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Diatoms as an Indicator of Water Quality in the Kuils River, Western Cape, South Africa

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Abstract: Rivers are crucial hydrological cycle components, supporting ecosystems and human activities. Managing and protecting river water quality is essential. Diatoms, microscopic algae, are widespread and sensitive to changes in water quality, making them effective bioindicators. This study focused on the Kuils River in the Western Cape, South Africa, where diatom sampling was conducted at four sites. Their communities are affected by different physicochemical parameters, such as changes in pH, salinisation, eutrophication, and organic enrichment. A total of 98 diatom species were identified. The Omnidia software was used to calculate the Generic Diatom Index, Specific Pollution Index, and Trophic Diatom Index. Historical physicochemical data such as pH, chemical oxygen demand, and phosphorus were obtained from the Department of Water and Sanitation between 2019 and 2021 and were used to provide a reference for comparing diatoms. The results showed clear signs of pollution, as shifts in diatom species composition were observed. Pollution-tolerant species such as the *Nitzschia palea* (Kützing) W Smith 1856, *Navicula viridula* (Kützing) Ehrenberg 1838, *Eunotia bilunaris* (Ehrenberg) Schaarschmidt 1880, and *Ulnaria ulna* (Nitzsch) Compère 2001 dominated, while less pollution-tolerant species like *Gomphonema parvulum f.saprophilum* Lange-Bertalot and E. Reichardt 1993 and *Stephanocylus meneghinianus* (Kutzing) Kulikovskiy, Genkal, and Kociolek 2022 were less abundant. Both diatom indices and physicochemical data indicated poor water quality in the Kuils River. The study concludes that diatoms are a valuable tool for biomonitoring river water quality and recommends their use alongside traditional physicochemical methods for future assessments of river systems.

Keywords: diatoms; biomonitoring; physicochemical parameters; water quality; Kuils River

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1. Introduction

Increased pollution growth and high water demand have caused a decline in freshwater availability [1]. This has caused a scarcity in water resources. In South Africa, a semi-arid country, freshwater quality deterioration continues to be a major problem. It is estimated that about 55.5% of rivers in the City of Cape Town metropole are polluted [2]. Multiple sources of pollution, such as industrial effluents, agricultural runoff, catchment land-use changes, chemical contaminations, litter, freshwater flow modifications, commercial and domestic sewage, abstraction, and the introduction of alien vegetation species, are responsible for this deterioration [3]. Therefore, proper assessment of a water resource requires regular monitoring. Water quality monitoring in South Africa has mostly been carried out using physicochemical parameters, and where biomonitoring has been used as a monitoring tool, macroinvertebrates have been used [4]. In addition to diatoms, other bio-monitoring indicators, such as fish and macroinvertebrates, have also been used [5].

The study aimed at using diatoms as an indicator of water quality. This study also used historical physicochemical parameters obtained from the Department of Water and

Sanitation from 2019 to 2021 as a reference of water quality conditions along the Kuils River. According to [6], both diatoms and macroinvertebrates can equally predict water quality. However, diatoms are better associated with water quality, while macroinvertebrates are regarded as better indicators of disturbances (land use, dissolved oxygen, and acid and ammonia contamination) in river systems [5,7]. Diatoms are sensitive to a variety of ecological conditions. The tolerance and preference levels of diatoms to pH, conductivity, salinity, humidity, organic matter, trophic state, oxygen, and current velocity in freshwater resources make them good indicators [8]. Diatoms live in different types of aquatic habitats because of their ubiquitous nature. They are a well-defined group of algae belonging to the *Bacillariophyceae* class. Their cell walls (skeletons) are made of silica, which looks like a glass house, and they are both unicellular and autotrophic organisms [9].

Diatoms require limited specialised equipment, which makes water quality monitoring using diatoms cost-effective [10]. For the quality of an environment in biology to be shown, a scale is used known as an index. Indices show the quality of an environment by indicating the types of organisms as well as the abundance of a particular organism in a representative environmental sample [11]. Therefore, indices are commonly used to assess water quality in aquatic ecosystems (mainly marine and freshwater). An advantage of using diatom indices is that they summarise information (ecological and hydrological) that is given by diatom assemblages [12].

In this study, Omnidia 6.1; Omnidia, Monbazillac, France, 2016, a diatom database and software identification tool, was used to identify diatom indices. The software generated 17 diatom indices, of which only 3 indices were used. All the developed indices have different functions. The trophic diatom index (TDI), the specific pollution index (SPI), and the generic diatom index (GDI) were used. These indices were chosen as they included more than 50% of the taxa that generated index values at all sites. The TDI measures inorganic nutrient concentrations, whereas SPI and GDI are indices that measure organic pollution [13]. This study used the following physicochemical parameters: pH, chemical oxygen demand, and phosphorus. These parameters were used as a reference for the conditions that commonly occur in the Kuils River.

The trophic diatom index, the generic diatom index, and the specific pollution index have been widely used in water quality monitoring to assess the ecological health of different aquatic ecosystems [14]. Europe has applied TDI to monitor nutrient pollution. One such example is a study that was conducted in the United Kingdom, which assessed the impact of agricultural runoff on river systems. According to [15], TDI was effective in correlating high nitrogen and phosphorus concentrations with eutrophication. Furthermore, some studies in China have shown that TDI is capable of identifying pollution fluctuations in urbanised river basins [16].

The SPI has been used in studies that have assessed organic pollution and heavy metal contamination. One such example is a study that was conducted in French rivers whereby the SPI distinguished pollution from urban runoff and industrial discharge [17]. Additionally, the SPI gave an indication of significant correlations with biological oxygen demand when it was used in Turkey to assess organic pollution in reservoirs [18].

Lastly, the GDI has been proven to be effective in detecting organic pollution and assessing different ecological aquatic environments [19,20]. For example, the GDI indicated strong correlations between diatom assemblages and physicochemical parameters after it was used to assess pollution levels in the Great Fish River in South Africa. In one such study [20], it was demonstrated that GDI showed similar results in the Mediterranean regions when it was used to track organic pollution from untreated sewage.

This study examined whether the trophic diatom index (TDI), the generic diatom index (GDI), and the specific pollution index (SPI) were effective in reflecting seasonal water quality variations along the Kuils River during the wet (rainy) and dry seasons between 2019 and 2021. Additionally, the study proposed that these indices be correlated strongly with physicochemical parameters that are common indicators of water quality. Physicochemical parameters include pH, chemical oxygen demand, and phosphorus concentrations. Factors affecting seasonal variations, such as increased pollutant concentration during dry periods and runoff during the wet (rainy) season, were expected to influence these indices.

