ZIBELINE INTERNATIONAL™ PUBLISHING
ISSN: 2523-5664 (Print)
ISSN: 2523-5672 (Online)
CODEN: WCMABD

Water Conservation & Management (WCM)

DOI: http://doi.org/10.26480/wcm.02.2025.402.413

CrossMark

RESEARCH ARTICLE

REASSESSING ENVIRONMENTAL FACTORS INFLUENCING DIATOMS IN THE KAROO – IMPROVING THE BASELINE FOR KAROO WATERS.

Marion Holmes[a,b]*, Eileen E. Campbell[a], Jonathan C. Taylor[c,d]

[a]*Department of Botany, Nelson Mandela University, Gqeberha South Africa*
[b]*Africa Earth Observatory Network (AEON) - Earth Stewardship Science Research Institute (ESSRI), Nelson Mandela University, Gqeberha, South Africa.*
[c]*Unit for Environmental Sciences and Management (UESM), North-West University, Potchefstroom, South Africa.*
[d]*South African Institute for Aquatic Biodiversity (SAIAB), P Bag 1015, Makhanda 6140, South Africa*
**Corresponding Author Email: karoocats007@gmail.com*

Article History:

Received 14 March 2025
Revised 02 April 2025
Accepted 25 April 2025
Available online 30 May 2025

ABSTRACT

Biomonitoring tools are constantly evolving, with South Africa being no exception. This study aims to improve the knowledge of ecological preferences and tolerances of diatom species in the Karoo and to expand upon the diatom baseline for this semi-arid area. The natural water of the Karoo has wide ranges within its chemistry – often with extremely high concentrations of dissolved salts. The diatom community composition of natural Karoo waters was sampled (n = 510). A total of 543 diatom species were recorded, but the 322 species with abundance over 1% were used in the analyses. The significant environmental drivers that potentially influence these communities included electrical conductivity, calcium, chloride, oxidised nitrogen and strontium. Multivariate analysis (CCA) revealed the three main measured variables influencing diatom community composition to be lithium, chloride and oxidized nitrogen. These were followed by sulphate, selenium, silica and scandium. Several diatom taxa (as yet unidentified) have tolerances to the extremely saline conditions of the natural springs and ground water of the Karoo.

KEYWORDS

Karoo, diatom, biomonitoring, ecology, water quality, bioindicators

1. INTRODUCTION

The semi-arid Eastern Karoo region of South Africa is a unique and environmentally sensitive area facing the dual pressures of increasing climatic variability and significant anthropogenic influences (Conradie et al., 2019; Timm Hoffman et al., 2021; Ziervogel et al., 2022). Freshwater systems in this region are critically scarce, and most of its inhabitants, encompassing human populations, diverse fauna, and unique flora, depend almost exclusively on groundwater that often emerges as springs (Braune and Xu, 2008; Hobbs et al., 2016). Many of the region's rivers, such as the Great Fish River, are intrinsically linked to these spring-fed origins, highlighting the paramount importance of groundwater resources. Both surface and subsurface water bodies are susceptible to the impacts of episodic runoff events following rainfall (Nyawo 2017; Geris et al., 2022). While droughts are a recurring feature of the Karoo climate, the period between 2011 and 2019 witnessed an exceptionally severe and prolonged drought, leading to a significant decline in rainfall and, consequently, reduced groundwater recharge (Archer et al., 2022; Mathivha et al. 2024). This underscores the vulnerability of the region's aquatic ecosystems to climatic extremes.

Ecological theory posits that significant alterations in biotic and abiotic environmental factors inevitably lead to changes within an ecosystem potentially impacting its resilience to disturbances such as drought (Bornette and Puijalon, 2011; Niinemets et al., 2017). The groundwater resources of the Karoo exhibit considerable spatial and temporal variability in their hydrochemistry with systems dominated by calcium, chloride, or a mixture of ions (Stroebel et al., 2019). Adding another layer of complexity, recent proposals for natural gas exploitation through hydraulic fracturing in the region have spurred extensive transdisciplinary baseline research initiatives, including the study presented here, which formed part of the broader Karoo Shale Gas Project undertaken by the Africa Earth Observatory Network (AEON). This context highlights the urgent need for robust environmental monitoring to establish baseline conditions and detect potential impacts from both natural and anthropogenic stressors.

