted site}}{\text{total } n \text{ taxa}} \times 100$	Decrease
% Reference individuals found in reference sites that	Relative abundance of individuals (valves) found in reference sites that also occurred in impacted sites:	Decrease

occurred in impaired sites	$\frac{n \text{ individuals found in reference sites as well as impacted sites}}{\text{total } n \text{ valves}} \times 100$	
Number of distinct reference taxa	Number of taxa found in reference sites, not in impaired sites.	Decrease
Functional group		
Mobile % taxa	Relative abundance of mobile (free moving) taxa: $= \frac{n \text{ mobile taxa}}{\text{total } n \text{ taxa}} \times 100$	Decrease
Adnate % taxa	Relative abundance of adnate taxa- attached by the valve face to substrates, growing parallel to the surface: $= \frac{n \text{ adnate taxa}}{\text{total } n \text{ taxa}} \times 100$	Decrease
Pad % taxa	Relative abundance of taxa attached to substrates by a mucilage pad-growing in upright orientation to substrates: $= \frac{n \text{ pad taxa}}{\text{total } n \text{ taxa}} \times 100$	Increase
Non-colonial % taxa	Relative abundance of taxa in a sample that occur individually, not in colonies: $= \frac{n \text{ non-colonial taxa}}{\text{total } n \text{ taxa}} \times 100$	Decrease
Ribbon % taxa	Relative abundance of taxa that occur in ribbon-like colonies: $= \frac{n \text{ ribbon taxa}}{\text{total } n \text{ taxa}} \times 100$	Increase
Ribbon % individuals	Relative abundance of individuals that occur in ribbon-like colonies: $= \frac{n \text{ ribbon individuals}}{\text{total } n \text{ valves}} \times 100$	Increase
High profile guild % taxa	Relative abundance of taxa in the high-profile guild- such as filamentous, branched, chainlike or tubular colonies: $= \frac{n \text{ high-profile taxa}}{\text{total } n \text{ taxa}} \times 100$	Increase
Taxonomic composition		
% Encyonopsis taxa	Relative abundance of taxa belonging to the genus <i>Encyonopsis</i> :	Increase

	$= \frac{n \text{ Encyonopsis taxa}}{\text{total } n \text{ taxa}} \times 100$	
% Cocconeis taxa	Relative abundance of taxa belonging to the genus <i>Cocconeis</i> : $= \frac{n \text{ Cocconeis taxa}}{\text{total } n \text{ taxa}} \times 100$	Increase
% Craticula taxa	Relative abundance of taxa belonging to the genus <i>Craticula</i> : $= \frac{n \text{ Craticula taxa}}{\text{total } n \text{ taxa}} \times 100$	Decrease
% Ctenophora taxa	Relative abundance of taxa belonging to the genus <i>Ctenophora</i> : $= \frac{n \text{ Ctenophora taxa}}{\text{total } n \text{ taxa}} \times 100$	Increase
% Gomphonema taxa	Relative abundance of taxa belonging to the genus <i>Gomphonema</i> : $= \frac{n \text{ Gomphonema taxa}}{\text{total } n \text{ taxa}} \times 100$	Variable
% Nitzschia individuals	Relative abundance of taxa belonging to the genus <i>Nitzschia</i> : $= \frac{n \text{ Nitzschia taxa}}{\text{total } n \text{ taxa}} \times 100$	Decrease

Each MMI consists of different combinations of metrics from the list above. The composition of each MMI is shown in Figure 2.

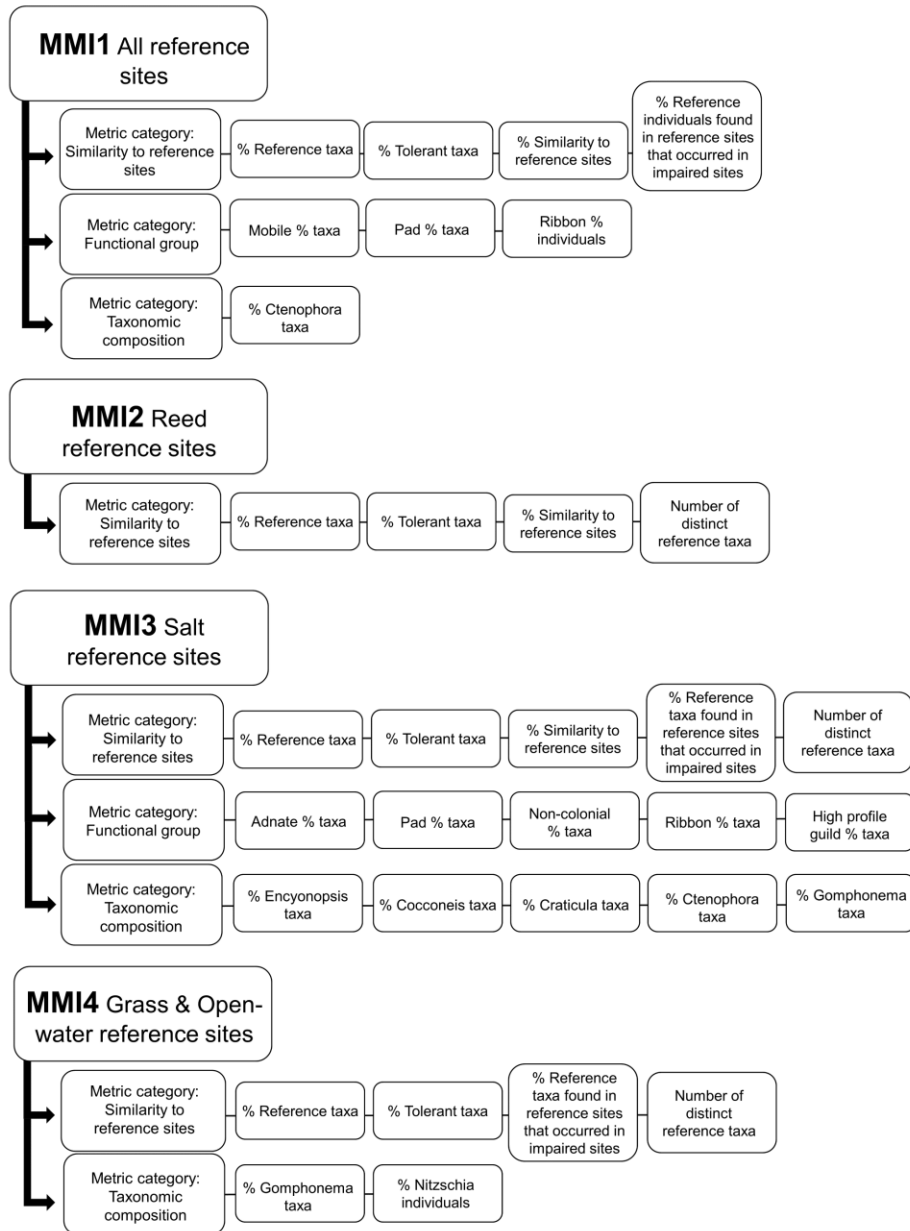


Figure 2: Metric categories and metrics calculated in each multimetric index

Once all the metrics in each MMI were calculated as described above, the metrics were rescaled to values between one and zero. Excluding the number of distinct reference taxa, all metrics are initially calculated as percentages. To rescale these scores, the 5th and 95th percentiles of all the sites' scores were calculated for each metric as did Riato *et al.* (2017a) following Blocksom (2003):

$$\text{Rescaled metric value} = \frac{\text{original metric value}}{95\text{th} - 5\text{th}}$$

Values less than zero were adjusted to zero and values greater than one adjusted to the maximum of one. The scale was inverted for metrics which increase with increases AMD-impact by subtracting the rescaled metric value from one. Higher values would thus correspond with less disturbance and better ecological condition.

2.6 Index score calculations

2.6.1 MMI score calculation

After the metric values were rescaled, the mean of the rescaled metric values within each metric category was calculated. The mean of the rescaled metric values represents the combined category value (composite metrics). The final MMI score on a scale of 0-100 equates to the mean of all the composite metrics multiplied by 100. Each metric category contributes equally to the MMI score regardless of the number of metrics it contains. MMI2 has only one metric category; therefore, the MMI score was determined as the mean of the rescaled metric values multiplied by 100. The 5th and 25th percentiles of the reference site MMI score distribution were subsequently calculated as points of reference by which to classify the conditions of the pans by their MMI scores. Scores above the 25th percentile of the reference site MMI score distribution represent a good ecological condition. MMI scores between the 25th and 5th percentiles indicate a fair condition and scores below the 5th percentiles indicate a poor condition.

2.6.2 Conventional diatom indices

For the purpose of comparing the multimetric index for acid mine drainage with diatom indices previously applied to South African wetlands, the diatom community counts were entered into OMNIDIA version 3.1 (Lecointe *et al.*, 1993) and the scores of its various diatom indices calculated. Though initially designed for rivers, the majority of indices include cosmopolitan taxa with well-established correspondences to environmental parameters and are capable of indicating water quality in wetlands. The correlations of index scores and ecological condition classes obtained from the MMIs and traditional diatom indices to water chemistry variables of each sample were determined and compared.

2.7 Data analysis

Diatom assemblage data were analysed using PRIMER version 6 software (Clarke and Gorley, 2006). Similarity Percentage (SIMPER) analysis was used on square root transformed data to determine the intra- and infra-group similarity among the samples created according

to three factors: disturbance level (AMD), pan type and season, while the variance caused by these factors were determined using permutational multivariate analysis of variance (PERMANOVA).

Kent (2012) defines correlation analysis as an assortment of methods used to determine the robustness of relationships between variables and many studies on the application of diatom indices employ CCA as a means of establishing the effects of various water quality variables on the structure of diatom communities (Taylor *et al.*, 2007b; Matlala *et al.*, 2011; Bere *et al.*, 2014; Dalu *et al.*, 2016; Musa and Greenfield, 2018). To ascertain the relationship between diatom assemblages and measured environmental variables Canonical Correspondence Analysis (CCA) was used. This was accomplished in CANOCO version 4.5 (Ter Braak and Smilauer, 1998). Results were depicted by means of biplots on which each point represents an individual species. Environmental variables are represented by arrows, the direction of which signifies the direction of a given variable's maximum change while the arrow's length is comparable to the magnitude of change. Thus, variables represented by longer arrows are responsible for variation within communities to a greater degree than variables represented by shorter arrows.

Correlation analyses provide a statistic valued between -1.0 and +1.0. Values of either -1.0 or +1.0 indicate perfect negative and positive relationships between variables, respectively. Weaker correlations between variables are represented by values closer to zero with zero indicating no relationship. Excluding pH, which is represented on a logarithmic scale, the chemical data was log₁₀ transformed and Pearson's correlation coefficient was calculated to establish whether significant correlations exist between calculated index scores and the measured water quality variables using STATISTICA version 13 (StatSoft, Inc., 2012).

The percentage of correctly classified instances (CCI) of every calculated index was determined as a measure of the indices' accuracy in distinguishing non-impacted from moderately and highly impacted sites. The percentages of sites that were rated the same- as being impacted or non-impacted- by the indices being compared were calculated and Cohen's Kappa coefficient (K) was used as a measure of the agreement between indices in rating ecological condition and establish the frequency with which different indices give similar ratings. Specifically, to measure the agreement between the multimetric indices and established indices as well as among the different MMIs. Cohen's Kappa statistic produces values between zero and one and representing increasing levels of agreement (Cohen, 1960). Negative values may also be obtained representing no-agreement/ disagreement.

CHAPTER 3: RESULTS AND DISCUSSION- PHYSICAL AND CHEMICAL VARIABLES

Physical and chemical variables such as those discussed below determine the quality of water and consequently the ecological condition of aquatic ecosystems. Nutrient surpluses or deficits and pollution can have devastating effects on aquatic biota. In view of diatoms having long been used to indicate organic pollution, salinity, eutrophication, acidification and water quality in general, the following variables were determined for each type of pan and compared between reference and impacted sites. Understanding the constitution of aquatic environments is key to interpreting diatom community composition and vice versa.

3.1 Temperature

All samples were taken at a depth ranging from 0.1 m to not more than 0.5 m. Figure 3 below presents the mean temperatures of reference and disturbed pans sampled during the winter and summer seasons. Temperatures did not differ significantly between pan types, but according to season. Except for the open water pan- which is highly disturbed, shallow and displayed higher temperatures during both seasons. All sites displayed normal rises in temperature due to seasonal change.

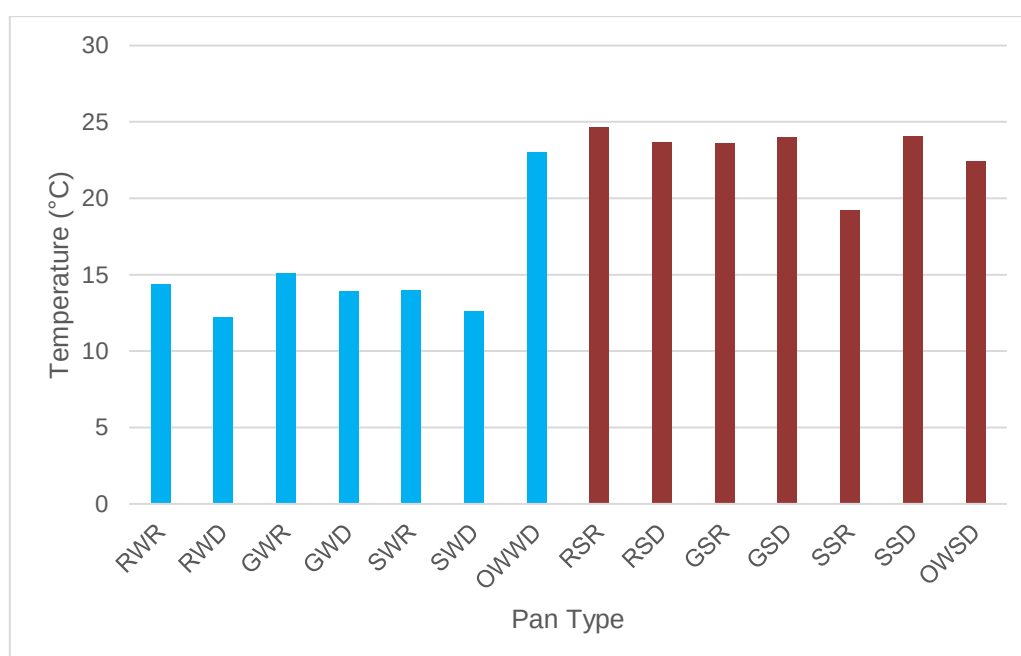


Figure 3: Mean temperatures of reference and disturbed reed, grass and salt pans during winter (blue) and summer seasons (red).

Increases in temperature have been known to contribute to increased metabolic rates in aquatic organisms and reduce the solubility and availability of dissolved oxygen in aquatic

habitats. Consequently, blooms of cyanobacteria may occur, replacing native diatoms and green algae may occur. Floating green mats of cyanobacteria were present at the open water pan during dry season sampling (represented as OWWD in the figure above).

3.2 pH

The acidity of water is determined by the concentration of hydrogen ions which is represented on a logarithmic scale as: $\text{pH} = \log_{10} [\text{H}^+]$. The higher the concentration of hydrogen ions, the lower the pH and the more acidic the solution. Pure water without any solutes has a pH of seven and is considered neutral. Values below seven signify acidity and values between seven and fourteen represent increasing levels of alkalinity (Dallas and Day, 2004).

Among reed pans, pH is seen to drop slightly during the summer. Minimum, maximum and mean pH values remain very similar between reference and disturbed sites in both seasons. Grass and salt pans also displayed a decrease in pH in the summer. Disturbed grass pans exhibited the overall highest pH values (10.6). Disturbed salt pans showed the lowest pH values (7.1). At values of between 8.0 and 8.4, the open water pan's pH values remained consistent throughout both seasons. During both seasons disturbed salt pans had lower minimum pH values and higher maximum values than reference pans. Reference salt pans showed less difference in minimum and maximum pH values than disturbed salt pans. The pH of the different pan types reflects more alkaline conditions overall. Grass pans are usually more acidic compared to other pan types and in the present study some grass pans were near neutral in pH, though they usually tend towards pH levels of around six.

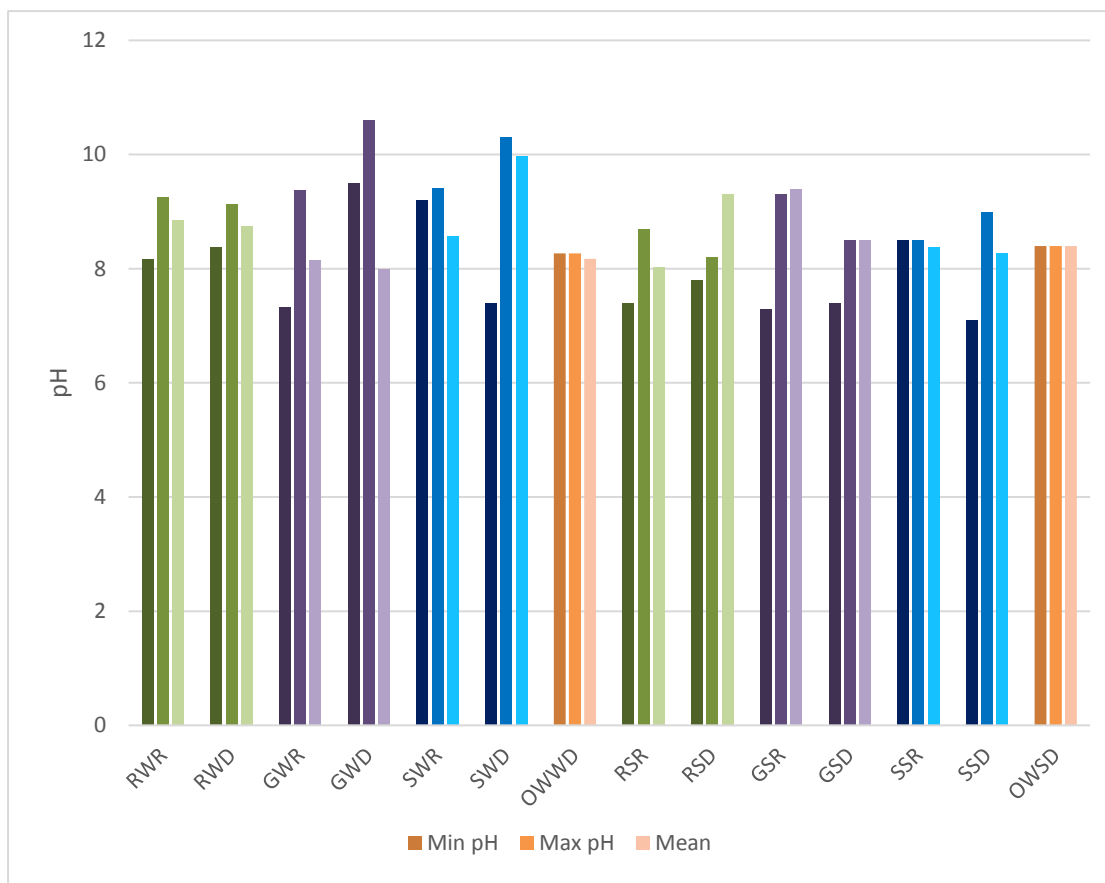


Figure 4: Minimum, maximum and mean pH values recorded among different pan types during the winter and summer seasons. The darkest bars signify minimum values, the lighter bars maximum values and mean values are represented by the lightest bars.

Riato *et al.* (2014) ascribe the higher pH levels of depressional wetlands in the Mpumalanga Highveld to the dolomitic nature of the region's groundwater and mentions that low pH values might be indicative of anthropogenic impacts such as AMD. However, no low pH values were recorded which might indicate severe acidification. The alkaline and slightly saline nature of the pans serves to increase their natural buffering capacity. Though direct measurements of pH may be misleading, the impacts of coal mining activities may be reflected by other variables which will be discussed in the following sections.

3.3 Alkalinity

Alkalinity has been described as analogous to “acid neutralizing capacity” and is mostly representative of the carbonate and bicarbonate concentrations in water (Dallas and Day, 2004). Other ions may contribute to alkalinity but usually very minimally. Whereas pH refers to the concentration of hydrogen ions, alkalinity is constituted all together by the anions of

weaker acids, hydroxyl ions and bicarbonate. The alkalinity of each site was determined as mg/L of calcium carbonate and Figures 5 and 6 below, the mean alkalinity of all pan types showed a decrease from winter to summer. Disturbed reed pans displayed the lowest mean alkalinity values during both seasons (132 mg/L and 65 mg/L respectively). As expected, the highest alkalinity values were recorded in salt pans. However, this was not the case during the summer where reference salt pans were exceeded in alkalinity by reference reed pans, disturbed grass pans and the open water pan. This may be due to dilution as the Lake Chrissie area had received substantial amounts of rain in December 2019 and January 2020 and only two of the original three reference sites could be sampled.

In Figure 5 below, the highest alkalinity value (5 650mg/L) was recorded in site S21, a site classified as moderately disturbed, which was only sampled in the dry season. All the salt pans had much lower alkalinity values during the summer, the highest recorded value being that of site S20 (539 mg/L). Regardless of the absence of S21 in the summer, all salt pans showed a drop in alkalinity similar to that witnessed in other pan types.

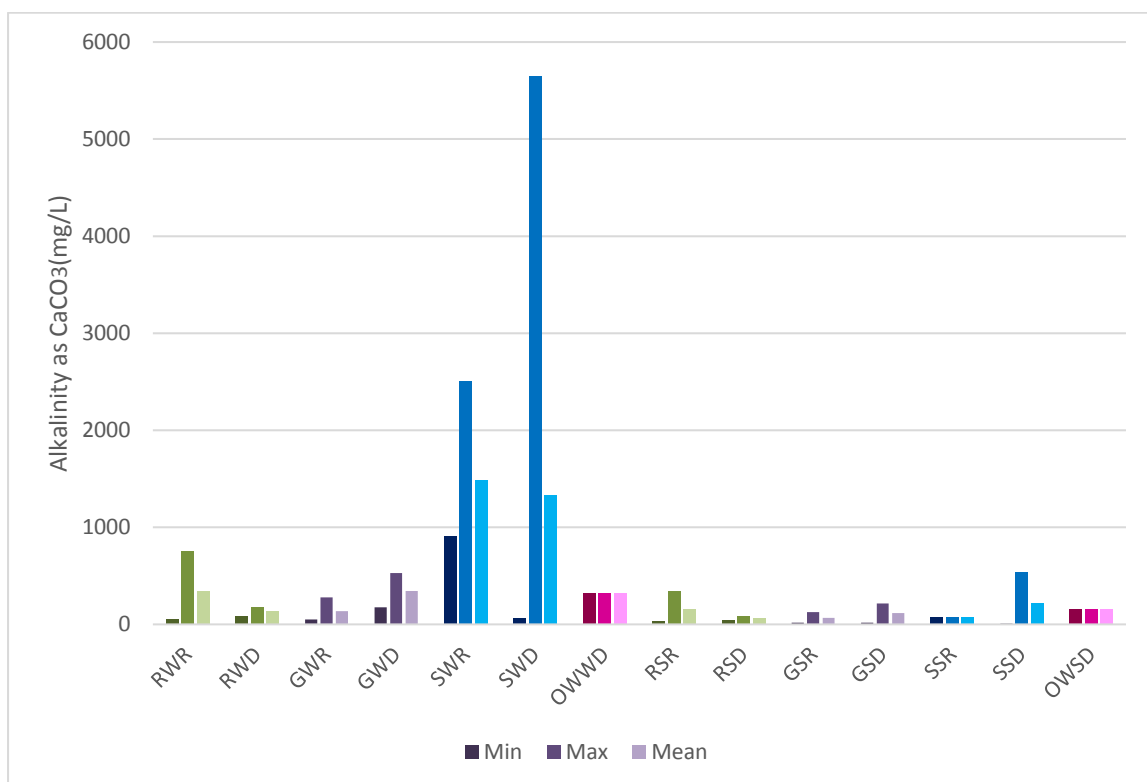


Figure 5: Minimum, maximum and mean alkalinity values for reference and disturbed reed, grass, salt and open water pans.

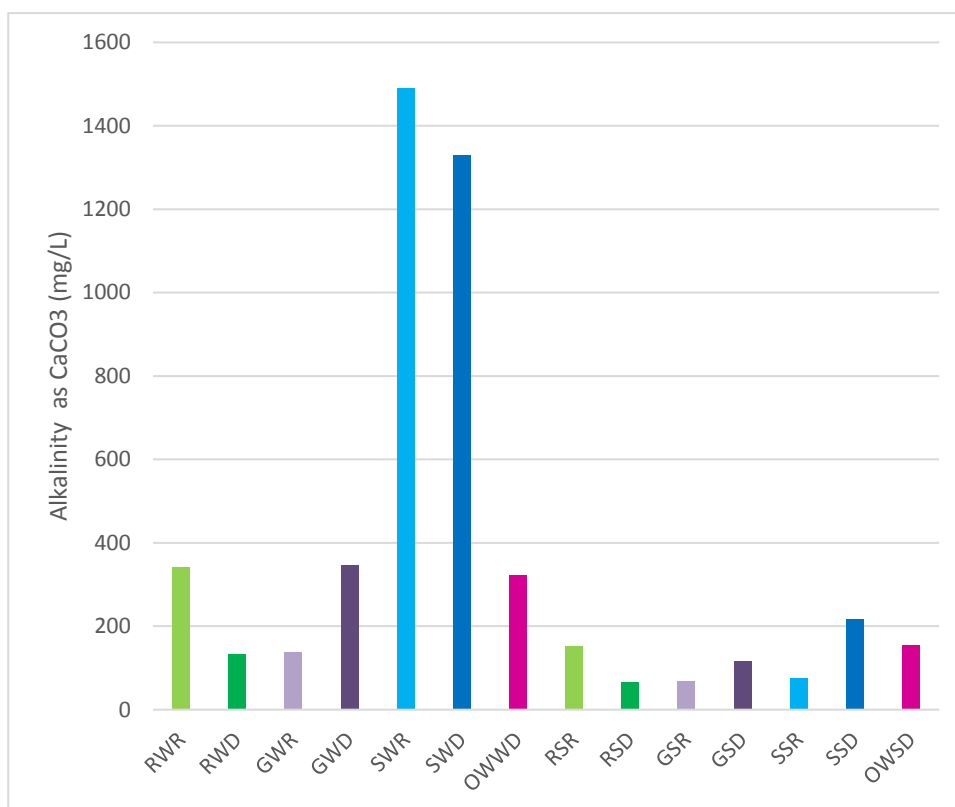


Figure 6: Mean alkalinity in mg/L CaCO₃ of reed, grass, salt and open water depressions, first in winter then in summer.

3.4 Hardness

Similar to alkalinity, hardness and magnesium hardness was measured as milligrams of CaCO₃ per litre (mg/L). Hardness is results from dissolved polyvalent metallic ions, most notably calcium and magnesium (World Health Organisation, 2011). As mentioned, acidification such as that caused by AMD may increase the availability and toxicity of various heavy metals. Hardness also lends a certain buffer capacity to water in that hardness decreases metal toxicity (Borgmann *et al.*, 2005 and Yim *et al.*, 2006). This may be by means of forming insoluble carbonates or the adsorption of metal ions to precipitated calcium carbonate. In Figure 7 below, the mean hardness of all pan types is seen to increase from reference sites to disturbed sites during both seasons, except for the open water pan which decreases slightly in summer. Magnesium hardness (Figure 8) follows a similar trend with the exception of reed pans, where values slightly decrease in the dry season.

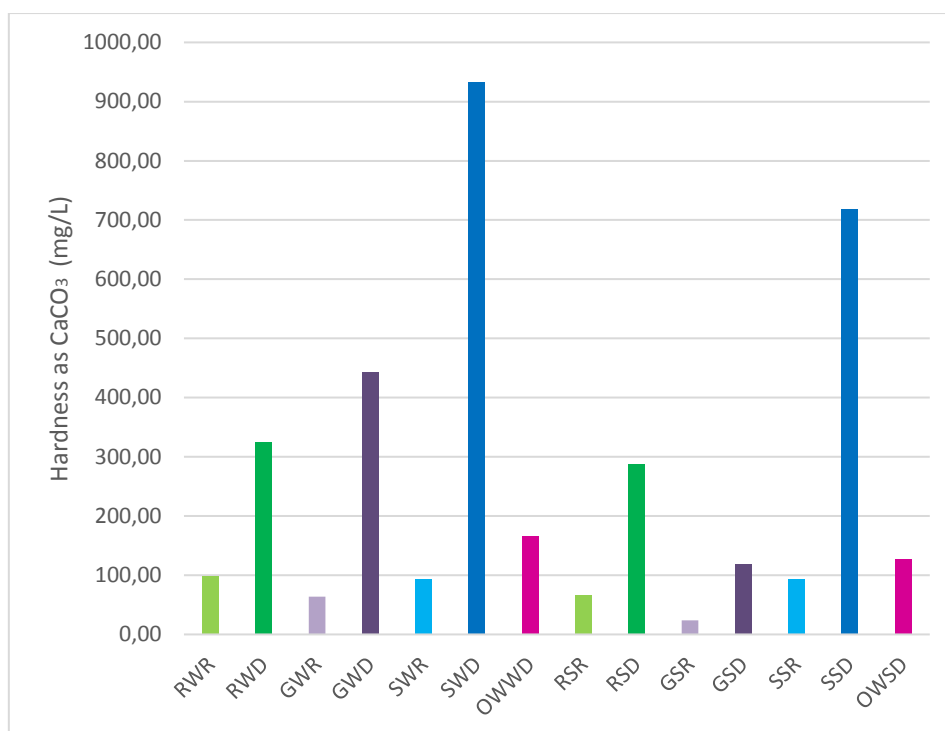


Figure 7: Mean hardness measured as CaCO₃ of reed, grass, salt and open water depressions, first in winter then in summer.

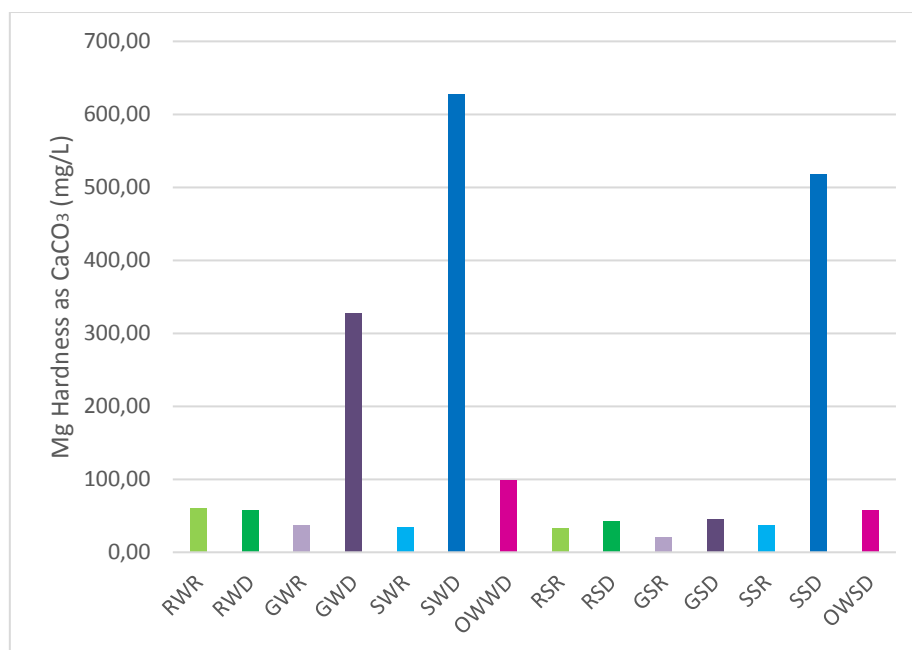


Figure 8: Mean magnesium hardness measured as CaCO₃ of reed, grass, salt and open water depressions, first in winter then in summer.

Despite grass pans demonstrating higher pH levels overall, salt pans display greater alkalinity as well as hardness. During the dry season, disturbed grass pans also displayed elevated

magnesium and total hardness. In the cases of reed, grass and open water pans, mean pH levels increase slightly in the summer while mean alkalinity, total hardness and magnesium hardness decrease for all pan types in the summer. Though alkalinity and hardness decreased in the summer, the pH of reed and grass pans increased, which can be partially attributed to an increase in photosynthesis during the wet season (Dallas and Day, 2004). Salt pans have little to no vegetation, due to elevated levels of dissolved salts and alkalinity.

In summary, the higher pH values of these wetlands, their characteristic alkalinity (concentrations of OH^- , HCO_3^- and CO_3^{2-}) and hardness serve as natural buffering systems. Conversely, alkaline conditions have been found to cause ammonium (NH_4^+) toxicity (Boyd *et al.*, 2016). For most plants, ammonium is the most important source of nitrogen and is not toxic but at a pH 8 and above, ammonium converts to unionized ammonia (NH_3) which is highly toxic (Dallas and Day, 2004, 49).

3.5 Conductivity and Total Dissolved Solids (TDS)

The concentration of dissolved materials in water has definite effects on water quality. Usually measured and described as total dissolved solids (TDS), salinity or electrical conductivity (EC), the total amount of dissolved solids includes all soluble solid substances. TDS is mostly comprised of cations such as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) and anions of bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), sulphate (SO_4^{2-}) and chloride (Cl^-). The electrical conductivity of water is used as a measure of the total quantity of dissolved solids as these increase the electrical conductivity of a solution.

Conductivity and TDS was measured in mS/cm and mg/L, respectively. In Figures 9 and 10 below all pan types showed a decrease in conductivity and TDS in the summer. The highest EC values in both seasons were obtained in the salt pans. The reference salt pan, S7 had the highest EC value (18,5 mS/cm). As reported by De Klerk *et al.* (2016), reed pans demonstrated EC values ranging from 0.2 mS/cm to 2 mS/cm. Grass pans tend to have the lowest EC values and nutrient concentrations of all pan types. This is the case among reference sites during both seasons. However, in the winter disturbed grass pans had higher EC values than the reed and open water pans.

