Benthic Diatoms as Indicators of Groundwater-Surface Water Connectivity in Coal Mining Wetlands: Case of depression wetlands in Belfast, Mpumalanga Province, South Africa

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Abstract

The applicability of diatoms as bioindicators to assess hydrological connectivity between groundwater and surface water in wetlands affected by coal mining was examined. Benthic diatoms were identified as effective indicators due to their sensitivity to water chemistry, flow, and nutrient dynamics. Field and lab analyses of hydrochemistry, isotopes, and diatom assemblages revealed that wetlands near mining activities had acidic water, higher EC, and diatoms indicative of mining, suggesting mine-affected groundwater influence (i.e. *Craticula Budari*). In contrast, distant wetlands showed nutrient-associated diatoms and atmospheric recharge signatures. This multidisciplinary approach demonstrates the value of diatoms in monitoring ecosystem health and managing wetlands in mining regions.

Keywords: Diatoms, groundwater-surface water interaction, coal mining, wetlands, hydrological connectivity, bio-indicators, hydrochemistry, isotopes, mine pollution.

Introduction

Coal mining can disrupt wetland ecosystems, affecting groundwater-surface water interactions and water quality. While chemical and isotopic analyses are commonly used to study these connections (Madlala et al. 2021), the potential of benthic diatoms as biological indicators remains largely underexplored. Limited research exists on their effectiveness in detecting groundwater influence in mining-impacted wetlands (Pfister et al. 2017; Oberholster 2019; Oberholster et al. 2020). The purpose of this study was to determine whether benthic diatoms, in conjunction with chemical and isotopic techniques, could accurately detect groundwater-surface water connection in wetlands that were used for coal mining. Understanding these dynamics is crucial for wetland conservation and sustainable water management in mining areas.

Methods

Study site description

The Exxaro Belfast Implementation Mining Rights Area is in the headwaters of the Komati River catchment in Mpumalanga Province, South Africa. The coal mine is situated 55 km east of Middelburg and 20 km southwest of Belfast, south of the N4 highway (Fig. 1). The area includes two catchments with southward-flowing streams, namely the Witkloofspruit and the Klein Komati, along with various wetland types such as depressional, valley bottom, and hillslope wetlands (Golder Associates Africa 2011).

Wetland occurrence in mining areas like the Exxaro Belfast Implementation

Mining Rights Area is shaped by geology and hydrogeology, which control water availability, flow, and storage. The area's Karoo Supergroup sedimentary rocks (sandstone, shale, and coal) along with dolerite dykes and sills (Steenekamp 2009), influence water movement and accumulation in the subsurface. Sandstone and shale act as water-bearing layers or confining units, while coal seams, with high porosity, can store and release groundwater, supporting wetlands. Dolerite dykes create barriers that force groundwater to the surface, aiding wetland formation. However, mining operations that remove these geological layers can disrupt natural hydrological balance, draining wetlands or altering their water supply.

The hydrogeology of the area includes two key aquifer systems. The shallow, semi-confined weathered zone aquifer, with static water levels of 0.2–35 mbgl and low groundwater yields (<0.3 l/s), sustains surface-dependent wetlands, like depressional and valley-bottom wetlands. The deeper fractured aquifer, yielding up to 2 l/s, supports wetlands relating to groundwater discharge (Golder Associates Africa 2018). Groundwater follows the Klein Komati River's flow, linking riparian and floodplain wetlands. Fractures and faults in the Karoo bedrock can create groundwater pathways, supplying wetlands even where surface water is limited.

Land use in the mining rights area is primarily cattle grazing, crop cultivation, and coal mining. Natural grasslands provide habitats for wildlife, including the endangered African Grass Owl. Vegetation consists of Mesic and Dry Highveld Grasslands, with indigenous grasses, biesies (*Juncus effuses*), and cattails (*Typha capensis*). Alien invasive species, including blue gum (*Eucalyptus globulus*) and poplar (*Populus sp.*), are also present.

The climate of the region is characterized by summer rainfall (October–April), with the highest temperatures occurring in wet summer months and lower temperatures in dry winter periods. Rainfall is influenced by convective storm systems.