Traditional water quality monitoring programmes have historically relied heavily on the measurement of physicochemical variables (Matlala et al., 2011; Dalu and Froneman, 2016). While providing valuable snapshot data of immediate conditions, these methods often fail to capture the cumulative and long-term effects of pollution or the influence of chronic, low-level contamination (Dallas and Day, 2004; Lavoie et al., 2006; Matlala et al., 2011). Furthermore, they typically assess only a limited suite of potential pollutants, potentially overlooking synergistic or antagonistic effects of complex contaminant mixtures (Wolska et al., 2007; Garg et al., 2022). In contrast, biological indicators, particularly diatoms, offer a more integrated perspective on environmental health, reflecting long-term impacts, the influence of subtle chronic contamination, and complex ecological interactions (Dalu and Froneman, 2016; Barinova, 2017, Soininen et al., 2025). Changes in water quality are often reflected in shifts in the structure and composition of biological communities (Smol and Stoermer, 2010; Gholizadeh 2021). Tracking these biological changes over extended periods allows for a better understanding of the environmental

Quick Response Code	Access this article online	
	Website: www.watconman.org	**DOI:** 10.26480/wcm.02.2025.402.413

Cite The Article: Marion Holmes, Eileen E. Campbell, Jonathan C. Taylor (2025). Sessing Environmental Factors Influencing Diatoms In The Karoo – Improving The Baseline For Karoo Waters. *Water Conservation & Management, 9(2): 402-413.*

tolerances of individual taxa within the Karoo.

Diatoms, as primary producers forming the base of aquatic food webs, play a crucial role in maintaining ecosystem health and productivity (Serôdio and Lavaud, 2022; B-Béres et al., 2023). Their rapid response to environmental change makes them particularly valuable indicators of water quality alterations (Dalu and Froneman, 2016; Serôdio and Lavaud, 2022). Found across a wide range of aquatic habitats, their unique siliceous cell walls, characterized by species-specific ornamentation, enable identification even after cell death, preserving a historical record within sediments (Matlala et al., 2011; Pandey et al., 2017; Serôdio and Lavaud, 2022). This characteristic makes diatoms ideal for paleolimnological reconstructions of past environmental conditions, forensic investigations, and long-term environmental monitoring programmes (Cantonati et al., 2022; Holmes et al., 2022, Hwang et al., 2023; Cantonati et al., 2024; Verma, 2013, Zhou et al., 2020; Ludes et al., 2024; Ncube, 2021; Gnjato et al., 2025; Zalat et al., 2025). The creation of permanent diatom slides allows for the potential hindcasting of water quality parameters (Taylor et al., 2004).

Diatom assemblages are integral components of numerous biomonitoring programmes worldwide (Bere and Tundisi, 2010 in Brazil; Szczepocka and Żelazna-Wieczorek, 2018 in Poland; Masouras et al., 2021 in Europe; Costa and Schneck, 2022 with a global overview). However, a significant gap exists in the biomonitoring of underground water resources that are stored above ground, such as reservoirs, particularly within the South African context. Current diatom-based biomonitoring practices globally often rely on European indices developed for the specific environmental conditions of that continent (Taylor et al., 2007c; Coste et. al. 2009; Dalu et al., 2016, Blanco, 2024, Mertens et al., 2025). While these indices can be useful in South Africa, studies have shown that they do not always accurately reflect the prevailing local environmental conditions and the responses of South African diatom communities (Bate et al., 2002; Taylor et al., 2007c; Dalu et al., 2016; Mangadze et al., 2019; Holmes, 2022) Recognizing this limitation, this study does not directly apply these indices as definitive guides to species preferences and tolerances but rather utilizes the underlying autecological information upon which these indices were originally based. It is increasingly recognized that the environmental preferences of even cosmopolitan diatom species can exhibit biogeographical variation highlighting the need for continuous refinement of the South African Diatom Index (SADI), which was modified from the Specific Pollution Sensitivity Index (SPI) for use in South Africa (Harding and Taylor, 2011; Dalu and Froneman, 2016; Soininen et al., 2016; Jyrkänkallio-Mikkola et al., 2017; Holmes, 2022).