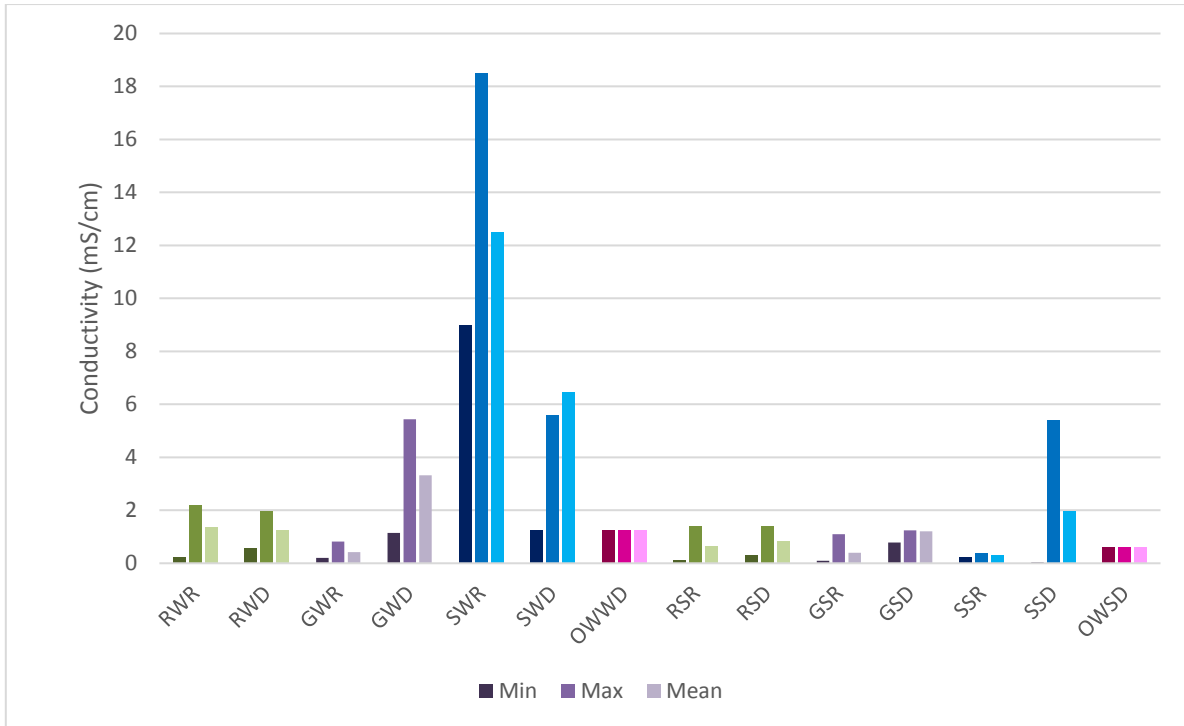


Figure 9: Minimum, maximum and mean conductivity values for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

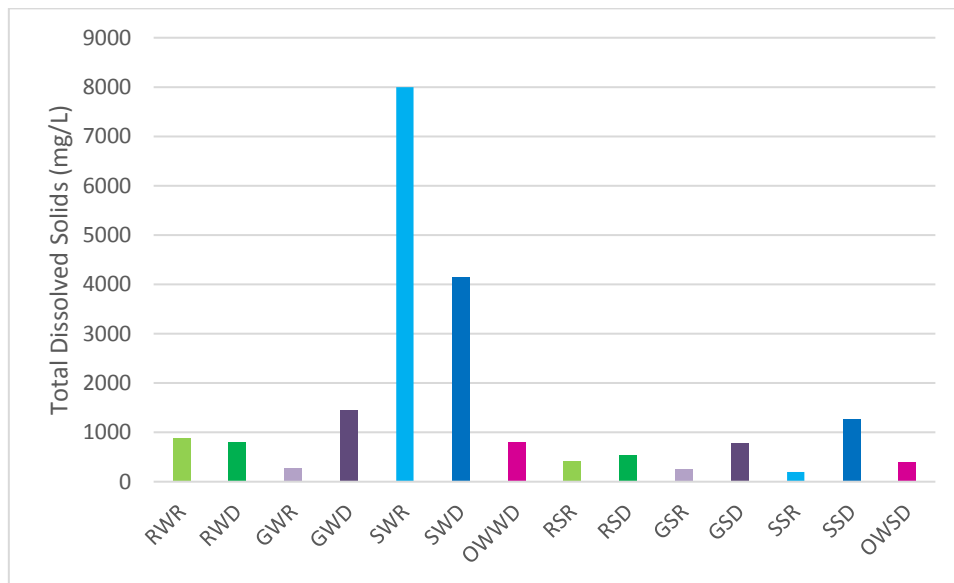


Figure 10: Mean TDS values for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

3.6 Cations and anions

In addition to EC and TDS, the balance of cations and anions was measured (in meq/L) to determine the reliability of the major ion analyses. Milliequivalents of major cations and anions proved to be nearly equal which indicates accurate analyses. The EC and TDS values in

Figures 9 and 10 are corroborated by total ion concentrations in different pan types and presented in Figures 11 and 12.

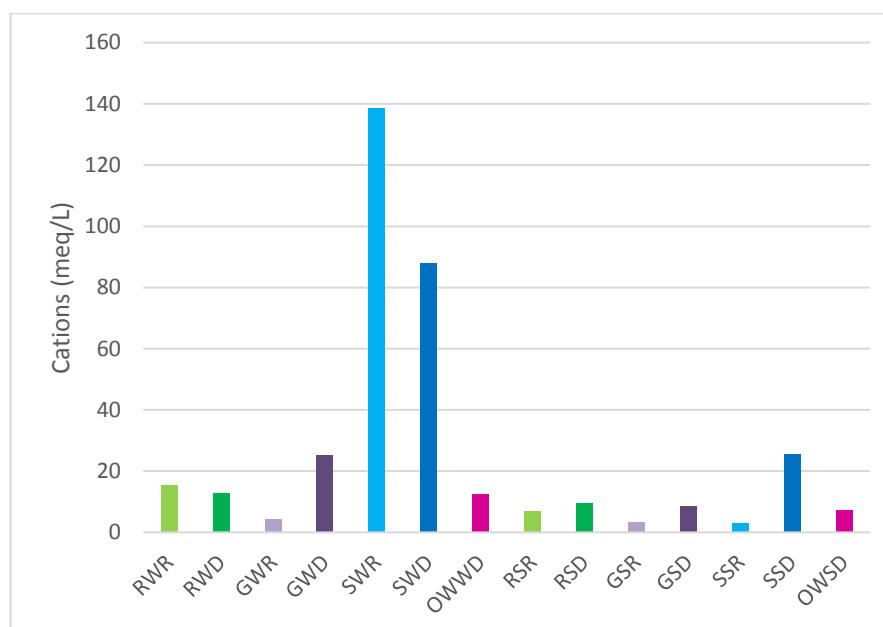


Figure 11: Mean measures of cations (milliequivalents per litre) for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

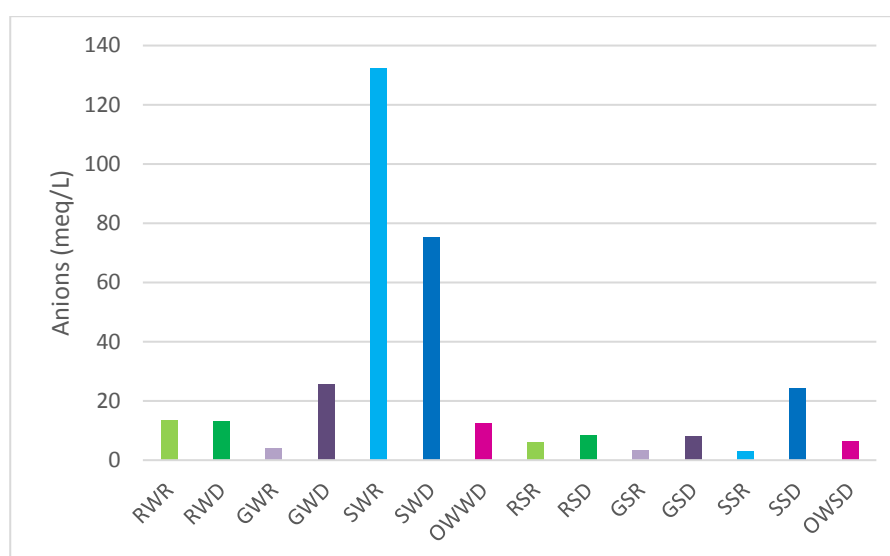


Figure 12: Mean measures of anions (milliequivalents per litre) for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

Calcium, sodium, potassium and magnesium, which generally constitute the bulk of dissolved cations in water, were also measured in each pan, as were concentrations of chloride and sulphate which will be discussed subsequently.

3.7 Calcium (Ca²⁺)

Calcium is often the major cation in inland waterbodies and the chief component responsible for the hardness of water. Calcium is essential to living organisms. It plays a vital role in various biochemical interactions and production of mollusc shells, crustacean exoskeletons as well as bones and teeth (Dallas and Day, 2004). The calcium concentration for each pan was determined as mg/L. Disturbed sites of all pan types displayed greater calcium concentrations compared to reference sites (Figure 13). Mean concentrations decreased in the summer but remained higher in disturbed pans. Disturbed grass pans displayed the highest calcium concentrations among highly disturbed sites (1 056 mg/L at S24) whereas reference grass pans revealed the lowest calcium concentrations.

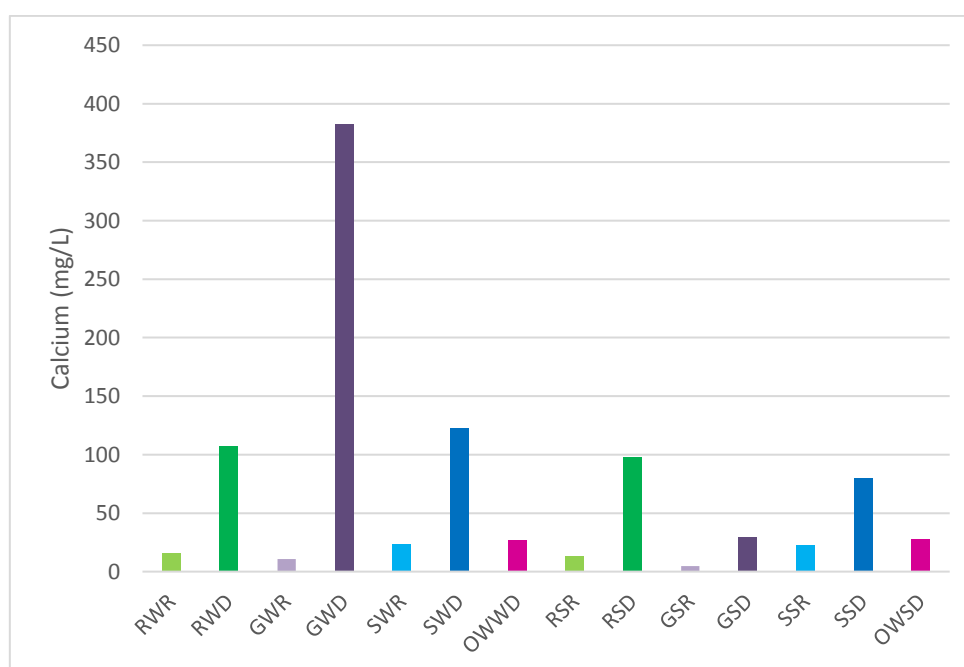


Figure 13: Mean calcium concentrations for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

Riato *et al.* (2017) used calcium as one of two principal coal mining-related stressors in pans by reason of lime, composed of calcium hydroxide, being used to remedy AMD and also reported substantially higher concentrations in AMD-disturbed sites compared to reference sites. The present study witnessed the same tendency among disturbed sites. Disturbed grass sites had a mean calcium concentration of nearly 30 times that of reference sites. The open water pan had much lower concentrations of calcium compared to other disturbed sites.

3.8 Sodium (Na^+)

Sodium is another major contributor to the TDS in fresh, saline and oceanic water. Much like calcium, it plays an important role in various biochemical interactions and is essential to living organisms. Sodium and calcium sulphates as well as sodium carbonate salts are especially common in salt pans (Seaman *et al.*, 1991). Figure 14 shows the mean sodium concentrations for each of the different pan types. Reference salt pans had the highest mean sodium concentration (3 098 mg/L) of all pan types. The highest recorded value is 7 548 mg/L in S21, a disturbed salt site. Salt pans maintained higher sodium concentrations in the summer as well, though levels in all pans had dropped substantially as a result of seasonal dilution.

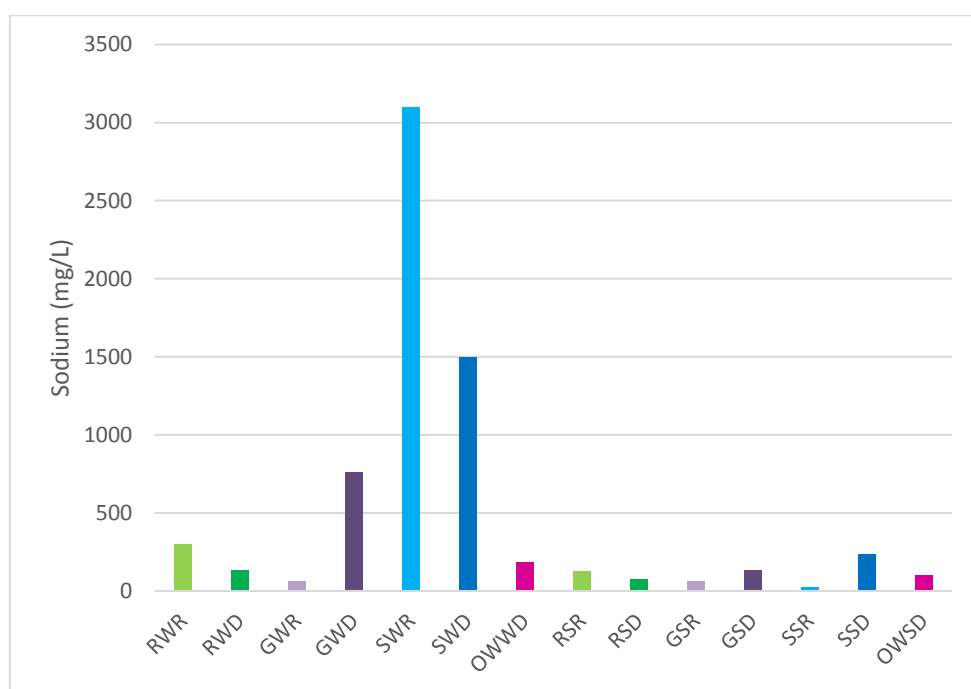


Figure 14: Mean sodium concentrations for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

3.9 Potassium (K^+)

Though occurring in natural waterbodies in much lower concentrations than sodium and calcium, potassium is likewise imperative to living organisms and is often a limiting factor with regards to plant growth (Hoffer, 1938; De Turk, 1941 and Dallas and Day, 2004). Below, in Figure 15, is displayed the mean concentrations of potassium. During the dry season, potassium concentrations were highest in salt pans and among salt pans, higher in disturbed sites. The potassium concentration in the open water pan was higher than that of disturbed

salt pans during the wet season. All pan types exhibited higher concentrations of potassium in disturbed sites compared to reference sites.

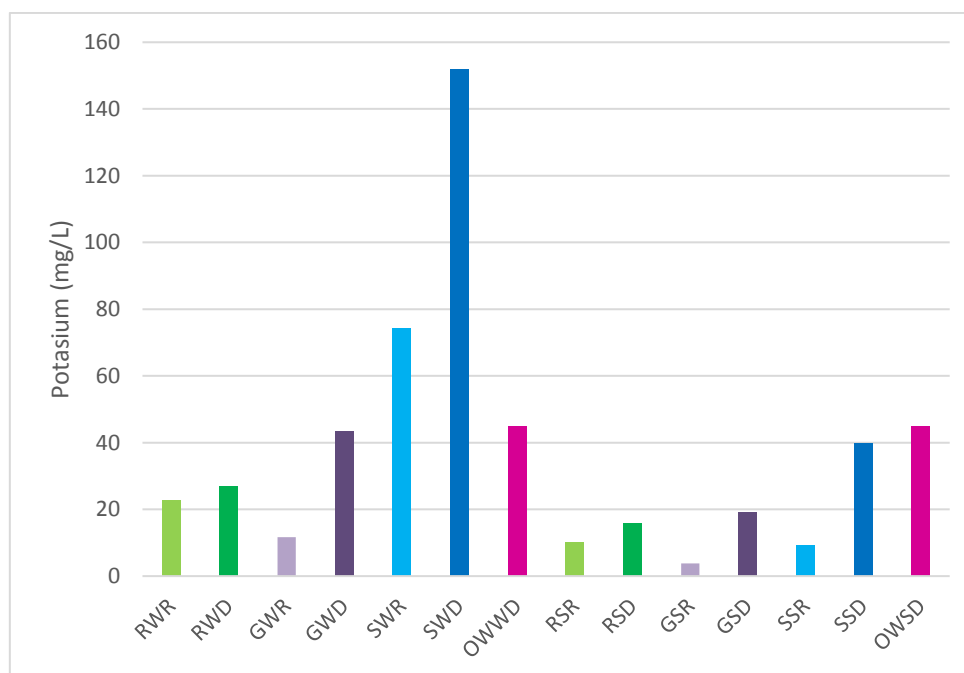


Figure 15: Mean potassium concentrations for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

3.10 Magnesium (Mg^{2+})

Next to calcium, magnesium is another principal element in the hardness of water. Magnesium is biologically important as a component of chlorophyll as well as of various enzymes (Dallas and Day, 2004). In Figure 16, distinctly elevated magnesium concentrations are apparent in disturbed grass and salt pans during the winter and again in disturbed salt pans during the summer. The highest concentration of magnesium among grass pans was recorded in S24 (298 mg/L), a highly disturbed site, during the dry season. The highest concentration of magnesium in a salt pan was recorded in S23 (751 mg/L), also a disturbed site and during the dry season. Reed pans exhibit low concentrations in both seasons, the lowest being measured in the summer. Reference grass pans exhibit the lowest magnesium concentrations overall, possibly as a result of absorption by vegetation.

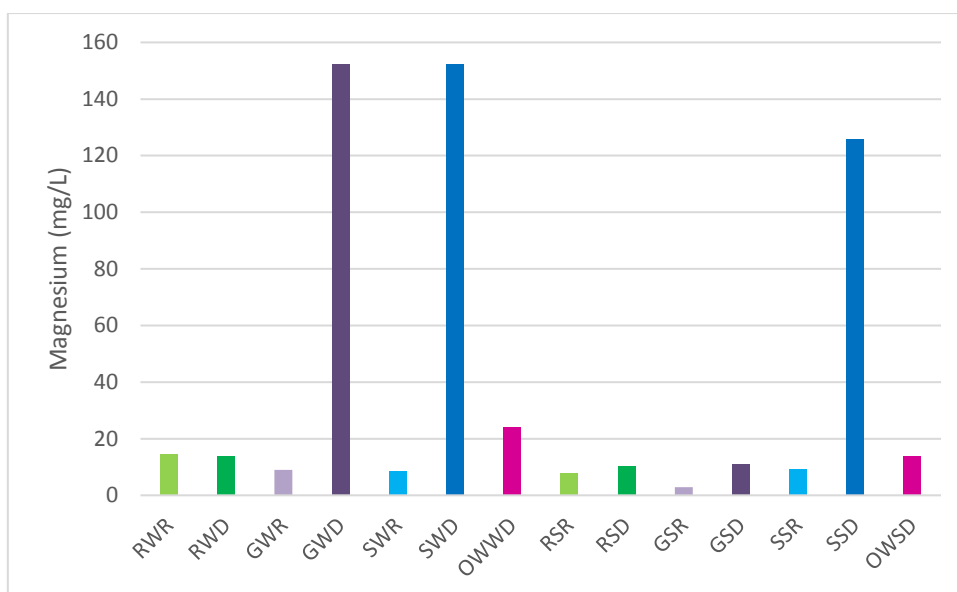


Figure 16: Mean magnesium concentrations for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

3.11 Chloride (Cl)

Chloride is one of the principal anions constituting the TDS of natural aquatic ecosystems and another primary ion necessary in biochemical interactions and the proper functioning of regulation systems in living organisms. According to Seaman *et al.* (1991), among other ions, salt pans have characteristically high chloride concentrations in comparison to other types of depressional wetlands. Figure 17 shows the mean chloride concentrations obtained for the different pan types. Reference salt pans had the highest mean chloride concentration of all the pan types, followed by disturbed grass and disturbed salt pans. The reference salt pan, S7 had the highest chloride concentration, 4080 mg/L during the dry season. Similar to sodium and magnesium, chloride concentrations decrease significantly in the wet season. Mean chloride concentrations of disturbed salt pans decreased from 629.63 mg/L to 99.61mg/L in the summer. Comparatively, the mean concentration of reference salt pans decreased from 2 986.67 mg/L in the dry season to 22.5 mg/L in the wet season. During both seasons, reference reed pans displayed higher chloride concentrations than disturbed reed pans as was the case for sodium concentrations in reed pans.

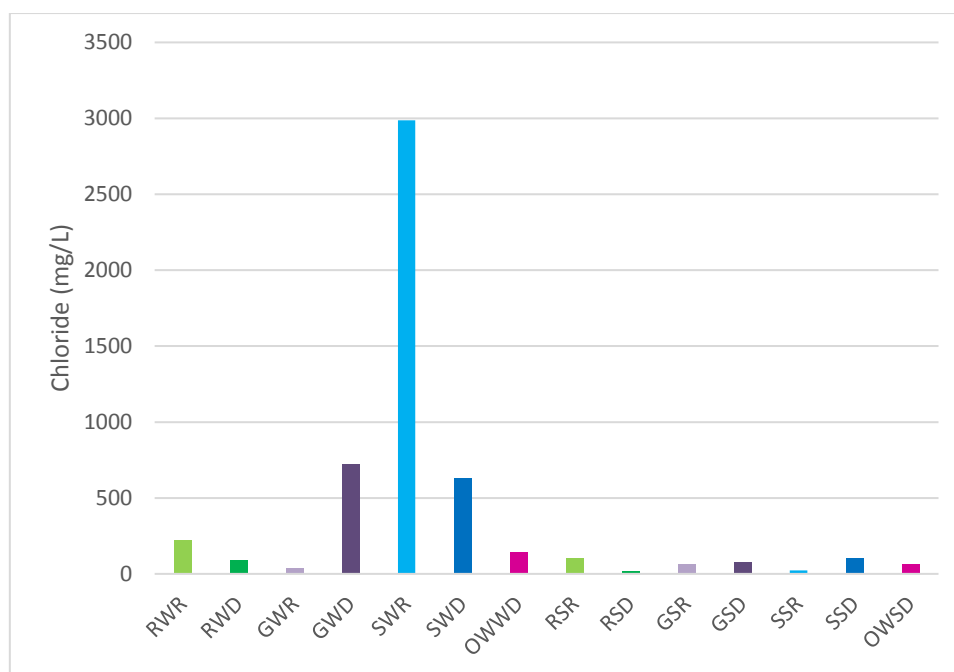


Figure 17: Mean chloride concentrations for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

3.12 Sulphate (SO_4^{2-})

Sulphate is another principal anion most commonly contributing to the TDS of natural waterbodies but generally does not occur in the same high concentrations as chloride and bicarbonate. Sulphate is biologically important as a component of proteins (De Turk, 1941). The problem of acidification occurs when sulphates are excessively present and sulphuric acid is produced. The oxidation of sulphur bearing minerals due to mining cause AMD seepage which can cause severe damage to aquatic ecosystems. In addition to high conductivity, high sulphate concentrations serve as significant evidence of AMD contamination.

The mean sulphate values for each type of reference and disturbed pan are presented in Figure 18. Across all pan types, sulphate concentrations were higher in disturbed pans during both seasons. Overall, concentrations are lower in the wet season, yet still substantially higher in disturbed sites.

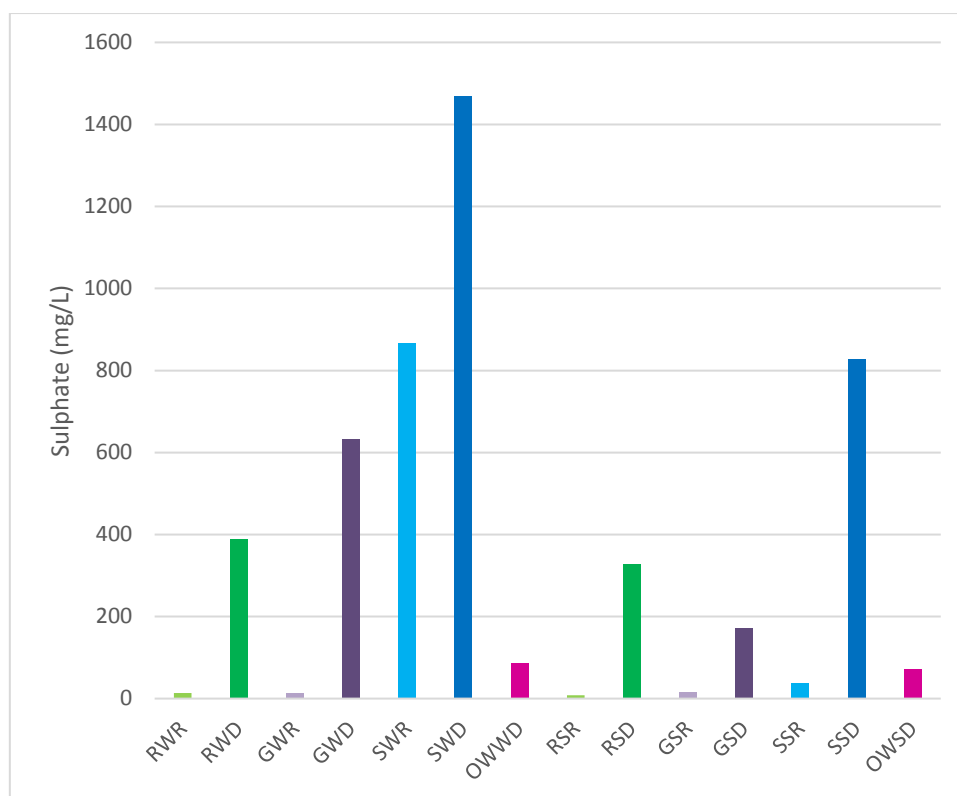


Figure 18: Mean sulphate concentrations for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

Salt pans naturally contain higher levels of sulphates than other pan types and sulphate concentrations in reference salt pans can exceed those in other pan types that are highly disturbed by AMD. For this reason, Riato *et al.* (2017) did not consider high sulphate concentrations as reliable evidence of AMD-disturbance in depressional wetlands. Though sulphate concentrations in reference salt sites are higher than in non-salt disturbed sites, salt pans exhibit the same trend as other pan types in that disturbed sites far exceed reference sites in sulphate concentrations.

3.13 Ratio of Sulphate to Chloride (% SO_4^{2-} : Cl^-)

Riato *et al.* (2017) identified the ratio of sulphate to chloride as being consistently lower among reference sites in comparison to disturbed sites. The SO_4^{2-} to Cl^- ratio was calculated as a percentage and is shown in Figure 19. Disturbed sites demonstrate notably higher ratios among reed, grass and salt pans, across both seasons. Despite being classified as disturbed, the open water pan exhibited much lower sulphate to chloride ratios. This pan also displayed considerably lower chloride and sulphate concentrations and lower conductivity than other disturbed sites.

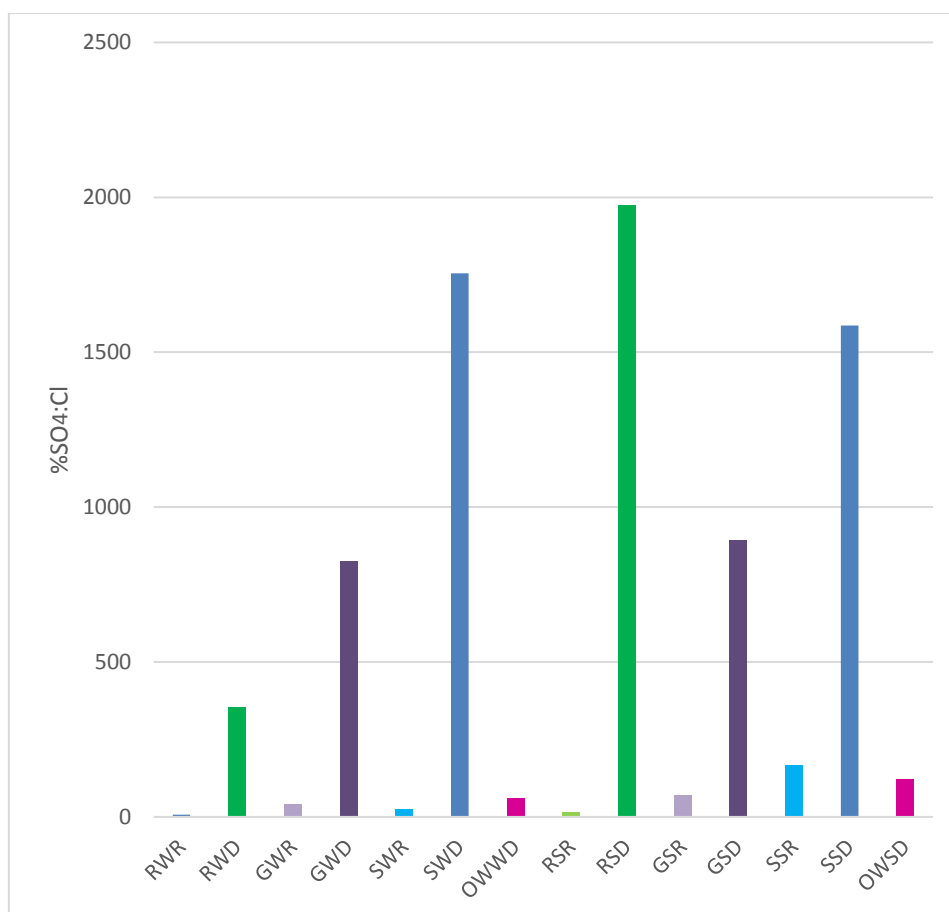


Figure 19: Mean sulphate to chloride ratios for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

Instead of exclusively employing sulphate as an indication of AMD, the ratio of sulphate to chloride is a valuable variable for indicating AMD-disturbance in unique aquatic ecosystems such as salt pans, which have natural high levels of sulphate that could be misleading.

3.14 Ammonia (NH₃)

To non-nitrogen fixating aquatic vegetation, ammonium and nitrate are the most important sources of essential dissolved inorganic nitrogen. At pH levels of eight or higher, hydrogen concentrations are low and ammonium converts to ammonia which is toxic (Wurts, 2003). Figure 20 presents the mean concentrations of ammonia measured in reference and disturbed pans. Disturbed salt pans had the highest concentrations of ammonia in the winter. A concentration of 38 mg/L was recorded in site S21 during the dry season. In the summer, ammonia concentrations increased in reference salt pans but decreased in disturbed salt pans. During both seasons, reference reed pans had higher ammonia concentrations than disturbed reed pans and levels in reference and disturbed pans were elevated in the summer.

Reference grass pans had similar ammonia concentrations during both seasons. During the summer however, ammonia concentrations were higher in disturbed grass pans. Regarding the open water pan, ammonia concentrations increased during the wet season.

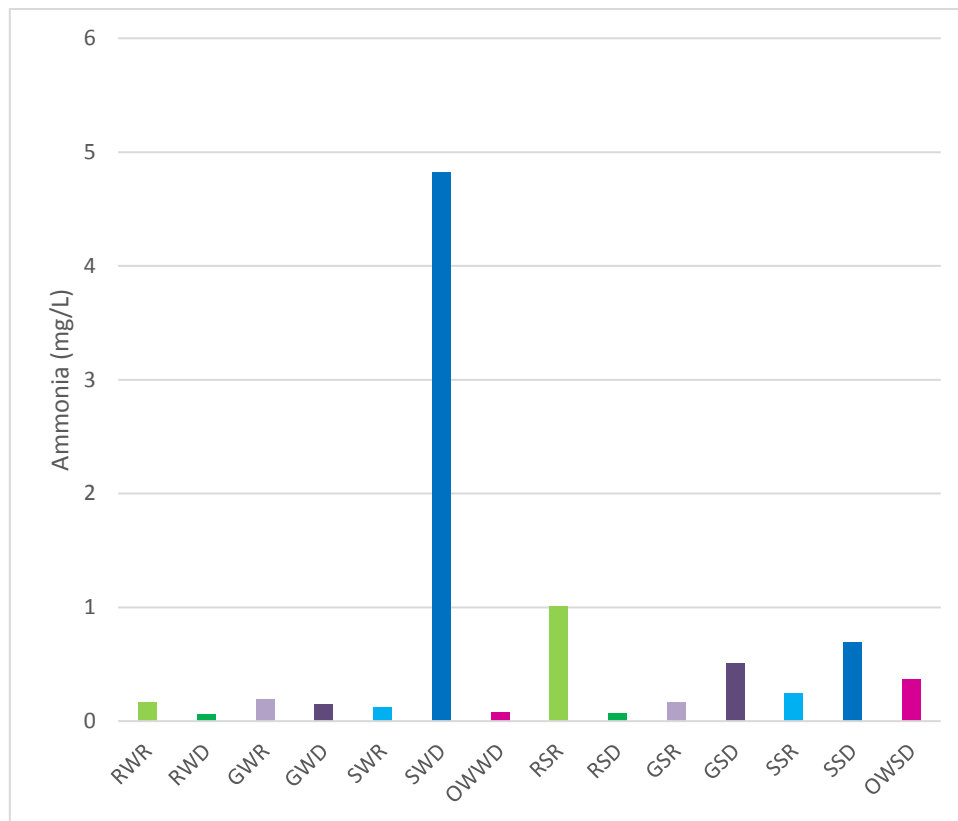


Figure 20: Mean concentrations of ammonia for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

3.15 Nitrate (NO_3^-)

Any element necessary to maintain the normal growth and reproduction of plants is considered to be a nutrient. Major nutrients like carbon, nitrogen, phosphorus, potassium, calcium, magnesium and sulphate are needed in large quantities. However, nutrient enrichment, particularly nitrogen (N) and phosphorus (P) enrichment, reduce water quality by causing eutrophication. Nitrate is a major nutrient needed to sustain life and is the principal form of inorganic nitrogen in oxygen rich water. In excess, nitrates cause eutrophication and consequential algal blooms, impaired water quality and reduced ecological condition (Parmar *et al.*, 2016).

The mean nitrate concentrations for different pan types are displayed in Figure 21. Mean nitrate concentrations for all pan groups except disturbed grass pans were found to be under 2 mg/L and not indicative of pollution (WRC, 1998). During the dry season, the nitrate

concentration of site S24 the most disturbed grass pan was measure as 32 mg/L. Nitrate concentrations were higher in reference reed pans than disturbed reed pans as well as in reference salt pans compared to disturbed salt pans. Across all pans, nitrate concentrations were lower in the summer, in most case lower than 0.05 mg/L.