Data Collection Methods

A comprehensive approach to assess the physicochemical and biological characteristics of water systems, focusing on wetlands, piezometers was followed. In situ physicochemical parameters, including pH, electrical conductivity, and total dissolved solids (TDS), were measured using a handheld Hanna HI991300™ multi-meter, depending on water availability at

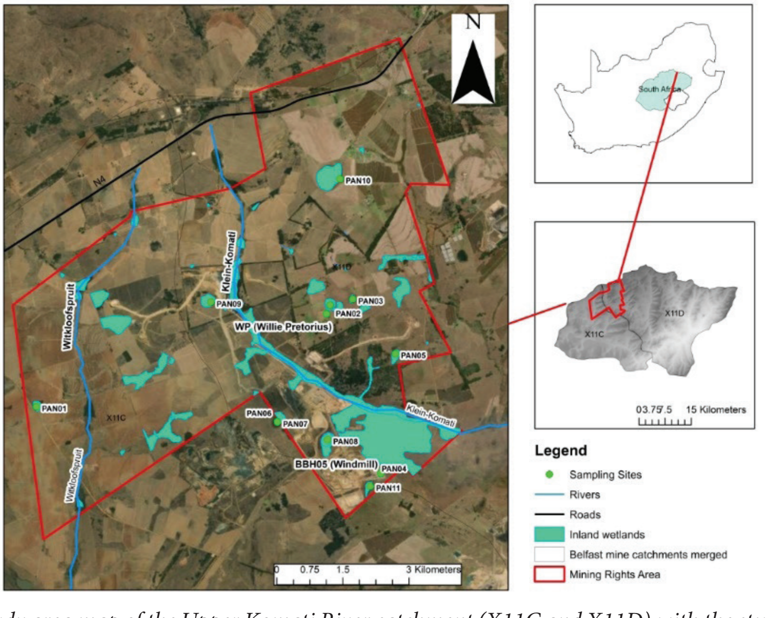

Figure 1 Study area map of the Upper Komati River catchment (X11C and X11D) with the study mine (red box) Belfast Implementation Mining Rights Area.

the sampling location. Water samples for hydrochemical analysis were collected using a grab sampling technique, with 1L acid-washed polyethylene bottles used to obtain representative samples from flowing water within wetlands. Sampling points were carefully selected to avoid stagnant water, and bottles were rinsed three times with site water prior to collection. For piezometers and boreholes, a bailer was used to collect samples after purging at least three well volumes to ensure representative groundwater and subsurface water samples (Brassington 2007). A total of 27 water samples were collected from various sources, including pan wetland surface water, rainwater, and piezometers, to identify the sources sustaining the pan wetlands (Table 3 in Appendix). The collected samples were immediately labelled, stored in a cooler box with ice to minimize chemical alterations, and transported to the CSIR chemistry laboratory in Stellenbosch, South Africa for analysis of major ions, metals, and nutrients to characterize water types and assess spatial variations in water chemistry.

Environmental isotope analysis was conducted to complement hydrochemical data. Water samples for isotopic analysis were collected in 50-100 mL double-sealed polyethylene bottles and transported to iThemba Labs at the University of Witwatersrand for analysis using mass spectrometry. To maintain consistency, both hydrochemical and isotopic samples were collected from the same water sources, ensuring comparability and minimizing variability in results.

Benthic microalgal communities, categorized as epipelic (motile) or epipsammic (attached to sand or rock surfaces), were sampled to evaluate their ecological role. Epilithic and epipsammic algae were collected from submerged substrates at depths of 5-20 cm using a hand auger, following established protocols (Oberholster 2019). Five random samples per site were pooled into composite samples, which were then divided into subsamples for specific analyses: (a) unpreserved samples for culturing and identification of filamentous algae, (b) preserved samples for microscopic identification of soft algae, and (c) unpreserved samples for diatom identification. Soft algae subsamples were fixed in 2.5% glutaraldehyde in the field and stored under cold, dark conditions to preserve integrity until laboratory analysis. This multi-faceted sampling and analytical approach ensured a robust characterization of the aquatic environment, integrating physicochemical, isotopic, and biological data to provide a comprehensive understanding of the studied ecosystems.

Data Analysis Methods

Water samples were analysed for major ions, nutrients, and dissolved metals at the CSIR SANAS-accredited laboratory following standard procedures (Younger 2007). Chemical analysis included cations (Ca^{2+}, Mg^{2+}, K^+, and N^+) and anions (HCO_3^-, Cl^-, SO_4^{2-}), nutrients (nitrates and phosphates), and dissolved metals (Al, Ba, Fe, Mn, Si, Sr). Analytical techniques included Inductive Coupled Plasma Optic Emission Spectroscopy (ICP-OES), Inductive Coupled Plasma Mass Spectroscopy (ICP-MS), and Flow Injection Colorimetric detection using a HACH D3900 Spectrophotometer.