2. Materials and Methods

2.1. Study Area

The Kuils River is located in the Western Cape Province of South Africa (Figure 1). The area is in a Mediterranean climate with a mean annual precipitation of between 500 and 600 mm per year [21]. The river is approximately 30 km long, flows southward, and passes through the residential and industrial areas of Bellville and Kuils River downstream. The main tributary of the Kuils River is the Bottelary River. Land use is influenced by agricultural, residential, commercial, and industrial activities along the catchment. This river also receives treated and untreated wastewater from the Scottsdene, Bellville, and Zandvliet Wastewater Treatment Works [21]. Upstream, the river is made up of the sedimentary Malmesbury group of rocks, while downstream, it is made up of deposits of loose sand and dune formations underlain by extensive clay lenses [22]. This study was conducted during both the dry (low water flow) and wet (high water flow) seasons.

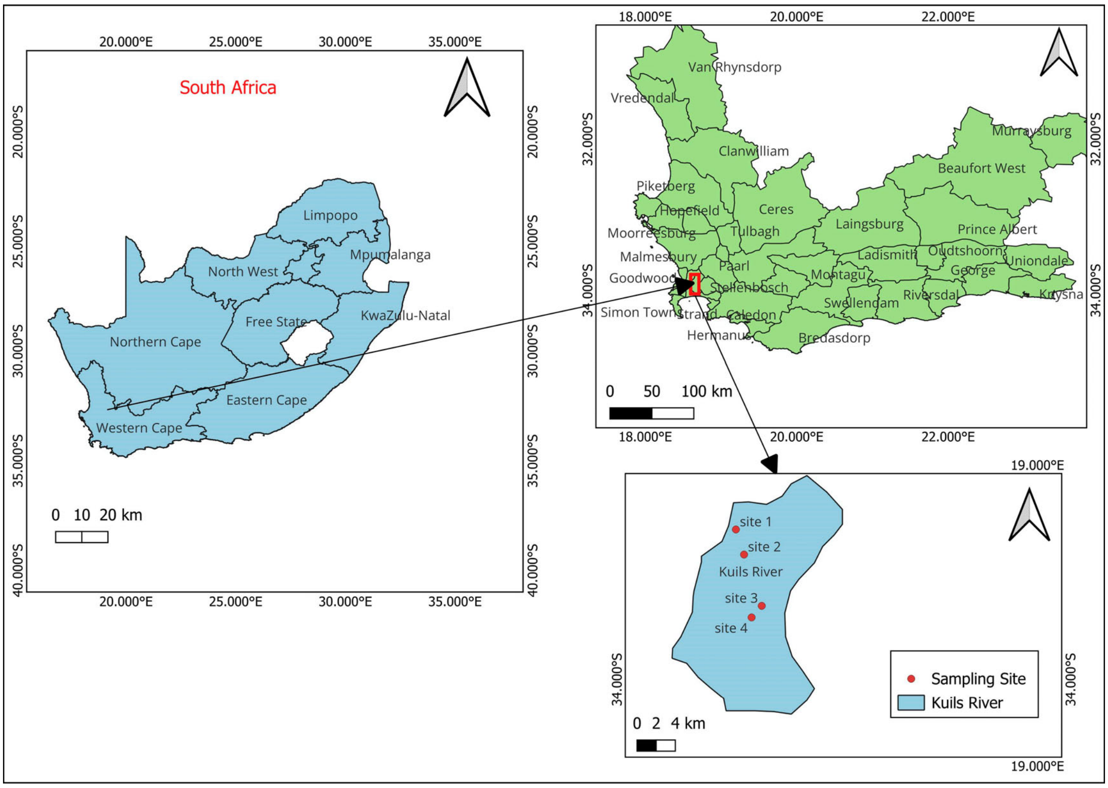

Figure 1. Location of the Kuils River catchment, Western Cape, South Africa.

2.2. Sample Collection

The study used the same four monitoring sampling points that the Department of Water and Sanitation uses for physicochemical sampling to collect diatom samples. The diatom samples at all four sites were collected on the same day. Two to three submerged rocks or cobbles were randomly collected at each site. Diatoms were removed by scrubbing the upper surface of the substratum with a clean toothbrush to dislodge the diatom community. Only the upper side (the side most exposed to flowing water) of the rock was scrubbed to avoid contamination with sediment that might be present on the bottom part of the rock. The resulting diatom suspension, after scrubbing, was poured into a labelled 100 mL sample (Schott) bottle. Ethanol was used to preserve the diatom samples, which were then taken to the laboratory for processing and analysis.

2.3. Physicochemical Analysis

The physical and chemical data used in the study were obtained from the National Water Monitoring Database, which is owned by the Department of Water and Sanitation. The historical data obtained were from 2019 to 2021. The physical and chemical parameters measured at the four sampling sites included chemical oxygen demand, pH, and phosphorus. The data received from the Department of Water and Sanitation were used to calculate the arithmetic mean for each parameter in this study. Graphs were generated, making it easier to compare the change in water quality over time in the Kuils River.

2.4. Diatom Sample Preparation

The diatom samples were allowed to settle for 48 h in the laboratory, after which the supernatant was discarded and the remaining sample was mixed well. Then, 10 mL of the diatom suspension was placed in a clean beaker, and 10 mL of potassium permanganate ($KMnO_4$) was added to the sample. This solution was left to settle for 24 h to allow for the oxidation of the organic material present in the sample. After 24 h, 10 mL of concentrated hydrochloric acid was added to the beaker containing the sample. The beakers were placed in a water bath at 90 °C for 2 h to speed up the digestion process of potassium permanganate by the hydrochloric acid. The solutions were slowly heated until they cleared to a pale yellow colour. To check if the oxidation reaction was complete and that no organic matter remained in the sample, 1 mL of hydrogen peroxide was added. The observation of foam upon the addition of hydrogen peroxide was used to determine whether organic material was still present within the sample. If still present, the process had to be repeated again. When oxidation was complete, the samples were left to cool to room temperature before they were centrifuged.

