# A DIATOM-BASED INDEX FOR ACID MINE DRAINAGE IMPACTED PERMANENT PANS IN THE MPUMALANGA HIGHVELD REGION

Final Report March 2021

Prepared for: COALTECH RESEARCH ASSOCIATION


Melanie Erasmus
melanieerasmus12@gmail.com
Supervisor: Prof Jonathan Taylor

## Summary

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).

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. 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.

# Table of Contents

## List of Figures

## List of tables



# Introduction

## 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.* (2012) 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*., 2012). 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).

### 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:

$$2FeS_2\ (s) + 7O_2\ (aq) + 2H_2O \rightarrow 2Fe^{+2} + 4SO_4^{-2} + 4H^{+};$$

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

$$2Fe^{+2} + \frac{1}{2}\ O_2 + 2H^{+} \rightarrow 2Fe^{+3} + H_2O$$

(Akcil and Koldas, 2006; Pinetown *et al.*, 2007). Smucker and Vis (2009) list high conductivity, low pH and high sulphate ($SO4^{2-}$) concentration as significant evidence of AMD contamination. Acid mine drainage contamination affects fresh water 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.

## 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.

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 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.

## 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, 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 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 Valves (%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 and their ecological requirements.

## Research aim and objectives

### 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.

### 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 have to 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 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.

# Materials and methods:

## 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.

## **Classification of Depressional Wetlands in the Mpumalanga** Highveld

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) recommends 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 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 s*p.) 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 seems due to the vegetation within the pans and seasonal dilution. Values as low as 0.16 mScm$^{-1}$ where observed by Hoffman (2012) ranging to o 0.25 mScm$^{-1}$.

## 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 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.

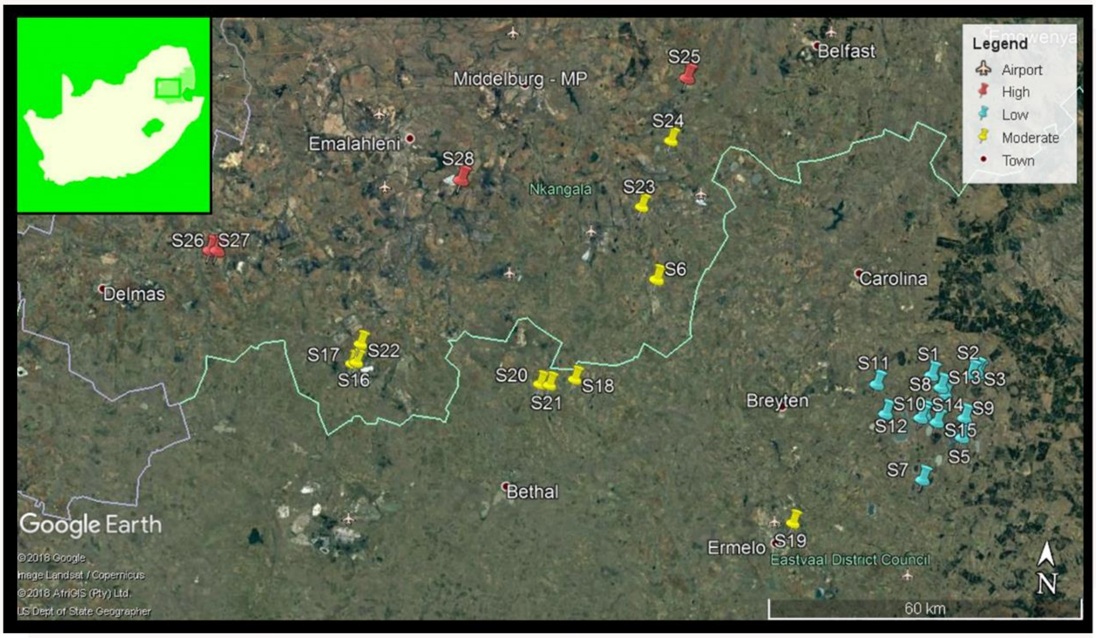


**Figure 1: Distribution of pan sites across the Mpumalanga Highveld region**.

**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 30°20'36.37"E | Reed | Low | 02/06/2019 | 17/01/2020 |
| **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 | Grass | Low | 02/06/2019 | 16/01/2020 |

| | | | | | |
|---|---|---|---|---|---|
| | 30° 9'17.00"E | | | | |
| **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 30°14'52.80"E | Grass | Low | 04/06/2019 | 16/01/2020 |
| **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 |

## Field procedures

### 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.

### Diatom sampling

Epiphytic diatoms were sampled 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 50cm 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.

### 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 500ml- 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).

## Laboratory procedures

### 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

### 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.

### Equipment necessary for preparation of diatom slides

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



### 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.

### 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).

## 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.

## 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 refence taxa: $= \frac{n\ reference\ taxa}{total\ n\ taxa}$ x 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: $= \frac{n\ tolerant\ taxa}{total\ n\ taxa}$ x 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: $= \frac{n\ taxa\ found\ in\ reference\ sites\ as\ well\ as\ impacted\ site}{total\ n\ taxa}$ x 100 | Decrease |
| % Reference individuals found in reference sites that occurred in impaired sites | Relative abundance of individuals (valves) found in reference sites that also occurred in impacted sites: $\frac{n\ individuals\ found\ in\ reference\ sites\ as\ well\ as\ impacted\ sites}{total\ n\ valves}$ x 100 | Decrease |
| 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: $= \frac{n\ mobile\ taxa}{total\ n\ taxa}$ x 100 | Decrease |
| Adnate % taxa | Relative abundance of adnate taxa- attached by the valve face to substrates, growing parallel to the surface: $= \frac{n\ adnate\ taxa}{total\ n\ taxa}$ x 100 | Decrease |
| Pad % taxa | Relative abundance of taxa attached to substrates by a mucilage pad- growing in upright orientation to substrates: $= \frac{n\ pad\ taxa}{total\ n\ taxa}$ x 100 | Increase |
| Non-colonial % taxa | Relative abundance of taxa in a sample that occur individually, not in colonies: $= \frac{n\ non-colonial\ taxa}{total\ n\ taxa}$ x 100 | Decrease |
| Ribbon % taxa | Relative abundance of taxa that occur in ribbon-like colonies: $= \frac{n\ ribbon\ taxa}{total\ n\ taxa}$ x 100 | Increase |
| Ribbon % individuals | Relative abundance of individuals that occur in ribbon-like colonies: $= \frac{n\ ribbon\ individuals}{total\ n\ valves}$ x 100 | Increase |
| High profile guild % taxa | Relative abundance of taxa in the high-profile guild- such as filamentous, branched, chainlike or tubular colonies: $= \frac{n\ high-profile\ taxa}{total\ n\ taxa}$ x 100 | Increase |
| **Taxonomic composition** | | |
| % Encyonopsis taxa | Relative abundance of taxa belonging to the genus *Encyonopsis*: $= \frac{n\ Encyonopsis\ taxa}{total\ n\ taxa}$ x 100 | Increase |
| % Cocconeis taxa | Relative abundance of taxa belonging to the genus *Cocconeis*: $= \frac{n\ Cocconeis\ taxa}{total\ n\ taxa}$ x 100 | Increase |
| % Craticula taxa | Relative abundance of taxa belonging to the genus *Craticula*: $= \frac{n\ Craticula\ taxa}{total\ n\ taxa}$ x 100 | Decrease |
| % Ctenophora taxa | Relative abundance of taxa belonging to the genus *Ctenophora*: $= \frac{n\ Ctenophora\ taxa}{total\ n\ taxa}$ x 100 | Increase |
| % Gomphonema taxa | Relative abundance of taxa belonging to the genus *Gomphonema*: | Variable |

| | | |
|---|---|---|
| | $=\frac{n\ Gomphonema\ taxa}{total\ n\ taxa} \times 100$ | |
| % Nitzschia individuals | Relative abundance of taxa belonging to the genus *Nitzschia*: $=\frac{n\ Nitzschia\ taxa}{total\ n\ taxa} \times 100$ | Decrease |