Stable hydrogen ($^2H/^1H$) and oxygen ($^{18}O/^{16}O$) isotope ratios were analysed at iThemba Laboratories, Gauteng, using a Los Gatos Research (LGR) Liquid Water Isotope Analyzer. Laboratory standards were calibrated against international reference materials, with analytical precision of ±0.5‰ for $\Delta^{18}O$ and ±1.5‰ for Δ^2H. Results were expressed in delta-notation (‰ deviation from Standard Mean Ocean Water, SMOW)

Diatom samples were processed by removing organic matter with potassium dichromate and sulfuric acid, followed by rinsing and mounting in Pleurax medium for microscopic analysis. Identification was performed at 1250 × magnification (Carl Zeiss, Germany) following taxonomic references (Van Vuuren 2006; Taylor et al. 2007; Oberholster et al. 2022). Species abundance was categorized from rare (≤50 cells/5 cm²) to predominant (5,001–25,000 cells/5 cm²). Only diatom species classified as scarce to predominant were selected for statistical analysis. To explore groundwater-surface water interactions, Canonical

Correspondence Analysis (CCA) was used to assess relationships between diatom community composition and water chemistry in coal mining environments.

Results and Discussion

The hydrochemical analysis of sampled water revealed a freshwater system dominated by naturally occurring constituents, suggesting minimal anthropogenic influence. Major ions such as Na, SO_4^{2-}, Cl^-, and HCO_3^- dominated the total dissolved components in boreholes, wetland surface waters, and piezometers, resulting from natural processes like dissolution, ion exchange, and subsurface biological activity. Metal concentrations varied significantly (Table 2 in Appendix: Al ranged from 0.04 to 62 mg/L (mean = 5.89 mg/L), B from 0.02 to 0.08 mg/L (mean = 0.03 mg/L), Ba from 0.03 to 0.7 mg/L (mean = 0.24 mg/L), Fe from 0.34 to 71 mg/L (mean = 10.43 mg/L), Mn from 0.01 to 2.4 mg/L (mean = 0.45 mg/L), and Si from 1.1 to 81 mg/L (mean = 12.01 mg/L).

Comparison with South African Water Quality Guidelines revealed that while most parameters were within permissible limits for aquatic ecosystems, industrial, irrigation, domestic, and livestock uses, certain elements exceeded thresholds (Table 2 in Appendix). TDS, Cl^-, Fe, and Si surpassed industrial use limits; Al, Fe, and Mn exceeded irrigation and livestock watering standards; and Al, Fe, and Mn were above domestic use limits. Al and Mn also exceeded aquatic ecosystem guidelines. Notably, groundwater exhibited higher metal concentrations than wetland piezometers and surface water, with elevated levels of Fe, Al, and Mn likely linked to mining activities, particularly overburden stripping during mine development, which impacts wetland water quality. These findings highlight the need for monitoring and mitigation to address metal contamination and ensure water suitability for various uses.

Water samples from wetlands, piezometers, and groundwater exhibited similar mineralization, dominated by Na, Ca, K, and HCO_3^-, indicating prolonged subsurface residence times and extensive water-rock interactions. Ion exchange processes facilitated the leaching of these geologically derived elements. While HCO_3^- presence could suggest geological or anthropogenic buffering of natural acidity,

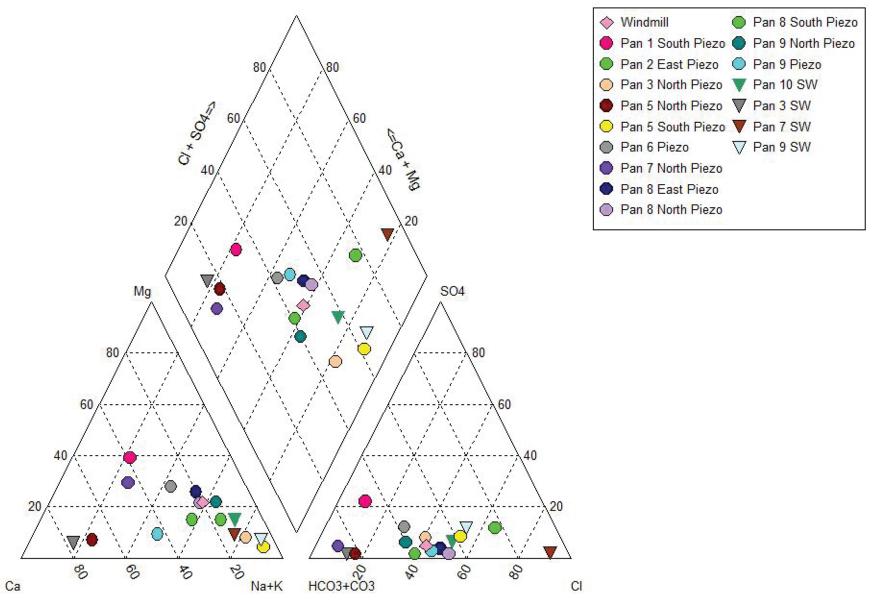

Figure 2 *Piper diagram showing hydrochemical facies of sampled water.*

pH levels in groundwater and surface water ranged from 5.58 to 6.69, reflecting naturally acidic conditions. However, biological activity, geological heterogeneity (e.g., coal seams), and varying soil buffering capacities resulted in circumneutral pH ranges (6–8). This suggests the upwelling and discharge of more basic deeper groundwater into the wetlands, consistent with findings by (Marques et al. 2004)