The cooled samples were vigorously swirled and transferred into the centrifuge tubes. Thereafter, the samples were rinsed by centrifuging them with distilled water at 2500 rpm for 7 min. After centrifugation, the supernatant was decanted, and the washing of the diatoms was repeated twice until the sample became circumneutral (clear). After the rinsing process was completed, the clear supernatant was removed from the testing tubes. The diatoms and small particles that settled at the bottom of the tube were resuspended by means of a jet of distilled water from the wash bottles. More distilled water was added until the test tubes were full. Furthermore, after the last wash, the diatoms were loosened again with distilled water, and the vortex mixer was used to mix and resuspend diatom samples. The final sample solution was poured into glass cylinders bearing the necessary sample information. After the centrifugation process was complete, slides and coverslips were thoroughly cleaned with detergent soap and stored in ethanol until needed. A portion of the cleaned sample was drawn from the numbered diatom suspension using a micropipette. The cleaned diatom suspension was then diluted until it appeared slightly cloudy to the naked eye. A single drop of ammonium peroxide (10% NH_4) was added to neutralise electrostatic charges on the suspended particles and reduce aggregation. Then, 1.5 mL of this cleaned diatom suspension was placed on a clean, dry coverslip and allowed to dry out overnight.

The dried coverslips were placed on a hot plate for approximately 2 min to remove the remaining ammonium peroxide. After the coverslips were cooled, they were briefly examined under the 40× objective lens to determine if the concentration of diatoms in the solution was adequate. According to [23], at least 10, but not more than 40, valves should be visible per field. This is so that when the sample is finally viewed under the 100× objective lens, the diatom valves ranging between 5 and 15 should be visible per field of view [23]. Thus, if the diatom concentration was too high or too low, a new diatom slide was prepared and the whole process was repeated. Once the concentration of diatom samples present on the coverslips was found to be correct or adequate, a previously dried and cleaned slide was lowered onto the coverslips, and Sellotape was used to attach the coverslip to the slide. The completed slides were then ready for microscopic examination.

2.5. Diatom Counting and Data Processing

Diatom species were counted using a light microscope (Olympus CX21 LED, Olympus Corporation, Tokyo, Japan). Diatoms were identified at the species level using the diatom guide that was developed by [23]. Where possible, 300 diatoms were counted on each slide and recorded. The recorded information was then uploaded into the Omnidia 6.1 software, Omnidia, Monbazillac, France, 2016. Seventeen different index values were calculated in the software. Only three indices were used in this study because they included more than 50% of the taxa, which generated index values at all sample sites [24]. The three chosen indices were the following: the generic diatom index (GDI), the specific pollution index (SPI), and the trophic diatom index (TDI).

2.6. Statistical Analysis (Redundancy Analysis)

In an aquatic environment, varied species have different requirements or specific optimum survival conditions. Diatoms are no exception, as they survive in certain conditions in aquatic waters. According to [4], dominant species can be taken to represent their environment. This part of the study examined the relationship between diatoms and the water quality parameters (pH, COD, and phosphorus). According to [25], redundancy analysis is a technique that is multivariate and used in determining the response of diatoms to environmental variables. In this study, the redundancy technique was used to determine the response of diatom species to pH, chemical oxygen demand, and phosphorus. One study [25] stated that redundancy analysis can detect patterns in community composition that can be elaborated best by the environmental variables.

3. Results

3.1. Physicochemical Parameters

Tables 1 and 2 represent the average historical physicochemical values that were obtained from the Department of Water and Sanitation over a period of 3 years. These values were obtained from the same sampling points that were used to obtain diatom samples. The pH levels during both high flow and low flow ranged between 6 and 8. The highest pH levels were recorded at Site 1, which had a pH of 8.3 (during high flow), and Site 4, which had a pH of 8.5 (during low flow). The lowest pH was 7.5 (Site 1) during high flow and 7.4 (Site 1) during low flow in 2019. These pH values fall within the range of natural conditions. Therefore, the Kuils River maintained a slightly alkaline water system over 3 years.

Table 1. Historical physicochemical data obtained from the Department of Water and Sanitation during high flow.

Variables	Unit	Sampling Year	Site 1	Site 2	Site 3	Site 4
pH	-	2019	7.5	7.7	7.7	7.7
	-	2020	8.3	8	8	8
	-	2021	7.9	7.7	7.7	7.9
Chemical oxygen demand (COD)	mg/L	2019	24	25	25	26
	mg/L	2020	54	32	32	0
	mg/L	2021	64	34	34	48
Phosphorus (PO_4^{3-})	mg/L	2019	0.13	0.08	0.08	0.55
	mg/L	2020	0.17	0.33	0.33	0
	mg/L	2021	0.1	0.22	0.22	1.14

Table 2. Historical physicochemical data obtained from the Department of Water and Sanitation during low flow.

Variables	Unit	Sampling Year	Site 1	Site 2	Site 3	Site 4
pH	-	2019	7.4	8.2	8.2	8.5
	-	2020	7.8	8.1	8.1	8.5
	-	2021	8.1	7.8	7.8	8.2
Chemical oxygen demand (COD)	mg/L	2019	55	39	39	45
	mg/L	2020	41	35	35	57
	mg/L	2021	84	35	35	40
Phosphorus (PO_4^{3-})	mg/L	2019	0.2	0.2	0.2	0.49
	mg/L	2020	0.13	0.1	0.1	0.82
	mg/L	2021	1.7	0.12	0.12	1.2

The phosphate concentrations were slightly consistent and lower upstream during both high flow and low flow and slightly increased downstream (Tables 1 and 2). The lower phosphate concentration upstream results from the Wastewater Treatment Works discharging effluent into the Kuils River, located further downstream. This means that effluent, which is discharged into the river, contains pollutants that will cause a rise in phosphate levels downstream (Site 4). The presence of phosphorus in the Kuils River is a result of natural processes, such as the Malmesbury group of rocks that make up this catchment. Rocks like shale from the Malmesbury group contain phosphate minerals and organic matter from agricultural activities that enter the Kuils River through weathering.