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

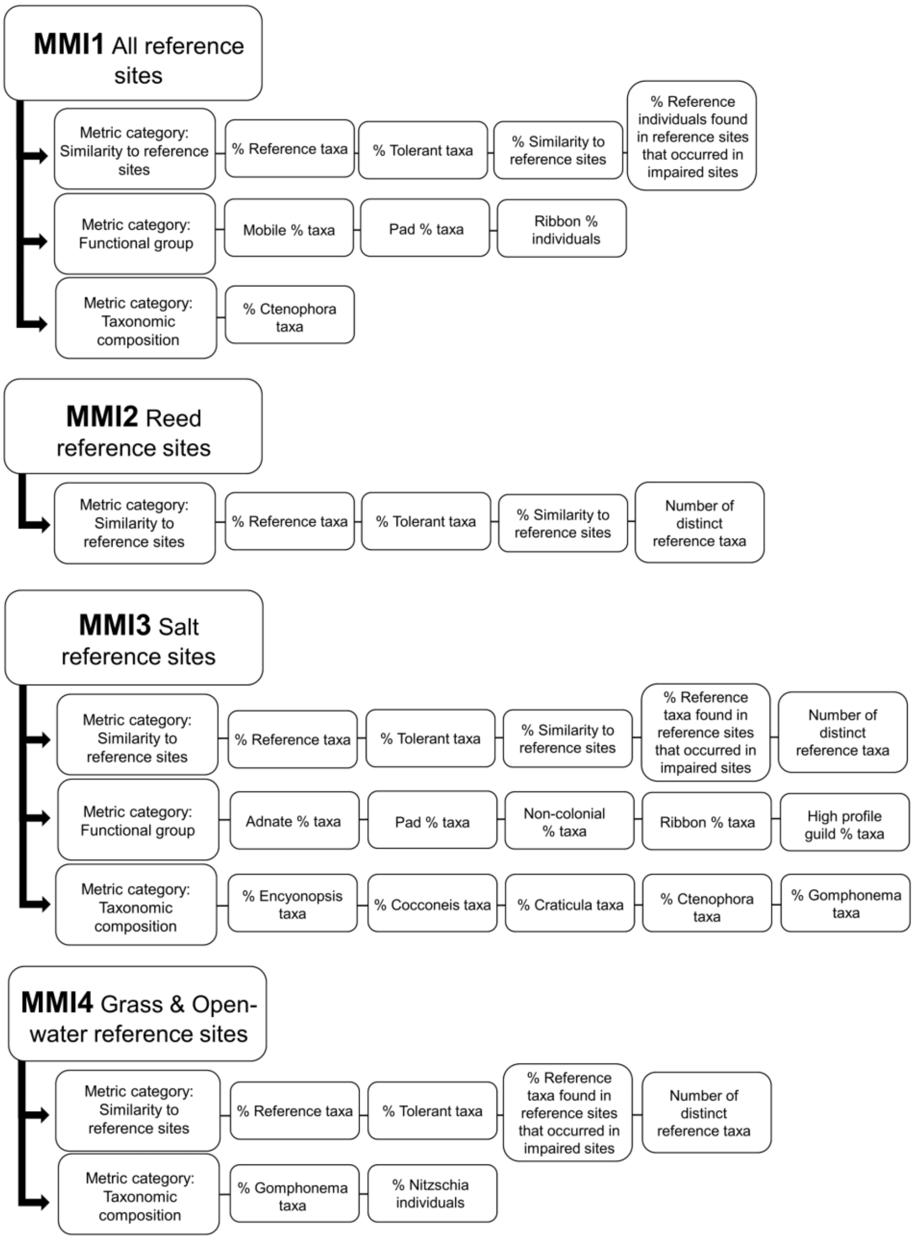


**Figure 2: Metric categories and metrics calculated in each multimetric index**

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 5$^{th}$ and 95$^{th}$ 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.

## Index score calculations

### 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.



### 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 if 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.

# 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

## Results and discussion

### pH

The acidity of water in determined by the concentration of hydrogen ions which is represented on a logarithmic scale as: pH = log10 [$H^+$]. 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.

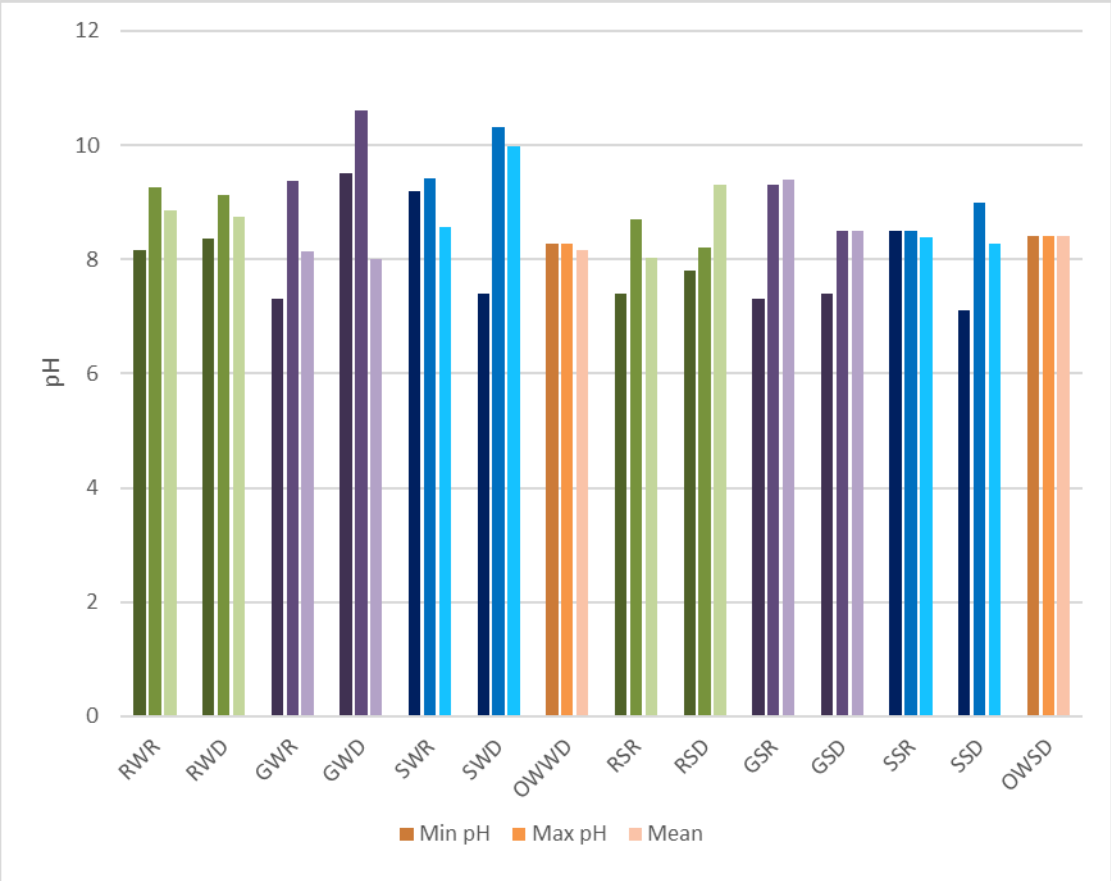


**Figure 3: Minimum, maximum and mean pH values recorded among different pan types. The darkest bars signify minimum values and the lighter bars maximum and mean values**

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.

## Conductivity and Total Dissolved Solids (TDS)

The amount 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 by 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.

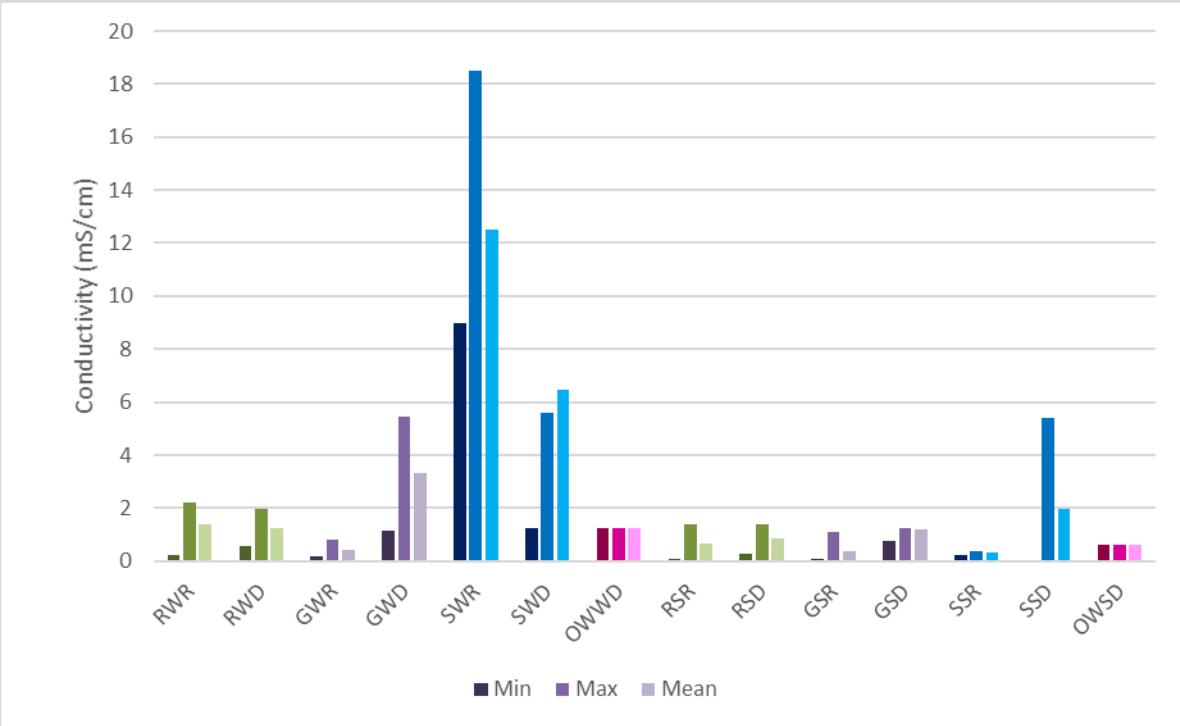




**Figure 4: Minimum, maximum and mean conductivity values for reference and disturbed reed, grass, salt and open water pans.**