The isotopic variations in $\Delta^{18}O$ and $\Delta^{2}H$ across different sampling sites indicate that some wetlands were sustained by depleted subsurface water, while others received enriched atmospheric water. During the dry season, isotopic signatures showed that certain piezometers had been submerged and exhibited enrichment due to evaporative effects, suggesting a mix of surface and subsurface water. Few samples aligned with the evaporation line, indicating reduced evaporative influence on water sources. The clustering of surface water with pan piezometers and groundwater sources suggests groundwater exfiltration into the wetlands, with delayed discharge attributed to subsurface flow paths.

Diatoms are a highly diverse group of algae, often the most species-rich component of aquatic ecosystems, making them valuable biological indicators due to their distinct habitat preferences. Their diversity enhances ecological assessments by providing redundancy in data, increasing confidence in environmental inferences. Three dominant diatom functional groups in the depression wetlands were identified: mobile, motile, and high-profile taxa, with *Nitzschia tripunctata*, *Navicula rhynchocephala*, and *Fragilaria tenera* being the most abundant species. High-profile diatoms, particularly *Gomphonema* species, were highly sensitive to environmental disturbances, including fluctuating water levels, elevated ionic concentration, and nutrient scarcity, aligning with findings from previous studies (Stenger-

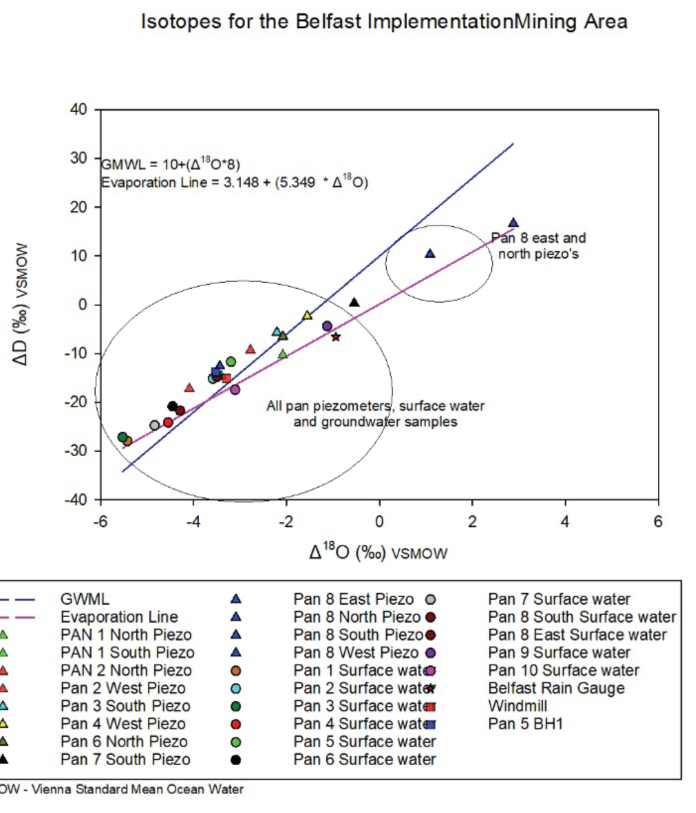

Figure 3 *Biplot showing the Isotope ratios for Pans and local groundwater against the Global Meteoric Water Line and Evaporation Line (Craig 1961).*

Kovács et al. 2018; Oberholster et al. 2022). The phytoplankton community (Table 4 in Appendix) was dominated by *Bacillariophyta* (diatoms), *Euglenophyta* (small flagellates), and *Chlorophyta* (green algae), with *Euglenophyta* flourishing in wetlands enriched with organic matter from decaying macrophytes, which contributed to water coloration and lower pH levels. The seasonal presence of *Trachelomonas* suggested elevated organic matter and nutrient concentrations in the dry season, mirroring trends observed in other eutrophic wetland ecosystems.