3.2. Diatom Species Composition

Table 3 indicates the dominant diatom species (>5%) that were observed during the study and their relative abundance at different sites. Since all four sites had alkaline waters, diatom species such as *Ulnaria acus* (Kützing) Aboal 2003 were present and dominant at all the sites. This type of diatom is found all over the world and favours alkaline environments. *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot 1999 and *Cocconeis engelbrechtii* Cholnoky 1955 are examples of some of the diatom species that were in abundance at all four sites. The presence of these diatom species indicates that water conditions in the Kuils River are alkaline, as these species favour such conditions. In addition, most diatoms are known to be sensitive to high levels of COD and organic pollution. In this study, the presence of dominant diatom species such as *Nitzschia palea* (Kützing) W Smith 1856 and *Ulnaria ulna* (Nitzsch) Compère 2001 across all four sites indicates waters with moderate to high COD levels. *Nitzschia palea* (Kützing) W Smith 1856 and *Ulnaria ulna* (Nitzsch) Compère 2001 are known as pollution-tolerant species and commonly dominate rivers with moderate to high COD levels. Therefore, the presence of these species in the Kuils River across all four sites indicates that water within the Kuils River contains increased levels of COD. These species also indicated organic pollution in the Kuils River as they reproduce and increase in abundance under moderate to high COD conditions. *Stephanocyclus meneghinianus* (Kützing) Kulikovskiy, Genkal, and Kociolek 2022 is a dominant diatom species that was found at Sites 2 and 3. The presence of this diatom gives an indication of low phosphorus concentrations at these sites, as this species favours such conditions, whereas diatoms of the genera *Nitzschia* and *Navicula* were dominant at Site 4. These species flourish under high phosphorus concentrations as they prefer nutrient-rich environments. Hence, their presence in abundance (*Nitzschia* and *Navicula*) at Site 4 gives an indication of increased phosphorus concentrations at Site 4.

Table 3. Dominant diatoms at each site with their relative abundance (%).

Taxon	Distribution at Sample Site	Relative Abundance (%)
Navicula viridula (Kützing) Ehrenberg 1838	Site 1 and 4	30.8
Nitzschia frustulum (Kützing) Grunow 1880	Site 4	25.5
Planothidium rostratum (Østrup) Lange-Bertalot 1999	All Sites	18.5
Nitzschia perspicua Cholnoky 1960	Site 4	19.4
Planothidium engelbrechtii (Cholnoky) Round and Bukhtiyarova 1996	All Sites	15.3
Nitzschia frustulum (Kützing) Grunow 1880	All Sites	24.5
Gomphonema pseudoaugur Lange-Bertalot 1979	All Sites	9.9
Nitzschia archibaldii Lange-Bertalot 1980	All Sites	19.9
Stephanocyclus meneghinianus (Kützing) Kulikovskiy, Genkal, and Kociolek 2022	Site 2 and 3	18.5
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot 1999	All Sites	20.4
Gomphonema saprophilum (Lange-Bertalot and E.Reichardt) Abraca, R. Jahn, J. Zimmermann, and Enke 2014	Site 2 and 3	12.5
Platessa oblongella (Østrup) C. E. Wetzel, Lange-Bertalot, and Ector 2017	All Sites	7.5
Craticula acidoclinata Lange-Bertalot and Metzeltin 1996	All Sites	9.0
Gogorevia exilis (Kützing) Kulikovskiy and Kociolek 2020	All Sites	20.5
Navigeia decussis (Østrup) Bukhtiyarova 2013	All Sites	5.4
Cocconeis lineata Ehrenberg 1849	All Sites	8.0
Eunotia rhomboidea Hustedt 1950	All Sites	15.0
Eunotia formica Ehrenberg 1843	All Sites	6.6
Nitzschia communis Rabenhorst 1860	All Sites	8.0
Nitzschia palea (Kützing) W. Smith 1856	Site 4	35.6
Gyrosigma acuminatum (Kützing) Rabenhorst 1853	Site 4	5.5
Cocconeis engelbrechtii Cholnoky 1955	All Sites	27.8
Craticula ambigua (Ehrenberg) D.G.Mann 1990	All Sites	10.5
Nitzschia filiformis (W. Smith) Van Heurck 1896	All Sites	19.4
Craticula cuspidata (Kutzing) D. G. Mann 1990	All Sites	20.4
Nitzschia heufleriana Grunow 1862	All Sites	22.4
Cymbopleura amphicephala (Nägeli ex Kützing) Krammer 2003	All Sites	10.5
Eunotia bilunaris (Ehrenberg) Schaarschmidt 1880	All Sites	11.6
Eunotia flexuosa (Brébisson ex Kützing) Kützing 1849	All Sites	9.9
Ulnaria biceps (Kützing) Compère 2001	All Sites	8.5
Fragilaria tenera (W. Smith) Lange-Bertalot 1980	All Sites	8.0
Ulnaria ulna (Nitzsch) Compère 2001	All Sites	24.5
Ulnaria acus (Kützing) Aboal 2003	All Sites	7.2
Nitzschia umbonata (Ehrenberg) Lange-Bertalot 1978	All Sites	6.3
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot 1999	All Sites	23.4
Tabularia fasciculata (C. Agardh) D.M.Williams and Round 1986	All Sites	12.5
Tryblionella coarctata (Grunow) D. G. Mann 1990	All Sites	5.4
Tryblionella littoralis (Grunow) D. G. Mann 1990	All Sites	6.4

Index Scores

The Omnidia 6.1 software, Omnidia, Monbazillac, France, 2016calculates index scores for different indices. This software was developed because of the importance of indices to be designed in an easy manner, which enables data interpretation to be translated into information useful for management purposes [26] Tables 4 and 5 give an indication of how diatom index scores are interpreted as calculated by the Omnidia software. The range of the scores varies, with SPI and GDI (Table 3) indicating bad water quality when the score is at zero, while TDI (Table 4) indicates good water quality at a score of zero [4,27]).

Table 4. Water quality categories indicating different classes of water quality based on the GDI and SPI [28].

Class	The Interpretation of the Index Scores (Index Score)
Bad	<9
Poor	9–12
Satisfactory	12–15
Good	15–17
Very good	>17

Table 5. TDI scores and their corresponding trophic status [13].

Trophic Category of Water	The Interpretation of the Index Scores (Index Value Ranges)
Oligotrophic	0–20
Oligo-mesotrophic	21–40
Mesotrophic	41–60
Meso-eutrophic	61–80
Eutrophic	>80

Figures 2–4 show index scores (SPI, GDI, and TDI) that are interpreted according to Tables 4 and 5. The SPI graphs (Figure 2) indicate how both high flow and low flow values remained constant as they ranged between 7 and 9 in this study. A diminished water quality was observed across all the sites during all the seasons, as the values obtained were less than 9, as indicated in Table 4. The water quality showed an improvement during the low flow season at Site 2 with a value of 11.7, even though the water quality remained poor at this site. Poor water quality in the high-flow season could be associated with the heavy rains that might have caused an increase in suspended solids and sediment yields [25]. Factors such as runoff from stormwater drains and discharge from sewage pipes also contribute to the poor water quality in the river.