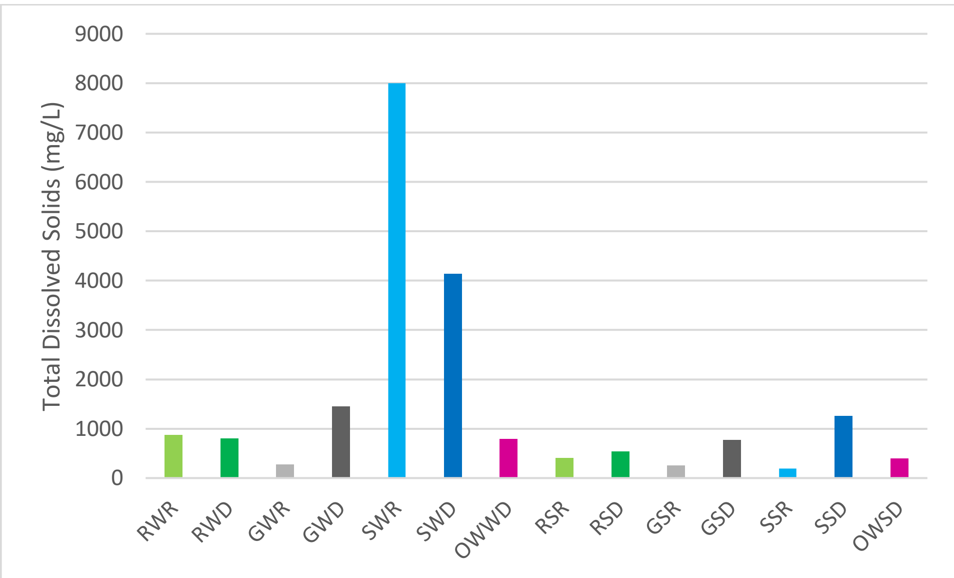


**Figure5: Mean TDS values for reference and disturbed reed, grass, salt and open water pans.**

## Calcium ($Ca^{2+}$)

Calcium is often the major cation in inlands 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 6). 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.

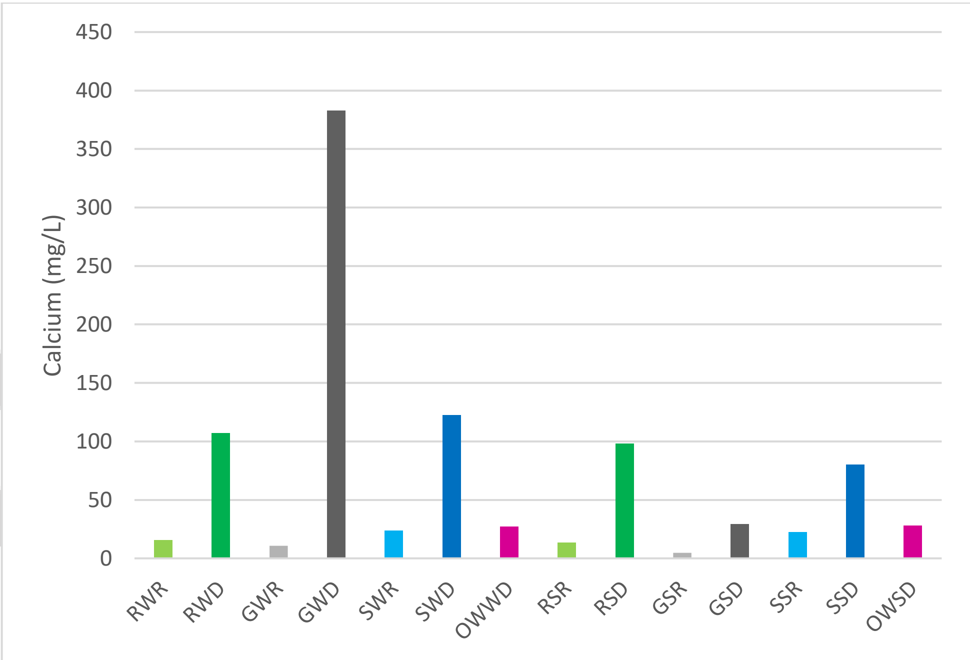


**Figure 6: Mean calcium concentrations for reference and disturbed reed, grass, salt and open water pans.**

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.

## 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. 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 7. Across all pan types, sulphate concentrations are higher in disturbed pans during both seasons. Overall, concentrations are lower in the wet season, yet still substantially higher in disturbed sites.

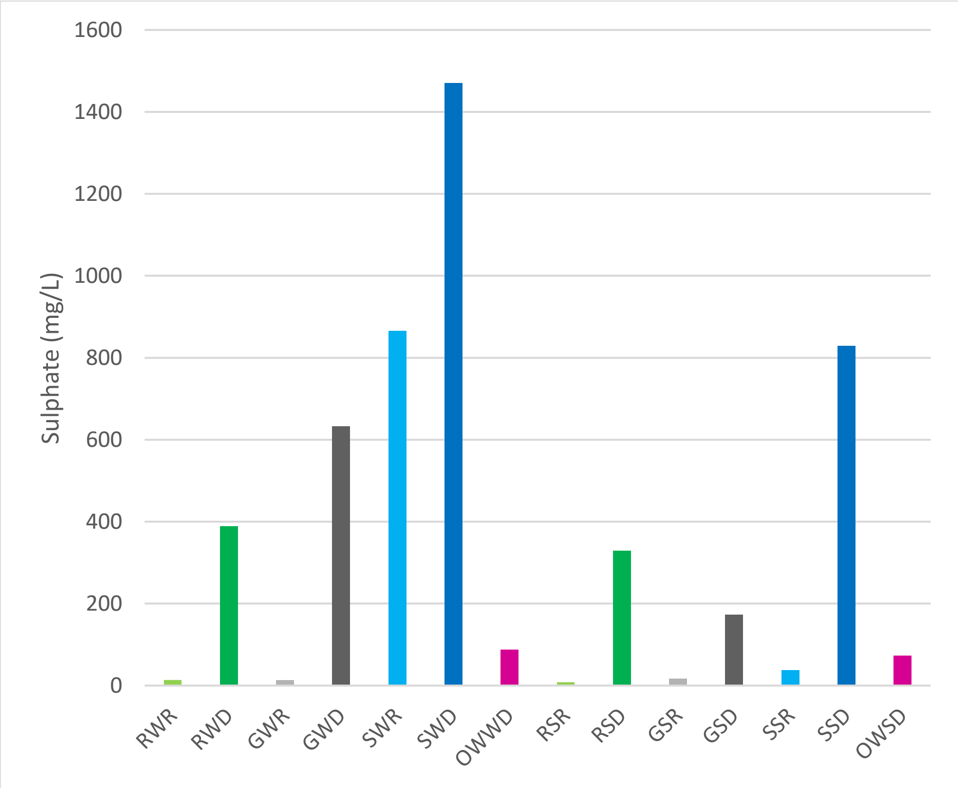


**Figure 7: Mean sulphate concentrations for reference and disturbed reed, grass, salt and open water pans.**

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.