Unlike many depression wetlands in Mpumalanga, which experience seasonal hydroperiods, the studied wetlands remained inundated throughout the year, indicating passive groundwater contributions during the dry season. The detection of *Craticula buderi* at Pan 7 (near the abandoned mine) suggested potential contamination, as this taxon is commonly linked to mine water seepage (Oberholster et al. 2022). CCA ordination (See Fig. 1 in Appendix) revealed that and associated with agricultural activity were closely associated with diatom species indicative of nutrient enrichment, while species resistant to elevated electrolyte concentrations were associated with mining activity diatom indicators.

Conclusion

Hydrochemical, isotopic and benthic phytoplankton analyses confirmed the influence of mining on the wetland's hydrology. A total of 65 phytoplankton taxa were identified, with 25 species indicating eutrophic conditions likely driven by agricultural activities, including organic matter accumulation, fertilizer runoff, and cattle grazing. The presence of Craticula buderi and isotopic indications of groundwater influx at a wetland near an abandoned mining site suggests its impact on wetland hydrology. These findings highlight the necessity of an integrated monitoring framework to evaluate wetland ecological integrity, considering both groundwater-surface water interactions and anthropogenic influences on phytoplankton dynamics.

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Appendix

Table 1 *Results from hydrochemical analysis of surface water sampled from wetlands at the Belfast Implementation mine site used for Canonical Correspondence Analysis (CCA). Table shows the concentrations for Potassium (K), Sodium (Na), Calcium (Ca), Magnesium (Mg), Sulfate (SO4-), Chloride (Cl), Bicarbonate (HCO3-),*

Parameter	K	Na	Ca	Mg	SO_4^-	Cl	HCO_3^-	TN	EC mS/m	pH	P	Al	Fe	Si
Pan 1	1.5	11	18	11	26	9.2	102.48	1.1	24	8	0.025	7.8	14	15
Pan 2	37	72	12	10	16	97	136.64	0.8	60	7.3	0.025	1.4	1.8	11
Pan 3	16	7.2	3.4	1.9	5.8	24	14.64	3.3	4	6.1	0.22	0.08	0.58	3.1
Pan 4	0.4	14	56	2.6	2.7	18	192.76	1.1	36	7.3	0.22	5.6	1.8	6.5
Pan 5	0.6	15	39	2.4	2.1	17	140.3	0.8	27	7.4	0.17	2.8	1.9	4.3
Pan 6	5.3	4.9	0.4	0.2	2.3	11	13.42	16	5	6	1.2	5	7.4	6.2
Pan 7	5.4	8.6	6.8	4	6.5	12	39.04	1.8	12	7.1	0.23	0.12	0.57	4.8
Pan 8	0.05	19	6.2	4.8	2.6	25	42.7	1.2	16	6.9	0.22	0.04	0.34	2.1
Pan 9	24	13	5.4	4.9	4.8	20	61	6.4	19	6.7	0.94	0.66	24	2.5
Pan 10	31	213	11	10	67	227	250.1	5.5	114	8	1.2	33	32	69

Table 2 Descriptive statistics of hydrochemical analysis for water samples collected from boreholes, wetland piezometers and wetland surface water.

Statistic	Units	Minimum	Maximum	Mean	Standard deviation (n-1)	Industrial	Irrigation	Livestock	Domestic	Aquatic Ecosystems
K	mg/l	132	0.1	11.99	22.01	*	*	*	>50	*
Na	mg/l	334	2	33.13	59.15	*	>70	>2000	>100	*
Ca	mg/l	56	0.4	12.45	13.08	*	*	>1000	>32	*
Mg	mg/l	25	0.2	5.34	4.71	*	*	>500	>30	*
SO4	mg/l	245	0.1	19.65	39.50	>30	*	>1000	>200	*
Cl	mg/l	426	2	40.45	72.87	>20	>100	>3000	>100	*
HCO3	mg/l	309.88	6.831	75.18	75.01	*	*	*	*	*
EC	µS/cm	250	2	30.61	40.84	>150	>400	*	>70	*
pH	mg/l	8.2	6	7.00	0.55	7.0-9.0	6.5-8.4	*	6.0-9.0	*
TDS	mg/l	1418	5	185.44	240.12	>100	*	>2000	>450	*
Al	mg/l	62	0.04	5.89	11.39	*	>5	>5	>0.15	>0.1
B	mg/l	0.08	0.02	0.03	0.01	*	>0.5	>5	*	*
Ba	mg/l	1.2	0.03	0.24	0.24	*	*	*	*	*
Fe	mg/l	71	0.34	10.43	13.76	>0.1	>5	>10	>0.1	*
Mn	mg/l	2.4	0.01	0.45	0.61	>0.5	>0.02	>10	>0.05	>0.18
Si	mg/l	81	1.1	12.01	17.25	>0.5	*	*	*	*

Table 3 ΔD (‰) and δ18O (‰) SMOW isotope ratios for water samples collected from boreholes, wetland piezometers and wetland surface water.