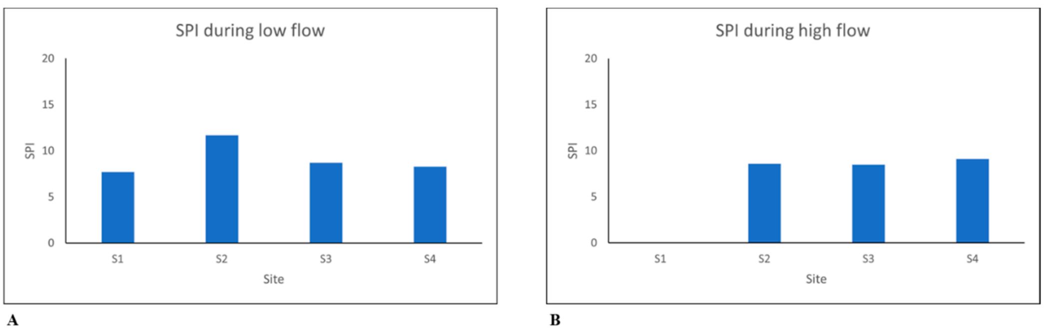

Figure 2. (**A**) Specific pollution index (SPI) values during low flow along the sampled sites on the Kuils River. (**B**) High flow specific pollution index (SPI) values during high flow along the sampled sites on the Kuils River (Site 1 SPI value was not recorded because of high water levels at the time of diatom sampling, which made the site inaccessible).

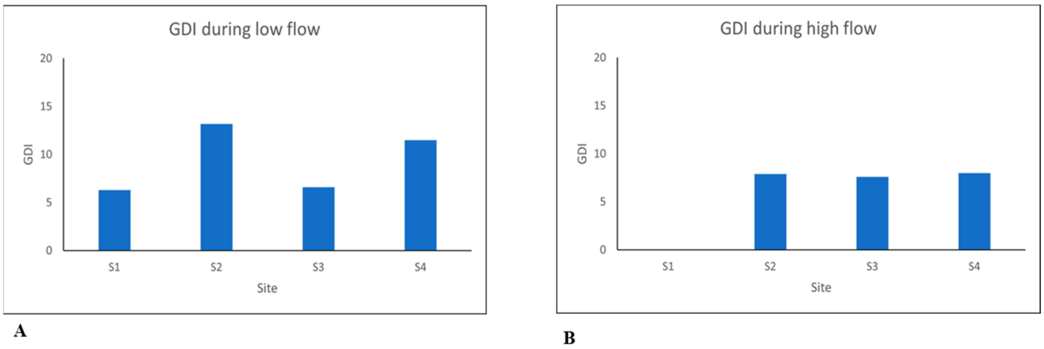

Figure 3. (**A**) Generic diatom index (GDI) values during low flow along the sampled sites on the Kuils River. (**B**) Generic diatom index (GDI) values during high flow along the sampled sites on the Kuils River (Site 1 GDI value was not recorded because of high water levels at the time of diatom sampling, which made the site inaccessible).

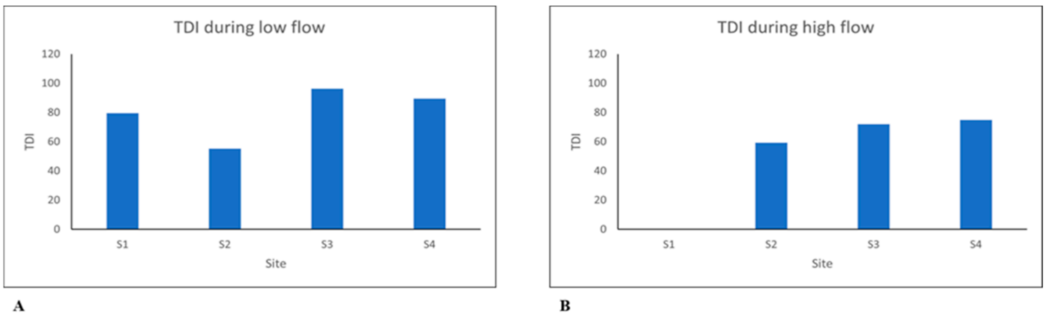

Figure 4. (**A**) Trophic diatom index (TDI) values during low flow along the sampled sites on the Kuils River. (**B**) Trophic diatom index (TDI) values during high flow along the sampled sites on the Kuils River (Site 1 TDI value was not recorded because of high water levels at the time of diatom sampling, which made the site inaccessible).

The GDI values in Figure 3 indicate poor water quality at all sites across the river. The values are greater than 9, with the lowest value being 6.3 at Site 1. However, there was a slight change during the low flow season in which water quality improved to moderate quality, with values ranging between 12 to 15. This improvement occurred at Site 2 and could have been influenced by the presence of different diatom species that were encountered during the study in the low-flow season compared to those found in the high-flow season. The GDI accounts for the genera of taxa in which a genus may have different species with regard to their optimal conditions [25]. The high flow season gives an indication of bad water quality in the river, as the values in the graph (Figure 3B) are not greater than 9. According to [22], GDI levels less than 9 are classified as bad water quality. The findings of GDI in this study corresponded to the findings from physicochemical parameters, as both results indicated the presence of pollutants in the river.

The TDI values were the lowest during low flow, with a value of 55.2 at Site 2 (Figure 5). Some of the highest recorded TDI values were 71.9, 74.9, 79.6, and 89.4. All these values indicate that the water quality during both high flow and low flow ranged between mesotrophic and eutrophic conditions. A rise in the TDI concentration was also noticeable as one moved downstream from one site to the next (Figure 4). The high concentration in the TDI indicated increased pollution along the river as one moved downstream. The findings from the TDI, when compared to those obtained from physicochemical parameters, indicate similar conditions. Both results indicate poor water quality along the Kuils River and also show that the pollutant concentrations increased downstream. The increased pollution inputs can be attributed to runoff from residential areas or the dilution of diatoms in the water taking place during high flow [25]. Another reason for poor water quality could be nutrients entering the Kuils River through discharge from wastewater facilities, as well as farming activities along the catchment.