The limnological conditions within the Karoo (geology, hydrology, and climatic regimes), are dynamic and subject to change (Harkness et al., 2018; Stroebel et al., 2019). These evolving environmental conditions are expected to exert a significant influence on the structure and composition of diatom assemblages. Therefore, data collected from this ecologically important region can contribute valuable insights to expand our understanding of the specific ecological preferences of diatom species in the Karoo context, ultimately improving their efficacy in future regional monitoring efforts.

Recent studies have highlighted the importance of sustainable water resource management in semi-arid regions like the Karoo. For instance, Le Maitre et al. (2009) discuss the challenges of sustainable development in water-scarce areas and emphasize the need for improved water management practices to enhance water infiltration and reduce soil erosion. In the context of biomonitoring, recent advancements have been made in the use of diatoms as bioindicators. Masouras et al. (2021) review the implementation of benthic diatoms in river biomonitoring under the European Water Framework Directive, highlighting their effectiveness in detecting eutrophication, organic pollution, and acidification. Costa and Schneck (2022) provide a global overview of the use of benthic diatoms in lotic environments, emphasizing the need for low-cost and time-efficient biological assessment strategies.

This study aims to build upon the baseline data established by previous research and to further elucidate the relationship between environmental factors and diatom distribution by analysing a significantly larger dataset (n=510) compared to the 290 samples reported in (Holmes et al., 2023). The earlier study indicated that community composition was significantly influenced by electrical conductivity, lithium, sulphate, and oxidized nitrogen. By expanding the spatial and temporal scope of the investigation, this research seeks to provide a better understanding of the key environmental drivers shaping diatom communities in the Karoo.

Objectives

1. To improve the baseline data from previous by using a larger dataset (510 samples compared to 290) research (Holmes et al., 2023).

2. Identify the key environmental variables that significantly correlate with diatom community composition in these reservoirs, utilizing a larger dataset (n=510) compared to previous research.

3. Determine the relative influence of electrical conductivity, lithium, sulphate, and oxidized nitrogen (identified in previous research) on diatom distribution within the expanded dataset.

4. Improve ecological understanding of the tolerance and preferences of local diatom taxa to enable better region-specific biomonitoring approaches.

5. Contribute toward the refinement of the South African Diatom Index by providing updated autecological data for cosmopolitan and locally significant species

2. Materials And Methods

This study took place in the eastern part of the Karoo in South Africa. Nine spring/surface water sites and 56 reservoirs (and their boreholes) were included in this study (Fig. 1).

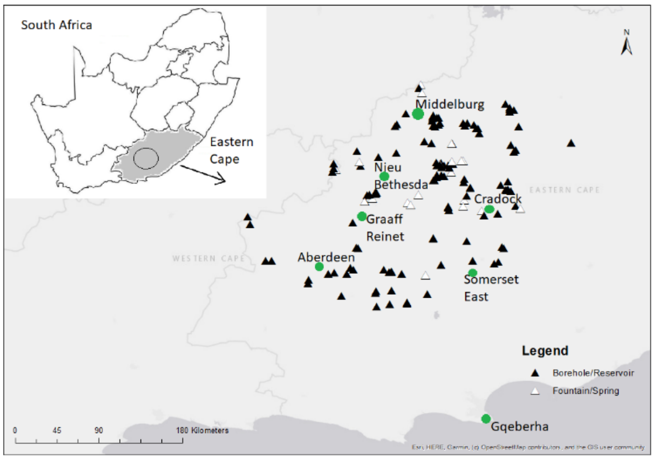

Plate 1: Typical example of a windmill that pumps the underground water into the associated reservoir for storage commonly found in the Karoo.

2.1 Field Work

Fieldwork was carried out between September 2015 and December 2017 as part of a long-term monitoring programme across the Eastern Karoo. The objective was to sample each site three times over an 18-month period, aiming to capture both seasonal and inter-annual variability in diatom assemblages and water quality. While this temporal design intended to include one sample per year and one from the opposing season, logistical constraints (such as site inaccessibility or equipment loss) occasionally prevented full adherence to this schedule. At each site, diatom and water samples were collected simultaneously to ensure comparability between biotic and abiotic data. Sites were categorised into two primary freshwater system types: reservoirs (man-made above-ground storage fed by groundwater) and springs (natural groundwater-fed surface flows).