Sample	ΔD (‰) SMOW	$\Delta^{18}O$ (‰) SMOW	GWML	EL
PAN 1 NP	-10.30	-2.08	-6.699	-10.989
PAN 1 SP	-14.59	-3.40	-17.304	-18.063
PAN 1 SW	-28.0	-5.4	-33.514	-28.874
PAN 10 SW	-17.45	-3.11	-14.929	-16.479
PAN 2 NP	-17.18	-4.09	-22.821	-21.742
PAN 2 SW	-15.18	-3.59	-18.778	-19.046
PAN 2 WP	-9.27	-2.78	-12.288	-14.717
PAN 3 EP	-11.91	-3.13	-15.140	-16.619
PAN 3 SP	-5.69	-2.21	-7.709	-11.663
PAN 3 SW	-27.18	-5.53	-34.341	-29.425
PAN 4 WP	-2.36	-1.56	-2.491	-8.183
PAN 4 SW	-24.16	-4.55	-26.493	-24.191
PAN 5 SW	-11.74	-3.20	-15.633	-16.948
PAN 6 NP	-6.56	-2.08	-6.691	-10.984
PAN 6 SW	-20.86	-4.45	-25.709	-23.668
PAN 7 South/Open	0.29	-0.55	5.613	-2.778
PAN 7 SW	-24.77	-4.84	-28.811	-25.737
PAN 8 EP	16.62	2.88	33.109	15.561
PAN 8 NP	10.27	1.09	18.712	5.959
PAN 8 RG	-6.57	-0.94	2.433	-4.899
PAN 8 South 8	-14.79	-3.50	-18.076	-18.578
PAN 8 SP	-12.62	-3.44	-17.566	-18.237
PAN 8 SW	-21.75	-4.29	-24.418	-22.807
PAN 8 WP	-13.78	-3.50	-18.099	-18.593
PAN 9 SW	-4.41	-1.13	0.957	-5.883
Windmill	-15.03	-3.29	-16.399	-17.459
BH 1 Belfast	-13.76	-3.52	-18.254	-18.696

Table 4 Composition of epilithic algal communities of the pan sites at the Belfast Implementation mine site. The relative abundance of each epilithic algal taxa was grouped as follows: + = ≤ 50 (rare); ++ = 51- 250 (scarce); +++ = 251-1000 (common); ++++ = 1001-5000 (abundant); and +++++ = 5001-25 000 (predominant) cells/5 cm^2..

Species	Autecology of common benthic algae	Pan 1	Pan 2	Pan 3	Pan 4	Pan 5	Pan 6	Pan 7	Pan 8	Pan 9	Pan 10
Bacillariophyta											
Cocconeis pediculus	Water with a moderated to high electrolyte content. Taylor et al. (2007)		++								
Craticula buderi	Occurs in mine effluent with moderated to elevated electrolyte content. Taylor et al. (2007)						+	++++			
Craticula cuspidate	Eutrophic water with moderate to high electrolyte content Taylor et al. (2007)		++								++
Cyclotella meneghiniana	Eutrophic water. Taylor et al. (2007)		++							++	++
Cocconeis placentula	Meso –to eutrophic standing water. Taylor et al. (2007)		+++							+++	++
Diploneis elliptica	Oligotrophic standing water with moderated electrolyte content. Taylor et al. (2007)						+++	++			
Eunotia pectinalis var. undulata	Found in circumneutral to weakly acidic, electrolyte-poor waters. Taylor et al. (2007)							++	+++		
Eunotia bilunaris	Oligotrophic standing waters. Taylor et al. (2007)						++		+++		
Encyonopsis leei var. sinensis	Oligo- to mesotrophic water with low to moderate electrolyte content. Taylor et al. (2007)							++	+++		
Flagilaria ulna	Mesotrophic to eutrophic alkaline water. Taylor et al. (2007)	++	+++								++
Fragilaria tenera	Meso- to eutrophic water. Taylor et al. (2007).		+++							++++	++
Frustulia vulgaris	Oligotrophic to highly polluted water. Taylor et al. (2007)						++	+++			
Gomphonema laticollum	Slightly eutrophic water. Taylor et al. (2007).	++				+++					
Gomphonema venusta	Oligo- to mesotrophic water with low to moderated electrolyte content. Taylor et al. (2007).					++	++++	++			