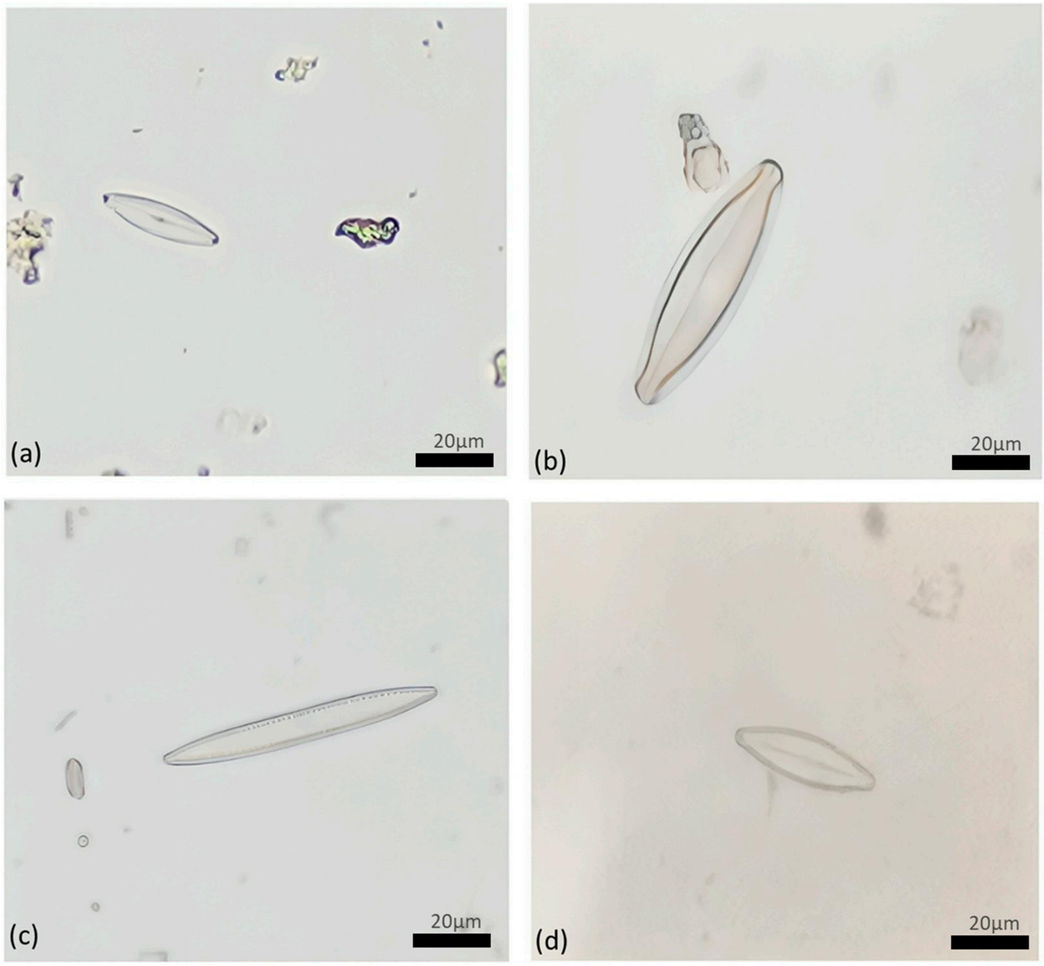

Figure 5. Photomicrographs of diatom species of (**a**) *Craticula cuspidata* (Kutzing) D.G.Mann 1990; (**b**) *Navicula viridula* (Kützing) Ehrenberg 1838; (**c**) *Nitzschia perspicua* Cholnoky 1960; and (**d**) *Nitzschia communis* Rabenhorst 1860.

3.3. Observed Diatom Species

Photomicrographs of different types of diatoms observed in the samples are shown in Figures 5 and 6. These photomicrographs show the diatom silica structure (cell wall or frustule). Centric and pennate diatoms are two groups of diatoms commonly found in freshwater systems [23]. Centric diatoms are adapted to live in the water as part of phytoplankton, while pennate diatoms live in benthic habitats (on occasion temporarily resuspended in the water column) [23]. Figures 5 and 6 indicate diatoms belonging to the pennate group. *Craticula cuspidata* (Kutzing) D.G.Mann 1990 (Figure 5a), which commonly occurs in eutrophic and brackish waters, is distinguished by parallel transverse striation. Additionally, *Craticula cuspidata* (Kutzing) D.G.Mann 1990 tolerates critical to heavy pollution levels [23]. *Nitzschia perspicua* Cholnoky 1960 (Figure 5b) contains weakly silicified frustule (cell wall), while its striae are not easily observable in light microscopy. *Navicula viridula* (Kützing) Ehrenberg 1838 (Figure 5c) favours eutrophic waters and tolerates critical levels of pollution. *Nitzschia communis* Rabenhorst 1860 (Figure 5d) contains striae that are observable under light microscopy. It occurs in electrolyte-rich as well as brackish waters and is tolerant of extremely polluted conditions [23].