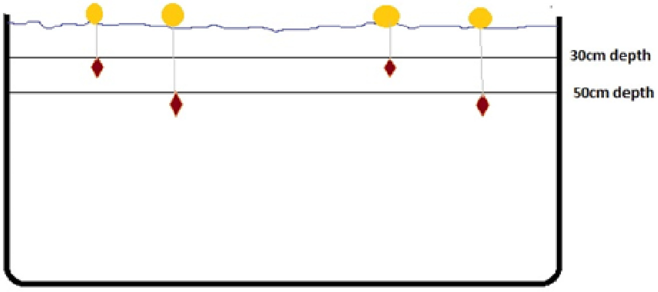

Figure 2: The positioning of tiles using floatation devices within the reservoirs.

2.1.1 Diatom sampling - reservoirs

Reservoirs in the Karoo are typically fed by underground water, extracted via windmill or solar pump systems and stored in above-ground tanks (Plate 1). Water turnover rates within these reservoirs varied depending on local use patterns, affecting nutrient and substrate stability.

Initial diatom samples were taken from the submerged surfaces of the reservoir walls or floors (commonly concrete).

To standardise substrate colonisation and allow for community succession studies, four unglazed ceramic tiles (15 × 15 cm) were deployed at each site, attached to plastic flotation devices to ensure consistent submersion and positioning within the water column (Figure 2). These tiles were left in place for at least six months to allow for adequate biofilm development.

Follow-up samples, where possible, were taken from both the original reservoir substrate and the colonised tiles (Plate 2). Additional samples were also collected from any biofilm-covered aquatic vegetation or the plastic flotation apparatus itself. These were used for comparative analysis of diatom communities on different substrates. However, sample recovery from tiles was not always feasible, primarily due to disturbance or removal by wildlife such as monkeys and baboons.

Plate 2: An example ceramic tiles with biofilm (top) and floatation devices (ball valves, bottom) after six months in the reservoirs in the Karoo.

2.1.2 Diatom sampling – springs

The sampled springs, all groundwater-fed, are hydrologically dependent on seasonal and interannual rainfall for recharge (Owen et al., 2004). In the Karoo, these springs typically emerge as shallow pools (1–30 cm depth) and often form isolated microhabitats (Plate 3).

Sampling at spring sites focused on riffle zones, where flow over substrates was sufficient to support benthic diatom colonisation. From each site, five cobble- or pebble-sized rocks were selected. Each rock was gently rinsed to remove inorganic sediment and then scrubbed using a soft-bristled toothbrush to collect the epilithic biofilm, following Taylor et al. (2007b). Additionally, macrophytes and filamentous algae bearing visible biofilm were sampled where present to assess species composition across substrate types.

Plate 3: Example of a spring site in the Eastern Karoo sampled during the period 2015 to 2017.

2.2 Diatoms - Processing and Analysis

Upon collection, all diatom samples were kept cool during transport to minimise cellular degradation. Within 24 hours of sampling, a subsample was examined using light microscopy to assess the proportion of live cells based on chloroplast visibility (Taylor et al., 2007b).

Samples were then subjected to oxidative cleaning using the hot potassium permanganate–hydrochloric acid ($KMnO_4$–HCl) method as described by (Von Stosch, 1974). This procedure removed organic matter, leaving clean frustules for identification. Processed material was mounted onto permanent slides using Pleurax mounting medium, in accordance with protocols detailed in (Taylor et al., 2007b).

Photomicrographs were taken using either a Nokia E100 microscope fitted with a Nikon 100× 1.25 phase contrast objective and a 1/2.5-Inch 5Mp CMOS Digital Image Sensor (camera) with images captured using the software IC Measure (The Imaging Software Company). Alternately, photomicrographs were taken using an Olympus BX51 microscope fitted with Olympus 100×/1.30 N Differential Interference Contrast objective. Photographs and measurements of cells were taken using the analySIS Image processing software.