Gomphonema italicum	Found in slightly eutrophic habitats. Taylor et al. (2007).		+++							+++	++
Gomphonema parvulum	Found in acidic, oligotrophic, electrolyte poor water. Taylor et al. (2007).							+++	++		
Hantzschia amphioxys	Favour periodically dry habitats. Taylor et al. (2007).										+++
Melosira variance	Eutrophic water. Taylor et al. (2007).		+++								
Navicula capitatoradiata	Tolerant of critical levels of pollution. Taylor et al. (2007).						++				
Navicula notha	Circumneutral, oligotrophic electrolyte poor water Taylor et al. (2007).				++++						
Navicula rhynchocephala	Oligo-to eutrophic water with low to moderated electrolyte content. Taylor et al. (2007).	++++			+++						
Navicula angusta	Weakly acidic, oligotrophic, clean, un-impacted water. Taylor et al. (2007).								+++		
Navicula riediana	Alkaline, eutrophic electrolyte-rich water. Taylor et al. (2007).		++				+				++
Navicula tripunctata	Good indicator of eutrophic water with a moderate to high electrolyte content. Taylor et al. (2007).		+++							++++	+
Nitzschia littorea	Mining effluent. Oberholster et al. (2017).							+++			
Nitschia pura	Weakly to moderately polluted waters with moderate electrolyte content. Taylor et al. (2007).	+++		++							
Nitzchia closterium	Brackish water. Taylor et al. (2007).	++									
Nitzschia intermedia	Eutrophic water with moderated to high electrolyte content. Taylor et al. (2007).		++							+++	
Nitzchia gracilis	Eutrophic, electrolyte rich water Taylor et al. (2007)									++	
Pinnularia viridiformis	Oligo-to mesotrophic water with low to moderate electrolyte content Taylor et al. (2007); Oberholster et al. (2010)		+++		++++	+++		+++	++++	++++	

Pinnularia divergens	Oligotrophic electrolyte poor water. Taylor et al. (2007).					+	++	++		
Pinnularia viridis	Found in circumneutral water with a low to moderated electrolyte content. Taylor et al. (2007)								+++	
Pinnularia subcapitata	Oligotrophic electrolyte poor water Taylor et al. (2007)		+++		+++					
Tabellaria flocculosa	This taxon flourish in oligotrophic, circumneutral or slightly acidic water. Taylor et al. (2007)								++	
Melosira variance	Eutrophic water. Taylor et al. (2007).		++							
Chlorophyta										
Closterium margaritiferum	Oligo-to mesotrophic water (Oberholster et al., 2010)								++	
Closterium peracerosum	Eutrophic water (Brook, 1965)				++				+++	
Cosmarium hammeri	Eutrophic water (Brook, 1965)						+++		++++	
Cosmarium pseudopraemorsium	Oligo-to mesotrophic water (Oberholster et al., 2010)			+++			++		+++	
Euastrum evolutum	Mesotropic water (Brook, 1965)				+++					
Actinastrum lagerheim	Not none				+++					
Cosmarium quadrum	Eutrophic water (Brook, 1965)								++	
Dictyosphaeium sp.	Not none			++++						
Eudorina elegans	Found in meso to eutrophic water. Van Vuuren et al .(2006)			+				+++	++	++
Eudorina unicocca	Found in meso to eutrophic water. Van Vuuren et al .(2006)									++++
Scenedesmus armatus	Found in meso to eutrophic water. Van Vuuren et al .(2006), Palmer, (1969)								++	
Oedegonium crassum	Eutrophic water (Simons, 1994)	++								+++
Spirogyra adnata	Oligo-to mesotrophic water (Oberholster., 2011)							+++	++	

Spirogyra reticulata	Oligo-to mesotrophic water (Oberholster, 2011)								+++		
Spirogyra rugulosa	Oligo-to mesotrophic water (Oberholster, 2011)						+++				
Staurastrum anatinum	Oligotrophic water (Brook, 1965)								+++		
Spondylosium sp.	Oligotrophic water (Brook, 1965)								+++		
Euglenophyta							++				
Euglena sociabilis	Organic pollution or brackish water rich in organic matter (Palmer, 1969; Effiong and Inyang, 2015)					++					
Euglena mutabilis	Organic pollution or brackish water rich in organic matter (Palmer, 1969; Effiong and Inyang, 2015)							+++			
Phacus acuminata	Organic pollution or indicator of moderate to strong pollution water (Effiong and Inyang, 2015); (Valadez et al., 2010)		+++								
Phacus pleuronectes	Organic pollution or indicator of moderate to strong pollution water (Effiong and Inyang, 2015); (Valadez et al., 2010)					+++		+++	+		
Trachelomonas intermedia	Eutrophic water (Wolowski and Grabowska, 2007)	++	++++	+++					+++++	+++	++++
Trachelomonas hispida	Eutrophic water (Effiong and Inyang, 2015)					+++					
Trachelomonas volvocina	Eutrophic water (Wolowski and Grabowska, 2007)			++++	+++						
Trachelomonas sp. 1				++					++		++
Cyanophyta											
Merismopedia punctate	Indicator of poor water quality (Begum and Hossain, 1993)									++	
Oscillatoria tenuis	Organic pollution, Eutrophic water (Palmer, 1969, Douterelo, I, Perona, E., Mateo, P. 2004)		++								
Microcystis aeruginosa	Eutrophic water (Oberholster et al., 2012)				+++						
Nostoc sp.	Oligotrophic water (Douterelo, I, Perona, E., Mateo, P. 2004)	++									