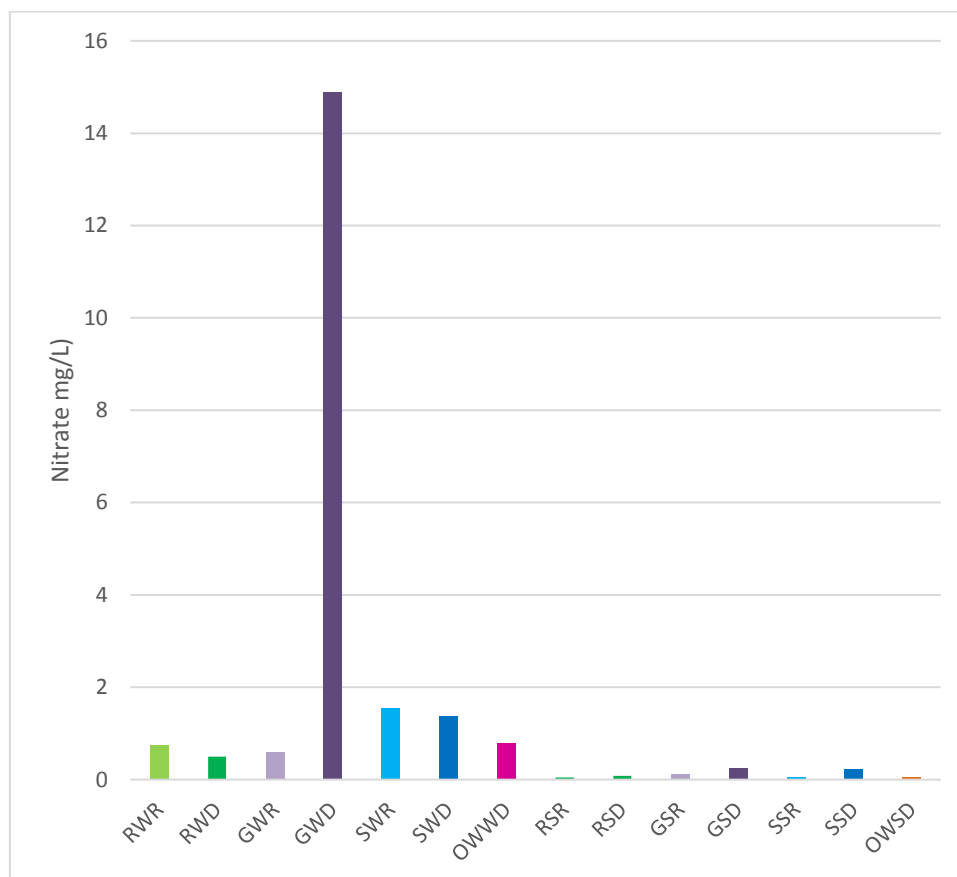


Figure 21: Mean concentrations of ammonia for reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

3.16 Orthophosphate (PO_4^{3-}) and Total Phosphorus (TP)

In addition to nitrogen, phosphorus is another major nutrient which can cause eutrophication when present in excessive quantities but is usually considered to be the limiting nutrient for primary producers in aquatic ecosystems Dallas and Day (2004). From Figures 22 and 23, mean phosphate and total phosphorus levels are observed to be low among reed and grass reference sites and disturbed reed pans as well. Reference salt pans had the highest phosphate and TP concentrations while the open water pan ranks second. Site S18, a disturbed salt pan had the greatest phosphate and TP concentrations of all pans (5.45 mg/L P and 15.04 mg/L TP). Salt pans do typically have significantly higher phosphate concentrations than other types of pans (De Klerk *et al.* 2016).

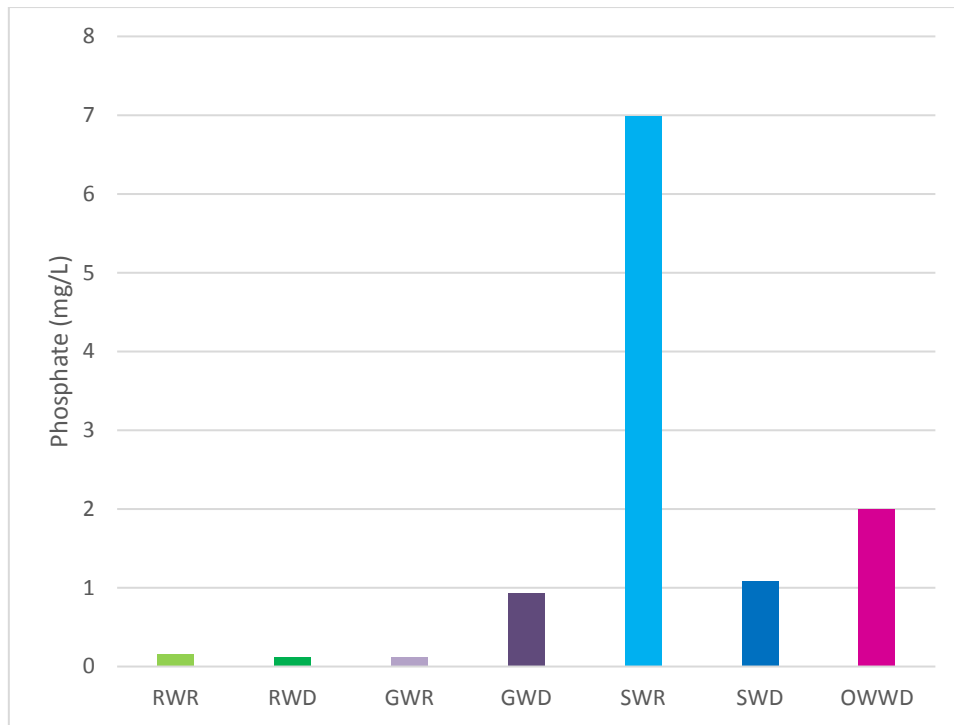


Figure 22: Mean orthophosphate concentrations of reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

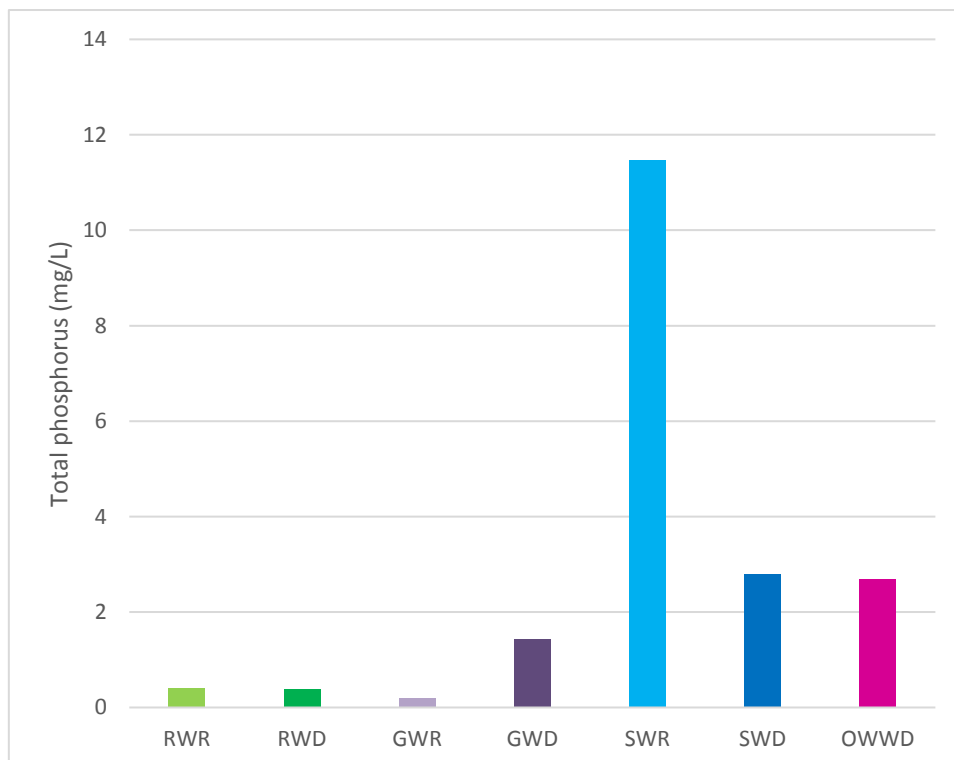


Figure 23: Mean total phosphorous concentrations of reference and disturbed reed, grass, salt and open water pans, first in winter then in summer.

3.17 Summary

Notable differences were found in the measured water quality variables of reference and disturbed conditions. During the dry season reference reed pans exhibited higher EC and alkalinity values. Hardness levels were higher in disturbed sites, as was calcium, sodium potassium and sulphate. EC, magnesium concentrations and nitrate concentrations were higher in disturbed sites in the wet season. Reference reed pans had higher concentration of nitrogen and phosphorous than disturbed sites.

Increased alkalinity, hardness and conductivity in disturbed grass pans reflected the higher concentrations of calcium, sodium, potassium, magnesium, chloride and sulphate found in disturbed sites. Reference sites had lower concentrations of nitrate, phosphate and total phosphorous as well. Ammonia was higher in reference sites in the winter but higher in disturbed pans during the summer. Reference grass pans displayed reduced EC values and nutrient concentrations in comparison with other pan types.

In contrast to the trends witnessed in grass pans, proportions of phosphate and total phosphorous were greater in reference salt depressions. Ammonia was present in larger quantities in disturbed sites, as was nitrate, though nitrate levels were higher in reference pans in the winter. Alkalinity and conductivity were higher in reference sites during the winter but higher in disturbed sites during the summer. Hardness, calcium, magnesium, sodium and potassium were present in larger amounts in disturbed pans. Chloride concentrations were higher in reference sites during the dry season but lower in reference sites and higher in disturbed sites in the summer. Impacted depressions contained greater sulphate concentrations and sulphate to chloride ratios.

In addition to the differences in physical appearance and vegetation, various pan types also display characteristic water quality features. Regardless of the type of pan, AMD disturbance is evident in impacted pans, which demonstrated elevated conductivity and concentrations of calcium and sulphate and higher ratios of sulphate to chloride.

CHAPTER 4: RESULTS AND DISCUSSION- DIATOM COMMUNITY COMPOSITION AND RELATION TO ENVIRONMENTAL VARIABLES

4.1 Diatom Community Composition

In total, 169 species of diatoms from 48 genera were identified from 28 depressional wetlands sampled from May 2019 to February 2020. As was the case in the study of Riato *et al.* in 2014, the genus *Nitzschia* was represented by the most taxa, followed by *Navicula*, and *Gomphonema*. The structures of diatom communities of reference and disturbed depressions differed significantly. Diatom assemblages varied amid distinct pan types as well.

Taxa found abundantly in reference reed sites that did not occur or very were scarce in disturbed sites include *Amphora veneta* Kützing, *Craticula buderi* (Hustedt) Lange-Bertalot, *Gomphonema angustatum* (Kützing) Rabenhorst, *Gomphonema gracile* Ehrenberg, *Gomphonema lagenula* Kützing, *Gomphonema parvulus* Lange-Bertalot & Reichardt, *Nitzschia amphibia* Grunow, *Nitzschia frustulum* (Kützing) Grunow, *Nitzschia liebertruthii* Rabenhorst, *Nitzschia radicola* Hustedt, *Nitzschia palea* (Kützing) W Smith, *Nitzschia pura* Hustedt, *Planothidium engelbrechtii* (Cholnoky) Round & Bukhityarova and *Sellaphora seminulum* (Grunow) DG Mann. These taxa distinguish reference sites from disturbed sites and were important in the calculation of AMD-MMI metrics. Disturbed pans contained taxa such as *Aulacoseira granulata* (Ehrenberg) Simonsen, which was also present only in disturbed grass pans and in the open water pan. Disturbed reed pans contained specimens of *Encyonopsis microcephala* (Grunow) Krammer and greater numbers of *Achnantheidium minutissimum* (Kützing) Czarnecki than reference sites. As determined by SIMPER analysis, reed pans displayed an average similarity of 26,49% according to the occurrence of diatom taxa: *Achnantheidium minutissimum* (Kützing) Czarnecki, *Nitzschia palea* (Kützing) W Smith, *Nitzschia liebertruthii* Rabenhorst, *Navicula recens* (Lange-Bertalot) Lange-Bertalot, *Nitzschia radicola* Hustedt, *Sellaphora seminulum* (Grunow) DG Mann, *Gomphonema parvulum* Kützing, *Nitzschia archibaldii* Lange-Bertalot and *Navicula veneta*.

The occurrence of *Navicula recens* and *Nitzschia liebertruthii* decreased in disturbed salt sites. Counts of *Amphora veneta* were higher in reference salt depressions. Two moderately disturbed sites (S17 and S20) had many individuals of *Amphora veneta* as well. *Nitzschia amphibia*, *Nitzschia frustulum* and *Nitzschia palea* occurred in both reference and disturbed sites, though in greater numbers in disturbed sites. Specimens of *Encyonopsis microcephala* (Grunow) Krammer, *Encyonopsis cesatii* (Rabenhorst) Krammer, *Cyclotella meneghiniana*

Kützing, *Nitzschia desertorum* Hustedt, *Nitzschia draveillensis* Coste & Ricard and *Nitzschia etoshensis* Cholnoky were absent from reference sites and thus served as indicators of disturbance contributing to the determination of MMI scores. A 20.27% similarity was found between the salt pans, which was mainly attributed to taxa such as *Amphora veneta*, *Nitzschia palea*, *Nitzschia frustulum*, *Navicula recens*, *Nitzschia amphibia*, *Nitzschia liebertruthii* and *Achnantheidium minutissimum*. The alkaline nature of the salt pans was reflected in the diatom community composition and the presence of taxa such as *Navicula radiosa*, *Navicula zanonii*, *Navicula cryptocephala*, *Pseudostaurosira brevistriata* and *Planothidium frequentissimum* in reference pans. Likewise, *Achnantheidium exiguum*, *Fragilaria ulna* var. *acus*, *Brachysira neoexilis* (Grunow) DG Mann, *Cymbella turgidula* Grunow and *Cymbella kolbei* Hustedt were found in disturbed sites and prefer alkaline waters.

Dominant diatom taxa found exclusively or predominantly in reference grass sites were *Achnantheidium minutissimum*, *Brachysira neoexilis*, *Caloneis molaris* (Grunow) Krammer, *Gomphonema* aff. *gracile*, *Gomphonema angustatum* (Kützing) Rabenhorst, *Gomphonema gracile* Ehrenberg, *Gomphonema lagenula* Kützing, *Gomphonema parvulum* Kützing, *Navicula radiosa* Kützing, *Nitzschia frustulum*, *N. gracilis*, *N. intermedia*, *N. liebertruthii*, *N. microcephala*, *N. nana*, *N. palea*, *N. radicula* and *Sellaphora seminulum*. Taxa that were characteristic of disturbed grass depressions include *Aulacoseira granulata* (Ehrenberg) Simonsen, *Gomphonema insigne*, *Navicula rostellata*, *Nitzschia aurariae*, *Nitzschia microcephala*. By using SIMPER analysis, it was determined that grass pans have 27.22% similarity according to their species composition. Taxa that contribute the most to similarity between pans are *N. palea*, *N. liebertruthii*, *A. minutissimum*, *Gomphonema* aff. *gracile*, *Gomphonema parvulum*, *Nitzschia radicula* Hustedt, *Gomphonema gracile*, *Navicula recens*, *Amphora veneta*, *Nitzschia frustulum* and *Nitzschia pura*. Diatom communities in the open water pan were dominated by *A. minutissimum*, *Encyonopsis microcephala*, *Fragilaria capucina* var. *rumpens* (Kützing) Lange-Bertalot, *Gomphonema parvulum* and *Navicula recens*.

4.2 Influence of Disturbance Level on Diatom Community composition

The SIMPER analysis is calculated from the Bray-Curtiss dissimilarity matrix and calculates the contribution of each species to the dissimilarity between reference and impacted sites or between pan types.

According to diatoms assemblages, a 30.55% similarity in community composition exist among reference sites. Sites classified as moderately impacted were 18.56% similar in their

composition. Highly disturbed sites showed a 26.83% similarity in diatom community composition.

Reference site community composition differed from those in moderately disturbed sites by 80.66% as is indicated in Table 3. Of this difference, 27.12% was caused by (in order of decreasing % contribution) *Amphora veneta*, *Nitzschia palea*, *Achnantheidium minutissimum*, *Nitzschia liebertruthii*, *Cyclotella meneghiniana*, *Nitzschia frustulum* and *Nitzschia amphibia*. The distributions of *A minutissimum*, *N palea*, *Amphora veneta*, *Aulacoseira granulata*, *Encyonopsis microcephala*, *Nitzschia liebertruthii* and *Gomphonema parvulum* accounted for 27.35% of the difference between reference and disturbed sites, which had a total dissimilarity in diatom community composition of 79.52%. *Achnantheidium minutissimum*, *Aulacoseira granulata*, *Encyonopsis microcephala*, and *Gomphonema parvulum* were more abundant in disturbed sites compared to sites of low and moderate impact. Higher abundances of these taxa correspond with higher magnitudes of AMD-pollution. Between moderately and highly disturbed sites, an 82.89% total dissimilarity was found. *Achnantheidium minutissimum*, *Encyonopsis microcephala*, *Amphora veneta*, *Aulacoseira granulata*, *Nitzschia palea* and *Cyclotella meneghiniana* collectively accounted for 27.62% of the variance between moderately and highly disturbed sites.

Table 3: The percentage dissimilarities in diatom community composition in non-impacted, moderately impacted and highly impacted depressions.

	Low	Moderate
Moderate	80,66%	
High	79,52%	82,89%

Taxa responsible for 25% of variance are indicated in Table 4. The taxa are ranked (from one up to seven) according to their contribution to variance between pan types.

Table 4: Taxa that cause at least 25% of variance between pans of various disturbance levels.

	Low & Moderate	Low & High	Moderate & High

<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki	3	1	1
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen		4	4
<i>Amphora veneta</i> Kützing	1	3	3
<i>Cyclotella meneghiniana</i> Kützing	5		6
<i>Encyonopsis microcephala</i> (Grunow) Krammer		5	2
<i>Gomphonema parvulum</i> Kützing		7	
<i>Nitzschia amphibia</i> Grunow	7		
<i>Nitzschia frustulum</i> (Kützing) Grunow	6		
<i>Nitzschia liebertruthii</i> Rabenhorst	4	6	
<i>Nitzschia palea</i> (Kützing) W Smith	2	2	5

Although the diatom assemblages of sites with different levels of disturbance vary distinctly, PERMANOVA was carried out to determine the degree to which the factor of AMD-impact level influences the diatom community composition. Three factors were analysed: disturbance level, pan type and season. The analysis established AMD-disturbance level as the factor that explained most of the variance in diatom community composition. Disturbance level generated 8.59% of the total variance.

4.3 Influence of Pan type on Diatom Community composition

The average dissimilarities in Table 5 indicates an 81.01% difference between the diatom communities in reed pans and those in salt pans. *Achnantheidium minutissimum*, *Amphora*

veneta, *Nitzschia palea*, *N. amphibia*, *N. frustulum* and *N. liebertruthii* together account for 25.48% of the difference. *Amphora veneta* occurs in much greater numbers in reference salt pans than in reed pans and is associated with increased conductivity.

Reed pans and grass pans have a 72.78% total difference. Taxa such as *Achnantheidium minutissimum*, *N palea*, *Nitzschia liebertruthii*, *Nitzschia pura*, *Amphora veneta*, *Nitzschia radricula* and *Cyclotella meneghiniana* are responsible for 25.58% of the difference.

Compared to open water pans, reed pans showed a 71.76% dissimilarity. In descending order, *Encyonopsis microcephala*, *Achnantheidium minutissimum*, *Nitzschia palea*, *Fragilaria capucina* and *Fragilaria capucina* var. *rumpens* contribute to 27.63% of the variance between reed and open water pans.

Table 5: Average dissimilarity between pan types

	Reed	Salt	Grass
Salt	81.01%		
Grass	72.78%	81.33%	
Open Water	71.69%	77.51%	77.17%

Salt depression diatom assemblages differ from grass pan communities by 81.33% and 77.51% from the open water communities. Species contributing to dissimilarity the most are *Amphora veneta*, *Achnantheidium minutissimum*, *Nitzschia palea*, *Nitzschia liebertruthii*, *Cyclotella meneghiniana*, *Nitzschia frustulum* and *Nitzschia amphibia* (which are responsible for 25.92% of the dissimilarity). *Encyonopsis microcephala*, *Achnantheidium minutissimum*, *Amphora veneta* and *Fragilaria capucina* var. *rumpens* account for 27.58% of the difference between salt and open water communities.

Grass and open water pans demonstrated a total difference of 77.17% in their diatom assemblages. Very similar to the variance from reed pans, *Encyonopsis microcephala*, *Achnantheidium minutissimum*, *Nitzschia palea*, *Fragilaria capucina* var. *rumpens* and *Fragilaria capucina* Desmazières contributed to 26.84% of the difference between grass and open water pans.

Though assorted pan types vary in terms of diatom community composition, taxa that are characteristic of the reference sites of all the different pan types are used in MMI1 to determine levels of AMD-impact. Taxa responsible for 25% of variance are indicated in Table 6. The taxa

are ranked (from one up to seven) according to their contribution to variance between pan types.

Table 6: Taxa that cause at least 25% of variance between pan types.

	Reed & Grass	Reed & Salt	Grass & Salt	Reed & Open water	Grass & Open water	Salt & Open water
<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki	1	1	2	2	2	2
<i>Amphora veneta</i> Kützing	5	2	1			3
<i>Cyclotella meneghiniana</i> Kützing	7		5			
<i>Encyonopsis microcephala</i> (Grunow) Krammer				1	1	1
<i>Fragilaria capucina</i> Desmazières				4	5	
<i>Fragilaria capucina</i> var. <i>rumpens</i> (Kützing) Lange-Bertalot				5	4	4
<i>Nitzschia amphibia</i> Grunow		4	7			
<i>Nitzschia frustulum</i> (Kützing) Grunow		5	6			
<i>Nitzschia liebertruthii</i> Rabenhorst	3	6	4			
<i>Nitzschia palea</i> (Kützing) W Smith	2	3	3	3	3	
<i>Nitzschia pura</i> Hustedt	4					
<i>Nitzschia radicola</i> Hustedt	6					

PERMANOVA indicated that next to AMD-disturbance level, pan type caused 7,85% of the total variance in diatom community composition in depressional wetlands. The influence of pan type on diatom community structure is accounted for in the AMD-MMIs. MMI1

incorporates reference and disturbed site taxa from all pan types. The results of MMI1 can be compared to those of MMI2, MMI3 and MMI4. A different MMI for each pan type prevents the interference that pan type has on diatom community composition and consequently on measures of ecological condition. MMI2 uses only reed pan diatom communities, MMI3 is designed for salt pans and MMI4 for grass and open water pans.

PERMANOVA tests further illustrated the importance of disturbance level and pan type by determining that the influence of both factors combined brought about 10.78% of the total variance.

4.4 Influence of Seasonal Change on Diatom Community composition

Among all sites sampled in the dry season there was a similarity in diatom assemblages of 20.89%. All sites sampled in wet season display a total similarity of 25.1%. The average dissimilarity between winter and summer diatom communities equates to 78.6%. In order of decreasing % contribution, more than 25% of the dissimilarity arises from the abundances of *Achnantheidium minutissimum*, *Amphora veneta*, *Nitzschia palea*, *Cyclotella meneghiniana*, *Nitzschia liebertruthii*, *Nitzschia frustulum* and *Nitzschia amphibia*. The factor of season explained 1.72% of the total variance in diatom community composition. The percentages of variance owing to different factors are presents in Figure 24.

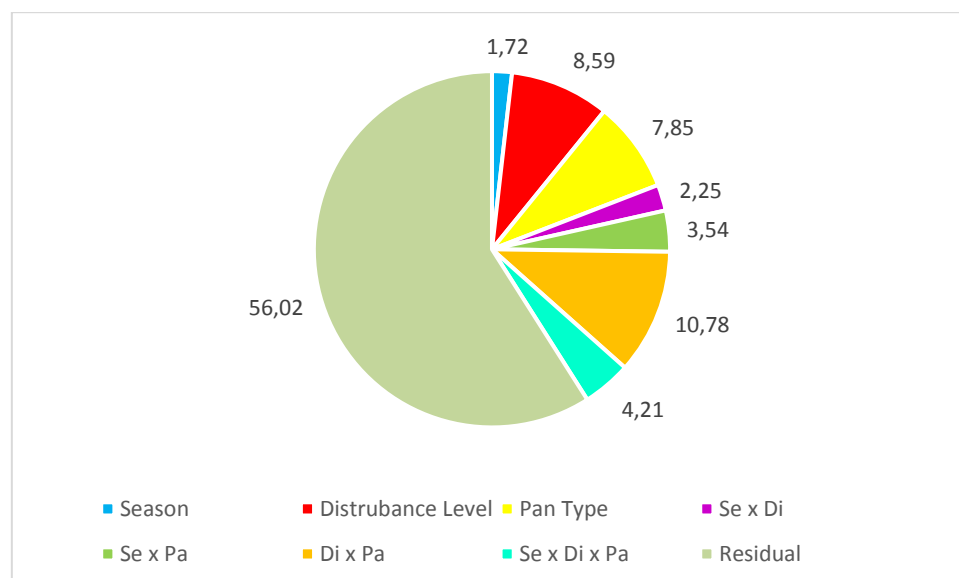


Figure 24: Taxa that cause at least 25% of variance between pan types. The combined effects of seasonal change (Se) and pan type (Pa) is represented by Se x Pa, the effects of disturbance level (Di) and pan type combined is represented by Di x Pa and the combined effect of all three factors by Se x Di x Pa.

Depressional wetlands were sampled during the dry and the wet season to determine the degree to which seasonal change alters diatom assemblages. Though results indicate little variance in diatom community composition due to seasonal variation, some variance does occur. Multimetric index scores were calculated twice for each site: once using data obtained from sampling done in the dry season and once from wet season data. The achieved MMI scores are thus not affected by seasonal variation and only represent disturbance levels by reason of the reference site data used to calculate the scores already being representative of each particular season.

The two sets of scores can be compared to determine if pan condition improved or deteriorated over time. The influence of seasonal change can be avoided by ensuring that samples for which MMI's are being calculated are compared to reference site data of the same season.

4.5 Diatom community composition in relation to environmental variables

The results of the Canonical Correspondence Analysis (CCA) display the correlations between diatom assemblages and the environmental variables of each pan type and are presented in biplots.

4.5.1 Reed depressions

In the bottom right corner of Figure 25, diatom species such as *Encyonopsis microcephala* (found in disturbed reed pans), and *Achnantheidium exiguum* are ordinated in conjunction with increased levels of water quality variables. Indicators of AMD disturbance (SO_4^{2-} , $\%\text{SO}_4^{2-}:\text{Cl}^-$ and Ca^{2+}) are ordered on the right-hand side and represented by vectors which, by their lengths, indicate greater degrees of influence on community composition. Higher concentrations of Na^+ and K^+ ordinated with *Amphora pediculus* (Kützing) Grunow and *Epithemia adnata* (Kützing) Brébisson which was dominant in S1. In the top right corner of Figure 25, coinciding with moderate to high amounts of electrolytes, are *Fragilaria capucina*, *Aulacoseira granulata* and *Fragilaria tenera* (WM Smith) Lange-Bertalot. These species in particular were found in the disturbed site S27.

Ordinated on the left are taxa inversely corresponding with $\%\text{SO}_4^{2-}:\text{Cl}^-$. Species such as, *Gomphonema* aff. *gracile*, *Gomphonema gracile*, *G. lagenula*, *G. angustatum*, *Nitzschia palea*, *N. archibaldii*, *N. amphibia*, *N. pura*, *N. frustulum*, *Planothidium engelbrechtii*, *Sellaphora seminulum*, *Caloneis molaris* and *N. intermedia* are predominant in reference reed sites. *Gomphonema parvulum* and *Navicula rostellata* occur in both reference and disturbed sites though more often in reference sites while *Encyonema minutum* (Hilse) DG Mann and

Fragilaria tenera more frequently occurred in disturbed sites. The results of the CCA are outlined in Table 7. The first four axes of the species-environment plot accounted for 56.7% of the total variance in reed pan diatom communities caused by environmental variables. Diatom species correlated well with environmental variables.

Table 7: Summary of the CCA for diatom community composition and measured environmental variables in reed pans

Axes	1	2	3	4	Total inertia
Eigenvalues:	0.567	0.460	0.395	0.265	3.029
Species-environment correlations:	1.000	1.000	1.000	1.000	
Cumulative percentage variance					
of species data:	18.7	33.9	47.0	55.7	
of species-environment relation:	18.7	33.9	47.0	55.7	
Sum of all eigenvalues					3.029
Sum of all canonical eigenvalues					3.029

Table 8: Summary of the CCA for diatom community composition and measured environmental variables between reed pans

Axes	1	2	3	4	Total inertia
Eigenvalues:	0.467	0.343	0.260	0.258	3.029
Species-environment correlations:	1.000	1.000	1.000	1.000	
Cumulative percentage variance					
of species data:	21.1	36.7	48.4	60.1	
of species-environment relation:	21.1	36.7	48.4	60.1	
Sum of all eigenvalues					2.208
Sum of all canonical eigenvalues					2.208

Figure 26 displays all reed sites in relation to environmental variables. Site 1 (S1) is located at the top right corner. This site had the highest EC value and SO_4^{2-} and Na concentrations among reference reed sites in the dry season. The remaining reference sites are ordinated in the centre of the graph to the left-hand side owing to diatom communities which denote lower levels of solutes. In the bottom right corner is site S28 (6=winter and 12= summer). This highly disturbed site corresponds with elevated levels of AMD-indicators. The second highly disturbed site, S27 (5=winter and 11=summer), is positioned far left on account of high magnesium concentrations. This site had higher concentrations of Ca^{2+} and SO_4^{2-} as well as % $\text{SO}_4^{2-}:\text{Cl}^-$ ratios than reference sites but less than S28. With the exception of site S1 in the winter, all reed sites remain comparable in terms of water quality variables across seasons. Based on environmental variables and reflected in diatom community compositions, disturbed and reference reed pans are distinctly apart in their arrangement. Variables that showed the strongest influence on diatom community composition include SO_4^{2-} , Ca^{2+} , EC and K^+ .

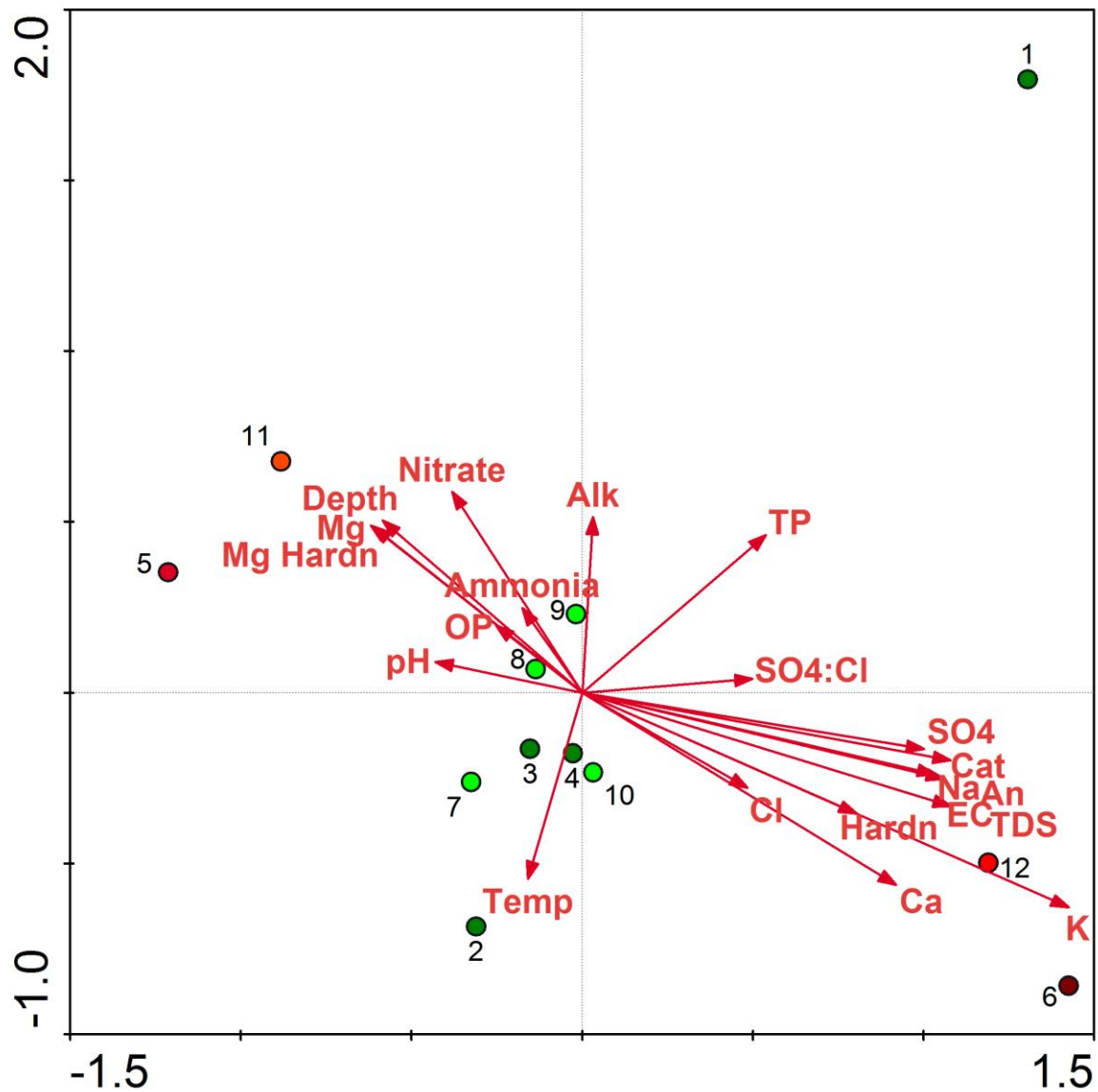


Figure 26: CCA biplot showing the inter sample relationship between the measured environmental variables and reed pan samples (based on diatom taxa from reed pans). Reference sites are labelled: 1=S1; 2=S2; 3=S3; 4=S4 (winter) and 7=S1; 8=S2; 9=S3; 10=S4 (summer). Disturbed sites are labelled: 5=S27; 6=S28 (winter) and 11=S27; 12=S28 (summer). Reference sites are light green (summer) and dark green (winter). Highly disturbed sites are red (summer) and dark red (winter).

4.5.2 Salt depressions

Diatom in salt depressions were well correlated with water quality variables. Eigenvalues, species-environment correlations and percentages variance of diatom communities in salt pans are given in Table 9. The first four axes of the species-environment plot explained 70.7% of the total variance in salt pan diatom communities.

Table 9: Summary of the CCA for diatom community composition and measured environmental variables between salt pans

Axes	1	2	3	4	Total inertia
Eigenvalues:	0.707	0.532	0.499	0.449	4.972
Species-environment correlations:	1.000	0.987	0.999	0.997	
Cumulative percentage variance					
of species data:	14.2	24.9	35.0	44.0	
of species-environment relation:	14.7	25.8	36.1	45.5	
Sum of all eigenvalues					4.972
Sum of all canonical eigenvalues					4.811

Among salt pans groups of diatom species are clustered in relation to various environmental variables. Figure 27 displays the relationship between the water quality variables and diatom taxa from salt pans. Ordered in the top left section are *Encyonopsis microcephala*, *Nitzschia pura* and *Achnanthyidium minutissimum*, all of which are characteristic of disturbed salt sites and especially correlate with high sodium concentrations. Also to the left, are taxa associated with brackish waters including *Fragilaria capucina* var. *rumpens*, *Aulacoseira granulata*, *Nitzschia draveillensis*, *Nitzschia clausii* Hantzsch, *Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski and *Achnanthyidium exiguum* that display strong relationships with sodium, chloride and nitrates. These species were also associated with disturbed pan sites.

Amphora veneta, *Craticula molestiformis*, *Cyclotella meneghiniana* and various *Nitzschia* species display association with moderate phosphate-phosphorous levels. Phosphorous and orthophosphate concentrations were higher in disturbed salt pans and these species occurred mostly if not only in disturbed pans. *Brachysira neoexilis*, *Encyonopsis cesatii*, *Navicymbula pusilla* (Grunow) Krammer and *Mastogloia elliptica* (Agardh) Cleve are clustered at the far

right of the figure, being associated with high magnesium concentrations. These four species in particular were abundant in S23 which had much higher magnesium levels than any other salt pan. Community composition is clearly influenced by water quality variables and can be considered representative of environmental conditions.