## 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 8. 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.

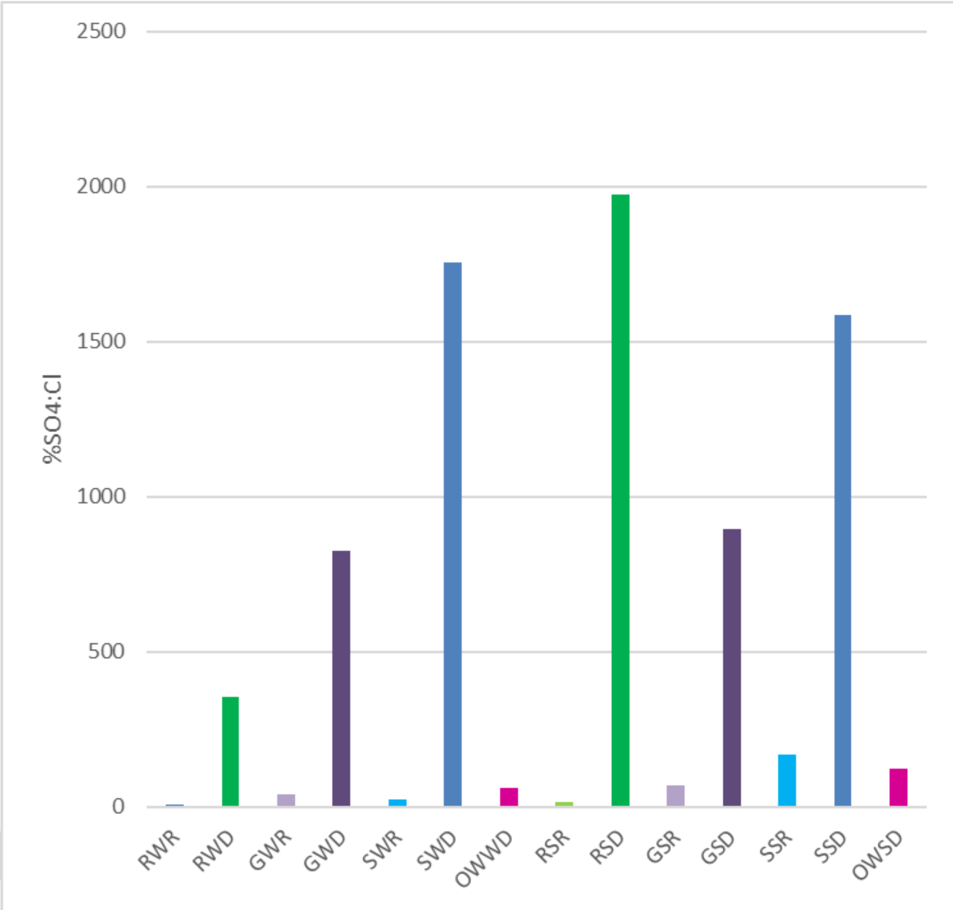


**Figure 8: Mean sulphate to chloride ratios for reference and disturbed reed, grass, salt and open water pans.**

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 a salt pans, which have natural high levels of sulphate that could be misleading.

## 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 which are known to be the lowest of the different 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.

## Diatom community composition and relation to environmental variables

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.

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. Secondly, 7.85% of the total variance was due to pan type. 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 9.

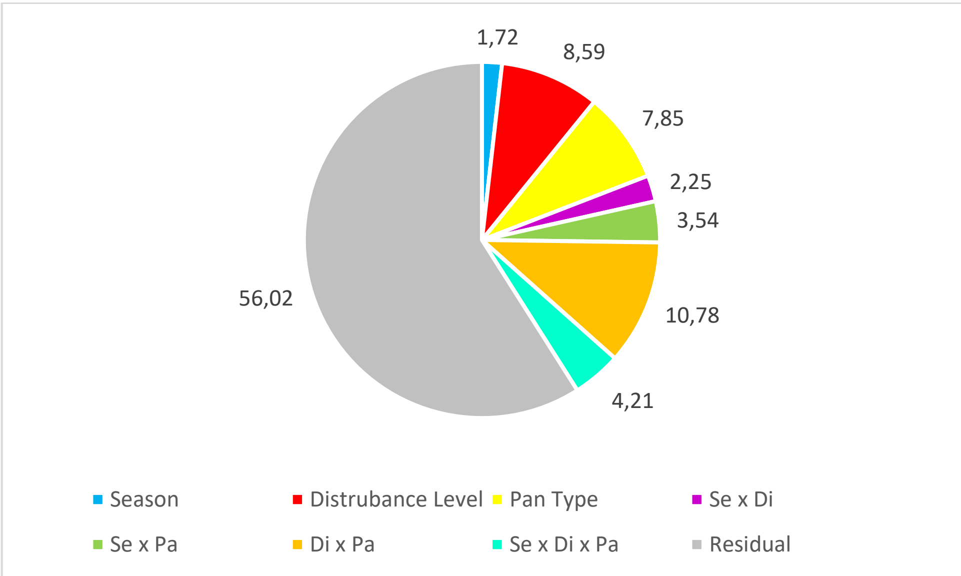


**Figure 9: Taxa that cause at least 25% of variance between pan types. The combined effects of seasonal change and pan is represented by Se x Pa, the effects of disturbance level and pan type combined is represented by Di x Pa and the combined effect of all three factors by Se x Di x Pa.**

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.

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 below. 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. In the figures below, 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.

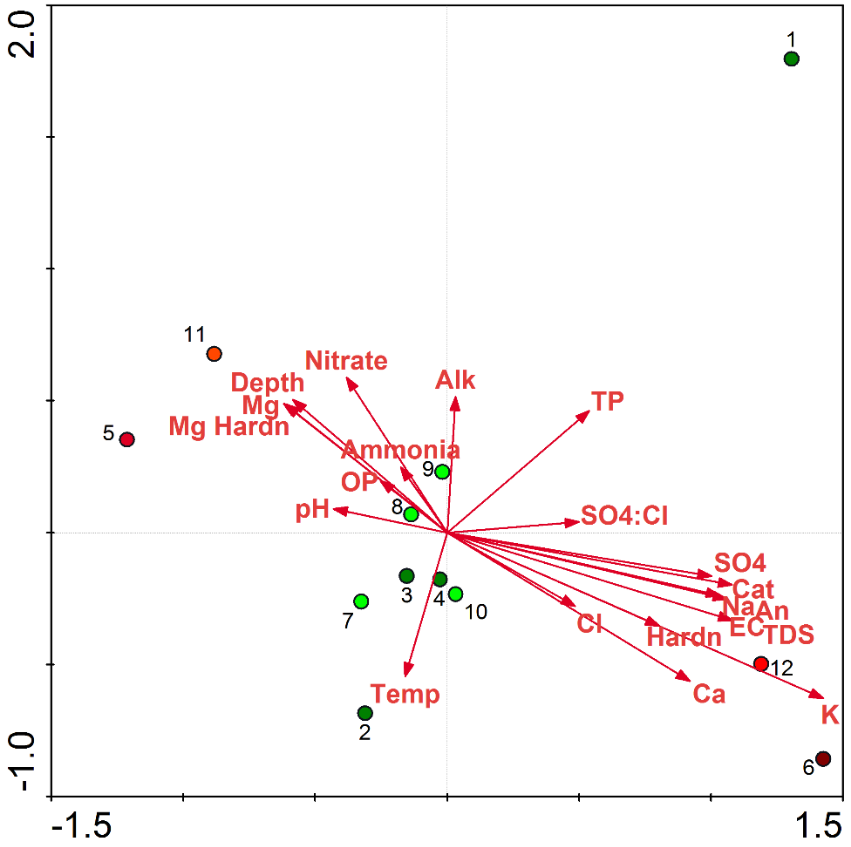


**Figure 10: CCA biplot showing the inter sample relationship between the measured environmental variables and reed pan samples (based on diatom taxa from reed pans). Reference sites are labelled: 1=S1; 2=S2; 3=S3; 4=S4 (winter) and 7=S1; 8=S2; 9=S3; 10=S4 (summer). Disturbed sites are labelled: 5=S27;6=S28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