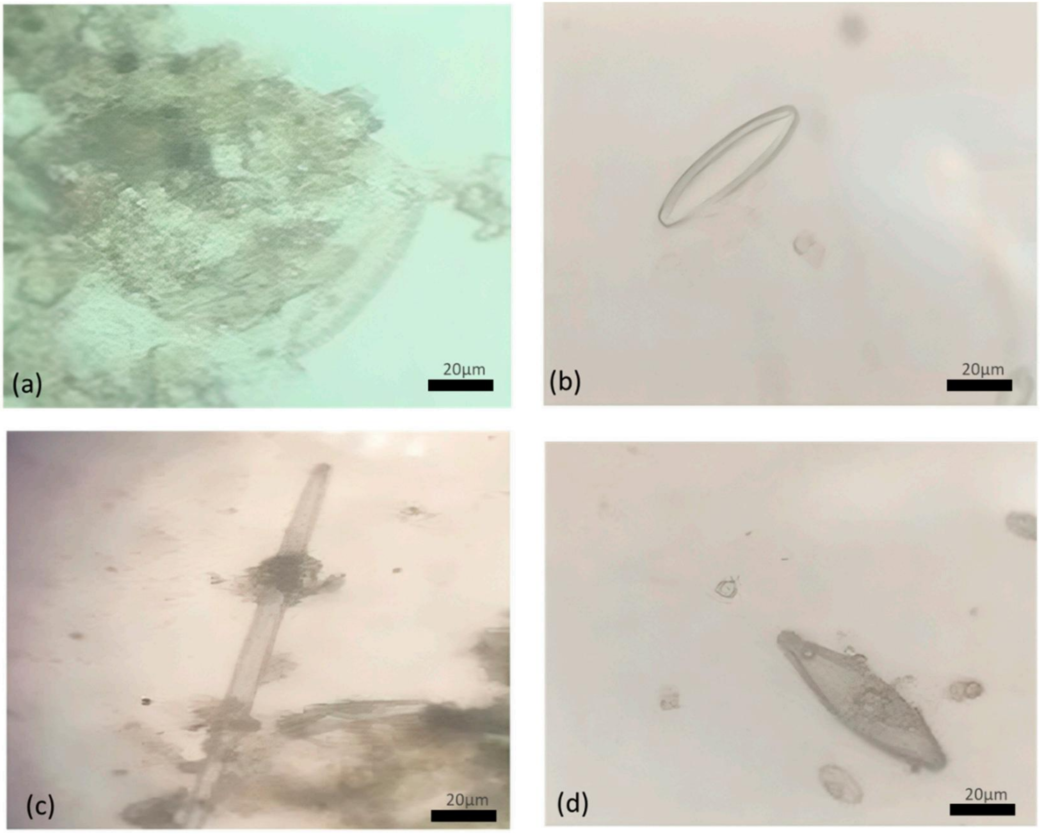

Figure 6. Photomicrographs of diatom species of (**a**) *Nitzschia palea* (Kützing) W Smith 1856; (**b**) *Eunotia rhomboidea* Hustedt 1950; (**c**) *Ulnaria acus* (Kützing) Aboal 2003; and (**d**) *Gomphonema pseudoaugur* Lange-Bertalot 1979.

Figure 6a illustrates *Nitzschia palea* (Kützing) W.Smith 1856, which is a type of diatom that is found all over the world. It thrives in eutrophic, heavily polluted to extremely polluted environments [23]. *Eunotia rhomboidea* Hustedt 1950 (Figure 6b) contains small valves, and the frustule is visible in light microscopy. They are found in oligotrophic, electrolyte-poor waters. *Ulnaria acus* (Kützing) Aboal 2003 (Figure 6c) is a cosmopolitan species that is found in the benthos of rivers and exists in alkaline freshwaters [23]. *Gomphonema pseudoaugur* Lange-Bertalot 1979 (Figure 6d) has an oval shape and occurs in eutrophic waters. This diatom type is sensitive to more critical levels of pollution [23].

3.4. Redundancy Analysis (RDA)

Figure 7 indicates the redundancy analysis graph. The graph has a central point that represents the mean or dominant diatom species. The central point represents the mean, or average, of the variables, whereas the arrows indicate the direction of maximum change in the value linked to the variable. The length of the arrows is related to the highest level of variation. According to [24], a positive correlation is observed when the response variable is in the same quadrant as the water quality parameter, whereas a negative correlation can be seen when the response variable is in the opposite direction as the water quality parameter. Class 1 represents diatoms that prefer low nutrient and COD levels, while Class 2 represents diatoms that are related to high pH levels. Class 3 displays diatom species that are linked to increased levels of nutrient concentration and COD, and lastly, Class 4 indicates diatoms that have low tolerance to pH variation.

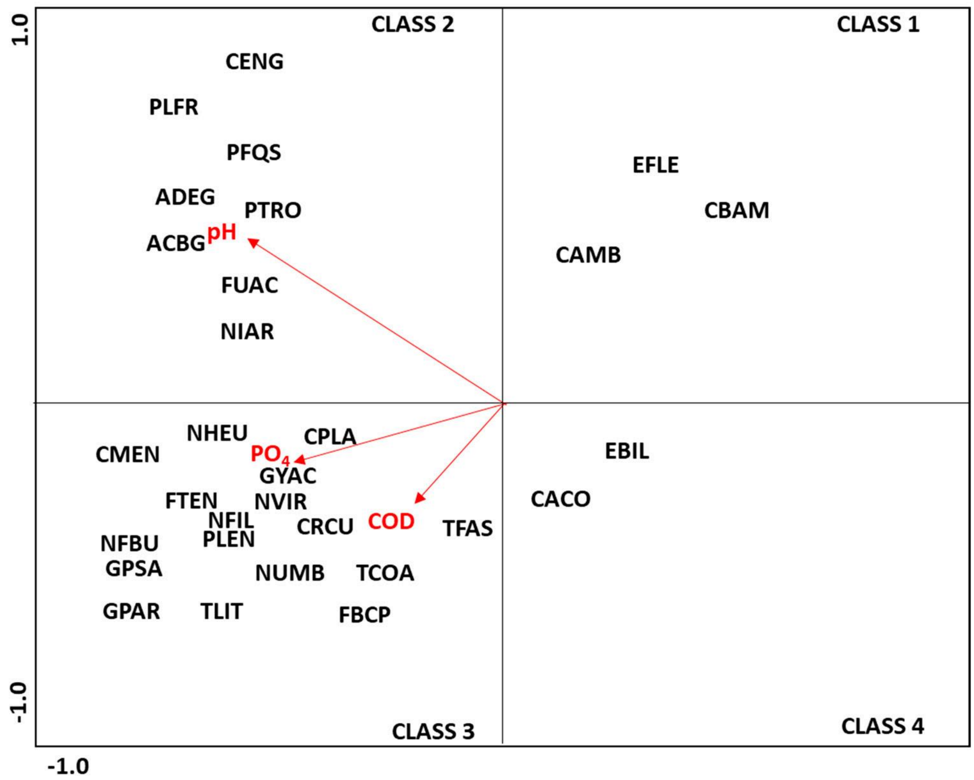

Figure 7. Redundancy analysis plot showing a relationship between dominant diatom taxa and environmental variables along the Kuils River.