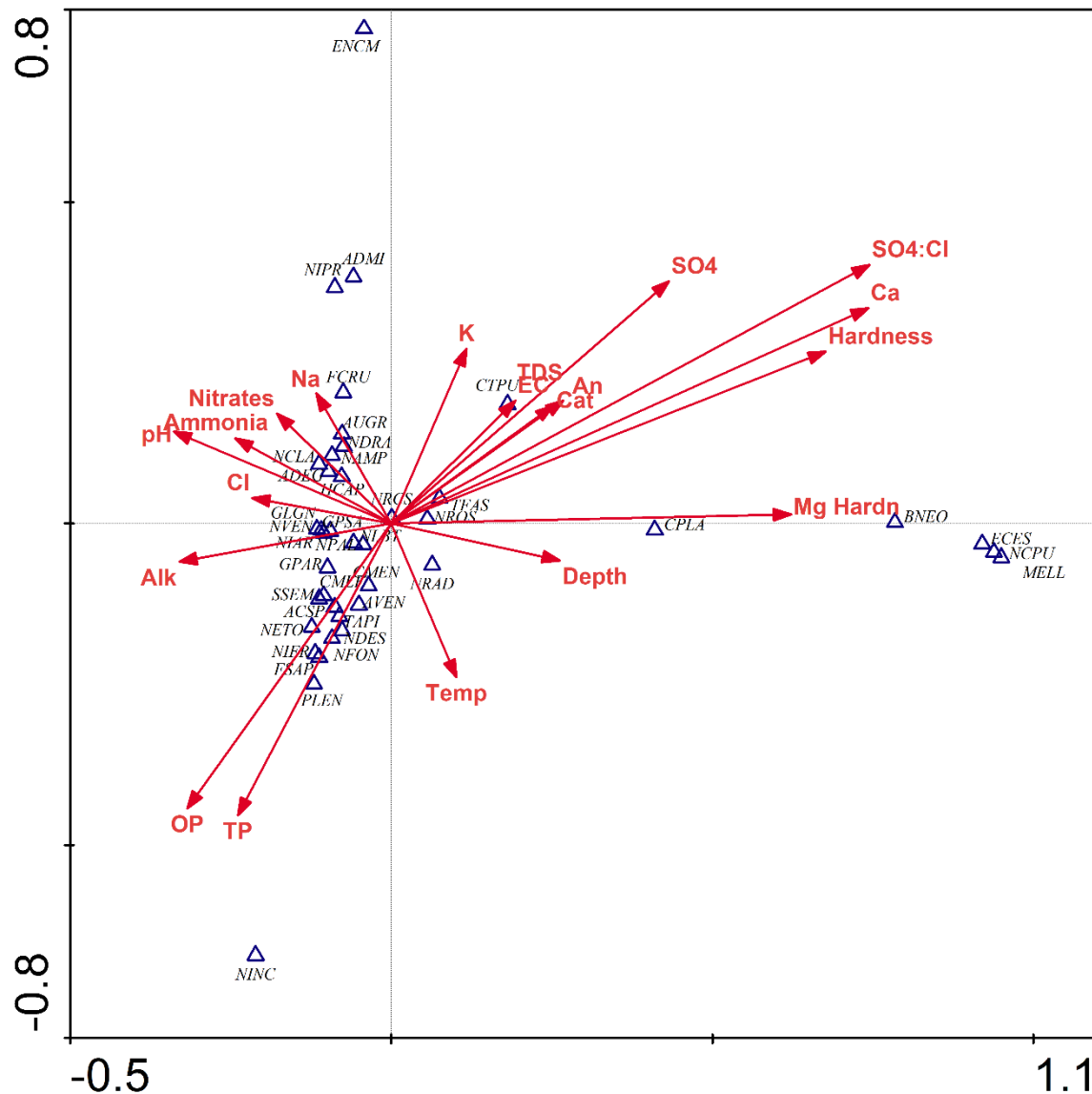


Figure 27: CCA biplot showing the relationship between the measured environmental variables and diatom taxa from salt pans during the wet and dry seasons.

Table 10: Summary of the CCA for diatom community composition and measured environmental variables between salt pans

Axes	1	2	3	4	Total inertia
Eigenvalues:	0.705	0.531	0.476	0.395	4.972
Species-environment correlations:	1.000	1.000	1.000	1.000	
Cumulative percentage variance					
of species data:	16.3	28.6	39.6	48.7	16.3
of species-environment relation:	16.3	28.6	39.6	48.7	16.3
Sum of all eigenvalues					4.320
Sum of all canonical eigenvalues					4.320

Figure 28 shows all salt pans in relation to environmental variables. The moderately disturbed site, S22 (number 9 and 18 in Figure 28) is ordinated according to species' preferences for high sodium concentrations. Similarly, S23 is ordinated on the right in accordance with high magnesium concentrations. Reference sites are ordinated around lower levels of nutrients. Increasing disturbance levels correlated with increased solutes. The vectors for Ca^{2+} , SO_4^{2-} and $\% \text{SO}_4^{2-} : \text{Cl}^-$ exceed others in length and are responsible for variation within communities to a greater degree than other variables. Site S18 had the highest phosphorous concentrations among disturbed salt pans and was orientated according to phosphorous levels. This was also reflected in the dominance of *Nitzschia inconspicua* Grunow in the site which correlated strongly with high phosphorous concentrations. Most disturbed salt pans could only be classified as moderately disturbed. Some pans show less signs of AMD-disturbance but are not in reference site condition (e.g. S17 and S20) and appear even less disturbed in the summer. Environmental variables that influenced diatom community composition the most are SO_4^{2-} , Ca^{2+} , $\% \text{SO}_4^{2-} : \text{Cl}^-$, hardness and Mg^{2+} .

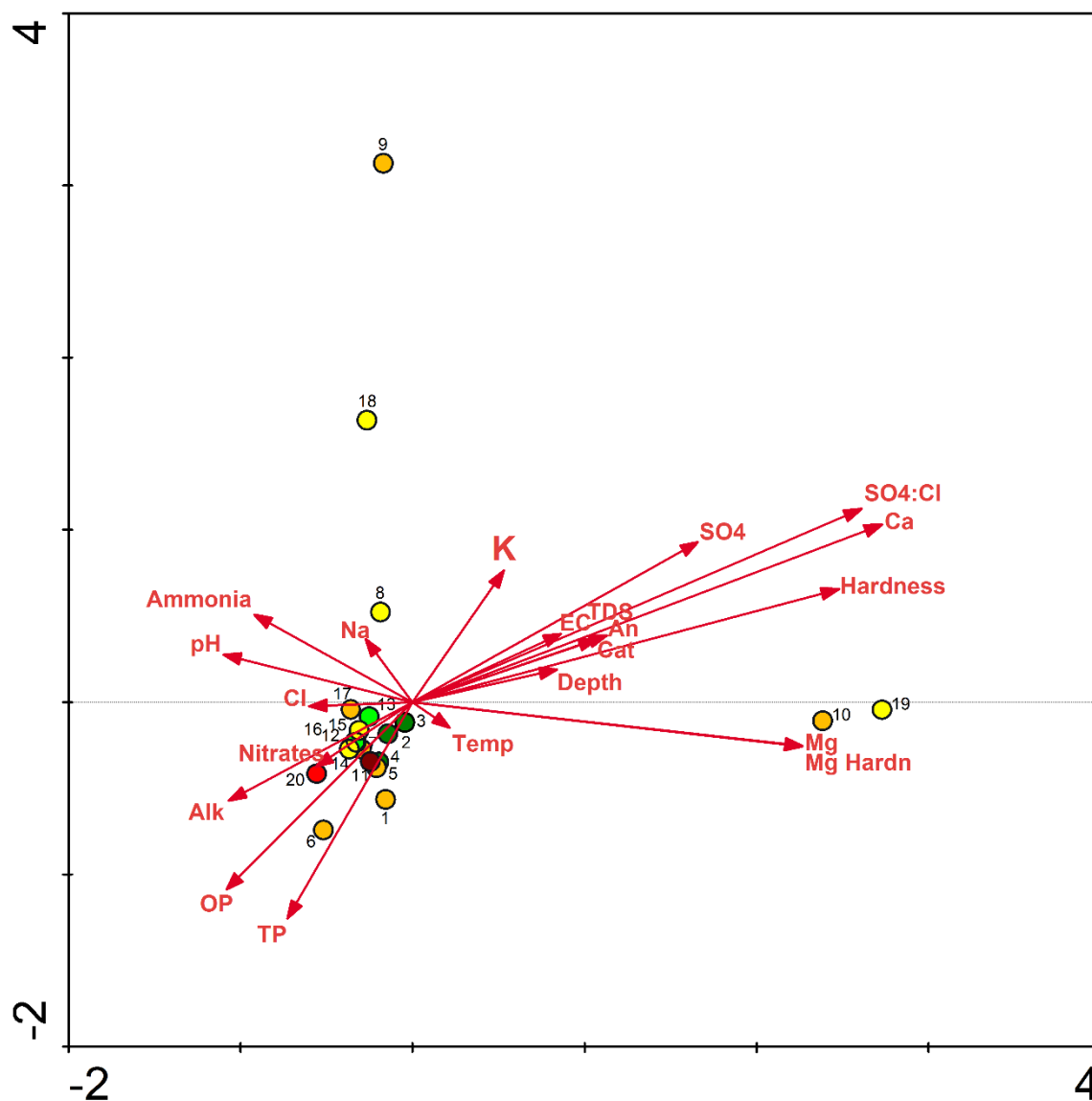


Figure 28: CCA biplot showing the inter sample relationship between environmental variables and salt pan samples. Dry season sites are labelled: 1=S6; 2=S7; 3=S9; 4=S10; 5=S17; 6=S18, 7=S20; 8=S21; 9=S22; 10=S23; 11=S26. Wet season sites are labelled: 12=S7; 13=S10; 14=S6; 15=S17; 16=S18; 17=S20; 18=S22; 19=S23 and 20=S26. Reference sites are light green (summer) and dark green (winter). Moderately disturbed sites are yellow (summer) and orange (winter). Highly disturbed sites are red (summer) and dark red (winter).

4.5.3 Grass depressions

It is illustrated in Table 11 that 46.9% of the total variance in grass pan diatom communities are accounted for by the first four axes. Species-environment correlations are strong in grass pans.

Table 11: Summary of the CCA for diatom community composition and measured environmental variables in grass pans

Axes	1	2	3	4	Total inertia
Eigenvalues:	0.468	0.394	0.351	0.281	3.512
Species-environment correlations:	0.996	0.997	0.993	0.972	
Cumulative percentage variance					
of species data:	13.3	24.5	34.5	42.5	
of species-environment relation:	14.0	25.7	36.1	44.5	
Sum of all eigenvalues					3.512
Sum of all canonical eigenvalues					3.354

The relationships between grass pan diatom communities and environmental variables are presented in Figure 29. Diatom species *Navicula veneta* Kützing, *Gomphonema pseudoaugur* Krammer and especially *Nitzschia aurariae* Cholnoky exhibited strong correlations with % $\text{SO}_4^{2-}:\text{Cl}^-$ and were numerous in disturbed sites. Variables that affected community composition to the greatest degree are SO_4^{2-} , Cl^- , % $\text{SO}_4^{2-}:\text{Cl}^-$, EC, Na^+ and alkalinity. Species corresponding with EC, Cl^- , Na^+ and Mg^{2+} are *Gomphonema parvulum* (Kützing) Kützing, *Encyonopsis microcephala* (Grunow) Krammer, *Craticula buderii* (Hustedt) Lange-Bertalot, *Navicula rostellata* Kützing, *Nitzschia microcephala* Grunow, *Nitzschia amphibia* Grunow and *Planothidium engelbrechtii* (Cholnoky) Round & Bukhityarova. These taxa are more typical of disturbed grass depressions.

On the left in Figure 29 are ordinated species characteristic of reference sites: *Caloneis molaris*, *Brachysira neoexilis*, *Eunotia bilunaris*, *Encyonema mesianum*, *Encyonema minutum*, *Frustulia crassinervia* (Kützing) Cleve, *Gomphonema* aff. *gracile*, *Gomphonema gracile*, *Nitzschia palea*, *N. nana*, *N. archibaldii*, *N. pura*, *N. radicola*, *N. frustulum*, *N. liebertruthii*, *Navicula zanonii* and *Navicula cryptocephala*. Reference site taxa clearly correlate with cleaner water quality conditions in contrast to taxa found in disturbed sites, which correspond with elevated levels of salts.

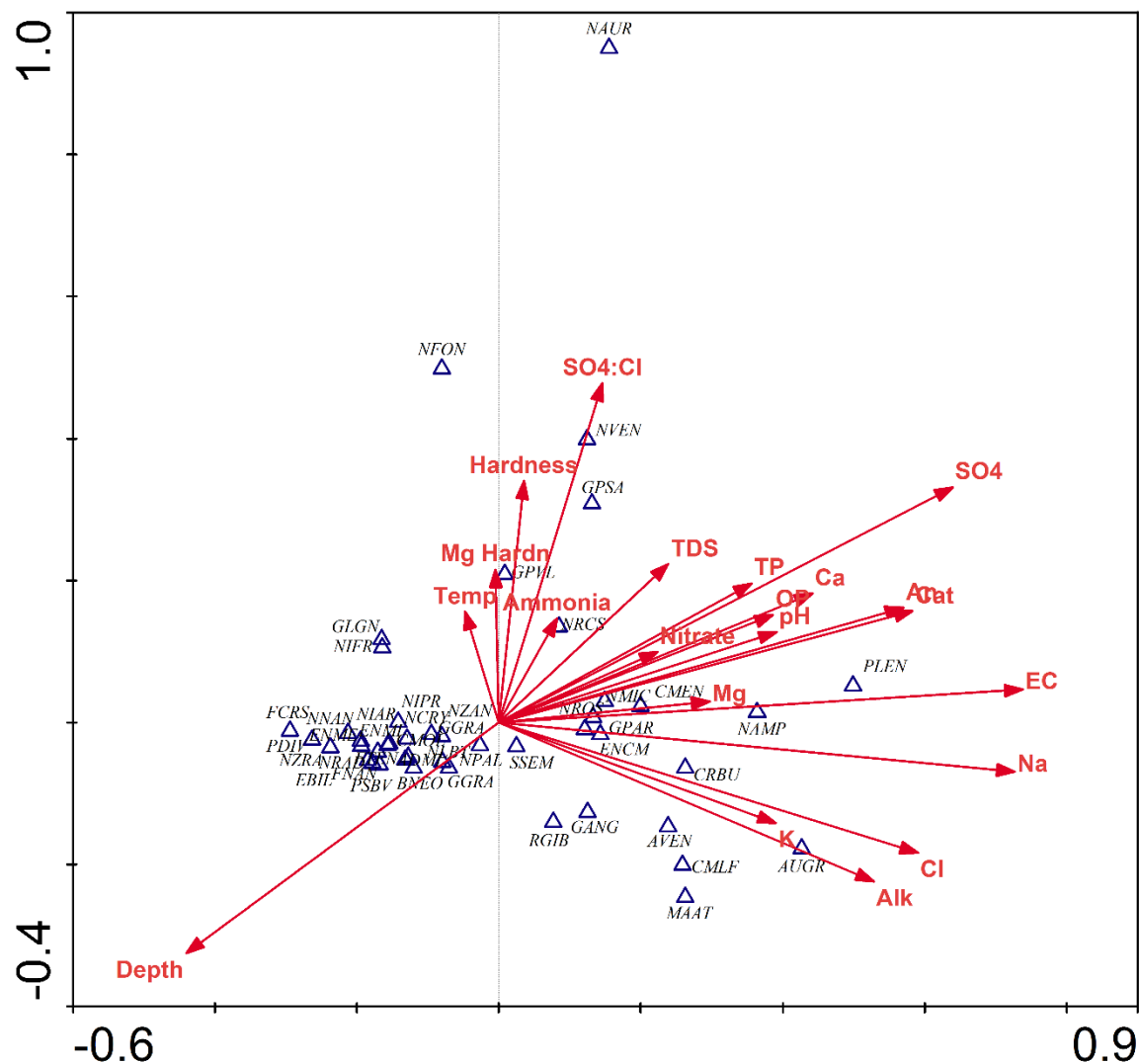


Figure 29: CCA biplot showing the relationship between the measured environmental variables and diatom taxa from grass pans during the wet and dry seasons.

Eigenvalues, species-environment correlations and percentages variance of diatom communities in grass pans are presented in Table 12. The first four axes of the species-environment plot explained 41.5% of the total variance among grass pan diatom communities which showed very strong correlations with environmental variables.

Table 12: Summary of the CCA for diatom community composition and measured environmental variables between grass pans

Axes	1	2	3	4	Total inertia
Eigenvalues:	0.415	0.386	0.310	0.274	3.512
Species-environment correlations:	1.000	1.000	1.000	1.000	
Cumulative percentage variance					
of species data:	13.7	26.4	36.7	45.7	
of species-environment relation:	13.7	26.4	36.7	45.7	
Sum of all eigenvalues					3.030
Sum of all canonical eigenvalues					3.030

In Figure 30, reference sites can be seen to have ordinated in the centre in correlation with lower levels of environmental variables and display a negative relationship with SO_4^{2-} , Na^+ and EC. The highly disturbed site S24 (10=winter and 21=summer) correlates strongly with alkalinity and elevated levels of solutes. Though highly disturbed, diatom species in S25 (11=winter and 22=summer) do not correlate with variables indicative of AMD-disturbance. The higher temperatures of this pan may contribute to the occurrence of cyanobacteria at this site. Site S16 (8=winter and 19=summer) was dominated by *Nitzschia aurariae* in the winter, which exhibited strong correlations with $\% \text{SO}_4^{2-}:\text{Cl}^-$. This site contained more individuals of *Nitzschia amphibia* and *Planothidium engelbrechtii* in the summer which correlates with higher EC levels. Differences in reference and disturbed pan water quality are clearly demonstrated by differences in diatom community composition.

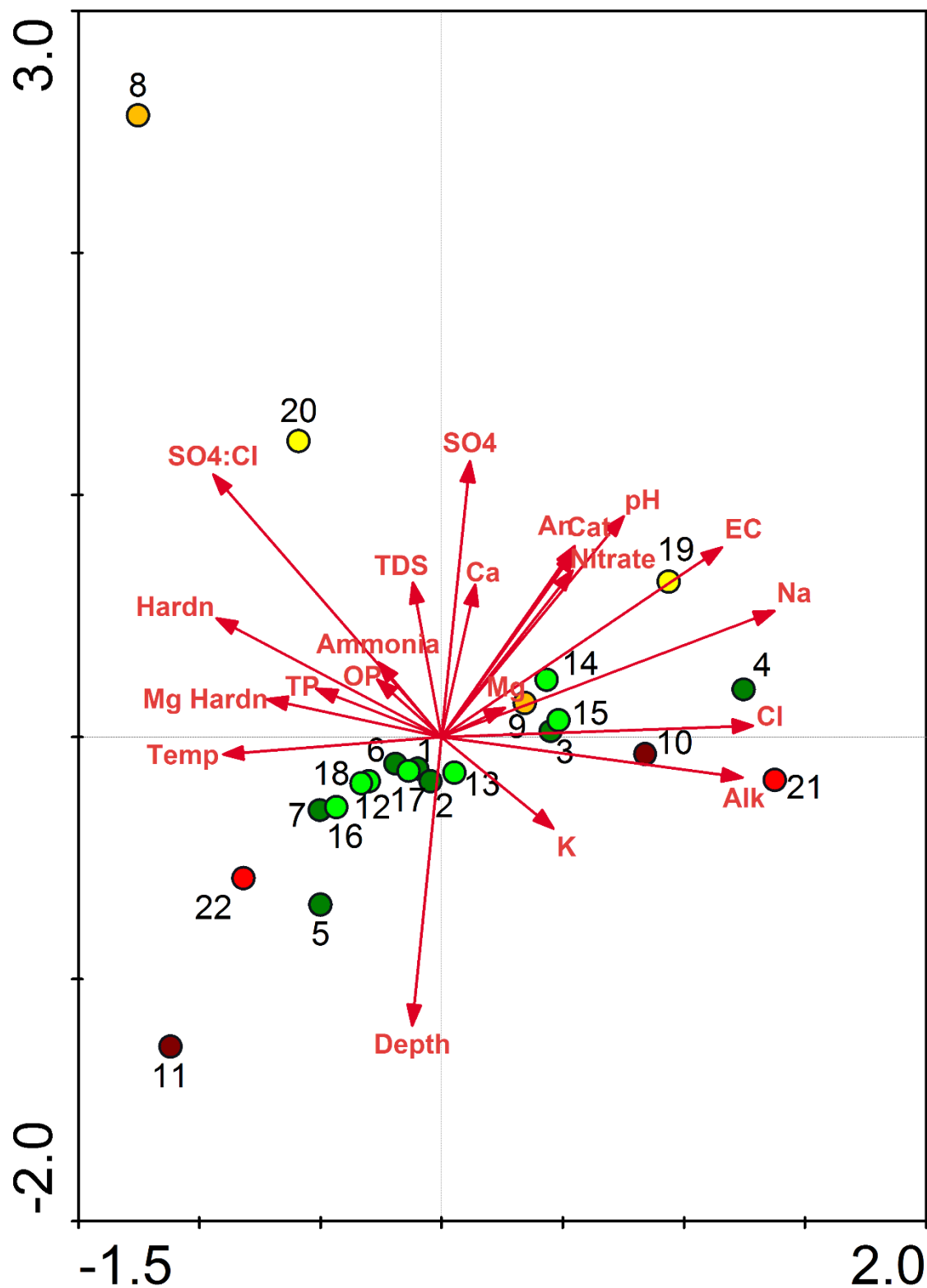


Figure 30: CCA biplot showing the relationship between the measured environmental variables and diatom taxa from salt pans.

Diatom communities varied greatly between different pan type and between reference and disturbed sites. Different pan types have particular water chemistry and distinct reference and disturbed communities. Among all pan types, diatom communities proved very responsive to water quality variables. Reference and disturbed sites were ordinated according to environmental variables based on diatom community compositions. Sites with different disturbance levels are easy to distinguish from one another. It is based on the composition differences that diatom indices can be used to distinguish healthy systems from those in poor condition.

CHAPTER 5: RESULTS AND DISCUSSION- APPLICATION AND TESTING OF DIATOM INDICES

Following the determination of the levels of AMD-disturbance, based on the water chemistry data, the scores from the various indices were calculated. Each index ranks the condition of a site according to the score obtained, placing a given site in a corresponding category of water quality. AMD-MMIs classify the ecological condition of pans as good, fair or poor based on the 5th and 25th percentile of reference site scores. Riverine indices classify sites as high quality (17-20), good quality (15-17), moderate quality (12-15), poor quality (9-12) or bad quality (9 or less). Table 14 shows the water quality classes obtained from various diatom indices.

The composition of diatom communities changes in response to shifts in environmental variables. To increase the ease with which the composition of diatom communities can be interpreted, AMD MMIs use diatom life forms and genera that represent the same ecological conditions rather than examining the whole community of every site. Riverine indices (mostly European) and MMI scores were calculated and are presented in Appendix 4. Scores obtained from riverine indices were rescaled to 0-100 for the purposes of comparison. The open water site (S25) was omitted in the calculation of MMIs as it lacked sufficient evidence of AMD-pollution. The ranking of pan sites by the AMD-MMI's and then the riverine indices were compared to the determined levels of disturbance and are as follows:

5.1.1 Classification of pan ecological condition according to AMD-MMIs

5.1.1.1 Classification of reed pan ecological condition according to MMI1

In the first AMD multimetric index, the first category of metrics- similarity to reference sites, compares a given pan to all reference pans. Though all four reference reed pans obtained high index scores, one site was initially classified as poor due to its score (75.193) being less than the 5th percentile of the reference sites score distribution (75.283). When the scores and distributions are rounded to down the nearest integer, the site is classified as fair, i.e. between the 5th and 25th percentiles of the reference distribution. Based on similarity to of reference sites, functional group and taxonomic composition, both disturbed sites were classified as poor in the winter. Results were similar in the summer as three reference pans displayed good quality, the other fair and both disturbed sites poor condition.

5.1.1.2 Classification of salt pan ecological condition according to MMI1

Reference salt pans displayed good (two) and fair (one) conditions. Six moderately disturbed sites were classified as poor and one as good during the winter. The highly disturbed site was classified as being in poor condition in both seasons and good condition was maintained by reference sites in the summer as well. Moderately disturbed sites had equal amounts of sites in poor, fair and good condition. S20 is a moderately disturbed salt pan of which the diatom community composition differed less from those of reference sites than other disturbed sites. Among disturbed sites, S20, S18 and S6 had the lowest % $\text{SO}_4^{2-}:\text{Cl}^-$ values. S20 showed good condition in the winter and fair in the summer. S18 and S6 showed poor condition in the winter but good condition in the summer in comparison to all reference sites.

5.1.1.3 Classification of grass pan ecological condition according to MMI1

Two reference grass pans were classified as fair and five as good during the winter season. One moderately disturbed site was rated as poor and the other as fair condition. The highly disturbed site displayed fair condition in comparison with all reference sites. During the next season five sites maintained a good condition, S8 maintained a fair condition and S5 was reduced from fair to poor. Moderately disturbed sites maintained their conditions, poor and fair, respectively. The highly disturbed site was in poor condition.

5.1.1.4 Classification of reed pan ecological condition according to MMI2

The only category of metrics in MMI2 is similarity to reference sites. This index is only for reed pans and compares a given pan to reference reed pans. In both seasons all reference pans except one which one? were rated as being in good condition. The sites were classified as poor due to their score being slightly less than the 5th percentile of the reference sites score distribution, despite scoring three times higher than disturbed sites. All disturbed reed pans achieved scores lower than the 5th percentile of reference site scores and were classified as being in poor condition.

5.1.1.5 Classification of salt pan ecological condition according to MMI3

Of the three reference salt pans, one (S7) achieved a score slightly less than the 5th percentile of the reference site score distribution according to which the condition thereof was classified as poor. S7 was the only reference salt pan in which *Achnantheidium minutissimum* and *Aulacoseira granulata* occurred and had the least amount of *Amphora veneta* of all reference sites. Another reference site was classified as fair and the last as good in the winter. Six

moderately disturbed pans displayed poor condition and one fair condition according to index scores. The highly disturbed site's score indicated poor condition. In the summer both reference sites displayed good quality. Moderately disturbed sites' scores were equally distributed into all categories, i.e. two in poor, fair and good condition while the highly disturbed site's condition remained poor. According to the MMI for salt pans, S20 was in fair condition during both seasons. S17 and S18 went from poor condition in the winter to fair condition in the summer. The occurrence of *Amphora veneta* increased notably in S17, S18 and S20 in the summer thus increasing their similarity to reference sites.

5.1.1.6 Classification of grass pan ecological condition according to MMI4

Five reference grass pans obtained scores indicative of good condition. One reference pan's score suggests fair condition and the last (S13) signifies poor condition. S13 was dominated by *Achnanthidium minutissimum* and contained less *Gomphonema* taxa and *Nitzschia* individuals than other reference sites. Both moderately disturbed pans displayed poor condition, the highly disturbed site displayed fair condition. Similar results were obtained in the summer as five reference pans showed good condition, one demonstrated fair condition and one poor condition. Moderately disturbed pans displayed poor condition and good condition, respectively. The highly disturbed site scored below the 5th percentile of reference site score distribution, thus indicating poor condition.

5.2 Classification of pan ecological condition according to riverine indices

5.2.1 Trophic diatom index (TDI) and % pollution tolerant valves (PTV)

The TDI which was developed in the UK for monitoring sewage pollution from WWTW (Kelly and Whitton, 1995) has two components, an index score for overall impacts of inorganic nutrients (especially soluble reactive P) coupled with the percentage of pollution tolerant valves (%PTV) which is used to help interpret TDI scores. Different percentages of pollution tolerant valves given in Table 13, indicate to what extent organic pollution may be present in an ecosystem (the trophic levels of a system). The TDI is a five-point scale that represents inorganic nutrient concentrations.

Table 13: Levels of organic pollution indicated by TDI and percentage of pollution tolerant valves

%PTV			TDI	
1	<20	Organic Pollution Free	1	Low Nutrient Concentrations
2	21-40	Some Organic Pollution	2	Moderate Nutrient Concentrations
3	41-60	Significant Organic Pollution	3	Considerable Nutrient Concentrations
4	>61	Heavy Organic Pollution	4	High Nutrient Concentrations
			5	Very High Nutrient Concentrations

5.2.1.1 Reed pan ecological condition according to TDI and %PTV

According to the scores obtained from the TDI and % PTV, all disturbed reed pans in both seasons were classified as high quality, i.e. free of both organic pollution and inorganic nutrients. During the winter only one reference pan was found to be free of organic pollution. Other reference sites were classified as bad, poor and moderate respectively. All reference sites were classified as bad or poor in the summer and regarded as being heavily or significantly polluted. The TDI score for S1 was 16.9 which was the highest of all sites. S1 had the highest concentration of phosphorous and was ordinated in Figure 25 as correlating strongly to high TP levels. The percentage of pollution tolerant valves in S1 was 0.7%. Site

S2, also a reference reed pan, had a TDI score of 5.3 but 77.9% pollution tolerant values. Disturbed reed pans had TDI score of 13.9 and 16.2 in the winter but with 3.3 and 0 %PTV. The conditions of these pans are high in terms of organic pollution though disturbed by AMD-pollution. TDI scores were lowered in the summer and %PTV increased but remained under 20%.

5.2.1.2 Salt pan ecological condition according to TDI and %PTV

During the winter, two of the three reference salt pans were classified as having bad water quality according to TDI scores, one as high, while both reference sites were classified as high quality during the summer. Site S9 had the lowest PO_4^{2-} and TP levels among reference salt pans though still much higher than in reed or grass pans but had a very small percentage of pollution tolerant taxa. Sites S7 and S10 had higher %PTV values than disturbed sites. Three moderately disturbed sites were classified as bad and one as moderate quality. Three disturbed salt pans were designated as high quality. The highly disturbed pan was classified as being of moderate quality. Three moderately disturbed sites were labelled as moderate and three as high during the following season. In the summer, the TDI and %PTV classified the highly disturbed pan as bad.

5.2.1.3 Grass pan ecological condition according to TDI and %PTV

Out of seven reference grass pans, two were classified as bad quality, two as poor and three as high. The seven reference sites were rated as bad (3) and moderate (4) during the summer. The two moderately disturbed sites were characterized as bad and poor, but high and moderate in the summer. The highly disturbed site was ranked as bad. During the summer the site was classified as poor.

Overall, many AMD-disturbed reed, salt and grass pans were deemed to be in good condition according to the TDI and %PTV. The influence of AMD may limit the diatom community to those taxa that are not tolerant to organic pollution. Disturbed reed sites were dominated by *Achnanthes minutissimum*, which occurred in disturbed salt pans (especially moderately disturbed pans) to a greater extent than in reference salt pans as well and was strongly associated with greater sulphate to chloride ratios. *Achnanthes minutissimum* is somewhat sensitive to organic pollution (Sonneman *et al.*, 2000) and reflects lower nutrient conditions, causing riverine indices to classify sites in which this species was prevalent as being in good condition.

5.2.2 Generic diatom index (GDI)

5.2.2.1 Reed pan ecological condition according to GDI

The Generic Diatom Index (GDI) is an index for which diatoms need only be identified to genus level. This index that uses 174 taxa to ascertain water quality according to five sensitivity classes (Coste and Ayphassoro, 1991) and offers a reliable representation of general riverine water quality (Wu, 1999; Taylor *et al*, 2007b, Taylor *et al.*, 2007c). According to the scores generated by this index, three quarters of the reference reed pans could be classified as bad quality with the remaining quarter classified as good quality. One disturbed site was classified as having water of moderate quality while the other as having water of high quality during the dry season. All reference sites displayed bad quality during the wet season. Simultaneously, both disturbed sites were ranked as moderate. Though impacted by AMD, disturbed reed sites were classified by the GDI as being in better condition than reference sites.

5.2.2.2 Salt pan ecological condition according to GDI

During the winter, all three reference salt pans had GDI scores ranking them as bad quality. Four moderately disturbed sites were characterized as bad, one as poor and two as high while the highly disturbed pan was characterized as bad. The moderately disturbed sites, S22 and S23, that were categorised as high, were dominated by *Encyonopsis microcephala* and *Encyonopsis cesatii*, respectively. Nitrates and phosphorous concentrations were lower compared to other disturbed sites though the % $\text{SO}_4^{2-}:\text{Cl}^-$ in these sites were the highest. The reference sites were ranked as bad and poor in the wet season. Four moderately disturbed sites were characterized as bad, one as poor and one as moderate. The highly disturbed pan was again characterized as bad.

5.2.2.3 Grass pan ecological condition according to GDI

Three reference sites were ranked according to GDI scores as bad, two as poor, one as moderate and one as good quality. Site S13 was the only reference site to show good quality. In the winter S13 had the highest pH and the greatest number of *Achnanthyidium minutissimum* individuals among all reference grass pans. Second to S13 in the number of *A minutissimum*, was S15 which had been classified as having moderate condition. Moderately disturbed sites (based on water chemistry data) were classified as bad (two) and poor (one). The highly disturbed site displayed poor quality. Subsequently, three reference sites were classified as bad and four as poor during the summer. The index ranked both moderately disturbed sites as bad. The highly disturbed site displayed bad quality in the summer. *A minutissimum*

occurred less frequently in reference sites during the summer and was absent from disturbed sites.

5.2.3 Specific Pollution Sensitivity Index (SPI)

5.2.3.1 Reed pan ecological condition according to SPI

The Specific Pollution Sensitivity index (SPI) is known to correlate significantly with riverine water quality variables (Kwardans *et al.*, 1998; Taylor, 2004; Matlala *et al.*, 2011). Similar to the GDI, the first reference reed pan was classified as moderate quality, the remaining three sites as bad quality. Disturbed sites were likewise characterized as good and high quality respectively. In the summer all reference pans were ranked as bad and both disturbed sites as poor quality. Disturbed reed pans showed less evidence of organic pollution according to the SPI and were dominated by *Achnantheidium minutissimum* during both seasons.

5.2.3.2 Salt pan ecological condition according to SPI

Analogous to the GDI, all three reference salt pans achieved scores ranking them as bad quality during the winter. Of the moderately disturbed pans, four were classified as bad, one as poor, one as good and one as high (S22 and S23). The highly disturbed site was classified as poor by the SPI. During the summer all reference salt pans were still characterized as being of bad quality. A single moderately disturbed pan was ranked as good, one as poor and four as bad. The highly disturbed pan was again ranked as bad quality.

5.2.3.3 Grass pan ecological condition according to SPI

The SPI classified three reference grass pans as poor, two as bad and two as high quality (S13 and S15). Moderately disturbed pans were ranked bad quality. The highly disturbed site was characterized as bad. In the summer, four reference sites displayed SPI score indicative of bad quality. The remaining three were characterized as poor. The moderately disturbed sites were classified as poor and bad, respectively and the highly disturbed site was again ranked as being in bad condition based on the scores obtained.

5.2.4 Biological Diatom Index (BDI)

5.2.4.1 Reed pan ecological condition according to BDI

Concerning samples taken during the dry season, the Biological Diatom Index (BDI) classified one reference reed site as bad quality, one as moderate and two as poor. Disturbed sites displayed good and high quality, respectively. In the summer reference sites were respectively classified as high, moderate, poor and bad while the two disturbed sites achieved a rating of moderate quality.