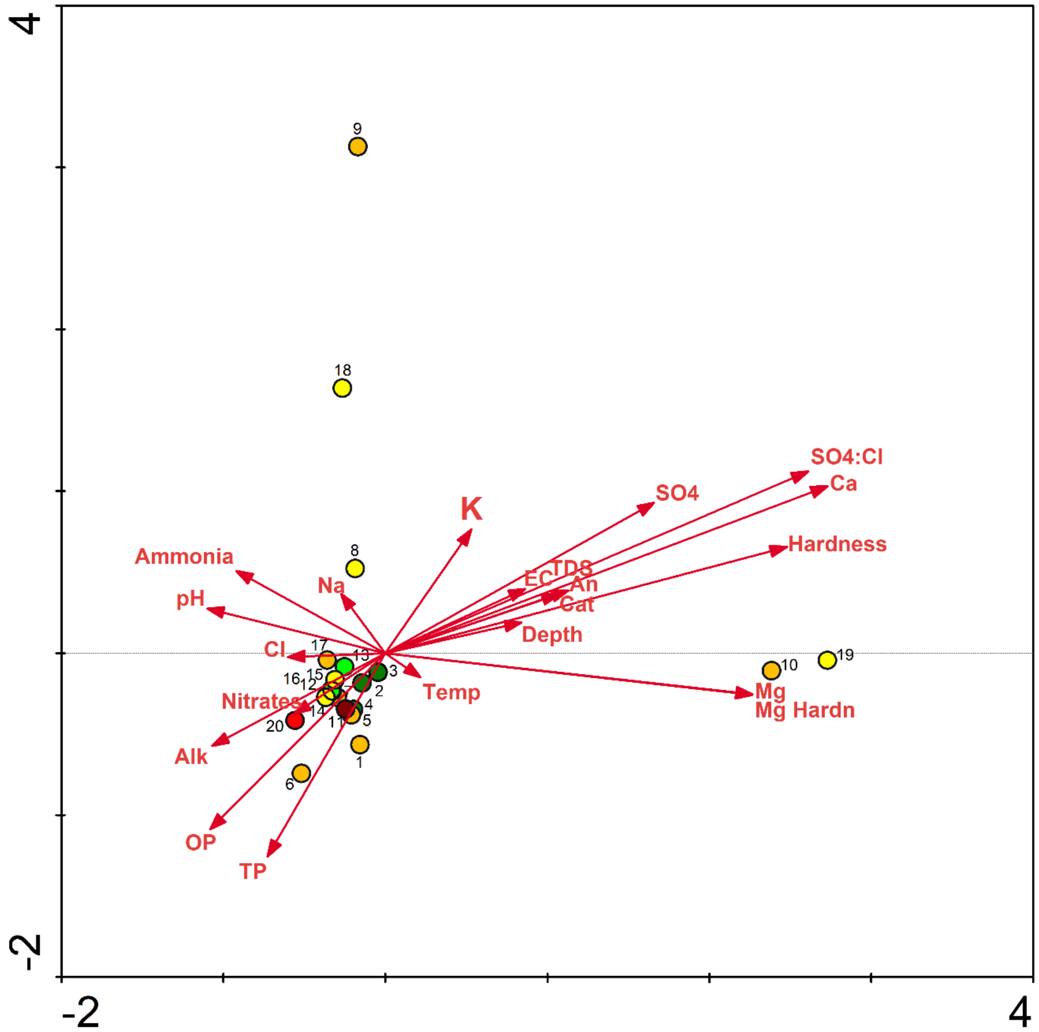


**Figure 11: CCA biplot showing the inter sample relationship between environmental variables and salt pan samples. Dry season sites are labelled: 1=S6; 2=S7; 3=S9; 4=S10; 5=S17; 6=S18, 7=S20; 8=S21; 9=S22; 10=S23; 11=S26. Wet season sites are labelled: 12=S7; 13=S10; 14=S6; 15=S17; 16=S18; 17=S20; 18=S22; 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)**.

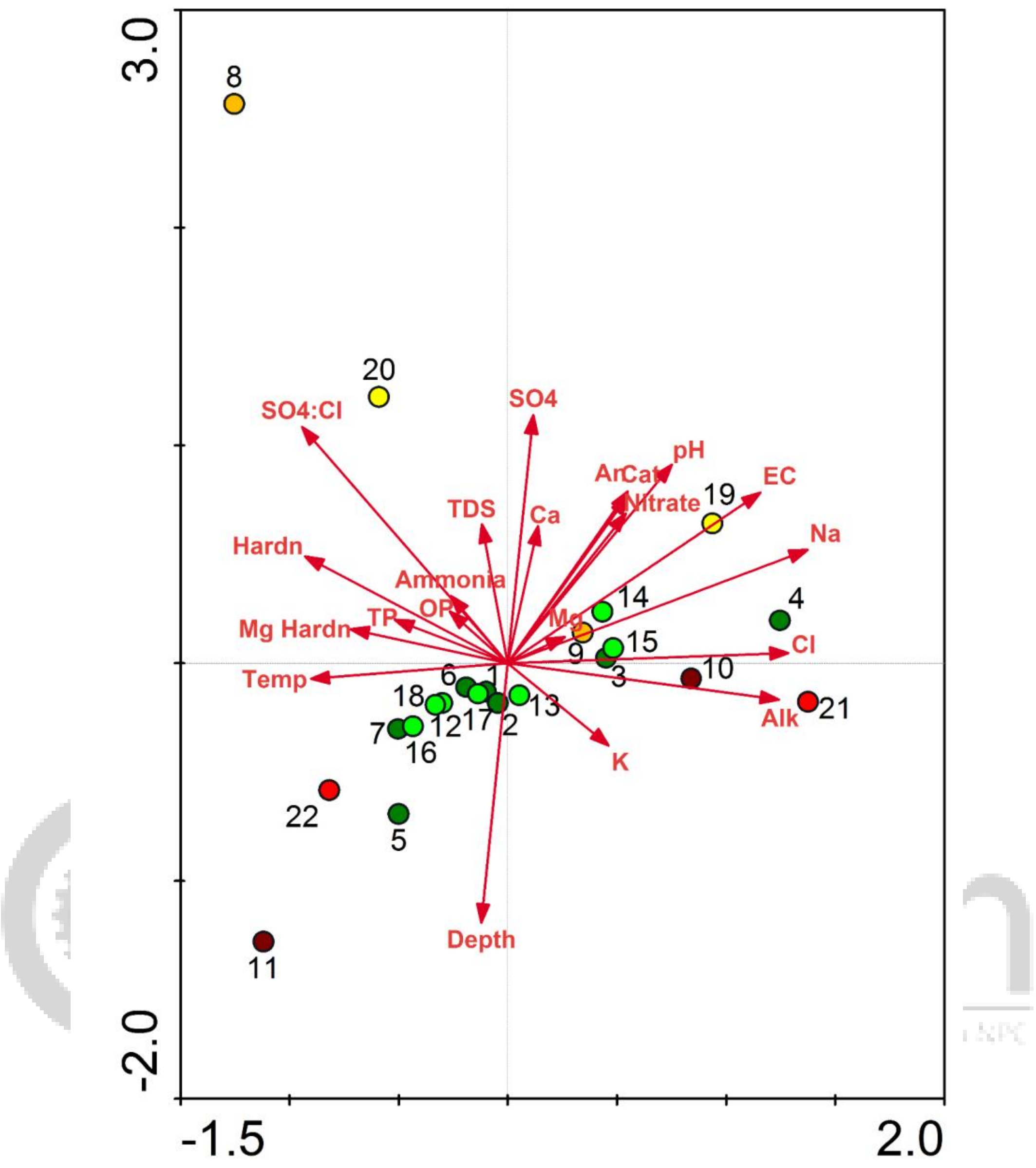


**Figure 12: CCA biplot showing the relationship between the measured environmental variables and diatom taxa from grass pans.**

## 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 the 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 3 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.

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 3 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.

**Table 3: 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 |

## 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 3: Percentages of reed, grass and salt pans disturbance levels classified correctly**

| | MMI1 | MMI2(Reed)<br>MMI3(Salt)<br>MMI4(Grass) | TDI<br>&%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 5$^{th}$ 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 % $SO_4^{2-}$:$Cl^-$ 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.

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. Table 5 shows Cohen's Kappa coefficient calculated for comparison between MMIs and riverine indices and between different MMI's.

### Table5: 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 |

## Correlation between different diatom indices and water quality variables

**Table 6: 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 %$SO_4{}^{2-}$:$Cl^-$ (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 %$SO_4{}^{2-}$:$Cl^-$ 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 %$SO_4{}^{2-}$:$Cl^-$.

All indices had significant correlation with $SO_4{}^{2-}$, %$SO_4{}^{2-}$:$Cl^-$. However, riverine indices corresponded positively and MMIs correlated negatively and more strongly. MMIs indicate AMD-impact correctly as evidenced by strong negative correlations with %$SO_4{}^{2-}$:$Cl^-$ 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 %$SO_4{}^{2-}$:$Cl^-$. 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 $\%SO_4^{2-}:Cl^-$. 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 $Ca^{2+}$, $SO_4^{2-}$, $\%SO_4^{2-}: Cl^-$, TDS, hardness, ammonia and total phosphorous. $Ca^{2+}$, $SO_4^{2-}$, $\%SO_4^{2-}:Cl^-$ and EC are positively correlated with pan type and negatively with disturbance level. Variables such as $Ca^{2+}$, $SO_4^{2-}$, $\%SO_4^{2-}:Cl^-$ 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 agriculture may be seen especially in reference pans that are 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).

# Conclusions and recommendations

## 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) revealed strong correlations between environmental variables and diatom species. Among all three pan types, characteristic reference site taxa were ordinated in correspondence with better water quality and tolerant taxa set disturbed sites apart. Seasonal change did not significantly influence diatom community composition in depressional wetlands according the SIMPER analysis. 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 Valves (%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

## 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 S*ellaphora 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.

# References


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., 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.

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'appreciation 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 á l'aide des communautés de Diatomées benthiques (application des indices diatomiques). Rapport Cemagref Bordeaux - Agence de I'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)

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, 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

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. 2012. 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.

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.

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.

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

Hudon C, Legendre P (1987) The ecological implications of growth forms in epibenthic diatoms. Journal of Phycology 25: 434-441. doi:10.1111/j.1529-8817.1987.tb02529.x

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).

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.

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.

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. Inland salt waters of southern Africa. Hydrobiologia 210, 75 (1991). 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.