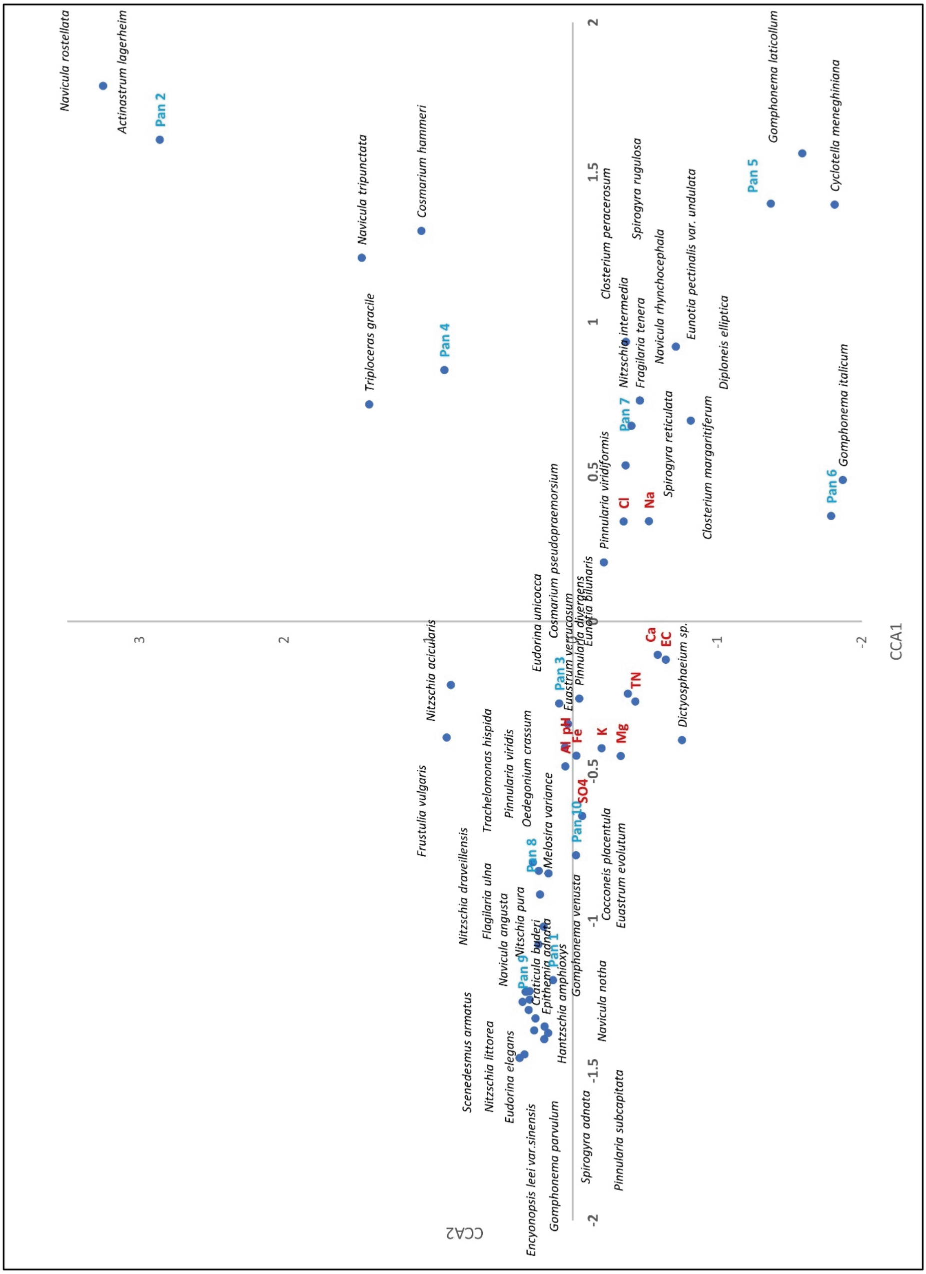

Figure 1 Canonical Correspondence Analysis of benthic diatoms and water quality parameters sampled from wetlands at the Belfast Implementation mine site.