5.2.4.2 Salt Pans pan ecological condition according to BDI

One reference salt pan obtained index scores ranking it as being of bad quality, the other as poor quality. Also in the winter, three moderately disturbed sites were classified as bad, one as poor, one as good and two as high. Reference sites displayed bad and poor quality again in the summer. Four moderately disturbed sites were classified as bad. One displayed good quality and one high quality. The highly disturbed site displayed bad quality based on the BDI scores thereof.

5.2.4.3 Grass Pans pan ecological condition according to BDI

Winter reference grass pans were labelled as high (two), moderate (three), poor (one) and bad (one) according to obtained index scores. Both moderately disturbed sites displayed scores indicative bad quality. The highly disturbed site was ranked as bad. Index scores from samples taken in the summer classify two reference sites as bad, one as poor, three as moderate and one as high. Moderately disturbed sites were classified as poor and bad, whilst the highly disturbed site displayed bad quality in each instance.

Overall, the BDI was similar to the SPI in its classification of pan ecological condition. Sites such as S13, S15, S22 and S23 obtained higher scores. The BDI classified grass pans that were found by the GDI and SPI to be in poor or bad condition, as being in moderate condition.

5.2.5 Eutrophication/ Pollution Index (EPI)

5.2.5.1 Reed pan ecological condition according to EPI

The Eutrophication/Pollution Index (EPI) is useful for determining eutrophication levels. The classifications of pans condition according to the EPI was very similar to those of the BDI.

In the winter two reference reed sites achieved scores indicating moderate quality with regards to eutrophication. One site displayed poor quality and the other bad quality. Disturbed sites were rated as being of good and high quality, respectively. Later, in the summer, three reference sites were classified as bad quality and one as poor. Both disturbed sites were ranked as moderate quality.

5.2.5.2 Salt pan ecological condition according to EPI

Two reference salt pans were classified as possessing bad quality and one as moderate in the winter. Four moderately disturbed sites also indicated bad quality. The remaining moderately disturbed sites individually were of moderate, good and high quality. Both reference sites were characterized as bad quality in the summer in addition to four moderately disturbed sites. One moderately disturbed site was of poor quality, the other of good quality. Finally, the highly disturbed salt pan's quality lowered from poor in the winter, to bad in the summer.

5.2.5.3 Grass pan ecological condition according to EPI

According to levels of eutrophication in reference grass pans, one pan was distinguished at possessing bad quality, one as poor, three as moderate, one as good and the last as high quality. Both moderately disturbed sites displayed bad quality with regard to eutrophication. The highly disturbed site was classified as bad in both seasons. During the summer a single reference site displayed bad quality, three displayed poor quality and three exhibited moderate quality.

5.2.6 Trophic Diatom Index for Lakes (TDIL)

5.2.6.1 Reed pan ecological condition according to TDIL

Another trophic diatom index, Stenger-Kovacs and Padisak's Trophic Diatom Index for Lakes (2007) rated one reference reed pan as poor quality and the remaining three as bad in both the dry and wet seasons. Disturbed sites exhibited moderate and good water quality in the winter which shifted to poor and bad, respectively in the summer.

5.2.6.2 Salt pan ecological condition according to TDIL

In the winter as well as in the summer, all reference salt pans were classified as bad quality. Four moderately disturbed pans were ranked as bad, one as poor, one as moderate and the last as high. Five of the moderately disturbed pans were classified as bad in the summer and the last as high quality. The highly disturbed site displayed bad quality in both seasons.

5.2.6.3 Grass pan ecological condition according to TDIL

According to index scores, five reference grass pans were classified as being of bad quality in terms of trophic state whereas two sites were of moderate quality. Both moderately disturbed sites exhibited moderate quality in the winter. The highly disturbed pan was characterized as bad quality. During the summer five reference grass pans were classified as being of bad quality, one poor and one moderate. The moderately disturbed sites exhibited bad quality in the summer, as did the highly disturbed site.

The TDIL categorised most pans as bad or poor. Only highly disturbed reed and moderately disturbed salt pans were ranked as good or high. In contrast, many disturbed sites and a few reference sites, especially salt pans, were regarded as being in very good condition (high) by the TDI and %PTV though it was developed of rivers and pans are more comparable to lakes. This may be due to the considerable number of *Amphora veneta* and *Cyclotella meneghiniana* in salt pans and *Achnanthes minutissimum* in reed and grass pans (Stenger-Kovács *et al.*, 2007). Nitrogen and phosphorous levels were higher in reference reed pans and phosphorous levels were the highest in reference salt pans.

Table 14: Water quality classes obtained from various diatom indices.

	Disturbance level	MMI1	MMI2;3;4	TDI%PT	GDI	SPI	BDI	EPI	TIDL
S1_R_Wint	Low	Poor	Poor	High	Good	Moderate	Moderate	Moderate	Poor
S2_R_Wint	Low	Good	Good	Bad	Bad	Bad	Bad	Bad	Bad
S3_R_Wint	Low	Good	Good	Poor	Bad	Bad	Poor	Moderate	Bad
S4_R_Wint	Low	Good	Good	Moderate	Bad	Bad	Poor	Poor	Bad
S27_R_Wint	High	Poor	Poor	High	Moderate	Good	Good	Good	Moderate
S28_R_Wint	High	Poor	Poor	High	High	High	High	High	Good
S1_R_Sum	Low	Good	Good	Bad	Bad	Bad	High	Bad	Poor
S2_R_Sum	Low	Fair	Good	Bad	Bad	Bad	Bad	Bad	Bad
S3_R_Sum	Low	Good	Good	Poor	Bad	Bad	Moderate	Poor	Bad
S4_R_Sum	Low	Good	Poor	Poor	Bad	Bad	Poor	Bad	Bad
S27_R_Sum	High	Poor	Poor	High	Moderate	Poor	Moderate	Moderate	Poor
S28_R_Sum	High	Poor	Poor	High	Moderate	Poor	Moderate	Moderate	Bad
S6_S_Wint	Med	Poor	Poor	Bad	Bad	Bad	Bad	Bad	Bad
S7_S_Wint	Low	Good	Poor	Bad	Bad	Bad	Bad	Moderate	Bad
S9_S_Wint	Low	Fair	Fair	High	Bad	Bad	Bad	Bad	Bad
S10_S_Wint	Low	Good	Good	Bad	Bad	Bad	Bad	Bad	Bad
S17_S_Wint	Med	Poor	Poor	Bad	Bad	Bad	Bad	Bad	Bad
S18_S_Wint	Med	Poor	Poor	Bad	Bad	Bad	Poor	Bad	Bad
S20_S_Wint	Med	Good	Fair	Moderate	Bad	Bad	Bad	Bad	Bad
S21_S_Wint	High	Poor	Poor	High	Poor	Poor	Good	Moderate	Poor
S22_S_Wint	Med	Poor	Poor	High	High	Good	High	Good	Moderate
S23_S_Wint	Med	Poor	Poor	High	High	High	High	High	High
S26_S_Wint	High	Poor	Poor	Moderate	Bad	Poor	Moderate	Poor	Bad
S6_S_Sum	Med	Good	Poor	Moderate	Bad	Bad	Bad	Bad	Bad
S7_S_Sum	Low	Good	Good	High	Poor	Bad	Poor	Bad	Bad
S10_S_Sum	Low	Good	Fair	High	Bad	Bad	Bad	Bad	Bad
S17_S_Sum	Med	Fair	Fair	Moderate	Bad	Bad	Bad	Bad	Bad
S18_S_Sum	Med	Good	Fair	High	Poor	Bad	Bad	Bad	Bad
S20_S_Sum	Med	Fair	Fair	Moderate	Bad	Bad	Bad	Bad	Bad
S22_S_Sum	Med	Poor	Poor	High	Bad	Poor	Good	Poor	Bad
S23_S_Sum	Med	Poor	Poor	High	Moderate	Good	High	Good	High
S26_S_Sum	High	Poor	Poor	Bad	Bad	Bad	Bad	Bad	Bad
S5_G_Wint	Low	Fair	Good	Bad	Bad	Poor	Moderate	Moderate	Bad
S8_G_Wint	Low	Fair	Good	Bad	Bad	Bad	Moderate	Poor	Bad
S11_G_Wint	Low	Good	Good	Poor	Poor	Poor	Poor	Moderate	Bad
S12_G_Wint	Low	Good	Good	High	Poor	Bad	Bad	Bad	Bad
S13_G_Wint	Low	Good	Poor	High	Good	High	High	High	Moderate
S14_G_Wint	Low	Good	Good	Poor	Bad	Poor	Moderate	Moderate	Bad
S15_G_Wint	Low	Good	Fair	High	Moderate	High	High	Good	Moderate
S16_G_Wint	Med	Poor	Poor	Bad	Bad	Bad	Bad	Bad	Bad
S19_G_Wint	Med	Fair	Poor	Poor	Bad	Bad	Bad	Bad	Bad
S24_G_Wint	High	Fair	Fair	Bad	Poor	Bad	Bad	Bad	Bad
S5_G_Sum	Low	Poor	Good	Bad	Bad	Bad	Poor	Moderate	Bad
S8_G_Sum	Low	Fair	Good	Moderate	Poor	Poor	High	Moderate	Poor
S11_G_Sum	Low	Good	Poor	Moderate	Poor	Bad	Bad	Bad	Bad
S12_G_Sum	Low	Good	Fair	Moderate	Poor	Poor	Moderate	Poor	Bad
S13_G_Sum	Low	Good	Good	Moderate	Poor	Poor	Moderate	Moderate	Moderate
S14_G_Sum	Low	Good	Good	Bad	Bad	Bad	Bad	Poor	Bad
S15_G_Sum	Low	Good	Good	Bad	Bad	Bad	Moderate	Poor	Bad
S16_G_Sum	Med	Poor	Poor	High	Poor	Poor	Poor	Bad	Bad
S19_G_Sum	Med	Fair	Good	Moderate	Bad	Bad	Bad	Bad	Bad
S24_G_Sum	High	Poor	Poor	Poor	Bad	Bad	Bad	Bad	Bad

In general, riverine indices tended to classify disturbed reed pans as good or high quality and reference reed pans as poor or bad. However, S1 was ranked as good by the TDI and GDI and moderate by the SPI, BDI and EPI. Altogether, reed sites showed a decline in conditions during the summer. The indices proclaimed most salt pans to be in bad condition with the exception of S22 and S23 which had good or high quality according to all indices in the winter and the majority of indices in the summer. Table 14 shows the level of AMD disturbance and the water quality classes obtained from various diatom indices. The AMD-MMIs yielded a greater number of water quality classifications that correspond with disturbance level than riverine indices.

5.2.7 Percentage of correctly classified instances

To compare accuracy of the ratings of ecological condition given by the indices to the reference and disturbed sites of each type of pan, the percentage of correctly classified instances (% CCI) of the indices are compared.

Table 15: Percentages of reed, grass and salt pans disturbance levels classified correctly

	MMI1	MMI2(Reed) MMI3(Salt) MMI4(Grass)	TDI &%PT	GDI	SPI	BDI	EPI	TDIL
Reed sites (12)	83.33	83.33	8.33	8.33	16.67	8.33	0	16.67
Salt sites (20)	80	85	40	20	15	5	10	20
Grass sites (20)	75	75	35	25	25	25	20	10

The above table exhibits the percentages of correctly classified instances, i.e. low disturbance (reference) sites classified as good or high quality by different indices, as well as the percentages of moderately disturbed sites rated as moderate or fair quality and highly disturbed sites as poor or bad quality. For example, if an index classifies only one reference site as good and two highly disturbed sites as poor and bad respectively, out of twelve reed sites, only three are considered correctly classified the percentage of CCI would be 25%.

Reed pans had very few correctly classified instances among riverine indices. Of these, the SPI and TIDL were most accurate but only classified nearly 17% of pans correctly. MMI1 and MMI2 characterized 83% of reed sites correctly. Though S1 lacks evidence of AMD disturbance, the diatom community in this reference site was dominated by *Epithemia adnata*. MMI2 consists of metrics from a single category, similarity to reference sites. S1 differed considerably from other reference sites in terms of % reference taxa and % similarity to reference sites which resulted in a lower score. Index scores for S1 were still notably higher than disturbed pan scores, which were especially influenced by higher % tolerant taxa in disturbed sites.

The TDI classified 40% of salt pans correctly. The GDI, SPI and the TIDL achieved better results than the EPI and BDI (5% CCI). MMI1 classified 80% of salt pans correctly. The reference site S9 was categorised as fair. Sites S6 and S20 was classified as good in the winter and S18 as good in the summer. MMI3 ranked S6 as poor in both seasons and ranked S18 and S20 as fair. S20 and S18 were the least disturbed AMD-impacted sites but were not similar enough to reference sites to be considered good condition. Only two reference sites were sampled in the summer and S10 was classified as fair due to having a lower score than S7 and thus representing the 5th percentile of reference site score. MMI3 classified 85% of salt pans correctly.

Riverine indices performed better among grass pans than reed or salt pans. The TDI distinguished 35% of pans correctly and the GDI, SPI and BDI each ranked 25% of grass pans correctly. The EPI was most inaccurate in reed pans, the BDI performed the worst in salt pans and the TIDL fared poorest in grass pans. In comparison with all reference sites, 75% of grass pans were classified correctly by MMI1. MMI4 ranked 75% of grass pans correctly. Reference sites S13 was classified as poor in the winter and S11 and S12 were ranked as poor and fair in the summer. S11 and S12 were the most alkaline grass pans and though not showing signs of AMD-impacts, their diatom communities correlated strongly with alkalinity and chloride and differed from those of other reference sites. S24 was ranked as fair in the winter. This site had high calcium concentrations and the % $\text{SO}_4^{2-}:\text{Cl}^-$ was lower due to a high concentration of

chloride and lower sulphate concentrations. The classification of S24 in the winter could then be considered correct.

Table 16 shows the percentages of correctly classified instances when considering the classification of reference sites as either high, good or fair to be acceptable or considered correct and the classification of moderately disturbed sites as moderate, poor or bad and highly disturbed sites as poor or bad quality. This was calculated simply to illustrate the differentiation of reference and disturbed sites with somewhat less emphases on the specific level of disturbance. All indices could be considered slightly more accurate in salt and grass pans.

Table 16: Percentages of reed, grass and salt pans disturbance levels classified correctly

	MMI1	MMI2(Reed) MMI3(Salt) MMI4(Grass)	TDI &%PT	GDI	SPI	BDI	EPI	TDIL
Reed sites (12)	91.67	83.33	8.33	8.33	16.67	8.33	0	16.67
Grass sites (20)	85	80	40	40	40	45	40	30
Salt sites (20)	85	95	55	65	60	45	55	65

The classification of moderately disturbed sites ranged from high to bad condition according to different indices. Table 17 below shows the percentages of moderately disturbed grass (4) and salt (12) pans classified as good or high, moderate, poor and bad. Reed pans have been excluded as all reed pans are either reference sites or highly disturbed.

Table 17: Percentages of moderately disturbed grass (4) and salt (12) pans classified as good or high, moderate, poor and bad.

	MMI1	MMI2(Reed) MMI3(Salt) MMI4(Grass)	TDI &%PT	GDI	SPI	BDI	EPI	TDIL
% of Moderately Disturbed Grass Pans Classified as:								
Good	0	25	25	0	0	0	0	0
Moderate	50	0	25	0	0	0	0	0
Poor	50	75	25	25	25	25	0	0
Bad			25	75	75	75	100	100
% of Moderately Disturbed Salt Pans Classified as:								
Good	25	0	41.67	16.67	25	33.33	25	16.67
Moderate	16.67	33.33	33.33	8.33	0	0	0	8.33
Poor	58.33	66.67	0	8.33	8.33	8.33	8.33	0
Bad			25	66.67	66.67	58.33	66.67	75

With the exception of the TDI, riverine indices classified moderately disturbed sites as bad quality. The TDI classified most moderately disturbed salt sites as good or moderately. All other indices classified most moderately disturbed salt sites as bad or poor.

Table 18 displays the percentages of sites which opposing indices rated correspondingly as non-impacted and impacted.

Table 18: Percentages of sites with the same classification according to different indices

	MMI1	MMI2	MMI3	MMI4
TDI & %PT	42.59	8.33	55	31.82
GDI	9.26	33.33	80	45.45
SPI	50.00	33.33	75	36.36
BDI	44.44	41.67	65	40.91
EPI	50.00	33.33	75	36.36
TDIL	51.85	41.67	80	50.00
MMI1		83.33	75	59.09

The TDI's classification of pan condition had a 43% correspondence with those of MMI1 and 55% correspondence with those of MMI3. Classifications of the TDI correspond least with those of MMIs for reed and grass pans. The GDI corresponded most with MMI3 (only in terms of ranking sites as disturbed or not). The SPI's classifications also coincided mostly with MMI3 and MMI1. The BDI corresponded the most with MMI3 as did the EPI and TIDL. Among the AMD-MMIs, the classifications of MMI1 corresponded most with those of MMI2 for reed pans and least with MMI4 for grass pans. Four grass pans that were rated as being in good condition by MMI4 were rated as fair by MMI1. Differences in the classification of water quality by the MMI's were primarily one class lower or higher (i.e. good and fair or fair and poor).

Table 19 shows Cohen's Kappa coefficient calculated for comparison between MMIs and riverine indices and between different MMI's.

Table 19: Cohen's Kappa coefficients

	MMI1	MMI2	MMI3	MMI4
TDI%PT	-0.19873	-0.83333	0.021739	-0.22642
GDI	-0.26355	-0.33333	-0.11111	-0.10092
SPI	-0.09208	-0.33333	-0.13636	-0.2037
BDI	-0.15372	-0.16667	-0.16667	-0.1215
EPI	-0.09208	-0.33333	-0.13636	-0.2037
TDIL	-0.11367	-0.16667	-0.11111	0
MMI1		0.666667	0.342105	0.1

As an additional measure of the agreement between riverine indices and MMIs, Cohen's Kappa coefficient (K) was calculated and mostly negative values were yielded, which signify disagreement. The highest value of agreement indicated substantial agreement between MMI1 and MMI2. MMI3 and MMI4 displayed fair and slight agreement with MMI1, respectively.

The various indices differ much in their classifications of pan ecological condition. AMD-MMI's displayed greater agreement in their classifications and were more in accord with levels of disturbance than riverine indices.

5.2.8 Correlation between different diatom indices and water quality variables

Table 20: Pearson correlations between water quality classifications revealed by diatom indices and environmental variables, and Pearson correlations between environmental variables and the factors pan type, season and disturbance level. Marked correlations are significant at $p < 0,05$.

	TDI %PT	GDI	SPI	BDI	EPI	TIDL	MMI1	MMI2;3;4	Pan type	Season	Disturbance level
Depth	0,125	0,285	0,271	0,288	0,254	0,274	0,204	0,301	-0,264	0,036	0,186
Temp	0,011	-0,254	-0,189	-0,062	-0,138	-0,107	0,072	0,059	-0,013	0,785	0,117
pH	-0,031	-0,028	-0,097	-0,233	-0,205	-0,070	-0,059	-0,231	0,177	-0,514	-0,139
K	0,135	0,183	0,164	0,083	0,098	0,174	-0,444	-0,440	0,354	-0,444	-0,406
Na	-0,011	0,013	-0,082	-0,181	-0,177	-0,034	-0,293	-0,374	0,289	-0,423	-0,255
Ca	0,292	0,454	0,364	0,184	0,222	0,414	-0,472	-0,423	0,149	-0,253	-0,521
Mg	0,123	0,193	0,198	0,037	0,076	0,337	-0,390	-0,309	0,273	-0,339	-0,415
Ammonia	-0,019	-0,216	-0,293	-0,100	-0,232	-0,218	-0,022	0,207	0,073	0,260	-0,076
SO4	0,274	0,286	0,217	0,090	0,110	0,317	-0,588	-0,553	0,442	-0,205	-0,551
Cl	-0,129	-0,079	-0,149	-0,245	-0,210	-0,116	-0,135	-0,241	0,253	-0,449	-0,113
Alkalinity	-0,106	-0,124	-0,179	-0,267	-0,232	-0,153	-0,136	-0,271	0,271	-0,492	-0,178
Nitrate	-0,198	0,018	0,071	-0,148	-0,028	-0,021	-0,220	-0,266	0,144	-0,720	-0,207
EC	0,075	0,116	0,039	-0,094	-0,064	0,138	-0,367	-0,415	0,326	-0,405	-0,318
TDS	0,177	0,083	0,094	0,010	0,030	0,166	-0,310	-0,368	0,303	-0,256	-0,117
Hardness	0,371	0,337	0,376	0,249	0,276	0,449	-0,479	-0,428	0,248	-0,202	-0,340
Cations	0,165	0,146	0,108	-0,004	0,022	0,205	-0,380	-0,439	0,340	-0,363	-0,236
Anions	0,164	0,145	0,112	-0,007	0,022	0,213	-0,375	-0,448	0,352	-0,368	-0,239
Mg Hardness	0,205	0,162	0,240	0,104	0,153	0,368	-0,361	-0,294	0,287	-0,228	-0,247
%SO4:Cl	0,407	0,377	0,361	0,304	0,295	0,443	-0,526	-0,400	0,269	0,152	-0,505
PO4	-0,194	-0,450	-0,497	-0,370	-0,478	-0,359	-0,007	-0,048	0,292	0,436	-0,052
TP	-0,226	-0,330	-0,368	-0,327	-0,376	-0,234	-0,120	-0,189	0,333	0,171	-0,110

The TDI exhibited significant ($p < 0,05$) correlations with indicators of AMD, especially $\text{SO}_4^{2-}:\text{Cl}^-$ (40.7%). Disturbed reed pans had very low percentages of pollution tolerant valves. Reference grass and salt pans likewise had higher percentages of pollution tolerant valves than disturbed grass pans and is the reason for the TDI's positive correlations with AMD-indicators. With the exception of S9, reference salt pans also displayed higher percentages of pollution tolerant valves than disturbed sites in the winter. In the summer, reference salt pans had lower %PTV than the highly disturbed pan but higher than moderately disturbed pans S22 and S23. These two sites and both reference salt sites were ranked as high quality by the TDI. AMD-impacted pans display low percentages of pollution tolerant valves that indicate limited nutrient enrichment, resulting in high TDI scores.

The GDI and SPI were very similar in their correlations with environmental variables and presented significant positive correlations with $\text{SO}_4^{2-}:\text{Cl}^-$ and Ca^{2+} . The GDI, SPI, BDI and EPI correlated negatively with ammonia, phosphates and total phosphorous (Bere *et al.*, 2014; Holmes and Taylor, 2015). The TDIL was similar to MMI1 in that it had significant correlations with Ca^{2+} , Mg^{2+} , SO_4^{2-} , hardness and $\text{SO}_4^{2-}:\text{Cl}^-$.

All indices had significant correlation with SO_4^{2-} , $\%\text{SO}_4^{2-}:\text{Cl}^-$. However, riverine indices corresponded positively and MMIs correlated negatively and more strongly. MMIs indicate AMD-impact correctly as evidenced by strong negative correlations with $\%\text{SO}_4^{2-}:\text{Cl}^-$ and Ca^{2+} . The AMD-MMIs demonstrated more and stronger correlations with environmental variables than other diatom indices. Particularly in the cases of EC, Ca^{2+} , SO_4^{2-} and $\%\text{SO}_4^{2-}:\text{Cl}^-$. MMI scores correlated negatively with Ca^{2+} , K^+ and Na^+ while riverine indices only corresponded negatively with Na^+ .

Evidence of the MMIs' effectiveness is confirmed by strong negative correlations between AMD-disturbance level and Ca^{2+} , SO_4^{2-} , EC and $\%\text{SO}_4^{2-}:\text{Cl}^-$. Pan type exhibited significant correlations with most environmental variables and only correlated negatively with depth and temperature though not substantially. Environmental variables that did not show significant correlations with seasonal change include Ca^{2+} , SO_4^{2-} , $\%\text{SO}_4^{2-}:\text{Cl}^-$, TDS, hardness, ammonia and total phosphorous. Ca^{2+} , SO_4^{2-} , $\%\text{SO}_4^{2-}:\text{Cl}^-$ and EC are positively correlated with pan type and negatively with disturbance level. Variables such as Ca^{2+} , SO_4^{2-} , $\%\text{SO}_4^{2-}:\text{Cl}^-$ are most strongly correlated with disturbance level and are indicative of AMD-impacts across pan types and seasons.

Though care was taken to avoid pans impacted by agricultural activities, agriculture is widespread in the Mpumalanga Highveld and very few ecosystems remain completely undisturbed by mining or agricultural activities (Ferreira *et al.*, 2012 and Riato *et al.*, 2014). The effects of agricultural activities may be seen especially in reference pans that are situated far from mines but surrounded by grazing cattle which may contribute to the classification of reference pans as poor or bad by riverine indices. High concentrations of nutrients, particularly nitrogen and phosphorous, have been observed in salt pans where flamingos were present (Riato, 2017).

CHAPTER 6: CONCLUSIONS AND RECOMENDATIONS

6.1 Conclusions

The objective of the present study was to determine whether the diatom based multimetric indices can be used to accurately distinguish sites impacted by coal mining activities from those impacted to a lesser degree or not at all. Diatom-based multimetric indices developed for reed, salt and grass pans were compared to well established riverine indices that have in previous studies been recommended for use in wetlands (Matlala *et al.*, 2011 and Olivier, 2016). In order to understand and interpret the results of the applied indices, the differences in physical and chemical variables and consequent differences in diatom community compositions of the various pan types were determined.

Reed, salt and grass pans each had characteristic water quality properties. Salt pans far exceeded other types of in pans in terms of their alkalinity and conductivity while grass pans had the lowest concentrations of solutes. The distinctive water quality features of the different pan types determine the composition of the diatom communities therein. The diatom communities of grass and reed pans were equally dissimilar (81%) from those in salt pans. The variance in properties of water quality of reed and grass pans was reflected in the structural heterogeneity of their diatom communities which was 73% dissimilar. Pan type was found to be second most important factor that determines diatom community composition.

Definite differences in the measures of water quality variables were evident between reference sites and AMD-disturbed. The quantities of ions such as SO_4^{2-} and Ca^{2+} and SO_4^{2-} to Cl^- ratios, that are indicators of AMD disturbance, were remarkably greater in disturbed pans, regardless of pan type or season. AMD-disturbance level was observed to be the factor most responsible for variance in diatom community compositions. Canonical Correspondence Analysis (CCA) (figures 25; 27 and 29) revealed strong correlations between environmental variables and diatom species. Among all three pan types, characteristic reference site taxa such as *Gomphonema* aff. *gracile* and *Sellaphora seminulum* were ordinated in correspondence with better water quality and tolerant taxa such as *Encyonopsis microcephala* and *Fragilaria capucina* set disturbed sites apart. Seasonal change did not significantly influence diatom community composition in depressional wetlands according the SIMPER analysis (figure 24). Decreases in the concentration of some water quality variables were observed due to seasonal dilution. However, the relative differences between reference and disturbed site concentrations were sustained.

Six riverine diatom indices were applied to the same wetlands as the AMD-MMI, including the Trophic Diatom Index (TDI) and percentage of Pollution Tolerant Values (%PTV), the Generic Diatom Index (GDI), the Specific Pollution sensitivity Index (SPI), the Biological Diatom Index (BDI), The Eutrophication/Pollution Index/ EPI and Stenger-Kovacs and Padisak's Trophic Diatom Index for Lakes (TIDL). Index scores and ecological condition classifications were compared to those of AMD-MMIs. The MMIs for different pans were eminently better at distinguishing reference sites from disturbed sites and more accurate in discerning moderately disturbed sites from reference and highly disturbed sites. Riverine indices showed a tendency to rate reference sites as being in poor or bad condition and many highly disturbed pans as good quality.

MMI1 uses data from salt, reed and grass reference pans to ascertain the quality of reference pans for comparison to disturbed pans. MMI1 was equally accurate in establishing level of AMD-disturbance as MMI2 for reed pans and MMI4 for grass pans, but slightly less than accurate than MMI3 for salt pans. The classifications attained by MMI1 were most in agreement with those attained by MMI2. Between the MMIs, MMI1 and MMI4 displayed the least agreement. In terms of classification of ecological condition, all MMIs demonstrated agreement and definite disagreement with riverine indices.

Higher MMI scores were observed in non-impacted pans. Lower scores in impacted sites indicates proper correspondence with heavier coal mining impacts. According to the calculated Pearson correlations, MMIs correlated more strongly and negatively with AMD-indicators which demonstrates correct and reliable responses of MMIs to increasing levels of AMD-disturbance. Of the three factors tested, environmental variables correlated the most with disturbance level, followed by pan type and least with season. Water quality variables are mainly affected by disturbance level and determines the composition of diatoms communities in the pans accordingly. Shifts in the community composition caused by coal mining disturbance are reflected well by MMIs. In contrast conditions in reference pans are indicated as being poor using riverine indices, which then cannot discriminate between reference sites and impacted sites.

Thus, differences in the properties of water quality and diatom communities of different pan types have been established. It was determined AMD-disturbance is most important factor that influences diatom community and that seasonal change has negligible effects on diatom communities. Though the MMIs were developed using only winter diatom community data, it is the case that AMD-MMIs can be applied in the wet season as well, as seasonal effects can be mitigated by using same-season reference site data. MMIs correlated strongly and

negatively with AMD indicators and were 75% or more accurate in determining AMD-disturbance level.

Depressional wetlands have characteristic water quality parameters that have been established to define a more natural and desired state. The AMD-MMI's measure deviation from a pan's defined natural state while indices such as the GDI and BDI reflect water quality in general according to a broader standard based on the ecological preferences of diatom species. The well-established ecological preferences of diatoms make these organisms excellent indicators of pollution and conditions in general, but they have also proven reliable in demonstrating deviation from reference conditions. In the case of water bodies such as pans, of which the various types have specific properties that make them different from other wetlands and rivers, it may be more beneficial to use comparisons to reference conditions rather than a general standard. This approach may be more appropriate when dealing with a specific kind of pollution

7.1 Recommendations

Riverine indices are not recommended for use in depressional wetlands. Due to the lentic nature of pans, nutrients accumulate to a degree that is considered badly polluted according to the majority of riverine indices. Diatom species such as *Nitzschia palea* and *Sellaphora seminulum* were characteristic of reference grass and reed pans. Diatom communities from disturbed pans lack taxa indicative of larger amounts of organic material and exhibit conditions of low trophic status. *Nitzschia amphibia* and *Nitzschia palea* were characteristic of disturbed salt pans but occurred predominantly in reference reed and grass pans.

The diatom-based index for acid mine drainage impacted permanent pans proved effective in distinguishing between sites that are not impacted and sites highly or moderately impacted by coal mining activities. Non-impacted or reference sites are imperative for the calculation of index scores and it is suggested that a database of information on the diatom assemblages of each of the types of pans Mpumalanga Highveld, in their undisturbed state, be compiled for ease of future utilization. The index was developed specifically for depressional wetlands and to indicate AMD, but similar indices may be designed for the various other wetland types in the region. Care should be taken to select reference pans that are not impacted by agricultural activities and in the case of salt pans, to take into account the nutrient loading effects of flamingos.

BIBLIOGRAPHY

Akcil, A. and Koldas, S. 2006. Acid Mine Drainage (AMD): causes, treatment and case studies. *Journal of Cleaner Production*. 14: 1139-1145.

Allan, D.G., 1987. Transvaal Highveld pans. *African Wildlife*. 41: pp. 233–235.

Allan, D. G., Seaman, M.T. and Kaletja, B. 1995. The endorheic pans of South Africa (In Cowan, G. I., ed. *Wetlands of South Africa*. Department of Environmental Affairs and Tourism. p 76, 93, 254).

Battarbee, R. W., Charles, D. F., Dixit, S. S. and Renberg, I. 2004. Diatoms as indicators of surface water acidity. (In E. F. Stoermer and J. P. Smol, *The Diatoms: Applications for the Environmental and Earth Sciences* (pp. 85–126).

Bere, T., Mangadze, T. and Mwedzi, T. 2014. The application and testing of diatom-based indices of stream water quality in Chinhoyi Town, Zimbabwe. *Water SA* 40: 3.

Bidlack, J. E. and Jansky, S. H. 2014. *Stern's Introductory Plant Biology* 13th ed. New York: McGraw-Hill.

Borgmann, U., Couillard, Y., Doyle, P. and Dixon, D. 2005. Toxicity of sixty-three metals and metalloids to *Hyalella azteca* at two levels of water hardness. *Environmental Toxicology and Chemistry*, 24(3): 641-652.

Boyd, C., Tucker, C. and Somridhivej, B. 2016. Alkalinity and Hardness: Critical but Elusive Concepts in Aquaculture. *Journal of the World Aquaculture Society*, 47(1): 6-41

Burliga, A. L. and Kociolek, J. P. 2016. Diatoms (Bacillariophyta) in Rivers. (In O. Necchi ed. *River Algae*. São José do Rio Preto, SP, Brazil: Springer. pp. 93–127).

Cemagref. 1982. Etude des méthodes biologiques quantitatives d'appréciation de la qualité des eaux. Rapport Division Qualité des Eaux Lyon - Agence Financière de Bassin Rhône-Méditerranée- Corse. Pierre-Bénite.

Coetzee, M. A. S. 1995. Water pollution in South Africa: its impact on wetland biota (In Cowan, G. I., ed. *Wetlands of South Africa*. Department of Environmental Affairs and Tourism. p255).

Cohen, J., 1960. A coefficient of agreement for nominal scales. *Educational and psychological measurement*, 20:1.

Collins, N.B. 2005. Wetlands: The basics and some more. Free State Department of Tourism, Environmental and Economic Affairs.

Convention on wetlands of international importance, especially as waterfowl habitat. 1971. Certified Copy, 13 July 1994.UNESCO Paris.

Couceiro, S., Hamada, N., Forsberg, B., Pimentel, T. and Luz, S. 2012. A macroinvertebrate multimetric index to evaluate the biological condition of streams in the Central Amazon region of Brazil. *Ecological Indicators*, 18: pp.118-125.

Coste, M. and Ayphassorho, H. 1991. Étude de la qualité des eaux du Bassin Artois Picardie à l'aide des communautés de Diatomées benthiques (application des indices diatomiques). Rapport Cemagref Bordeaux - Agence de l'Eau Artois Picardie, Douai.

Cowan, G. I. 1995. South Africa and the RAMSAR Convention (In Cowan, G. I., ed. *Wetlands of South Africa*. Department of Environmental Affairs and Tourism. p1)

D'Ippolito, G., Sardo, A., Paris, D., Vella, F., Adelfi, M., Botte, P., Gallo, C. and Fontana, A. 2015. Potential of lipid metabolism in marine diatoms for biofuel production. *Biotechnology for Biofuels*, 8(1): 28.

Dallas, H. F.1997. A preliminary evaluation of aspects of SASS (South African Scoring System) for the rapid bioassessment of water quality in rivers, with particular reference to the incorporation of SASS in a national biomonitoring programme. *Southern African Journal of Aquatic Sciences*, 23 (1): 79- 94

Dallas, H. and Day, J. 2004. The effect of water quality variables on aquatic ecosystems. Gezina, South Africa: Water Research Commission.

Dallas, H., Kennedy, M., Taylor, J., Lowe, S. and Murphy, K. 2010. SAFRASS: Southern African River Assessment Scheme WP4: Review of existing biomonitoring methodologies and appropriateness for adaptation to river quality assessment protocols for use in southern tropical Africa.

Dalu, T., Bere, T. and Froneman, P. 2016. Assessment of water quality based on diatom indices in a small temperate river system, Kowie River, South Africa. *Water SA*, 42(2), p.183. http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S1816-79502016000200002. Date of access: 8 May 2018.