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., Sincock, 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. Sûmilauer, 1998. CANOCO reference manual and user's guide to Canoco for Windows: Software for Cannonical 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.

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).

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

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.

Appendix A Field Notes:

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Site ID: | | | | | Date: | | | | | |
| Sample collected by: | | | | | Sample ID: | | | | | |
| Co-ordinates | | | | | Pan type: | | | | | |
| Elevation: | | | | | | | | | | |
| Depth: | | | | | Width: | | | | | |
| Substrate: | bedrock boulders | | cobbles pebbles | | gravel | | sand silt | | clay peat | |
| | Emergent aquatic macrophytes | | Submerged aquatic macrophytes | | | | | | | |
| Estimate percentage of boulders and cobbles covered by: | Filamentous algae: | | | | Other macrophytes: | | | | | |
| Shading along banks (record estimated percentage): | None | | | Broken | | | Dense | | | |
| Water clarity: | Clear | | Cloudy | | Turbid | | | | | |
| Bed stability: | Firm | | Stable | | Unstable | | Soft | | | |
| Notes; | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |

| | | | |
|---|---|---|---|
| Water temperature: | | | Sample ID: |
| Turbidity: | | Sulphates ($SO_4$) : (mg/L) | |
| pH: | | Dissolved Oxygen (DO) (mg/L) | |
| Conductivity:((µS/cm) | | Dissolved Organic Carbon (DOC) (mg/L) | |
| Salinity (%): | | Alkalinity | |
| Total dissolved solids (TDS) : | | Biological Oxygen Demand (BOD) | |
| Orthophosphate-phosphorus ($PO_4^-$ P) : (mg/L) | | | |
| Total phosphate (TP) : (mg/L) | | | |
| Ammonium-nitrogen (NH4-N) (mg/L): | | | |
| Nitrite-nitrogen ($NO_2^-$N) : (mg/L) | | | |
| Nitrate-nitrogen ($NO_3^-$):(mg/L) | | | |
| Total Kjeldahl nitrogen (TKN) : | | | |
| Calcium ($Ca^{2+}$):ppm | | | |
| Magnesium ($Mg^{2+}$):(mg/L) | | | |
| Potassium ($K^+$):ppm | | | |
| Sodium ($Na^+$):ppm | | | |
| Chloride ($Cl^-$):(mg/L) | | | |

Appendix B

| Assignment of taxa to life-forms and ecological guilds following Passy (2007a,b) and Rimet and Bouchez (2012b). Functional groups | Definition of functional group classification | Taxa Assigned |
|---|---|---|
| *Life-forms* | | |
| Mobile | Free moving e.g. some species vertically migrate into the sediments to acquire nutrients | *Achnanthes*, *Achnanthidium*, *Brachysira*, *Caloneis*, *Diadesmis*, *Encyonema*, *Eolimna*, *Eunotia*, *Frustulia*, *Gomphonema*, *Luticola*, *Mayamaea*, *Navicula*, *Nitzschia*, *Rhopalodia*, *Sellaphora*, *Stauroneis* |
| Pioneer | Species colonise bare substrates faster than other species | *Achnanthidium minutissima* var. *minutissima*, *A. minutissima* var. *affinis*, *A. saprophilum* |
| Tube-living | Species live in mucous substance within which they can move freely | *Frustulia*, *Encyonema mesianum* |
| Rosette colony | Species attached to substrate by a short stalk at one pole; colonies look fan-shaped | *Ulnaria acus* |
| Ribbon colony | Species attached to one another either by interlocking spines or by a layer of mucous on their valve face, forming long, ribbon-like colonies | *Eunotia bilunaris*, *Eunotia minor*, *Eunotia pectinalis* |
| Pedunculate | Species grows upright to substrate, attached either by a mucilage pad or by a stalk | *Achnanthes*, *Achnanthidium*, *Fragilaria*, *Ulnaria*, *Gomphonema* |
| Adnate | Species grows parallel to substrate, attached by their valve face | *Rhopalodia gibba* |

***Ecological guilds***

| | | |
|---|---|---|
| High profile | Species of tall stature, including erect, filamentous, branched, chain-forming, tube-forming, pedunculate, and colonial centrics | *Diadesmis*, *Encyonema mesianum*, *Eunotia*, *Fragilaria*, *Ulnaria Gomphonema* |
| Low profile | Species of short stature, including prostrate, adnate, small erect, solitary centrics, slow-moving species | *Achnanthes*, *Achnanthidium*, *Brachysira* |
| Motile | Fast-moving species | *Caloneis*, *Eolimna*, *Luticola*, *Mayamaea*, *Navicula*, *Nitzschia*, *Rhopalodia*, *Sellaphora*, *Stauroneis*, |
| Planktonic | Solitary or colonial centrics, pennates | *Cyclotella meneghiniana*, *Fragilaria tenera*, *Ulnaria acus*, *Nitzschia acicularis* |

Diatom Growth forms



| 2019 Diatom Species | 2020 Diatom Species | Growth form/ Lifeform |
|---|---|---|
| *Achnanthidium exiguum* (Grunow) Czarnecki | *Achnanthidium exiguum* (Grunow) Czarnecki | Motile<br><br>Solitary/ pairs<br><br>Apical mucilage stalk |
| *Achnanthidium minutissimum* (Kützing) Czarnecki | *Achnanthidium minutissimum* (Kützing) Czarnecki | Pedunculate<br><br>Mobile<br><br>Solitary/ pairs<br><br>Apical mucilage stalk |
| *Amphora coffeaeformis* (Agardh) Kützing | | |
| *Amphora pediculus* (Kützing) Grunow | *Amphora pediculus* (Kützing) Grunow | Pioneer<br><br>Mobile<br><br>Slow-moving<br><br>Solitary |

| | | |
|---|---|---|
| *Amphora veneta* Kützing | *Amphora veneta* Kützing | Pioneer<br>Solitary<br>Mobile<br>Slow-moving |
| *Anomoeneis sphaerophora* (Ehrenberg) Pfitzer | *Anomoeneis sphaerophora* (Ehrenberg) Pfitzer | Mobile motile<br>Solitary<br>Biraphid |
| *Anomoeneis sphaerophora* f. *costata* (Kützing) Schmid | | |
| *Aulacoseira granulata* (Ehrenberg) Simonsen | *Aulacoseira granulata* (Ehrenberg) Simonsen | Planktonic<br>Colonial chains |
| *Brachysira brebissonii* Ross | | |
| *Brachysira neoexilis* (Grunow) DG Mann | *Brachysira neoexilis* (Grunow) DG Mann | Solitary motile<br>Biraphid |
| *Caloneis bacillum* (Grunow) Cleve | | Solitary motile<br>Biraphid |
| *Caloneis molaris* (Grunow) Krammer | *Caloneis molaris* (Grunow) Krammer | Solitary motile<br>Biraphid |
| *Cocconeis pediculus* Ehrenberg | | |
| *Caloneis sp* | | Solitary motile<br>Biraphid |
| *Cocconeis placentula* Ehrenberg | *Cocconeis placentula* Ehrenberg | Solitary Adnate<br>monoraphid |
| *Cocconeis placentula* var. lineata (Ehr.) V.H. | *Cocconeis placentula* var. lineata (Ehr.) V.H. | Solitary Adnate<br>monoraphid |
| *Craticula ambigua* (Ehrenberg) DG Mann | | Solitary motile<br>Biraphid |

| | | |
|---|---|---|
| *Craticula buderi* (Hustedt) Lange-Bertalot | *Craticula buderi* (Hustedt) Lange-Bertalot | Solitary motile<br><br>Biraphid |
| *Craticula cuspidata* (Kützing) DG Mann | | Solitary motile<br><br>Biraphid |
| *Craticula halophila* (Grunow) DG Mann | | |
| *Craticula molestiformis* (Hustedt) Lange-Bertalot | *Craticula molestiformis* (Hustedt) Lange-Bertalot | Solitary motile<br><br>Biraphid |
| *Ctenophora pulchella* (Ralfs ex Kützing) Williams & Round | *Ctenophora pulchella* (Ralfs ex Kützing) Williams & Round | Solitary<br><br>Attached<br><br>Araphid→cells grow in tuft-like colonies, attached to surfaces by mucilage pads. |
| *Cyclostephanos sp 1* | | Planktonic Solitary |
| *Cyclotella meneghiniana* Kützing | *Cyclotella meneghiniana* Kützing | Solitary/pairs→ not chains |
| *Cymbella kolbei* Hustedt | | |
| *Cyclotella ocellata* Pantocsek | | Solitary/pairs→ not chains |
| *Cyclotella sp1* | | Solitary/pairs→ not chains |
| *Cymbella tumida* (Brébisson) Van Heurck | *Cymbella tumida* (Brébisson) Van Heurck | Solitary<br><br>Attached<br><br>Or free living motile<br><br>Biraphid |
| *Cymbella turgidula* Grunow | *Cymbella turgidula* Grunow | Solitary<br><br>Attached<br><br>Or free living motile<br><br>Biraphid |
| *Diadesmis confervacea* (Kützing) DG Mann | | Ribbon |
| *Diploneis puella* (Schumann) Cleve | | |