4. Discussion

Dominant diatom species that were found in this study, such as *Planothidium engelbrechtii* (Cholnoky) Round and Bukhtiyarova 1996, *Nitzschia frustulum* (Kützing) Grunow 1880, *Nitzschia heufleriana* Grunow 1862, and *Craticula cuspidata* (Kutzing) D.G.Mann 1990, are known to be tolerant to moderately critical pollution occurring in eutrophic waters as well as waters with high levels of pollution [12,23,25]. The values obtained from diatom indices (SPI, GDI, and TDI) and physicochemical parameters indicate that these tools can be used to determine the water quality of a river. The findings from this study indicate that diatoms are able to determine water quality based on their dominance and abundance at a particular site along the river. This information reflects the conditions present in the river. For the purposes of water quality analysis, when used together, physicochemical parameters and diatoms complement each other in giving an indication of water quality. This is because physicochemical parameters give an exact number of the conditions occurring at a particular site. For example, the physicochemical parameter method was able to record pH levels that ranged between 6 and 8 across the Kuils River, which indicated alkaline water, whereas the dominance of diatom species Fragilaria ulna var. indicated alkaline waters, as this species prefers environments with moderate to high pH levels. These conditions correspond to the natural pH level found in riverine systems [25]. The observed level of pH in the water is attributed to the sedimentary rocks of the Malmesbury group that make up this river. These rocks are carbonate-rich and are composed of calcium and magnesium carbonates that are naturally alkaline [29]. Carbonates from these rocks are dissolved when water comes into contact with these carbonate rocks. Therefore, the information from both physicochemical parameters and diatoms works well together, as they can determine the quality of water along the river.

The COD in uncontaminated waters is usually less than 20 mg/L, while water that receives effluents has a concentration of more than 200 mg/L [30]. However, in industrial wastewater, the values could be as high as 60,000 mg/L. With regards to the current study, Tables 1 and 2 show the highest COD concentration of 84 mg/L, recorded at Site 1 in

2021. This high COD value was recorded during the low flow season. According to [25], an increase in nutrients such as nitrates and phosphates is related to an increase in COD concentration. Thus, there is a link between COD and the input of organic matter and nutrients. The relationship between water flow fluctuations and nutrients proves that it is possible to use both these variables in determining the relationship with COD [25]. The COD levels during high flow and low flow across all the sites over the three-year period were all above 20 mg/L. This means that all the sampled sites were experiencing a moderate increase in COD concentrations during high flow at Site 1 from 2019 to 2021, whereas Sites 2 and 3 remained constant.

The moderate increase in COD levels in the Kuils River is because of the location of the river. This river is surrounded by vineyards and fruit-growing farms. As a result of these surroundings, agricultural runoff causes an increase in COD levels present in the river. The chemicals used during agricultural activities to grow the fruits, including fertilisers, herbicides, and pesticides, contain organic compounds that are transferred into the Kuils River through runoff when precipitation occurs. Thus, the surrounding farms are contributing to the increased levels of COD in the Kuils River. Effluent discharge from Scottsdene, Bellville, and Zandvliet Wastewater Treatment Works also contributes to increased levels of COD in the Kuils River. According to [21], these wastewater treatment plants discharge treated and untreated wastewater into the Kuils River. Untreated wastewaters contain organic compounds, and once discharged into the Kuils River, it causes COD levels to rise.

The sources of pollution in the Kuils River can be attributed to anthropogenic activities and natural processes. Anthropogenic activities such as agricultural runoff, industrial effluents, and informal and formal settlements may have contributed to the poor quality of the river. Natural processes in the Kuils River may have contributed to poor water quality through the introduction of pollutants from the river's bedrock, which causes groundwater and surface water interaction through seepage. The Malmesbury group of rocks, like shale, contains phosphate minerals and organic matter that enter the Kuils River through weathering. It is understood that this weathering occurring in the catchment increases the levels of phosphorus present in the river.

Different species have different preferences when it comes to conditions in which they survive, which is also evident in Figure 7. Diatoms, such as *Planothidium rostratum* (Østrup) Lange-Bertalot 1999, *Platessa oblongella* (Østrup) C. E. Wetzel, Lange-Bertalot, and Ector 2017, *Cocconeis engelbrechtii* Cholnoky 1955, found in Class 2, are associated with alkaline waters, i.e., waters with high pH levels. Class 3 contains diatom species, such as *Navicula viridula* (Kützing) Ehrenberg 1838, *Navigeia decussis* (Østrup) Bukhtiyarova 2013, *Fragilaria tenera* (W. Smith) Lange-Bertalot 1980, and *Stephanocyclus meneghinianus* (Kützing) Kulikovskiy, Genkal, and Kociolek 2022, which prefer increased nutrient concentrations and COD.

Furthermore, these species tolerate pollution and thus make good indicators of pollution in water sources. *Tabularia fasciculata* (C. Agardh) D. M. Williams and Round 1986 is known to favour wastewater containing pollutants. This species forms part of Class 3 and is also a good indicator of pollution. *Craticula acidoclinata* Lange-Bertalot and Metzeltin 1996, which forms part of the Class 4 diatom group, commonly appears in negative correlation to increased levels of pH. This means that *Craticula acidoclinata* Lange-Bertalot and Metzeltin 1996 prefers waters that are acidic.

5. Conclusions

The study examined whether diatom-based water quality assessments align with traditional physicochemical methods, given the scarcity of diatom studies in South Africa, particularly in the Kuils River. Since no prior diatom data existed for this river, historical water quality parameters were used for comparison. The study found that diatoms, which respond to pollution levels, are reliable bioindicators for water quality monitoring. The results showed poor water quality in the Kuils River, especially downstream, due to nutrient runoff from rainfall, agriculture, sewage leaks, and wastewater treatment plants.

The study confirmed that both diatom analysis and physicochemical measurements complement each other, providing a comprehensive understanding of environmental conditions. Diatom communities reflect changes in water quality, and shifts in their composition indicate the presence of pollutants. The findings suggest that diatoms can be used effectively to monitor water quality alongside traditional methods, with regular long-term monitoring recommended.

The conclusions from this study indicate that diatoms are present in the Kuils River, and their distribution is influenced by physicochemical factors at each site. Diatoms play a crucial role in assessing water quality, as the dominant species at a given location reflect the environmental conditions that allow them to thrive, providing insights into current water quality. In the Kuils River, specific diatom communities are linked to eutrophic conditions, where nutrient-rich environments support species adapted to such settings. The ease of collecting diatom samples from river systems makes them valuable bioindicators. The study recommends monitoring diatom communities over an extended period, ideally longer than a year, to track changes in water quality over time. Additionally, future research should incorporate diatom analysis alongside traditional water quality assessments.

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