Dalu, T. and Froneman, P. W. 2016. Diatom-based water quality monitoring in southern Africa: challenges and future prospects. *Water SA*: 42(4).

De Klerk, A. R., De Klerk, L. P., Chamier, J. and Wepener, V. 2012. Seasonal variations of water and sediment quality parameters in endorheic reed pans on the Mpumalanga Highveld. *Water SA*, 38.

De Klerk, A. R., De Klerk, L.P., Oberholster, P. J., Ashton, P. J., Dini, J.A. and Holness, S. D. 2016. A review of depressional wetlands (pans) in South Africa, including a water quality classification system. Water Research Commission, pp.1, 3.

De Klerk, A. R. and Wepener, V. 2013. Macroinvertebrate Assemblage Changes as an Indicator of Water Quality of Perennial Endorheic Reed Pans on the Mpumalanga Highveld, South Africa. *Journal of Environmental Protection*, 4(7): pp.10-21.

DeNicola, D. M. 2000. A review of diatoms found in highly acidic environments. *Hydrobiologia* 433: 111-122.

Descy, J.-P., and Coste, M. 1991. A test of methods for assessing water quality based on diatoms, (December 2015). <http://doi.org/10.1080/03680770.1989.11899905>.

Dickens, C. W. S. and Graham, P. M. 2002. The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers, *African Journal of Aquatic Science*, 27 (1): 1-10.

Dixit, S. S. and Smol, J. R. 1994 Diatoms as indicators in the environmental monitoring and assessment program-surface waters (EMAP-SW). *Environmental monitoring and Assessment*: 31: 275-306.

Donnenfeld, Z., Crookes, C. and Hedden, S. 2018. A delicate balance: Water scarcity in South Africa. Southern Africa Report 13. Institute for Security Studies.

Driver, A., Sink, K. J., Nel, J. L., Holness, S., Van Niekerk, L., Daniels, F., Jonas, Z., Majiedt, P. A., Harris, L. and Maze, K. 2011. National Biodiversity Assessment 2011: An assessment of South Africa's biodiversity and ecosystems. Synthesis Report. South African National Biodiversity Institute and Department of environmental Affairs, Pretoria.

Dungumaro, E. W. 2007. Socioeconomic differentials and availability of domestic water in South Africa. *Physics and Chemistry of the Earth* 32: 1141–1147.

Ferreira, M., Wepener, V. and Van Vuren, J. H. J. 2012. Aquatic Invertebrate Communities of Perennial Pans in Mpumalanga, South Africa: A Diversity and Functional Approach. *African Invertebrates* 53 (2): 751–768.

Fosso-Kankeu, E., Manyatshe, A. and Waanders, F. 2016. Mobility potential of metals in acid mine drainage occurring in the Highveld area of Mpumalanga Province in South Africa: Implication of sediments and efflorescent crusts. *International Biodeterioration & Biodegradation*, 119: pp.661-670.

Gala, T. S. and Young, D. 2015. Geographically Isolated Depressional Wetlands – Hydrodynamics, Ecosystem Functions and Conditions. *Applied Ecology and Environmental Sciences*, 3(4): 108-116.

Harding, W. R., Archibald, C. G. M., and Taylor, J. C. 2005. The relevance of diatoms for water quality assessment in South Africa: A position paper. *Water SA*: 31(1): p 41-46.

Harding, W. R. and Taylor, J. C. 2011. The South African Diatom Index (SADI) - a preliminary index for indicating water quality in rivers and streams in southern Africa. WRC Report No. 1707/1/11. Water Research Commission (WRC).

Hattikudur, R., Alakananda, B. and Ramachandra, T. V. 2014. Diatoms as Bioindicators. LAKE 2014: Conference on Conservation and Sustainable Management of Wetland Ecosystems in Western Ghats, (April 2015). <http://ces.iisc.ernet.in/energy>. Date of Access: 1 Apr 2018. Holmes, M. and Taylor, J. C. 2015. Diatoms as water quality indicators in the upper reaches of the Great Fish River, Eastern Cape, South Africa, *African Journal of Aquatic Science*, 40:4, 321-337. DOI: 10.2989/16085914.2015.1086722.

Hopkins, J. T. and Drum, R. W. 1966 Diatom motility: An explanation and a problem. *British Phycological Bulletin*, 3 (1): 63-67.

Hoffman, A. 2012. The application of diatoms as indicators of grass pan health in Mpumalanga, South Africa. Unpublished Mini-dissertation. University of Johannesburg, Johannesburg, South Africa.

Hudon, C. and Legendre, P. 1987. The ecological implications of growth forms in epibenthic diatoms. *Journal of Phycology* 25: 434-441. Kelly, M. G., Juggins S., Guthrie, R., Pritchard, S., Jamieson J., Rippey, B., Hirst, H. and Yallop, M. 2008. Assessment of ecological status in UK rivers using diatoms. *Freshwater Biology*, 53: 403-422.

Kelly, M. G. and Whitton, B. A. 1995. The Trophic Diatom Index: A New Index for Monitoring Eutrophication in Rivers. *Journal of Applied Phycology* 7:433-444.

Kent, M. 2012. Vegetation description and data analysis; a practical approach. 2nd. ed. Chichester, West Sussex: John Wiley & Sons, Ltd.

Kleynhans, C. J. 1999. The development of a fish index to assess the biological integrity of South African rivers. Department of Water Affairs and Forestry, Pretoria.

Kotze, D. C., Ellery W. N., Macfarlane D. M. and Jewitt, G. P. W. 2012. A rapid assessment method for coupling anthropogenic stressors and wetland ecological condition. *Ecological Indicators* 13: 284-293.

Kwandrans, J., Eloranta, and P., Kawecka, B. 1998. Use of benthic diatom communities to evaluate water quality in rivers of southern Poland. *Journal of Applied Phycology* 10: 193–201 . <https://doi.org/10.1023/A:1008087114256>.

Legendre, P. 1987. The ecological implications of growth forms in epibenthic diatoms. *Journal of Phycology* 25: 434-441.

Lobo, E. A., Heinrich, C. G., Schuch, M., Wetzel, C. E., and Ector, L. 2016. Diatoms as bioindicators in rivers. (In Necchi, O. ed. *River Algae*). São José do Rio Preto, SP, Brazil: Springer. <http://doi.org/10.1007/978-3-319-31984-1> Date of access: 31 Mar 2018.

Loy, C. W., and Moi, P. S. 1999. Epiphytic diatoms on *Enhalus acoroides* (L.F.) Royle. *Malaysian Journal of Science*, 18: 83–89.

Mann, D. G. 2002. Diatoms: organism and image. (In Bunke, H and Wang, P. S. P. eds. *Automatic diatom identification* (pp. 9–40)). Singapore: World Scientific Publishing.

Mann, D.G. and Droop, S. M. (1996). 3. Biodiversity, biogeography and conservation of diatoms. *Hydrobiologia*, 336(1-3): pp.19-32.

Matlala, M. D., Taylor, J. C. and Harding, W. R. 2011. Development of a diatom index for wetland health. WRC Report No KV 270/11. Water Research Commission (WRC).

Majewska, R., Gambi, M., Totti, C., Pennesi, C. and De Stefano, M. 2013. Growth form analysis of epiphytic diatom communities of Terra Nova Bay (Ross Sea, Antarctica). *Polar Biology*, 36(1): 73-86.

Mereta, S., Boets, P., De Meester, L. and Goethals, P. (2013). Development of a multimetric index based on benthic macroinvertebrates for the assessment of natural wetlands in Southwest Ethiopia. *Ecological Indicators*, 29: pp.510-521.

Munnik, V., Hochmann, G., Hlabane, M. and Law, S. 2010. The social and environmental consequences of coal mining in South Africa: a case study. Cape Town, South Africa & Amsterdam, The Netherlands. Environmental Monitoring Group & Both ENDS.pp.5.

Musa, R. and Greenfield, R. 2018. Use of diatom indices to categorise impacts on and recovery of a floodplain system in South Africa, *African Journal of Aquatic Science*, 43 (1): 59-69.

National Water Act, 1998. Act No. 36. Government Gazette No. 19182, Government Printer, Pretoria South Africa, pp9.

Nodine, E. R., and Gaiser, E. E. 2013. Distribution of Diatoms Along Environmental Gradients in the Charlotte Harbor, Florida (USA), Estuary and Its Watershed: Implications for Bioassessment of Salinity and Nutrient Concentrations. *Estuaries and Coasts*, 37: 864-79.

Ochieng, G. M., Seanego, E. S. and Nkwonta, O. I. 2010. Impacts of mining on water resources in South Africa: A review. *Scientific Research and Essays*, 5(22): pp. 3351-3357.

Olivier, N., 2016. The diatoms of the Western Cape and their use in monitoring wetland water quality. MSc. Thesis, North-West University. Potchefstroom Campus, Potchefstroom.

Ollis, D., Snaddon, K., Job, N. and Mbona, N. 2013. Classification System for Wetlands and other Aquatic Ecosystems in South Africa. User manual: Inland Systems. South African National Biodiversity Institute (SANBI). Pretoria.

Pan, Y. and Stevenson. R. J. 1996. 'Gradient analysis of diatom assemblages in western Kentucky wetlands, *Journal of Applied Phycology*, 32: 222-32.

Parisara, N., Narayana, J., Kiran, B. R. and Puttaiah, E. T. 2016. Ecology and distribution of diatoms in Konandur pond of Thirthahalli Taluk, Karnataka. *International Journal of Applied and Pure Science and Agriculture (IJAPSA)*, 2(4): pp.49-55.

Passy, S. 2007. Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquatic Botany*, 86(2): 171-178

Pinetown, K.L., Ward, C.R. and Van der Westhuizen, W.A., 2007. Quantitative evaluation of minerals in coal deposits in the Witbank and Highveld Coalfields, and the potential impact on acid mine drainage. *Int. J. Coal Geol.* 70: 166-183.

Rand, G. M., Wells, P. G. and McCarty, L. S. 1995. Introduction to aquatic toxicology (In Rand, G. M. ed. *Fundamentals of aquatic toxicology: effects, environmental fate, and risk assessment*. 2nd ed. Washington, D.C.: Taylor & Francis. p3-69).

Ratshomo, K. and Nembahe, R., 2016. South African coal sector report. Directorate: energy data collection, management and analysis. Pretoria: Department of Energy, p.1.

Riato, L. 2017. Development of diatom-based monitoring tools for assessing depressional wetland condition in the Mpumalanga Highveld region, South Africa. Ph.D. University of Pretoria.

Riato, L., Leira, M., Della Bella, V., and Oberholster, P. J. 2017a. Development of a diatom-based multimetric index for acid mine drainage impacted depressional wetlands. *Science of the Total Environment*, 612: 214–222.

Riato, L., Leira, M., Della Bella, V. and Oberholster, P. J. 2017b. Diatom-based models for inferring water chemistry and hydrology in temporary depressional wetlands. *Hydrobiologia* 797:127–143.

Riato, L., Van Ginkel, C. and Taylor, J. 2014. Zooplankton and diatoms of temporary and permanent freshwater pans in the Mpumalanga Highveld region, South Africa. *African Zoology*, 49(1): pp.113-127.

Rimet F. and Bouchez A., 2011. Use of diatom life-forms and ecological guilds to assess pesticide contamination in rivers: Lotic mesocosm approaches. *Ecological Indicators*, 11(2): 489-499.

Rimet, F., and Bouchez, A. 2012a. Biomonitoring river diatoms: Implications of taxonomic resolution. *Ecological Indicators* 15: 92–99.

Rimet, F. and Bouchez, A. 2012b. Life-forms, cell-sizes and ecological guilds of diatoms in European rivers. *Knowledge and Management of Aquatic Ecosystems* 01.

Rimet, F., Ector, L., Cauchie, H. M. and Hoffmann, L. 2009. Changes in diatom-dominated biofilms during simulated improvements in water quality: implications for diatom-based monitoring in rivers. *European Journal of Phycology*, 44: 567-577.

Sabater, S. 2009. Diatoms. *Algae (Incl. Cynobacteria)*. Girona, Spain: Elsevier Inc., pp.149-156.

Salomoni, S., Rocha, O., Hermany, G. and Lobo, E. 2011. Application of water quality biological indices using diatoms as bioindicators in the Gravataí river, RS, Brazil. *Brazilian Journal of Biology*, 71(4): pp.949-959.

Schoeman, F. R. 1979. Diatoms as indicators of water quality in the Upper Hennops River (Transvaal, South Africa). *Journal of the Limnological. Society of South Africa*. 5: 73-78.

Seaman, M.T., Ashton, P.J. and Williams, W.D. 1999 Inland salt waters of southern Africa. *Hydrobiologia* 210: 75 . <https://doi.org/10.1007/BF00014324>.

Shaw, P. A. and Thomas D. S. G. 1989. Playas, pans and salt lakes. (In Thomas, D. S. G. ed. *Arid zone geomorphology*). Belhaven Press, London, England.

Sieben, E. J. J., Collins, N. B., Kotze, D. C., Mofutsanyana, S. S. and Janks, M. 2017. Temperate grassy wetlands of South Africa: Description, classification and explanatory environmental factors. *South African Journal of Botany*, 113: 68-76.

Siemińska, J. 2015. On the age of fossil diatoms. *Acta Palaeobotanica*, 55(1): 116-118.

Smucker, N. and Vis, M. 2009. Use of diatoms to assess agricultural and coal mining impacts on streams and a multiassemblage case study. *Journal of the North American Benthological Society*, 28 (3): pp.659-675.

Sonneman, J. A., Sincok, A., Fluin, J., Reid, M., Newall, P., Tibby, J. and Gell, P. 2000. *An Illustrated Guide to Common Stream Diatom Species from Temperate Australia*. Cooperative Research Centre for Freshwater Ecology Identification Guide No 33, Hawking, J. H. (ed.). Thurgoona, NSW: Land & Water Resources Research & Development Corporation.

StatSoft, Inc. 2012. *Electronic Statistics Textbook*. Tulsa, OK: StatSoft. WEB: <http://www.statsoft.com/textbook/>. (Printed Version): Hill, T. & Lewicki, P. (2007). *STATISTICS: Methods and Applications*. StatSoft, Tulsa, OK.

Stenger-Kovács, C., Buczkó, K., Hajnal, É. and Padisák, J., 2007. Epiphytic, littoral diatoms as bioindicators of shallow lake trophic status: Trophic Diatom Index for Lakes (TDIL) developed in Hungary. *Hydrobiologia*, 589(1): pp.141-154.

Tang, T., Niu, S. Q. and Dudgeon, D. 2012. Responses of epibenthic algal assemblages to water abstraction in Hong Kong streams. *Hydrobiologia*, 703: 225-237.

Tapia, P. M. 2008. Diatoms as bioindicators of pollution in the Mantaro River, Central Andes, Peru. *International Journal of Environment and Health* 2 (1): pp. 82-91.

Taylor, J. C., 2004. The application of diatom-based pollution-indices in the Vaal catchment. Unpublished M.Sc. thesis, North-West University, Potchefstroom Campus, Potchefstroom.

Taylor, J. C. 2006. Towards the successful application of diatom-based biomonitoring in South Africa. Ph.D. North West University.

Taylor, J. C., Harding, W. R., and Archibald, C. G. M. 2007a. A Methods Manual for the Collection, Preparation and Analysis of Diatom Samples. Pretoria, South Africa: Water Research Commission.

Taylor, J. C., Harding, W. R. and Archibald, C. G. M. 2007b. An Illustrated Guide to Some Common Diatom Species from South Africa. Pretoria, South Africa: Water Research Commission.

Taylor, J.C., Janse van Vuuren, M. S. and Pieterse, A. J. H. 2007d. The application and testing of diatom-based indices in the Vaal and Wilge Rivers, South Africa. *Water SA*, 33: 51-60.

Taylor, J. C., Prygiel, J., Vosloo, A., De la Rey, P. A., & Van Rensburg, L. 2007c. Can diatom-based pollution indices be used for biomonitoring in South Africa? A case study of the Crocodile West and Marico water management area. *Hydrobiologia*, 592: 455–464. <http://doi.org/10.1007/s10750-007-0788-1>. Date of access: 8 May 2018.

Telford, R. J., Juggins, S., Kelly, M. G. and Ludes, B. 2002. Diatom Applications. (In Bunke, H & Wang, P. S. P. eds. *Automatic diatom identification* (pp. 41-54)). Singapore: World Scientific Publishing.

Ter Braak, C. J. F. and P. Sîmilauer, 1998. CANOCO reference manual and user's guide to Canoco for Windows: Software for Canonical Community Ordination (version 4). Microcomputer Power, New York.

Tooth, S. and McCarthy, T.S. 2007. Wetlands in drylands: geomorphological and sedimentological characteristics, with emphasis on examples from southern Africa. *Progress in Physical Geography* 31(1): 3–41.

Totti, C., Poulin, M., Romagnoli, T., Perrone, C., Pennesi, C. and De Stefano, M. 2009. Epiphytic diatom communities on intertidal seaweeds from Iceland. *Polar Biology*, 32(11): 1681-1691.

Trobajo, R., Rovira, L., Ector, L., Wetzel, C. E., Kelly, M. and Mann, D. G. 2013. Morphology and identity of some ecologically important small *Nitzschia* species, *Diatom Research*, 28:1, 37-59, DOI: 10.1080/0269249X.2012.734531.

Verb, R. G., and Vis, M. L. 2000. Comparison of benthic diatom assemblages from streams draining abandoned and reclaimed coal mines and non-impacted sites. *Journal of the North American Benthological Society*, 19(2): 274–288.

Wachnicka, A., Gaiser, E., Collins, L., Frankovich, T. and Boyer, J. 2010. Distribution of Diatoms and Development of Diatom-Based Models for Inferring Salinity and Nutrient Concentrations in Florida Bay and Adjacent Coastal Wetlands of South Florida (USA)', *Estuaries and Coasts*, 33: 1080-98.

Water Research Commission. 1998. Quality of domestic water supplies, Assessment Guide. WRC Report No: TT 101/98. Water Research Commission (WRC).

Wessels, K., Prince, S., Malherbe, J., Small, J., Frost, P. and Van Zyl, D. 2007. Can human-induced land degradation be distinguished from the effects of rainfall variability? A case study in South Africa. *Journal of Arid Environments*, 68(2): 271-297.

World Health Organization. 2011. Hardness in drinking-water: background document for development of WHO guidelines for drinking-water quality. WHO/HSE/WSH/10.01/10/Rev/1. https://www.who.int/water_sanitation_health/dwq/chemicals/hardness.pdf.

Wu, J. 1999. A generic index of diatom assemblages as bioindicator of pollution in the Keelung River of Taiwan. *Hydrobiologia*, 397: 79–87.

Wurts, W. 2003. Daily pH Cycle and Ammonia Toxicity. *World Aquaculture*, 34:20-21 ISBN No: 1 86845 416 9.

Yim, J., Kim, K. and Kim, S. 2006. Effect of hardness on acute toxicity of metal mixtures using *Daphnia magna*: Prediction of acid mine drainage toxicity. *Journal of Hazardous Materials*, 138(1): 16-21

Zalack, J., Smucker, N. and Vis, M. 2010. Development of a diatom index of biotic integrity for acid mine drainage impacted streams. *Ecological Indicators*, 10(2): pp.287-295.

Zelnik, I., Balanč, T. and Toman, M. 2018. Diversity and structure of the tychoplankton diatom community in the Limnocrene Spring Zelenci (Slovenia) in relation to environmental factors. *Water*, 10(4): p.361.

ANNEXURES

Appendix 1- List of species

Species	Code
<i>Achnanthes</i> sp. 1	ACSP
<i>Achnantheidium exiguum</i> (Grunow) Czarnecki	ADEG
<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki	ADMI
<i>Amphora coffeaeformis</i> (Agardh) Kützing	ACOF
<i>Amphora pediculus</i> (Kützing) Grunow	APED
<i>Amphora veneta</i> Kützing	AVEN
<i>Anomoeneis</i> sp.	ANOS
<i>Anomoeneis sphaerophora</i> (Ehrenberg) Pfitzer	ASPH
<i>Anomoeneis sphaerophora</i> f. <i>costata</i> (Kützing) Schmid	ASCO
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	AUGR

<i>Brachysira brebissonii</i> Ross	BBRE
<i>Brachysira neoexilis</i> (Grunow) DG Mann	BNEO
<i>Caloneis bacillum</i> (Grunow) Cleve	CBAC
<i>Caloneis molaris</i> (Grunow) Krammer	CMOL
<i>Caloneis</i> sp.	CALS
<i>Cocconeis pediculus</i> Ehrenberg	CPED
<i>Cocconeis placentula</i> Ehrenberg	CPLA
<i>Cocconeis placentula</i> var. <i>lineata</i> (Ehrenberg)	CPLI
<i>Craticula ambigua</i> (Ehrenberg) DG Mann	CAMB
<i>Craticula buderii</i> (Hustedt) Lange-Bertalot	CRBU
<i>Craticula cuspidata</i> (Kützing) DG Mann	CRCU

<i>Craticula halophila</i> (Grunow) DG Mann	CHAL
<i>Craticula molestiformis</i> (Hustedt) Lange-Bertalot	CMLF
<i>Ctenophora pulchella</i> (Ralfs) Williams & Round	CTPU
<i>Cyclostephanos</i> sp. 1	CYCS
<i>Cyclotella meneghiniana</i> Kützing	CMEN
<i>Cyclotella ocellata</i> Pantocsek	COCE
<i>Cyclotella</i> sp. 1	CYLS
<i>Cymbella kolbei</i> Hustedt	CKOL
<i>Cymbella tumida</i> (Brébisson) Van Heurck	CTUM
<i>Cymbella turgidula</i> Grunow	CTUR
<i>Diadesmis confervacea</i> (Kützing) DG Mann	DCOF
<i>Diploneis puella</i> (Schumann) Cleve	DPUE
<i>Discostella pseudostelligera</i> (Hustedt) Houk & Klee	DPST
<i>Encyonema mesianum</i> (Cholnoky) DG Mann	ENME

<i>Encyonema minutum</i> (Hilse) DG Mann	ENMI
<i>Encyonema silesiacum</i> (Bleisch) DG Mann	ESLE
<i>Encyonopsis cesatii</i> (Rabenhorst) Krammer	ECES
<i>Encyonopsis leei</i> var. <i>sinensis</i> Metzeltin & Krammer	ENLS
<i>Encyonopsis microcephala</i> (Grunow) Krammer	ENCM
<i>Encyonopsis minuta</i> Krammer & Reichardt	ECPM
<i>Encyonopsis subminuta</i> Krammer & Reichardt	ESUM
<i>Encyonopsis vandamii</i> Krammer	ECVD
<i>Eolimna minima</i> (Grunow) Lange-Bertalot	EOMI
<i>Eolimna subminuscule</i> (Manguin) Lange-Bertalot	ESBM
<i>Epithemia adnata</i> (Kützing) Brébisson	EADN
<i>Eunotia bilunaris</i> (Ehrenberg) Mills	EBIL
<i>Eunotia flexuosa</i> (Brébisson) Kützing	EFLE

<i>Eunotia formica</i> Ehrenberg	EFOR
<i>Eunotia minor</i> (Kützing) Grunow	EMIN
<i>Eunotia naegeli</i> Migula	ENAE
<i>Fistulifera saprophila</i> (Lange-Bertalot & Bonik) Lange-Bertalot	FSAP
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	FBCP
<i>Fragilaria capucina</i> Desmazières	FCAP
<i>Fragilaria capucina</i> var. <i>rumpens</i> (Kützing) Lange- Bertalot	FCRU
<i>Fragilaria capucina</i> var. <i>vaucheriae</i> (Kützing) Lange-Bertalot	FCVA
<i>Fragilaria nanana</i> Lange- Bertalot	FNAN
<i>Fragilaria tenera</i> (WM Smith) Lange-Bertalot	FTEN
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing) Lange-Bertalot	FUAC
<i>Frustululia crassinervia</i> (Kützing) Cleve	FCRS
<i>Gomphonema</i> aff. <i>gracile</i>	GGRA

<i>Gomphonema</i> aff. <i>lagenula</i>	
<i>Gomphonema affine</i> Kützing	GAFF
<i>Gomphonema angustatum</i> (Kützing) Rabenhorst	GANG
<i>Gomphonema auritum</i> A. Braun	GAUR
<i>Gomphonema exilissimum</i> Lange-Bertalot & Reichardt	GEXL
<i>Gomphonema gracile</i> Ehrenberg	GGRA
<i>Gomphonema insigne</i> Gregory	GINS
<i>Gomphonema italicum</i> Kützing	GITA
<i>Gomphonema lagenula</i> Kützing	GLGN
<i>Gomphonema laticollum</i> Reichardt	GLTC
<i>Gomphonema parvulus</i> Lange-Bertalot & Reichardt	GPVL
<i>Gomphonema parvulum</i> Kützing	GPAR

<i>Gomphonema parvulum</i> f. <i>saprophilum</i> Lange-Bertalot & Reichardt	GPAS
<i>Gomphonema pseudoaugur</i> Krammer	GPSA
<i>Gomphonema turris</i> Ehrenberg	GTUR
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	HAMP
<i>Hippodonta capitata</i> (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	HCAP
<i>Lemnicola hungarica</i> (Grunow) Round & Basson	LHUN
<i>Luticola kotschy</i> (Grunow)	LKOT
<i>Luticola mutica</i> (Kützing) DG Mann	LMUT
<i>Luticola nivalis</i> (Ehrenberg) DG Mann	LNIV
<i>Mastogloia dansei</i> (Thwaites) Thwaites	MDAN
<i>Mastogloia elliptica</i> (Agardh) Cleve	MELL
<i>Mastogloia smithii</i> Thwaites	MSMI
<i>Mayamaea atomus</i> (Kützing) Lange-Bertalot	MAAT

<i>Mayamaea atomus</i> var. <i>permitis</i> (Hustedt) Lange-Bertalot	MAPE
<i>Mayamaea</i> sp.	
<i>Melosira varians</i> Agardh	MVAR
<i>Navicula angusta</i> Grunow	NAAN
<i>Navicula arvensis</i> var. <i>maior</i> Lange-Bertalot	NAMA
<i>Navicula capitatoradiata</i> Germain	NCPR
<i>Navicula cincta</i> (Ehrenberg) Ralfs	NCIN
<i>Navicula cryptocephala</i> Kützing	NCRY
<i>Navicula joubaudii</i> Germain	NJOU
<i>Navicula longicephala</i> Hustedt	NLGC
<i>Navicula radiosa</i> Kützing	NRAD
<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	NRCS
<i>Navicula rhynchocephala</i> Kützing	NRHY
<i>Navicula rostellata</i> Kützing	NROS
<i>Navicula schroeteri</i> Meister	NSHR

<i>Navicula symmetrica</i> Patrick	NSYM
<i>Navicula tripunctata</i> (OF Müller) Bory	NTPT
<i>Navicula veneta</i> Kützing	NVEN
<i>Navicula zanonii</i> Hustedt	NZAN
<i>Navicymbula pusilla</i> (Grunow) Krammer	NCPU
<i>Nitzschia acidoclinata</i> Lange-Bertalot	NACD
<i>Nitzschia agnita</i> Hustedt	NAGN
<i>Nitzschia amphibia</i> Grunow	NAMP
<i>Nitzschia archibaldii</i> Lange-Bertalot	NIAR
<i>Nitzschia aurariae</i> Cholnoky	NAUR
<i>Nitzschia capitellata</i> Hustedt	NCPL
<i>Nitzschia clausii</i> Hantzsch	NCLA
<i>Nitzschia desertorum</i> Hustedt	NDES
<i>Nitzschia dissipata</i> var. <i>media</i> (Hantzsch) Grunow	NDME
<i>Nitzschia draveillensis</i> Coste & Ricard	NDRA

<i>Nitzschia elegantula</i> Grunow	NELE
<i>Nitzschia etoshensis</i> Cholnoky	NETO
<i>Nitzschia filiformis</i> (W Smith) Van Heurk	NFIL
<i>Nitzschia fonticola</i> Grunow	NFON
<i>Nitzschia frustulum</i> (Kützing) Grunow	NIFR
<i>Nitzschia gracilis</i> Hantzsch	NIGR
<i>Nitzschia heufleriana</i> Grunow	NHEU
<i>Nitzschia inconspicua</i> Grunow	NINC
<i>Nitzschia intermedia</i> Hantzsch	NINT
<i>Nitzschia iremissa</i> Cholnoky	NIRM
<i>Nitzschia lancettula</i> O Müller	NLTL
<i>Nitzschia liebertruthii</i> Rabenhorst	NLBT
<i>Nitzschia linearis</i> var. <i>subtilis</i> Grunow	NLSU
<i>Nitzschia microcephala</i> Grunow	NMIC

<i>Nitzschia nana</i> Grunow	NNAN
<i>Nitzschia palea</i> (Kützinger) W Smith	NPAL
<i>Nitzschia palea</i> var. <i>debilis</i> (Kützinger) Grunow	NPAD
<i>Nitzschia paleacea</i> (Grunow) Grunow	NPAE
<i>Nitzschia pura</i> Hustedt	NIPR
<i>Nitzschia pusilla</i> Grunow	NIPU
<i>Nitzschia radícula</i> Hustedt	NZRA
<i>Nitzschia reversa</i> W Smith	NREV
<i>Nitzschia sigma</i> (Kützinger) W Smith	NSIG
<i>Nitzschia subacicularis</i> Hustedt	NSUA
<i>Nitzschia supralittorea</i> Lange-Bertalot	NZSU
<i>Pinnularia acrosphaeria</i> W Smith	PACR
<i>Pinnularia borealis</i> Ehrenberg	PBOR
<i>Pinnularia divergens</i> var. <i>undulata</i> (Péragallo & Heribaud) Hustedt	PDUN
<i>Pinnularia divergens</i> W Smith	PDIV

<i>Pinnularia gibba</i> Ehrenberg	PGIB
<i>Pinnularia</i> sp. 1	PINS
<i>Pinnularia subbrevistriata</i> Krammer	PSBV
<i>Pinnularia subcapitata</i> Gregory	PSCA
<i>Pinnularia viridiformis</i> Krammer	PVIF
<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	PVIR
<i>Planothidium delicatulum</i> (Kützinger) Round & Bukhtiyarova	PTDE
<i>Planothidium engelbrechtii</i> (Cholnoky) Round & Bukhtiyarova	PLEN
<i>Planothidium frequentissimum</i> (Lange- Bertalot) Round & Bukhtiyarova	PLFR
<i>Planothidium</i> sp. 1	PTDS
<i>Pseudostaurosira</i> <i>brevistriata</i> (Grunow in van Heurk) Williams & Round	PSBR
<i>Rhoicosphenia abbreviata</i> (C.Agardh) Lange-Bertalot	RABB

<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	RGIB
<i>Rhopalodia operculata</i> (Agardh) Håkansson	ROPE
<i>Sellaphora pupula</i> (Kützinger) Mereschkowsky	SPUP
<i>Sellaphora seminulum</i> (Grunow) DG Mann	SSEM
<i>Stauroneis obtusa</i> Lagerstedt	SOBT
<i>Staurosirella pinnata</i> (Ehrenberg) Williams & Round	SRPI
<i>Surirella ovalis</i> Brébisson	SOVI
<i>Tabularia fasciculata</i> (Agardh) Williams & Round	TFAS
<i>Thalassiosira duostra</i> Pienaar	TDUO
<i>Tryblionella apiculata</i> Gregory	TAPI
<i>Tryblionella calida</i> (Grunow) DG Mann	TCAL
<i>Tryblionella hungarica</i> (Grunow) Frenguelli	THUN

Appendix 2- Diatom growth forms

Winter Diatom Species	Summer Diatom Species	Growth form/ Lifeform
<i>Achnantheidium exiguum</i> (Grunow) Czarnecki	<i>Achnantheidium exiguum</i> (Grunow) Czarnecki	Motile Solitary/ pairs Apical mucilage stalk
<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki	<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki	Pedunculate Mobile Solitary/ pairs Apical mucilage stalk
	<i>Amphora coffeaeformis</i> (Agardh) Kützing	
<i>Amphora pediculus</i> (Kützing) Grunow	<i>Amphora pediculus</i> (Kützing) Grunow	Pioneer Mobile Slow-moving Solitary
<i>Amphora veneta</i> Kützing	<i>Amphora veneta</i> Kützing	Pioneer Solitary Mobile Slow-moving
<i>Anomoeneis sphaerophora</i> (Ehrenberg) Pfitzer	<i>Anomoeneis sphaerophora</i> (Ehrenberg) Pfitzer	Mobile motile Solitary Biraphid

<i>Anomoeneis sphaerophora</i> f. <i>costata</i> (Kützing) Schmid		
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	Planktonic Colonial chains
	<i>Brachysira brebissonii</i> Ross	
<i>Brachysira neoexilis</i> (Grunow) DG Mann	<i>Brachysira neoexilis</i> (Grunow) DG Mann	Solitary motile Biraphid
<i>Caloneis bacillum</i> (Grunow) Cleve		Solitary motile Biraphid
<i>Caloneis molaris</i> (Grunow) Krammer	<i>Caloneis molaris</i> (Grunow) Krammer	Solitary motile Biraphid
	<i>Cocconeis pediculus</i> Ehrenberg	
<i>Caloneis</i> sp.		Solitary motile Biraphid
<i>Cocconeis placentula</i> Ehrenberg	<i>Cocconeis placentula</i> Ehrenberg	Solitary Adnate monoraphid
<i>Cocconeis placentula</i> var. <i>lineata</i> (Ehrenberg) Van Heurk	<i>Cocconeis placentula</i> var. <i>lineata</i> (Ehrenberg) Van Heurk	Solitary Adnate monoraphid
<i>Craticula ambigua</i> (Ehrenberg) DG Mann		Solitary motile Biraphid
<i>Craticula buderi</i> (Hustedt) Lange-Bertalot	<i>Craticula buderi</i> (Hustedt) Lange-Bertalot	Solitary motile

		Biraphid
<i>Craticula cuspidata</i> (Kützing) DG Mann		Solitary motile Biraphid
	<i>Craticula halophila</i> (Grunow) DG Mann	
<i>Craticula molestiformis</i> (Hustedt) Lange-Bertalot	<i>Craticula molestiformis</i> (Hustedt) Lange-Bertalot	Solitary motile Biraphid
<i>Ctenophora pulchella</i> (Ralfs) Williams & Round	<i>Ctenophora pulchella</i> (Ralfs) Williams & Round	Solitary Attached Araphid→cells grow in tuft-like colonies, attached to surfaces by mucilage pads.
<i>Cyclostephanos</i> sp. 1		Planktonic Solitary
<i>Cyclotella meneghiniana</i> Kützing	<i>Cyclotella meneghiniana</i> Kützing	Solitary/pairs→ not chains
	<i>Cymbella kolbei</i> Hustedt	
<i>Cyclotella ocellata</i> Pantocsek		Solitary/pairs→ not chains
<i>Cyclotella</i> sp. 1		Solitary/pairs→ not chains
<i>Cymbella tumida</i> (Brébisson) Van Heurck	<i>Cymbella tumida</i> (Brébisson) Van Heurck	Solitary Attached Or free living motile Biraphid