| | | |
|---|---|---|
| *Discostella pseudostelligera* (Hustedt) Houk & Klee | | Planktonic Solitary |
| *Encyonema mesianum* (Cholnoky) DG Mann | *Encyonema mesianum* (Cholnoky) DG Mann | Solitary / mucilage tubes /free living motile<br>Biraphid |
| *Encyonema minutum* (Hilse) DG Mann | *Encyonema minutum* (Hilse) D.G. Mann | Solitary / mucilage tubes /free living motile<br>Biraphid |
| *Encyonema silesiacum* (Bleisch) DG Mann | | Solitary / mucilage tubes /free living motile<br>Biraphid |
| *Encyonopsis cesatii* (Rabenhorst) Krammer | *Encyonopsis cesatii* (Rabenhorst) Krammer | Solitary/free living motile<br>Biraphid |
| *Encyonopsis leei* var. *sinensis* Metzeltin & Krammer | | |
| *Encyonopsis microcephala* (Grunow) Krammer | *Encyonopsis microcephala* (Grunow) Krammer | Solitary/free living motile<br>Biraphid |
| *Encyonopsis minuta* Krammer & Reichardt | | |
| *Encyonopsis subminuta* Krammer & Reichardt | *Encyonopsis subminuta* Krammer & Reichardt | Solitary/free living motile<br>Biraphid |
| *Encyonopsis vandamii* Krammer | | |
| *Eolimna minima* (Grunow) Lange-Bertalot | *Eolimna minima* (Grunow) Lange-Bertalot | Solitary/free living motile<br>Biraphid |
| *Eolimna subminuscula* (Manguin) Lange-Bertalot | *Eolimna subminuscula* (Manguin) Lange-Bertalot | Solitary/free living motile<br>Biraphid |
| *Epithemia adnata* (Kützing) Brébisson | *Epithemia adnata* (Kützing) Brébisson | Solitary/free living motile<br>Biraphid |
| *Eunotia bilunaris* (Ehrenberg) Mills | *Eunotia bilunaris* (Ehrenberg) Mills | Solitary motile<br>Ribbon colonies<br>Raphid→Taxa with a raphe on both valves |

| | | |
|---|---|---|
| | | (short raphe system extending from the valve face on to the valve mantle)<br>Prosrate |
| *Eunotia flexuosa* (Brébisson) Kützing | *Eunotia flexuosa* (Brébisson) Kützing | cells occur singly, free, or attached by mucilaginous stalks, or in long ribbon-like colonies |
| *Eunotia formica* Ehrenberg | *Eunotia formica* Ehrenberg | Ribbon colony |
| *Eunotia minor* (Kützing) Grunow | *Eunotia minor* (Kützing) Grunow | Ribbon colony |
| *Eunotia naegeli* Migula | | Weakly motile |
| *Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot | *Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot | Solitary/free living motile<br>Biraphid |
| *Fragilaria biceps* (Kützing) Lange-Bertalot | *Fragilaria biceps* (Kützing) Lange-Bertalot | Pedunculate →pad<br>Ribbon colony<br>Araphid |
| *Fragilaria capucina* Desmazières | *Fragilaria capucina* Desmazières | Ribbon colony |
| *Fragilaria capucina* var. *rumpens* (Kützing) Lange-Bertalot | *Fragilaria capucina* var. *rumpens* (Kützing) Lange-Bertalot | Ribbon colony |
| *Fragilaria capucina* var. *vaucheriae* (Kützing) Lange-Bertalot | *Fragilaria capucina* var. *vaucheriae* (Kützing) Lange-Bertalot | Ribbon colony |
| *Fragilaria nanana* Lange-Bertalot | *Fragilaria nanana* Lange-Bertalot | Pedunculate →pad<br>Planktonic |
| *Fragilaria tenera* (WM Smith) Lange-Bertalot | *Fragilaria tenera* (WM Smith) Lange-Bertalot | Pedunculate →pad<br>Planktonic |
| *Fragilaria ulna* var. *acus* (Kützing) Lange-Bertalot | | Pedunculate →pad |

| | | |
|---|---|---|
| *Frustulia crassinervia* (Kützing) Cleve | *Frustululia crassinervia* (Kützing) Cleve | Unattached Tube-Forming<br>Mobile<br>Biraphid |
| *Gomphonema aff. gracile* | *Gomphonema aff. gracile* | Mobile<br>Pedunculate (mucilage stalk)<br>High profile<br>Biraphid |
| *Gomphonema affine* Kützing | | |
| *Gomphonema* aff. *lagenula* | | Mobile<br>Pedunculate (mucilage stalk)<br>High profile<br>Biraphid |
| *Gomphonema angustatum* (Kützing) Rabenhorst<br>*Gomphonema angustatum* (Kützing) Rabenhorst | | Mobile<br>Pedunculate (mucilage stalk)<br>High profile<br>Biraphid |
| *Gomphonema auritum* A. Braun ex Kützing | | Solitary/free living motile or in pairs (mucilage stalk))<br>Mobile<br>Pedunculate (mucilage stalk)<br>High profile<br>Biraphid |
| *Gomphonema exilissimum* Lange-Bertalot & Reichardt | | |
| *Gomphonema gracile* Ehrenberg<br>*Gomphonema gracile* Ehrenberg | | Solitary/free living motile or in pairs (mucilage stalk))<br>Mobile<br>Pedunculate (mucilage stalk)<br>High profile<br>Biraphid |

| | | |
|---|---|---|
| *Gomphonema italicum* Kützing | | Solitary/free living motile or in pairs (mucilage stalk))<br>Mobile<br>Pedunculate (mucilage stalk)<br>High profile<br>Biraphid<br>attached to substrata by dichotomous mucilage stalks. |
| *Gomphonema lagenula* Kützing | *Gomphonema lagenula* Kützing | Solitary/free living motile or in pairs (mucilage stalk))<br>Mobile<br>Pedunculate (mucilage stalk)<br>High profile<br>Biraphid |
| *Gomphonema laticollum* Reichart | *Gomphonema laticollum* Reichart | Solitary/free living motile or in pairs (mucilage stalk))<br>Mobile<br>Pedunculate (mucilage stalk)<br>High profile<br>Biraphid<br>attached to substrata by dichotomous mucilage stalks. |
| *Gomphonema parvulius* Lange-Bertalot & Reichardt | *Gomphonema parvulius* Lange-Bertalot & Reichardt | Solitary/free living motile or in pairs (mucilage stalk))<br>Mobile<br>Pedunculate (mucilage stalk)<br>High profile<br>Biraphid |
| *Gomphonema parvulum* (Kützing) Kützing | *Gomphonema parvulum* (Kützing) Kützing | Solitary/free living motile or in pairs (mucilage stalk))<br>Mobile<br>Pedunculate (mucilage stalk) |

| | | |
|---|---|---|
| | | High profile<br><br>Biraphid |
| *Gomphonema parvulum* f. saprophilum Lange-Bertalot & Reichardt | | Solitary/free living motile or in pairs (mucilage stalk))<br><br>Mobile<br><br>Pedunculate (mucilage stalk)<br><br>High profile<br><br>Biraphid |
| *Gomphonema pseudoaugur* Krammer | *Gomphonema pseudoaugur* Krammer | Solitary/free living motile or in pairs (mucilage stalk))<br><br>Mobile<br><br>Pedunculate (mucilage stalk)<br><br>High profile<br><br>Biraphid |
| *Gomphonema turris* Ehrenberg | | Solitary/free living motile or in pairs (mucilage stalk))<br><br>Mobile<br><br>Pedunculate (mucilage stalk)<br><br>High profile<br><br>Biraphid |
| *Hantzschia amphioxys* (Ehrenberg) Grunow | *Hantzschia amphioxys* (Ehrenberg) Grunow | Solitary/free living motile<br><br>Biraphid |
| *Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski | *Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski | Solitary/free living motile<br><br>Biraphid |
| *Lemnicola hungarica* (Grunow) Round & Basson | *Lemnicola hungarica* (Grunow) Round & Basson | Solitary<br><br>Attached (adnate)<br><br>Monoraphid |
| | *Luticola kotschyi* (Grunow) | |

| | | |
|---|---|---|
| *Luticola mutica* (Kützing) DG Mann | *Luticola mutica* (Kützing) DG Mann | Solitary/free living motile<br>Biraphid |
| *Luticola nivalis* (Ehrenberg) D.G. Mann | *Luticola nivalis* (Ehrenberg) D.G. Mann | Solitary/free living motile<br>Biraphid |
| *Mastogloia dansei* (Thwaites) Thwaites | *Mastogloia dansei* (Thwaites) Thwaites | Motile or encased in mucilage<br>Solitary<br>Biraphid |
| *Mastogloia elliptica* (Agardh) Cleve | *Mastogloia elliptica* (Agardh) Cleve | Motile or encased in mucilage<br>Solitary<br>Biraphid |
| *Mastogloia smithii* Thwaites | *Mastogloia smithii* Thwaites | Motile or encased in mucilage<br>Solitary<br>Biraphid |
| *Mayamaea atomus* (Kützing) Lange-Bertalot | *Mayamaea atomus* (Kützing) Lange-Bertalot | Solitary/free living motile<br>Biraphid |
| *Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot | *Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot | Solitary/free living motile<br>Biraphid |
| *Mayamaea sp* | | Solitary/free living motile<br>Biraphid |
| *Melosira varians* Agardh | *Melosira varians* Agardh | Centric<br>Linked face-to-face by mucilage pads→ long chain colonies<br>High profile |
| | *Navicula angusta* Grunow | |
| | *Navicula arvensis* var. *maior* Lange-Bertalot | |
| *Navicula capitatoradiata* Germain | | Solitary/free living motile<br>Biraphid |

| | | |
|---|---|---|
| *Navicula cincta* (Ehrenberg) Ralfs in Pritchard | *Navicula cincta (Ehrenberg) Ralfs* | Solitary/free living motile<br>Biraphid |
| *Navicula cryptocephala* Kützing | *Navicula cryptocephala* Kützing | Solitary/free living motile<br>Biraphid |
| | *Navicula erifuga* (OF Müller) Bory | |
| *Navicula joubaudii* Germain | | Solitary/free living motile<br>Biraphid |
| *Navicula longicephala* Hustedt | | Solitary/free living motile<br>Biraphid |
| *Navicula radiosa* Kützing | *Navicula radiosa* Kützing | Solitary/free living motile<br>Biraphid |
| *Navicula recens* (Lange-Bertalot) Lange-Bertalot | *Navicula recens* (Lange-Bertalot) Lange-Bertalot | Solitary/free living motile<br>Biraphid |
| | *Navicula rhynchocephala* Kützing | |
| *Navicula rostellata* Kützing | *Navicula rostellata* Kützing | Solitary/free living motile<br>Biraphid |
| | *Navicula schroeteri* Meister | |
| | *Navicula symmetrica* Patrick | |
| *Navicula tripunctata* (OF Müller) Bory | | Solitary/free living motile<br>Biraphid |
| *Navicula veneta* Kützing | *Navicula veneta* Kützing | Solitary/free living motile<br>Biraphid |
| *Navicula zanonii* Hustedt | *Navicula zanonii Hustedt* | Solitary/free living motile<br>Biraphid |
| *Navicymbula pusilla* (Grunow) Krammer | *Navicymbula pusilla* (Grunow) Krammer | Solitary<br>Unattached |
| *Nitzschia acidoclinata* Lange-Bertalot | *Nitzschia acidoclinata* Lange-Bertalot | Solitary / free living motile /mucilage tubes<br>Biraphid |

| | | |
|---|---|---|
| *Nitzschia agnita* Hustedt | *Nitzschia agnita* Hustet | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia amphibia* Grunow | *Nitzschia amphibia* Grunow | Solitary / free living **motile** /mucilage tubes<br>Biraphid |
| *Nitzschia archibaldii* Lange-Bertalot | *Nitzschia archibaldii* Lange-Bertalot | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia aurariae* Cholnoky | *Nitzschia aurariae* Cholnoky | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia capitellata* Hustedt | *Nitzschia capitellata* Hustedt | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia clausii* Hantzsch | *Nitzschia clausii* Hantzsch | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia desertorum* Hustedt | *Nitzschia desertorum* Hustedt | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia dissipata* var. *media* (Hantzsch) Grunow | | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia draveillensis* Coste & Ricard | | Solitary / free living motile /mucilage tubes<br>Biraphid |

| | | |
|---|---|---|
| | *Nitzschia elegantula* Grunow | |
| *Nitzschia etoshensis* Cholnoky | *Nitzschia etoshensis* Cholnoky | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia filiformis* (W Smith) Van Heurk | *Nitzschia filiformis* (W Smith) Van Heurk | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia fonticola* Grunow | *Nitzschia fonticola* Grunow | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia frustulum* (Kützing) Grunow | *Nitzschia frustulum* (Kützing) Grunow | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia gracilis* Hantzsch | *Nitzschia gracilis* Hantzsch | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia heufleriana* Grunow | | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia inconspicua*Grunow | | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia intermedia* Hantzsch | *Nitzschia intermedia* Hantzsch | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia iremissa* Cholnoky | | Solitary / free living motile /mucilage tubes<br>Biraphid |
| | *Nitzschia lancettula* O Müller | |

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

| | | |
|---|---|---|
| | *Nitzschia reversa* W Smith | |
| | *Nitzschia supralitorea* Lange-Bertalot | |
| *Nitzschia sigma* (Kützing) W Smith | | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Nitzschia subacicularis* Hustedt | | Solitary / free living motile /mucilage tubes<br>Biraphid |
| *Pinnularia acrosphaeria* W Smith | *Pinnularia acrosphaeria* W Smith | Solitary / free living motile<br>Biraphid |
| | *Pinnularia borealis* Ehrenberg | |
| *Pinnularia divergens* var. undulata (Péragallo & Heribaud) | | Solitary / free living motile<br>Biraphid |
| *Pinnularia divergens* W Smith | *Pinnularia divergens* W Smith | Solitary / free living motile<br>Biraphid |
| *Pinnularia gibba* Ehrenberg | *Pinnularia gibba* Ehrenberg | Solitary / free living motile<br>Biraphid |
| *Pinnularia sp1* | | Solitary / free living motile<br>Biraphid |
| *Pinnularia subbrevistriata* Krammer | *Pinnularia subbrevistriata* Krammer | Solitary / free living motile<br>Biraphid |
| | *Pinnularia subcapitata* Gregory | |
| | *Pinnularia viridiformis* Krammer | |
| *Pinnularia viridis* (Nitzsch) Ehrenberg | *Pinnularia viridis* (Nitzsch) Ehrenberg | Solitary / free living motile<br>Biraphid |
| | *Planothidium delicatulum* (Kützing) Round & Bukhtiyarova | |

| | | |
|---|---|---|
| *Planothidium engelbrechtii* (Cholnoky) Round & Bukhityarova | *Planothidium engelbrechtii* (Cholnoky) Round & Bukhityarova | Solitary<br>Attached (adnate)<br>monoraphid |
| *Planothidium frequentissimum (Lange-Bertalot) Round & Bukhityarova* | | Solitary<br>Attached (adnate)<br>Monoraphid |
| *Planothidium sp1* | | Solitary<br>Attached (adnate)<br>monoraphid |
| | *Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot* | Solitary or in pairs<br>mucilage stalks at the basal pole |
| *Pseudostaurosira brevistriata* (Grunow in van Heurk) Williams & Round | | Ribbon colony<br>Araphid |
| *Rhopalodia gibba* (Ehrenberg) O Müller | *Rhopalodia gibba* (Ehrenberg) O Müller | Solitary / free living motile /adnate<br>Biraphid<br>Or attached with mucilage stalks |
| *Rhopalodia operculata* (Agardh) Håkansson | | Solitary / free living motile /adnate<br>Biraphid |
| *Sellaphora pupula* (Kützing) Mereschkowsky | *Sellaphora pupula* (Kützing) Mereschkowsky | Solitary / free living motile<br>Biraphid |
| *Sellaphora seminulum* (Grunow) DG Mann | *Sellaphora seminulum* (Grunow) DG Mann | Solitary / free living motile<br>Biraphid |
| *Stauroneis obtusa* | *Stauroneis obtusa* | Solitary / free living motile<br>Biraphid |
| *Staurosirella pinnata* (Ehrenberg) Williams & Round | | Ribbon colony<br>Araphid |
| *Surirella ovalis* Brébisson | | Solitary / free living motile |

| | | |
|---|---|---|
| | | Biraphid |
| *Tabularia fasciculata* (Agardh) Williams & Round | *Tabularia fasciculata* (Agardh) Williams & Round | Colonial, basally attached<br>Araphid<br>erect |
| | *Thalassiosira duostra* | Planktonic |
| *Tryblionella apiculata* Gregory | *Tryblionella apiculata* Gregory | Solitary / free living motile<br>Biraphid |
| *Tryblionella calida* (Grunow) DG Mann | | Solitary / free living motile<br>Biraphid |
| | *Tryblionella hungarica* (Grunow) Frenguelli | Solitary / free living motile<br>Biraphid |