<i>Cymbella turgidula</i> Grunow	<i>Cymbella turgidula</i> Grunow	Solitary Attached Or free living motile Biraphid
	<i>Diadismus confervacea</i> (Kützing) DG Mann	Ribbon
	<i>Diploneis puella</i> (Schumann) Cleve	
<i>Discostella pseudostelligera</i> (Hustedt) Houk & Klee		Planktonic Solitary
<i>Encyonema mesianum</i> (Cholnoky) DG Mann	<i>Encyonema mesianum</i> (Cholnoky) DG Mann	Solitary / mucilage tubes /free living motile Biraphid
<i>Encyonema minutum</i> (Hilse) DG Mann	<i>Encyonema minutum</i> (Hilse) DG Mann	Solitary / mucilage tubes /free living motile Biraphid
<i>Encyonema silesiacum</i> (Bleisch) DG Mann		Solitary / mucilage tubes /free living motile Biraphid
<i>Encyonopsis cesatii</i> (Rabenhorst) Krammer	<i>Encyonopsis cesatii</i> (Rabenhorst) Krammer	Solitary/free living motile Biraphid
	<i>Encyonopsis leei</i> var. <i>sinensis</i> Metzeltin & Krammer	
<i>Encyonopsis microcephala</i> (Grunow) Krammer	<i>Encyonopsis microcephala</i> (Grunow) Krammer	Solitary/free living motile Biraphid
	<i>Encyonopsis minuta</i> Krammer & Reichardt	

<i>Encyonopsis subminuta</i> Krammer & Reichardt	<i>Encyonopsis subminuta</i> Krammer & Reichardt	Solitary/free living motile Biraphid
	<i>Encyonopsis vandamii</i> Krammer	
<i>Eolimna minima</i> (Grunow) Lange-Bertalot	<i>Eolimna minima</i> (Grunow) Lange-Bertalot	Solitary/free living motile Biraphid
<i>Eolimna subminuscula</i> (Manguin) Lange-Bertalot	<i>Eolimna subminuscula</i> (Manguin) Lange-Bertalot	Solitary/free living motile Biraphid
<i>Epithemia adnata</i> (Kützing) Brébisson	<i>Epithemia adnata</i> (Kützing) Brébisson	Solitary/free living motile Biraphid
<i>Eunotia bilunaris</i> (Ehrenberg) Mills	<i>Eunotia bilunaris</i> (Ehrenberg) Mills	Solitary motile Ribbon colonies Raphid→Taxa with a raphe on both valves (short raphe system extending from the valve face on to the valve mantle) Prostrate
<i>Eunotia flexuosa</i> (Brébisson) Kützing	<i>Eunotia flexuosa</i> (Brébisson) Kützing	cells occur singly, free, or attached by mucilaginous stalks, or in long ribbon-like colonies
<i>Eunotia formica</i> Ehrenberg	<i>Eunotia formica</i> Ehrenberg	Ribbon colony
<i>Eunotia minor</i> (Kützing) Grunow	<i>Eunotia minor</i> (Kützing) Grunow	Ribbon colony
<i>Eunotia naegeli</i> Migula		Weakly motile

<i>Fistulifera saprophila</i> (Lange-Bertalot & Bonik) Lange-Bertalot	<i>Fistulifera saprophila</i> (Lange-Bertalot & Bonik) Lange-Bertalot	Solitary/free living motile Biraphid
<i>Fragilaria biceps</i> (Kützinger) Lange-Bertalot	<i>Fragilaria biceps</i> (Kützinger) Lange-Bertalot	Pedunculate →pad Ribbon colony Araphid
<i>Fragilaria capucina</i> Desmazières	<i>Fragilaria capucina</i> Desmazières	Ribbon colony
<i>Fragilaria capucina</i> var. <i>rumpens</i> (Kützinger) Lange-Bertalot	<i>Fragilaria capucina</i> var. <i>rumpens</i> (Kützinger) Lange-Bertalot	Ribbon colony
<i>Fragilaria capucina</i> var. <i>vaucheriae</i> (Kützinger) Lange-Bertalot	<i>Fragilaria capucina</i> var. <i>vaucheriae</i> (Kützinger) Lange-Bertalot	Ribbon colony
<i>Fragilaria nanana</i> Lange-Bertalot	<i>Fragilaria nanana</i> Lange-Bertalot	Pedunculate →pad Planktonic
<i>Fragilaria tenera</i> (WM Smith) Lange-Bertalot	<i>Fragilaria tenera</i> (WM Smith) Lange-Bertalot	Pedunculate →pad Planktonic
	<i>Fragilaria ulna</i> var. <i>acus</i> (Kützinger) Lange-Bertalot	Pedunculate →pad
<i>Frustulia crassinervia</i> (Kützinger) Cleve	<i>Frustulia crassinervia</i> (Kützinger) Cleve	Unattached Tube-Forming Mobile Biraphid
<i>Gomphonema</i> aff. <i>gracile</i>	<i>Gomphonema</i> aff. <i>gracile</i>	Mobile

		Pedunculate (mucilage stalk) High profile Biraphid
	<i>Gomphonema affine</i> Kützing	
<i>Gomphonema</i> aff. <i>lagenula</i>		Mobile Pedunculate (mucilage stalk) High profile Biraphid
<i>Gomphonema angustatum</i> (Kützing) Rabenhorst	<i>Gomphonema angustatum</i> (Kützing) Rabenhorst	Mobile Pedunculate (mucilage stalk) High profile Biraphid
<i>Gomphonema auritum</i> A. Braun		Solitary/free living motile or in pairs (mucilage stalk)) Mobile Pedunculate (mucilage stalk) High profile Biraphid
	<i>Gomphonema exilissimum</i> Lange-Bertalot & Reichardt	
<i>Gomphonema gracile</i> Ehrenberg	<i>Gomphonema gracile</i> Ehrenberg	Solitary/free living motile or in pairs (mucilage stalk)) Mobile

		Pedunculate (mucilage stalk) High profile Biraphid
<i>Gomphonema italicum</i> Kützing		Solitary/free living motile or in pairs (mucilage stalk)) Mobile Pedunculate (mucilage stalk) High profile Biraphid attached to substrata by dichotomous mucilage stalks.
<i>Gomphonema lagenula</i> Kützing	<i>Gomphonema lagenula</i> Kützing	Solitary/free living motile or in pairs (mucilage stalk)) Mobile Pedunculate (mucilage stalk) High profile Biraphid
<i>Gomphonema laticollum</i> Reichart	<i>Gomphonema laticollum</i> Reichart	Solitary/free living motile or in pairs (mucilage stalk)) Mobile Pedunculate (mucilage stalk) High profile Biraphid

		attached to substrata by dichotomous mucilage stalks.
<i>Gomphonema parvulus</i> Lange-Bertalot & Reichardt	<i>Gomphonema parvulus</i> Lange-Bertalot & Reichardt	Solitary/free living motile or in pairs (mucilage stalk)) Mobile Pedunculate (mucilage stalk) High profile Biraphid
<i>Gomphonema parvulum</i> Kützing	<i>Gomphonema parvulum</i> (Kützing) Kützing	Solitary/free living motile or in pairs (mucilage stalk)) Mobile Pedunculate (mucilage stalk) High profile Biraphid
<i>Gomphonema parvulum</i> f. saprophilum Lange-Bertalot & Reichardt		Solitary/free living motile or in pairs (mucilage stalk)) Mobile Pedunculate (mucilage stalk) High profile Biraphid
<i>Gomphonema pseudoaugur</i> Krammer	<i>Gomphonema pseudoaugur</i> Krammer	Solitary/free living motile or in pairs (mucilage stalk)) Mobile Pedunculate (mucilage stalk)

		High profile Biraphid
<i>Gomphonema turris</i> Ehrenberg		Solitary/free living motile or in pairs (mucilage stalk)) Mobile Pedunculate (mucilage stalk) High profile Biraphid
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	Solitary/free living motile Biraphid
<i>Hippodonta capitata</i> (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	<i>Hippodonta capitata</i> (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	Solitary/free living motile Biraphid
<i>Lemnicola hungarica</i> (Grunow) Round & Basson	<i>Lemnicola hungarica</i> (Grunow) Round & Basson	Solitary Attached (adnate) Monoraphid
	<i>Luticola kotschy</i> (Grunow)	
<i>Luticola mutica</i> (Kützing) DG Mann	<i>Luticola mutica</i> (Kützing) DG Mann	Solitary/free living motile Biraphid
<i>Luticola nivalis</i> (Ehrenberg) DG Mann	<i>Luticola nivalis</i> (Ehrenberg) DG Mann	Solitary/free living motile Biraphid
<i>Mastogloia dansei</i> (Thwaites) Thwaites	<i>Mastogloia dansei</i> (Thwaites) Thwaites	Motile or encased in mucilage Solitary

		Biraphid
<i>Mastogloia elliptica</i> (Agardh) Cleve	<i>Mastogloia elliptica</i> (Agardh) Cleve	Motile or encased in mucilage Solitary Biraphid
<i>Mastogloia smithii</i> Thwaites	<i>Mastogloia smithii</i> Thwaites	Motile or encased in mucilage Solitary Biraphid
<i>Mayamaea atomus</i> (Kützinger) Lange-Bertalot	<i>Mayamaea atomus</i> (Kützinger) Lange-Bertalot	Solitary/free living motile Biraphid
<i>Mayamaea atomus</i> var. <i>permitis</i> (Hustedt) Lange- Bertalot	<i>Mayamaea atomus</i> var. <i>permitis</i> (Hustedt) Lange- Bertalot	Solitary/free living motile Biraphid
<i>Mayamaea</i> sp. 1		Solitary/free living motile Biraphid
<i>Melosira varians</i> Agardh	<i>Melosira varians</i> Agardh	Centric Linked face-to-face by mucilage pads→ long chain colonies High profile
	<i>Navicula angusta</i> Grunow	
	<i>Navicula arvensis</i> var. <i>maior</i> Lange-Bertalot	
<i>Navicula capitatoradiata</i> Germain		Solitary/free living motile Biraphid

<i>Navicula cincta</i> (Ehrenberg) Ralfs in Pritchard	<i>Navicula cincta</i> (Ehrenberg) Ralfs	Solitary/free living motile Biraphid
<i>Navicula cryptocephala</i> Kützinger	<i>Navicula cryptocephala</i> Kützinger	Solitary/free living motile Biraphid
	<i>Navicula erifuga</i> (OF Müller) Bory	
<i>Navicula joubaudii</i> Germain		Solitary/free living motile Biraphid
<i>Navicula longicephala</i> Hustedt		Solitary/free living motile Biraphid
<i>Navicula radiosa</i> Kützinger	<i>Navicula radiosa</i> Kützinger	Solitary/free living motile Biraphid
<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	Solitary/free living motile Biraphid
	<i>Navicula rhynchocephala</i> Kützinger	
<i>Navicula rostellata</i> Kützinger	<i>Navicula rostellata</i> Kützinger	Solitary/free living motile Biraphid
	<i>Navicula schroeteri</i> Meister	
	<i>Navicula symmetrica</i> Patrick	
<i>Navicula tripunctata</i> (OF Müller) Bory		Solitary/free living motile Biraphid
<i>Navicula veneta</i> Kützinger	<i>Navicula veneta</i> Kützinger	Solitary/free living motile

		Biraphid
<i>Navicula zanonii</i> Hustedt	<i>Navicula zanonii</i> Hustedt	Solitary/free living motile Biraphid
<i>Navicymbula pusilla</i> (Grunow) Krammer	<i>Navicymbula pusilla</i> (Grunow) Krammer	Solitary Unattached
<i>Nitzschia acidoclinata</i> Lange-Bertalot	<i>Nitzschia acidoclinata</i> Lange-Bertalot	Solitary / free living motile /mucilage tubes Biraphid
<i>Nitzschia agnita</i> Hustedt	<i>Nitzschia agnita</i> Hustet	Solitary / free living motile /mucilage tubes Biraphid
<i>Nitzschia amphibia</i> Grunow	<i>Nitzschia amphibia</i> Grunow	Solitary / free living motile /mucilage tubes Biraphid
<i>Nitzschia archibaldii</i> Lange-Bertalot	<i>Nitzschia archibaldii</i> Lange-Bertalot	Solitary / free living motile /mucilage tubes Biraphid
<i>Nitzschia aurariae</i> Chohnoky	<i>Nitzschia aurariae</i> Chohnoky	Solitary / free living motile /mucilage tubes Biraphid
<i>Nitzschia capitellata</i> Hustedt	<i>Nitzschia capitellata</i> Hustedt	Solitary / free living motile /mucilage tubes Biraphid

<i>Nitzschia clausii</i> Hantzsch	<i>Nitzschia clausii</i> Hantzsch	Solitary / free living motile /mucilage tubes Biraphid
<i>Nitzschia desertorum</i> Hustedt	<i>Nitzschia desertorum</i> Hustedt	Solitary / free living motile /mucilage tubes Biraphid
<i>Nitzschia dissipata</i> var. <i>media</i> (Hantzsch) Grunow		Solitary / free living motile /mucilage tubes Biraphid
<i>Nitzschia draveillensis</i> Coste & Ricard		Solitary / free living motile /mucilage tubes Biraphid
	<i>Nitzschia elegantula</i> Grunow	
<i>Nitzschia etoshensis</i> Chohnoky	<i>Nitzschia etoshensis</i> Chohnoky	Solitary / free living motile /mucilage tubes Biraphid
<i>Nitzschia filiformis</i> (W Smith) Van Heurk	<i>Nitzschia filiformis</i> (W Smith) Van Heurk	Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia fonticola</i> Grunow	<i>Nitzschia fonticola</i> Grunow	Solitary / free living motile / <u>mucilage tubes</u>

		Biraphid
<i>Nitzschia frustulum</i> (Kützinger) Grunow	<i>Nitzschia frustulum</i> (Kützinger) Grunow	Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia gracilis</i> Hantzsch	<i>Nitzschia gracilis</i> Hantzsch	Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia heufleriana</i> Grunow		Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia inconspicua</i> Grunow		Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia intermedia</i> Hantzsch	<i>Nitzschia intermedia</i> Hantzsch	Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia iremissa</i> Cholnoky		Solitary / free living motile / <u>mucilage tubes</u> Biraphid
	<i>Nitzschia lancettula</i> O Müller	
<i>Nitzschia liebertruthii</i> Rabenhorst	<i>Nitzschia liebertruthii</i> Rabenhorst	Solitary / free living motile / <u>mucilage tubes</u> Biraphid

<i>Nitzschia linearis</i> var. <i>subtilis</i> Grunow		Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia microcephala</i> Grunow	<i>Nitzschia microcephala</i>	Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia nana</i> Grunow	<i>Nitzschia nana</i> Grunow	Solitary / free living motile / <u>mucilage tubes</u> Biraphid
	<i>Nitzschia obtusa</i> var. <i>kurzii</i> Rabenhorst	
<i>Nitzschia palea</i> (Kützing) W Smith	<i>Nitzschia palea</i> (Kützing) W Smith	Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia palea</i> var. <i>debilis</i> (Kützing) Grunow		Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia paleacea</i> (Grunow) Grunow	<i>Nitzschia paleacea</i> (Grunow) Grunow	Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia pura</i> Hustedt	<i>Nitzschia pura</i> Hustedt	Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia pusilla</i> Grunow	<i>Nitzschia pusilla</i> Grunow	Solitary / free living motile / <u>mucilage tubes</u>

		Biraphid
<i>Nitzschia radicula</i> Hustedt	<i>Nitzschia radicula</i> Hustedt	Solitary / free living motile / <u>mucilage tubes</u> Biraphid
	<i>Nitzschia reversa</i> W Smith	
	<i>Nitzschia supralitorea</i> Lange-Bertalot	
<i>Nitzschia sigma</i> (Kützing) W Smith		Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Nitzschia subacicularis</i> Hustedt		Solitary / free living motile / <u>mucilage tubes</u> Biraphid
<i>Pinnularia acrosphaeria</i> W Smith	<i>Pinnularia acrosphaeria</i> W Smith	Solitary / free living motile Biraphid
	<i>Pinnularia borealis</i> Ehrenberg	
<i>Pinnularia divergens</i> var. <i>undulata</i> (Péragallo & Heribaud)		Solitary / free living motile Biraphid
<i>Pinnularia divergens</i> W Smith	<i>Pinnularia divergens</i> W Smith	Solitary / free living motile Biraphid
<i>Pinnularia gibba</i> Ehrenberg	<i>Pinnularia gibba</i> Ehrenberg	Solitary / free living motile

		Biraphid
<i>Pinnularia</i> sp. 1		Solitary / free living motile Biraphid
<i>Pinnularia subbrevistriata</i> Krammer	<i>Pinnularia subbrevistriata</i> Krammer	Solitary / free living motile Biraphid
	<i>Pinnularia subcapitata</i> Gregory	
	<i>Pinnularia viridiformis</i> Krammer	
<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	Solitary / free living motile Biraphid
	<i>Planothidium delicatulum</i> (Kützing) Round & Bukhtiyarova	
<i>Planothidium engelbrechtii</i> (Cholnoky) Round & Bukhtiyarova	<i>Planothidium engelbrechtii</i> (Cholnoky) Round & Bukhtiyarova	Solitary Attached (adnate) monoraphid
<i>Planothidium frequentissimum</i> (Lange- Bertalot) Round & Bukhtiyarova		Solitary Attached (adnate) Monoraphid
<i>Planothidium</i> sp. 1		Solitary Attached (adnate) monoraphid

	<i>Rhoicosphenia abbreviata</i> (C.Agardh) Lange-Bertalot	Solitary or in pairs mucilage stalks at the basal pole
<i>Pseudostaurosira brevistriata</i> (Grunow) Williams & Round		Ribbon colony Araphid
<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	Solitary / free living motile / <u>adnate</u> Biraphid Or attached with <u>mucilage stalks</u>
<i>Rhopalodia operculata</i> (Agardh) Håkansson		Solitary / free living motile / <u>adnate</u> Biraphid
<i>Sellaphora pupula</i> (Kützing) Mereschkowsky	<i>Sellaphora pupula</i> (Kützing) Mereschkowsky	Solitary / free living motile Biraphid
<i>Sellaphora seminulum</i> (Grunow) DG Mann	<i>Sellaphora seminulum</i> (Grunow) DG Mann	Solitary / free living motile Biraphid
<i>Stauroneis obtusa</i>	<i>Stauroneis obtusa</i>	Solitary / free living motile Biraphid
<i>Staurosirella pinnata</i> (Ehrenberg) Williams & Round		Ribbon colony Araphid
<i>Surirella ovalis</i> Brébisson		Solitary / free living motile Biraphid
<i>Tabularia fasciculata</i> (Agardh) Williams & Round	<i>Tabularia fasciculata</i> (Agardh) Williams & Round	Colonial, basally attached

		Araphid erect
	<i>Thalassiosira duostra</i>	Planktonic
<i>Tryblionella apiculata</i> Gregory	<i>Tryblionella apiculata</i> Gregory	Solitary / free living motile Biraphid
<i>Tryblionella calida</i> (Grunow) DG Mann		Solitary / free living motile Biraphid
	<i>Tryblionella hungarica</i> (Grunow) Frenguelli	Solitary / free living motile Biraphid

Appendix 3- Water chemistry data

	Depth (cm)	Temp (°C)	pH	K ⁺ (mg/L)	Na ⁺ (mg/L)	Ca ²⁺ (mg/L)	Mg ²⁺ (mg/L)	Ammonia (mg/L)	SO ₄ ²⁻ (mg/L)	Cl ⁻ (mg/L)	Alkalinity as CaCO ₃ (mg/L)	Nitrate (mg/L)	EC (mS/cm)	TDS (mg/L)	Hardness as CaCO ₃ (mg/L)	Cations (meq/L)	Anions (meq/L)	Mg Hardness as CaCO ₃ (mg/L)	% SO ₄ :Cl ⁻	PO ₄ (mg/L)	TP (mg/L)
REED_W_Reference																					
Min	30,00	10,50	8,17	14,00	22,00	6,80	5,70	0,08	2,80	28,00	55,00	0,05	0,24	154,00	40,00	2,20	2,10	23,00	2,57	0,01	0,30
Max	50,00	18,40	9,25	33,00	597,00	25,00	22,00	0,38	31,00	381,00	754,00	1,60	2,20	1408,00	153,00	29,20	23,30	91,00	11,70	0,02	0,68
Mean	40,00	14,38	8,86	22,75	297,00	15,45	14,65	0,17	13,30	220,50	342,50	0,75	1,37	873,75	98,75	15,48	13,43	60,25	7,22	0,16	0,41
REED_W_Disturbed																					
Min	40,00	10,80	8,37	9,10	44,00	32,00	5,00	0,05	57,00	27,00	84,00	0,11	0,56	358,00	175,00	5,60	5,60	21,00	211,11	0,05	0,31
Max	40,00	13,60	9,13	45,00	217,00	182,00	23,00	0,07	719,00	145,00	180,00	0,89	1,95	1248,00	475,00	20,10	20,80	95,00	495,86	0,18	0,45
Mean	40,00	12,20	8,75	27,05	130,50	107,00	14,00	0,06	388,00	86,00	132,00	0,50	1,26	803,00	325,00	12,85	13,20	58,00	353,49	0,12	0,38
REED_S_Reference																					
Min	10,00	20,20	7,40	4,80	7,50	3,20	2,20	0,13	1,90	8,90	29,00	0,05	0,10	64,00	17,00	0,90	0,90	9,10	4,40	0,00	0,00

Max	40,0 0	27,6 0	8,70	17,00	314,00	22,00	13,00	2,20	16,00	234,00	346,00	0,05	1,40	896,00	108,00	16,20	13,90	54,00	25,56	0,00	0,00
Mean	27,5 0	24,6 3	8,15	10,08	122,13	13,55	7,98	1,01	8,03	100,48	152,75	0,05	0,64	411,25	66,50	6,98	6,08	32,78	14,54	0,00	0,00
REED_S_Disturbed																					
Min	10,0 0	22,6 0	7,80	5,90	20,00	29,00	5,80	0,07	66,00	14,00	42,00	0,05	0,29	186,00	134,00	3,70	3,50	24,00	3476,4 7	0,00	0,00
Max	40,0 0	24,7 0	8,20	26,00	132,00	167,00	15,00	0,07	591,00	17,00	88,00	0,12	1,40	896,00	441,00	15,20	13,60	62,00	471,43	0,00	0,00
Mean	25,0 0	23,6 5	8,00	15,95	76,00	98,00	10,40	0,07	328,50	15,50	65,00	0,09	0,85	541,00	287,50	9,45	8,55	43,00	1973,9 5	0,00	0,00
GRASS_W_Reference																					
Min	30,0 0	12,0 0	7,32	8,20	17,00	6,70	5,40	0,06	6,30	18,00	50,00	0,05	0,20	128,00	41,00	2,00	1,90	22,00	7,16	0,04	0,10
Max	50,0 0	18,5 0	9,38	22,00	151,00	14,00	13,00	0,79	32,00	88,00	277,00	1,20	0,82	525,00	83,00	8,50	7,90	54,00	88,89	0,25	0,25
Mean	38,5 7	15,1 0	8,57	11,63	64,00	10,64	9,03	0,19	12,46	39,14	136,86	0,59	0,43	272,43	63,57	4,39	4,16	37,00	40,77	0,13	0,20
GRASS_W_Disturbed																					
Min	5,00	5,40	9,50	15,00	202,00	38,00	9,00	0,08	185,00	68,00	177,00	0,70	1,14	730,00	132,00	11,90	11,50	37,00	9,19	0,13	0,50
Max	44,0 0	18,6 0	10,6 0	98,00	1546,0 0	1056,0 0	298,0 0	0,28	1480,0 0	2013,0 0	530,00	32,00	5,44	2176,0 0	753,00	38,40	39,40	618,00	2176,4 7	1,77	2,60

Mean	26,33	13,90	9,98	43,33	759,00	382,67	152,33	0,15	632,33	720,67	345,33	14,90	3,33	1453,00	442,50	25,15	25,45	327,50	824,03	0,93	1,44
GRASS_S_Reference																					
Min	10,00	19,80	7,30	2,10	5,00	2,30	0,80	0,05	2,20	5,40	18,00	0,05	0,09	58,00	10,00	0,90	0,80	3,30	5,37	0,00	0,00
Max	50,00	31,60	9,30	6,30	193,00	7,90	4,20	0,35	38,00	236,00	128,00	0,43	1,10	704,00	37,00	8,70	9,30	77,00	203,70	0,00	0,00
Mean	35,71	23,57	8,17	3,80	62,21	4,70	2,91	0,16	16,46	59,77	68,57	0,11	0,40	253,29	23,71	3,31	3,41	20,63	70,94	0,00	0,00
GRASS_S_Disturbed																					
Min	10,00	21,00	7,40	2,70	35,00	6,60	4,30	0,15	66,00	12,00	19,00	0,05	0,78	499,00	34,00	6,10	6,30	18,00	40,24	0,00	0,00
Max	50,00	25,90	8,50	29,00	224,00	56,00	20,00	1,20	266,00	164,00	215,00	0,66	1,24	1024,00	222,00	11,20	10,40	37,00	2216,67	0,00	0,00
Mean	30,00	23,97	8,03	19,23	133,33	29,20	11,10	0,51	173,00	73,33	115,33	0,25	1,21	772,33	118,33	8,70	8,03	45,67	893,97	0,00	0,00
SALT_W_Reference																					
Min	7,00	10,20	9,20	12,00	2110,00	16,00	6,30	0,10	331,00	2140,00	906,00	0,42	9,00	5760,00	66,00	93,40	90,60	26,00	12,08	5,88	6,10
Max	10,00	17,60	9,42	181,00	4912,00	34,00	6,80	0,16	1839,00	4080,00	2502,00	2,60	18,50	11840,00	113,00	220,60	203,50	49,00	45,07	9,00	16,50
Mean	8,33	13,97	9,31	74,33	3098,00	23,67	8,37	0,12	865,67	2986,67	1489,33	1,54	12,50	8000,00	93,67	138,53	132,20	34,33	25,70	6,99	11,48

SALT_W_Disturbed																					
Min	10,0 0	9,80	7,40	20,00	132,00	12,00	7,50	0,05	77,00	36,00	66,00	0,25	1,26	806,00	62,00	13,90	13,20	31,00	19,95	0,03	0,12
Max	50,0 0	16,2 0	10,3 1	527,0 0	7548,0 0	686,00	707,0 0	38,00	4782,0 0	2620,0 0	5650,0 0	3,40	5,60	16000, 00	4624,00	366,4 0	286,5 0	2911,00	12213, 90	5,45	15,0 4
Mea n	26,8 8	12,5 8	9,39	152,0 0	1498,8 8	122,38	152,2 8	4,82	1469,8 8	629,63	1330,6 3	1,37	6,46	4136,7 5	932,63	88,06	75,09	627,00	1754,4 8	1,08	2,80
SALT_S_Reference																					
Min	30,0 0	19,2 0	8,50	6,20	19,00	17,00	6,30	0,05	5,00	22,00	71,00	0,05	0,24	154,00	68,00	2,70	2,40	26,00	21,70	0,00	0,00
Max	30,0 0	28,8 0	8,50	12,00	27,00	28,00	12,00	0,44	69,00	23,00	80,00	0,05	0,37	237,00	119,00	3,60	3,50	49,00	313,60	0,00	0,00
Mea n	30,0 0	24,0 0	8,50	9,10	23,00	22,50	9,15	0,25	37,00	22,50	75,50	0,05	0,31	195,50	93,50	3,15	2,95	37,50	167,65	0,00	0,00
SALT_S_Disturbed																					
Min	10,0 0	19,2 0	7,10	0,80	1,80	1,60	0,70	0,07	1,30	2,30	9,10	0,05	0,03	19,00	7,00	0,20	0,30	2,90	51,53	0,00	0,00
Max	30,0 0	27,0 0	9,00	62,00	384,00	396,00	751,0 0	2,50	4090,0 0	196,00	539,00	1,10	5,40	3456,0 0	4081,00	89,00	87,20	3093,00	9088,8 9	0,00	0,00
Mea n	24,2 9	24,0 7	8,39	39,83	233,40	80,09	125,7 7	0,69	828,76	99,61	217,01	0,22	1,97	1258,0 0	717,86	25,56	24,41	517,99	1585,9 8	0,00	0,00
Open Water_W_Disturbed																					

Min	20,0 0	23,0 0	8,27	45,00	184,00	27,00	24,00	0,08	87,00	143,00	323,00	0,79	1,24	794,00	166,00	12,50	12,40	99,00	60,84	2,00	2,70
Max	20,0 0	23,0 0	8,27	45,00	184,00	27,00	24,00	0,08	87,00	143,00	323,00	0,79	1,24	794,00	166,00	12,50	12,40	99,00	60,84	2,00	2,70
Mea n	20,0 0	23,0 0	8,27	45,00	184,00	27,00	24,00	0,08	87,00	143,00	323,00	0,79	1,24	794,00	166,00	12,50	12,40	99,00	60,84	2,00	2,70
Open Water_S_Disturbed																					
Min	30,0 0	22,4 0	8,40	20,00	98,00	28,00	14,00	0,37	73,00	60,00	155,00	0,05	0,62	397,00	128,00	7,40	6,30	58,00	121,67	0,00	0,00
Max	30,0 0	22,4 0	8,40	20,00	98,00	28,00	14,00	0,37	73,00	60,00	155,00	0,05	0,62	397,00	128,00	7,40	6,30	58,00	121,67	0,00	0,00
Mea n	30,0 0	22,4 0	8,40	20,00	98,00	28,00	14,00	0,37	73,00	60,00	155,00	0,05	0,62	397,00	128,00	7,40	6,30	58,00	121,67	0,00	0,00

Appendix 4-Index scores

	MMI1	MMI2;3;4	TDI	%PT	GDI	SPI	BDI	TIDL	EPI-D
S1_R_Wint	75,19254	63,647705	16,9	0,7	15	13,2	13,9	9,1	13,8
S2_R_Wint	82,03667	87,54643	5,3	77,9	3,2	8,8	8,9	6,1	7,2
S3_R_Wint	81,09091	94,820086	9,2	55	7,3	7,8	11,8	6,4	12,3
S4_R_Wint	78,48927	87,93237	6,4	28	7,8	8,5	10,8	4,2	10,9
S27_R_Wint	45,01711	21,587456	13,9	3,3	14,4	15,8	16,7	12,2	15,7
S28_R_Wint	70,56336	0	16,2	0	17,8	18,1	20	15,1	17,2
S1_R_Sum	86,93061	83,031546	5,1	89,6	2	7,7	18,9	9,4	10,5
S2_R_Sum	77,37128	87,754298	3,6	69,5	4,7	3,8	7,4	5	6,7
S3_R_Sum	89,62592	74,453908	7,9	57,7	6,9	7,6	12,7	6,6	11,2
S4_R_Sum	87,94593	70,083819	5,3	59,7	5,1	4,4	9,8	5	8
S27_R_Sum	68,51742	14,640652	11,4	11,5	13,7	11,8	12,1	9,8	13,9
S28_R_Sum	49,69417	11,274282	13,4	14,6	14,2	11,3	14,9	8,8	14,2

S6_S_Wint	71,87812	50,239922	5,4	61,5	5,2	4,1	4,1	4,5	7,1
S7_S_Wint	82,30242	66,238604	5,2	66,7	3,4	7,5	7,9	5	9,3
S9_S_Wint	75,88871	79,908839	2,3	0,5	7,8	5,9	7,2	3,7	6,7
S10_S_Wint	85,04944	84,06711	1,3	77	6,2	5,2	4,1	5,6	3,9
S17_S_Wint	67,3154	47,098381	1,7	67,4	4,2	3,5	7,2	3,6	7,7
S18_S_Wint	69,03532	41,769511	2,3	90,1	1	8	9,4	4,7	8
S20_S_Wint	90,94768	70,48818	2,4	29,8	3,7	5,3	6,5	2,7	6,7
S21_S_Wint	64,03933	46,720647	11,9	10,9	10,7	10,3	15,9	9,2	13,8
S22_S_Wint	60,54528	29,489076	19	0,4	18,9	15,5	20	15,1	16
S23_S_Wint	64,84876	42,076856	13,8	0	17,1	18,5	20	17,4	19,3
S26_S_Wint	20,06678	35,573555	8,4	35	8,6	10,2	12,9	5,7	9,6
S6_S_Sum	83,09561	55,578001	2,4	35,9	7,6	3,5	6,4	5	5,7
S7_S_Sum	86,18911	88,057009	1,1	12	11	7,7	9,7	3,3	3,9
S10_S_Sum	86,91638	65,852557	2,6	15,3	7,2	2,4	4,2	2,5	3,8

S17_S_Sum	74,08415	67,780546	2,6	37	6,3	2,5	4,1	3,1	4,3
S18_S_Sum	81,94045	68,668849	4	19,1	10,8	5,1	6,7	5,8	6,3
S20_S_Sum	79,19351	74,555824	3,2	34,8	7,2	2,8	4,5	3,1	4,9
S22_S_Sum	24,35457	32,136424	10,9	7,7	9,2	11,3	16,6	5,6	11,1
S23_S_Sum	22,22067	40,008214	12,9	1,9	13,8	15,8	20	17	16,8
S26_S_Sum	20,67695	23,460686	5,6	67,2	5,9	9	6,5	2,8	6,9
S5_G_Wint	75,33165	67,241291	10,3	67,4	6,4	9,6	13	7,9	13,8
S8_G_Wint	77,7647	72,237532	9,5	62,7	6,4	6	13,1	6,3	11,1
S11_G_Wint	84,76231	62,297863	9,6	30,5	11	11	11,6	8,2	12
S12_G_Wint	81,12909	56,509145	5,6	10,9	10	7,8	7,4	4	7,6
S13_G_Wint	79,92228	30,401721	14,7	4	16,6	18,6	19,1	14,8	17,4
S14_G_Wint	82,38606	71,888231	7,6	55,6	7,4	10,8	11,8	7,8	13,3
S15_G_Wint	81,67636	53,243417	12,8	15,6	13,7	17,4	17	13,2	16,8
S16_G_Wint	71,27193	34,372269	6,8	72,1	3,8	3,3	3,2	3,6	7,9

S19_G_Wint	75,8964	32,772448	5,1	46,2	6,4	5,5	6,9	3,4	6,9
S24_G_Wint	76,02213	50,02053	2,6	61	9	4,1	6,5	2,9	6,7
S5_G_Sum	66,05584	58,126268	8,2	65,5	5,4	7,8	9,7	8	13,8
S8_G_Sum	76,20398	53,456869	13	33	11,8	10,3	19,6	11,3	12,3
S11_G_Sum	88,34235	35,858372	5,5	27,8	11,8	5,5	7,2	5,8	7,2
S12_G_Sum	91,02167	41,28276	9,2	29,2	9	11	14,1	7	10,7
S13_G_Sum	84,29439	45,17089	9,2	19,7	11,1	13,2	14,7	13,7	15,5
S14_G_Sum	79,38763	60,438665	5,1	63,3	5,8	5,3	8,6	5,7	9,6
S15_G_Sum	79,33887	67,711986	6,6	74,5	4,6	6,6	12,1	6,8	11,9
S16_G_Sum	55,22079	21,166667	5,2	3,7	10,5	10,9	9,9	2,9	7,5
S19_G_Sum	77,06516	51,133796	6,9	36,5	8,7	5,3	8,2	5,9	5,9
S24_G_Sum	59,52378	27,5331	3,6	46,2	8,3	3	5,2	4,7	5,8

Appendix 4- Index scores

	MMI1	MMI2;3; 4	TDI	%PT	GDI	SPI	BDI	TIDL	EPI
S1_R_Wint	75,2	63,6	16,9	0,7	15	13,2	13,9	9,1	13,8
S2_R_Wint	82,0	87,5	5,3	77,9	3,2	8,8	8,9	6,1	7,2
S3_R_Wint	81,1	94,8	9,2	55	7,3	7,8	11,8	6,4	12,3
S4_R_Wint	78,5	87,9	6,4	28	7,8	8,5	10,8	4,2	10,9
S27_R_Wint	45,0	21,6	13,9	3,3	14,4	15,8	16,7	12,2	15,7
S28_R_Wint	70,6	0,0	16,2	0	17,8	18,1	20	15,1	17,2
S1_R_Sum	86,9	83,0	5,1	89,6	2	7,7	18,9	9,4	10,5
S2_R_Sum	77,4	87,8	3,6	69,5	4,7	3,8	7,4	5	6,7
S3_R_Sum	89,6	74,5	7,9	57,7	6,9	7,6	12,7	6,6	11,2
S4_R_Sum	87,9	70,1	5,3	59,7	5,1	4,4	9,8	5	8
S27_R_Sum	68,5	14,6	11,4	11,5	13,7	11,8	12,1	9,8	13,9
S28_R_Sum	49,7	11,3	13,4	14,6	14,2	11,3	14,9	8,8	14,2
S6_S_Wint	71,9	50,2	5,4	61,5	5,2	4,1	4,1	4,5	7,1
S7_S_Wint	82,3	66,2	5,2	66,7	3,4	7,5	7,9	5	9,3
S9_S_Wint	75,9	79,9	2,3	0,5	7,8	5,9	7,2	3,7	6,7
S10_S_Wint	85,0	84,1	1,3	77	6,2	5,2	4,1	5,6	3,9
S17_S_Wint	67,3	47,1	1,7	67,4	4,2	3,5	7,2	3,6	7,7
S18_S_Wint	69,0	41,8	2,3	90,1	1	8	9,4	4,7	8
S20_S_Wint	90,9	70,5	2,4	29,8	3,7	5,3	6,5	2,7	6,7

S21_S_Wint	64,0	46,7	11,9	10,9	10,7	10,3	15,9	9,2	13,8
S22_S_Wint	60,5	29,5	19	0,4	18,9	15,5	20	15,1	16
S23_S_Wint	64,8	42,1	13,8	0	17,1	18,5	20	17,4	19,3
S26_S_Wint	20,1	35,6	8,4	35	8,6	10,2	12,9	5,7	9,6
S6_S_Sum	83,1	55,6	2,4	35,9	7,6	3,5	6,4	5	5,7
S7_S_Sum	86,2	88,1	1,1	12	11	7,7	9,7	3,3	3,9
S10_S_Sum	86,9	65,9	2,6	15,3	7,2	2,4	4,2	2,5	3,8
S17_S_Sum	74,1	67,8	2,6	37	6,3	2,5	4,1	3,1	4,3
S18_S_Sum	81,9	68,7	4	19,1	10,8	5,1	6,7	5,8	6,3
S20_S_Sum	79,2	74,6	3,2	34,8	7,2	2,8	4,5	3,1	4,9
S22_S_Sum	24,4	32,1	10,9	7,7	9,2	11,3	16,6	5,6	11,1
S23_S_Sum	22,2	40,0	12,9	1,9	13,8	15,8	20	17	16,8
S26_S_Sum	20,7	23,5	5,6	67,2	5,9	9	6,5	2,8	6,9
S5_G_Wint	75,3	67,2	10,3	67,4	6,4	9,6	13	7,9	13,8
S8_G_Wint	77,8	72,2	9,5	62,7	6,4	6	13,1	6,3	11,1
S11_G_Wint	84,8	62,3	9,6	30,5	11	11	11,6	8,2	12
S12_G_Wint	81,1	56,5	5,6	10,9	10	7,8	7,4	4	7,6
S13_G_Wint	79,9	30,4	14,7	4	16,6	18,6	19,1	14,8	17,4
S14_G_Wint	82,4	71,9	7,6	55,6	7,4	10,8	11,8	7,8	13,3
S15_G_Wint	81,7	53,2	12,8	15,6	13,7	17,4	17	13,2	16,8
S16_G_Wint	71,3	34,4	6,8	72,1	3,8	3,3	3,2	3,6	7,9
S19_G_Wint	75,9	32,8	5,1	46,2	6,4	5,5	6,9	3,4	6,9

S24_G_Wint	76,0	50,0	2,6	61	9	4,1	6,5	2,9	6,7
S5_G_Sum	66,1	58,1	8,2	65,5	5,4	7,8	9,7	8	13,8
S8_G_Sum	76,2	53,5	13	33	11,8	10,3	19,6	11,3	12,3
S11_G_Sum	88,3	35,9	5,5	27,8	11,8	5,5	7,2	5,8	7,2
S12_G_Sum	91,0	41,3	9,2	29,2	9	11	14,1	7	10,7
S13_G_Sum	84,3	45,2	9,2	19,7	11,1	13,2	14,7	13,7	15,5
S14_G_Sum	79,4	60,4	5,1	63,3	5,8	5,3	8,6	5,7	9,6
S15_G_Sum	79,3	67,7	6,6	74,5	4,6	6,6	12,1	6,8	11,9
S16_G_Sum	55,2	21,2	5,2	3,7	10,5	10,9	9,9	2,9	7,5
S19_G_Sum	77,1	51,1	6,9	36,5	8,7	5,3	8,2	5,9	5,9
S24_G_Sum	59,5	27,5	3,6	46,2	8,3	3	5,2	4,7	5,8

**Appendix 5- Plates illustrating diatom species most responsible for dissimilarities in
community composition**

Plate 1:

Figure 1-5: *Achnanthes* sp. 1

Figure 6-7: *Achnanthidium exiguum* (Grunow) Czarnecki

Figure 8-15: *Achnanthidium minutissimum* (Kützing) Czarnecki

Figure 16-22: *Amphora veneta* Kützing

Figure 23-26: *Anomoeneis sphaerophora* (Ehrenberg) Pfitzer

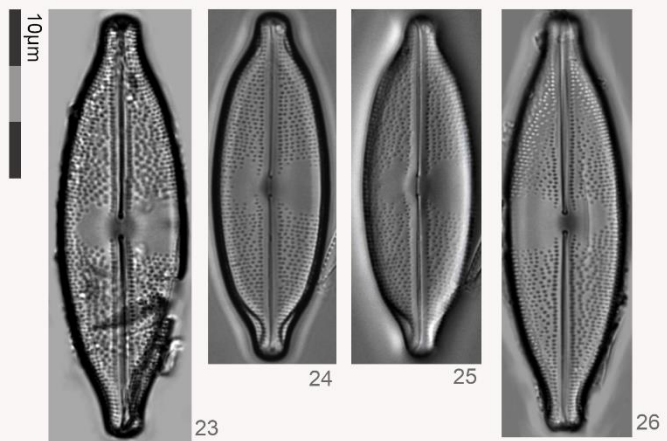
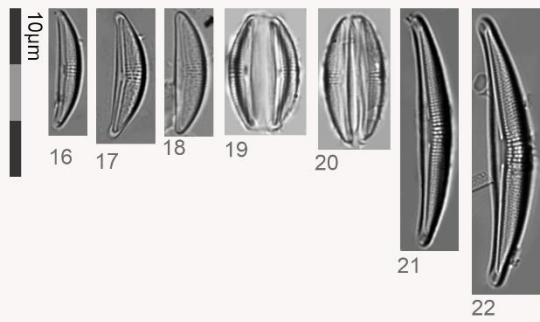
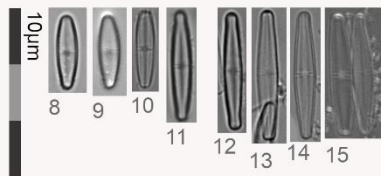
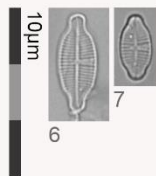
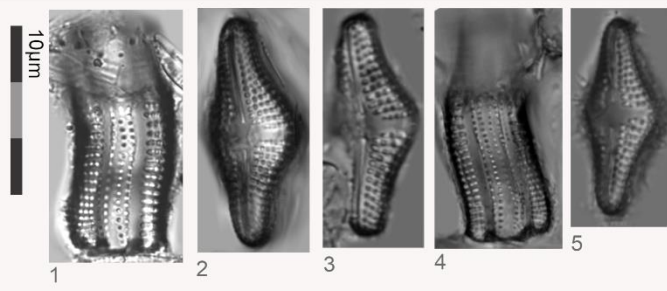


Plate 2

Figure 1-2: *Brachysira brebissonii* Ross

Figure 4-9: *Brachysira neoexilis* (Grunow) DG Mann

Figure 10-16: *Caloneis molaris* (Grunow) Krammer

Figure 17-23: *Cocconeis placentula* Ehrenberg

Figure 24: *Craticula buderii* (Hustedt) Lange-Bertalot

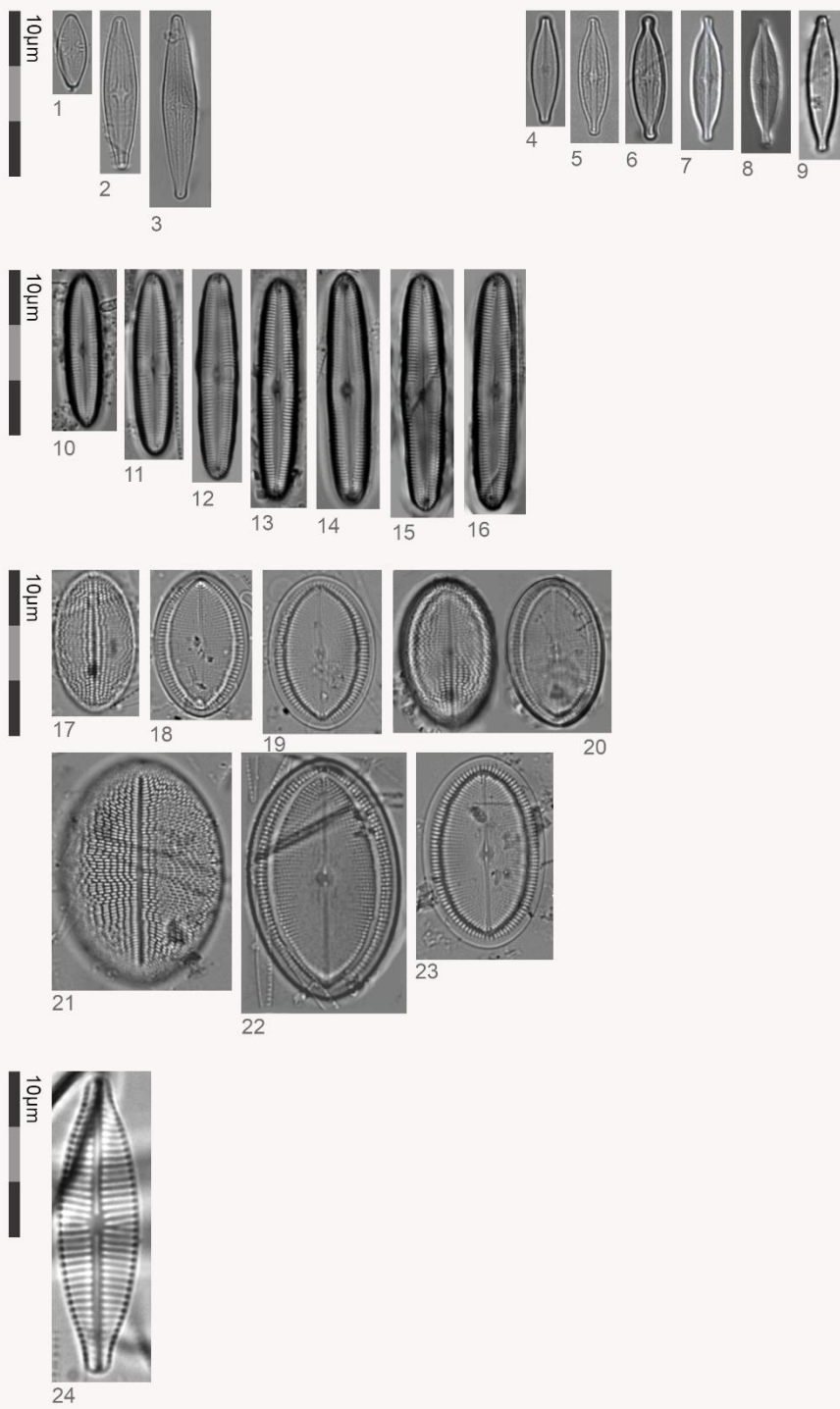


Plate 3

Figure 1-2: *Craticula cuspidata* (Kützinger) DG Mann

Figure 3-7: *Craticula halophila* (Grunow) DG Mann

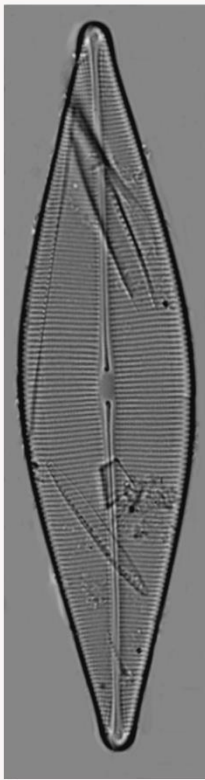
Figure 8-14: *Craticula molestiformis* (Hustedt) Lange-Bertalot

Figure 15-20: *Cyclotella meneghiniana* Kützinger

Figure 21-23: *Cymbella tumida* (Brébisson) Van Heurck

Figure 24-25: *Cymbella turgidula* Grunow

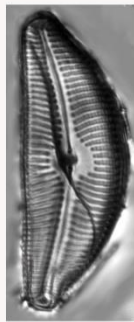
10µm



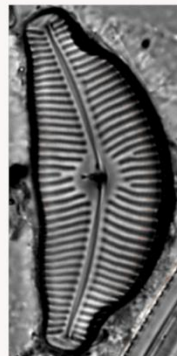
1



2



21

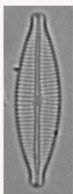


22



23

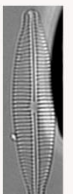
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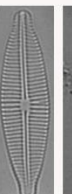
3



4



5



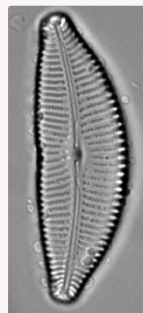
6



7



24



25

10µm



8



9



10



11



12

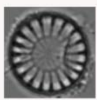


13

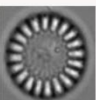


14

10µm



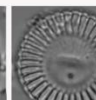
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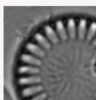
16



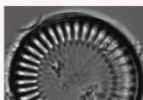
17



18



19



20

Plate 4

Figure 1-4: *Diadismus confervacea* (Kützing) DG Mann

Figure 5-13: *Encyonema mesianum* (Cholnoky) DG Mann

Figure 14-22: *Encyonema minutum* (Hilse) DG Mann

Figure 23-25: *Encyonema silesiacum* (Bleisch) DG Mann

Figure 26-31: *Encyonopsis cesatii* (Rabenhorst) Krammer

Figure 32-33: *Encyonopsis leei* var. *sinensis* Metzeltin & Krammer

Figure 34-38: *Encyonopsis microcephala* (Grunow) Krammer

Figure 39-40: *Encyonopsis subminuta* Krammer & Reichardt

Figure 41-43: *Encyonopsis vandamii* Krammer

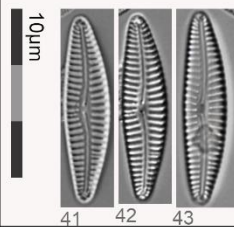
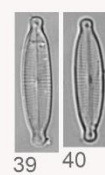
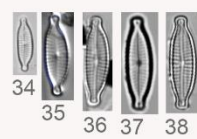
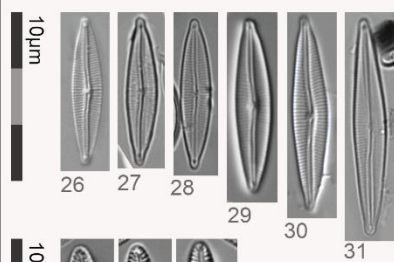
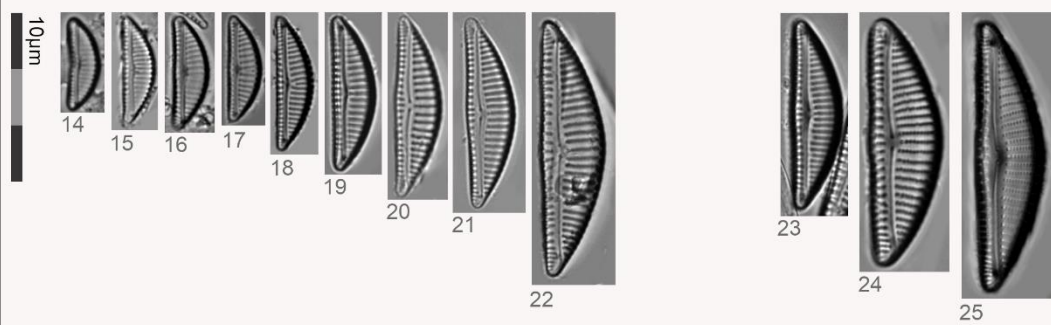
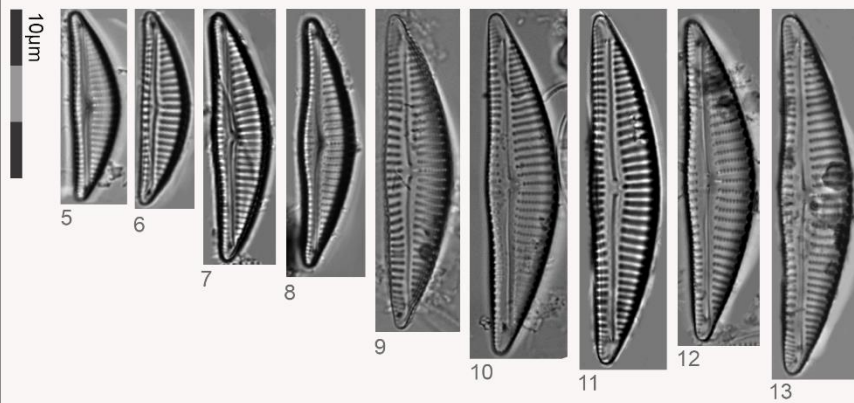
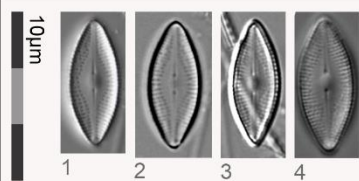


Plate 5

Figure 1-2: *Eolimna minima* (Grunow) Lange-Bertalot

Figure 3: *Eolimna subminuscula* (Manguin) Lange-Bertalot

Figure 4-11: *Epithemia adnata* (Kützing) Brébisson

Figure 12-16: *Eunotia bilunaris* (Ehrenberg) Mills

Figure 17-19: *Eunotia flexuosa* (Brébisson) Kützing

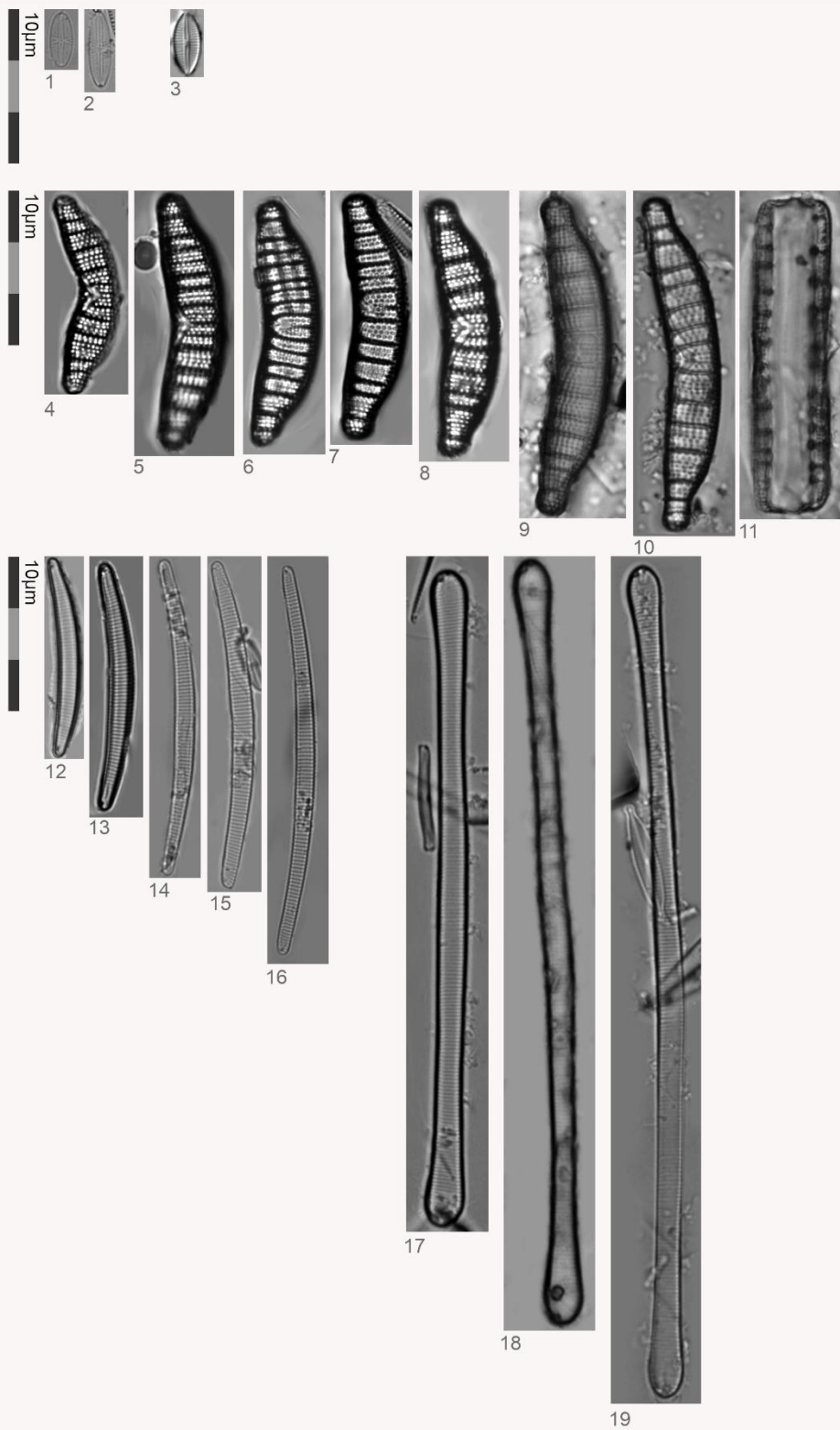


Plate 6

Figure 1: *Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot

Figure 2-6: *Fragilaria capucina* Desmazières

Figure 7-8: *Fragilaria capucina* var. *rumpens* (Kützing) Lange-Bertalot

Figure 9: *Fragilaria capucina* var. *vaucheriae* (Kützing) Lange-Bertalot

Figure 10-11: *Fragilaria tenera* (WM Smith) Lange-Bertalot

Figure 13-17: *Frustululia crassinervia* (Kützing) Cleve

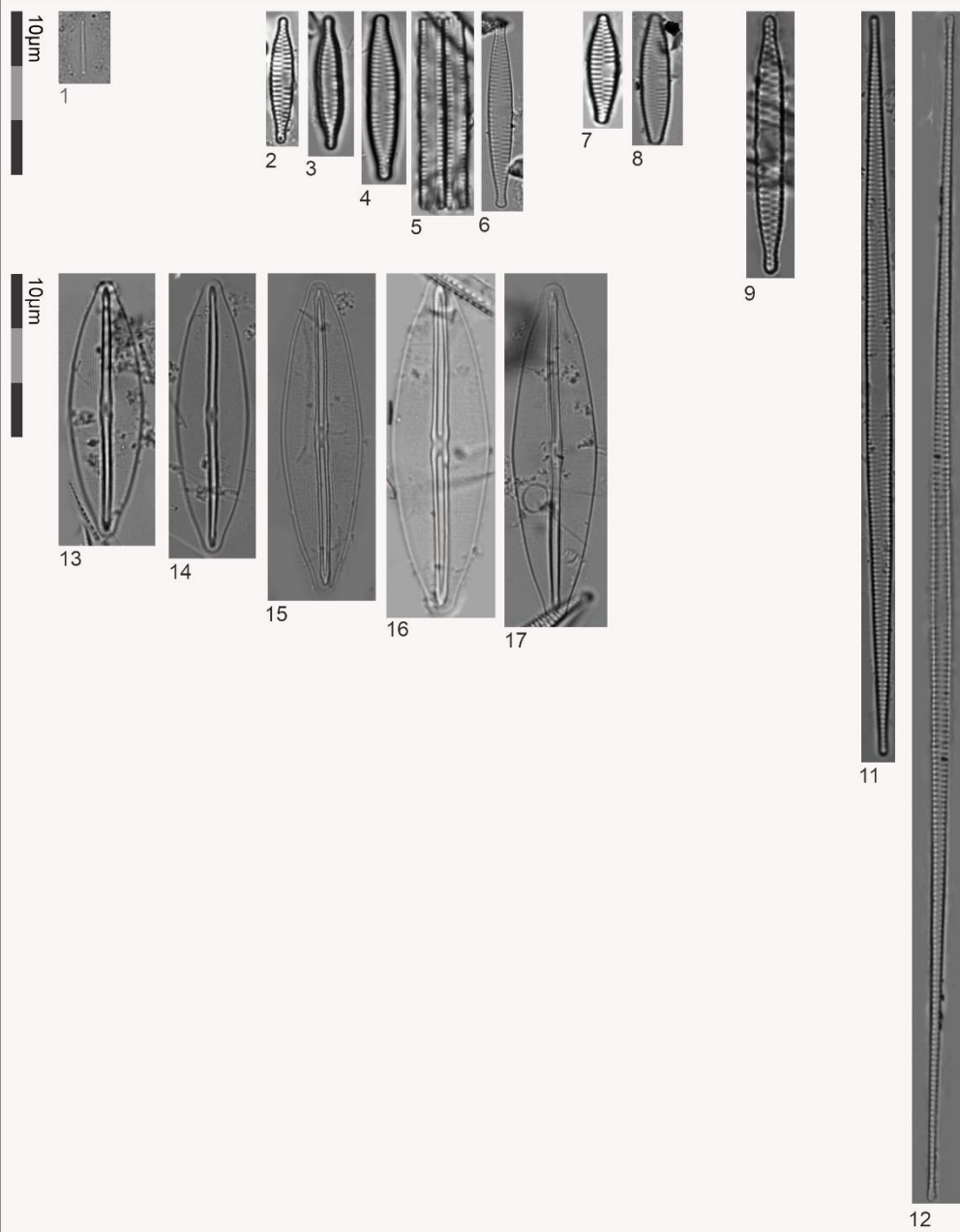


Plate 7

Figure 1-13: *Gomphonema* aff. *gracile*

Figure 21-30 *Gomphonema angustatum* (Kützing) Rabenhorst

Figure 14-20: *Gomphonema gracile* Ehrenberg

Figure 31-36: *Gomphonema insigne* Gregory

Figure 37-40: *Gomphonema lagenula* Kützing

Figure 41-45: *Gomphonema parvulus* Lange-Bertalot & Reichardt

Figure 46-50: *Gomphonema parvulum* (Kützing) Kützing

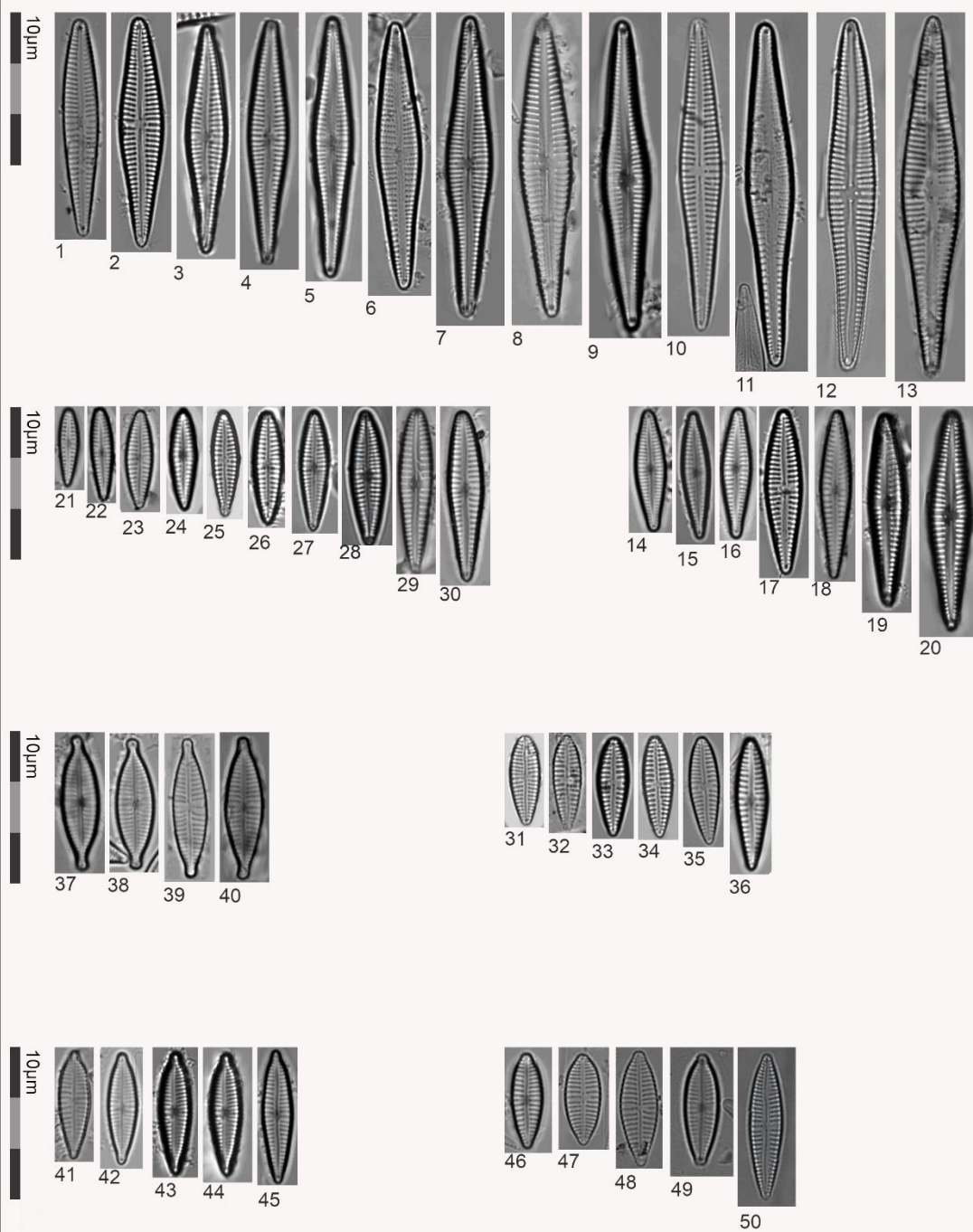


Plate 8

Figure 1-4: *Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski

Figure 5-13: *Mastogloia elliptica* (Agardh) Cleve

Figure 14-20: *Navicula radiosa* Kützinger

Figure 21-29: *Navicula recens* (Lange-Bertalot) Lange-Bertalot

Figure 30: *Navicula rostellata* Kützinger

Figure 31-35: *Navicymbula pusilla* (Grunow) Krammer

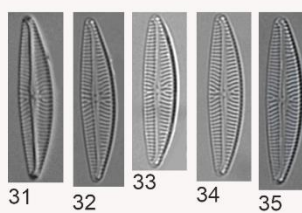
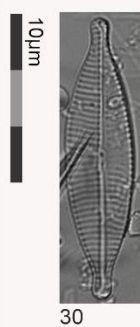
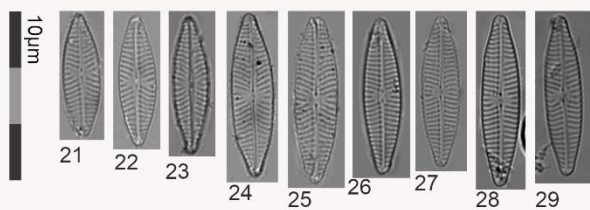
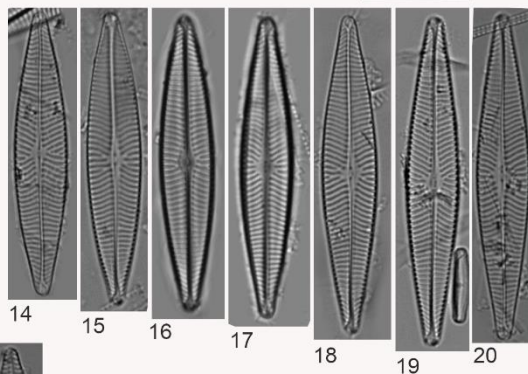
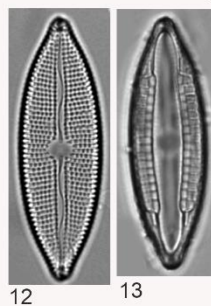
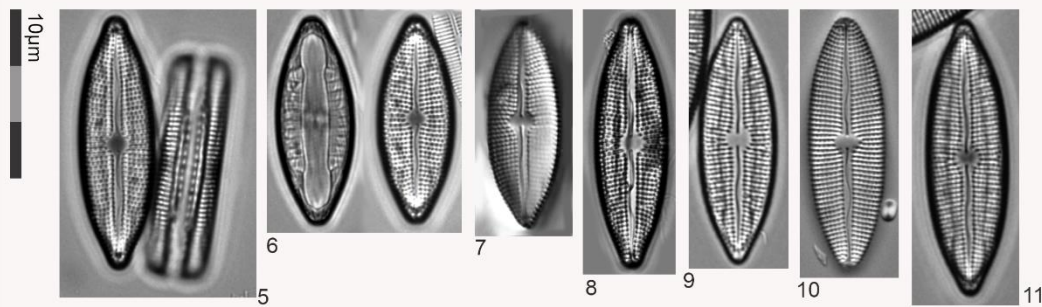
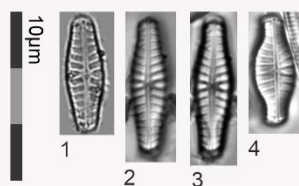


Plate 9

Figure 1-9: *Nitzschia amphibia* Grunow

Figure 7-8: *Nitzschia archibaldii* Lange-Bertalot

Figure 9-12: *Nitzschia aurariae* Cholnoky

Figure 13-15: *Nitzschia clausii* Hantzsch

Figure 16-18: *Nitzschia etoshensis* Cholnoky

Figure 19-22: *Nitzschia frustulum* (Kützing) Grunow

Figure 23-28: *Nitzschia liebertruthii* Rabenhorst

Figure 29-33: *Nitzschia microcephala* Grunow

Figure 37-40: *Nitzschia palea* (Kützing) W Smith

Figure 41-45: *Nitzschia pura* Hustedt

Figure 46-53: *Planothidium engelbrechtii* (Cholnoky) Round & Bukhityarova

Figure 54-57: *Rhopalodia gibba* (Ehrenberg) O Müller

Figure 58-64: *Sellaphora seminulum* (Grunow) DG Mann