Each sample count was 400 valves per slide with each species assigned the four-letter code from Omnidia v. 6 (Lecointe et al., 1993; 1999; 2016). For species that could only be identified to genus level, a unique code was assigned to each species group. For cells that could not be identified to a genus level, the code ZZZZ was assigned. Within the ZZZZ group each 'taxa' grouping was assigned the same code (e.g., ZZZZ1, ZZZZ2, ZZZZ3 etc). Deformed valves of each species were recorded and were allocated the code DEFO. The number of deformed cells was then added to the species code once the total of 400 cells had been counted in a sample allowing for the calculation of the percentage of deformed cells within a sample (Taylor et al., 2007b). If more than two-thirds of a broken valve was present and an identification to species level could be made, it was included in the count. Frustules in girdle view were counted if they could be identified to species level.

2.3 Water Chemistry

Physical water measurements (electrical conductivity, pH and temperature) were taken *in situ*, using a Hanna HI98129 meter. Dissolved oxygen of the water was measured *in situ*, using a Hanna HI98193 DO/BOD/OUR/SOUR meter.

Fourty ions (Table 1) and water alkalinity was measured in water samples. InnoVenton calculated CO3 and HCO3 alkalinity values after measuring P-alkalinity and T-alkalinity. Anion analysis was conducted at InnoVenton Laboratories, Gqeberha using a Metrohm IC 761 Compact (with external 5-point calibration) after which they were preserved with nitric acid to allow the samples to be transported to Sci-Ba Laboratory in Cape Town. Sci-Ba analysed the cations and metals using the Inductively Coupled Plasma Mass Spectrometer method (ICP-MS) with a detection standard. Metals and radioactive elements were analysed using a Perkin-Elmer Elan 6100 q-ICP-MS.

Table 1: Water Quality Variables Measured In Karoo Water Samples Taken During 2015 To 2017. Radioactive Elements Are In Bold.

Physical parameters	Electrical conductivity, pH, temperature, dissolved oxygen, CO_3 Alkalinity, HCO_3 Alkalinity, P Alkalinity, T Alkalinity
Anions	Cl^-, F^-, NO_2^-, NO_3^-, PO_4^-, SO_4^{2-}
Cations, Metals and radioactive elements (marked in bold)	Al^{3+}, As, B, Ba^{2+}, Be^{2+}, Ca^+, **Cd^{2+}**, **Ce^{3+}**, Co^{1+}, **Cr^+**, Cu^+, Fe^+, Hg^+, K^+, Li^+, Mg^{2+}, Mn^+, Mo^+, Na^+, Ni^{2+}, Pb^{2+}, **Rb^+**, **Rh^{3+}**, **Sb^+**, **Sc^{3+}**, Se, Si^{4+}, Sr^{2+}, **Th^{4+}**, Ti^+, **U^+**, **V**, **Y^{3+}**, Zn^{2+}

2.4 Statistical Analysis

A Shapiro Wilks test (Statistica 13.3; TIBCO Software, 2022) was used to analyse for normality in the water chemistry data. The data was non-normal and log transformed (with the exception of pH which is a log function of the H^+ ion) for further analysis using ordination (Bate et al, 2002; Taylor, 2004). For those chemistry results that were reported as less than, or greater than a threshold, to ensure a usable result a less than value was recorded as half the threshold while if the value was more than the threshold, 10% was added to the threshold value. The detection limit used by InnoVenton resulted in many nitrite (0.05 mg/L for NO_2^-) and phosphate (1 mg/L for PO_4^{3-}) levels being reported below the detection limit. The minimum detection limit for these two variables were divided by 2 and the nitrite (NO_2^-) results were combined with nitrate (NO_3^-) and reported as oxidized nitrogen.

Spearman's rank correlation coefficients were calculated using TIBCO Statistica. This was performed to explore potential between raw water chemistry variables and diatom indices or species This non-parametric method was chosen due to its suitability for non-normally distributed data and the ordinal nature of some biological indices.

A significance threshold of $p < 0.05$ was applied in all tests. While correlation does not imply causation, it serves as an effective tool to highlight potential relationships between environmental variables and biological responses that may warrant further investigation. To enhance analytical robustness and avoid skewing results due to infrequent taxa, only diatom species with a relative abundance greater than 1% within any given sample were included in the correlation analyses. This exclusion of rare species reduced statistical noise and focused the analysis on ecologically relevant taxa.

Although the autecological information found is based on diatoms in lakes and mild brackish waters in Europe, they were used as guidelines in the analysis in (Van Dam et al., 1994). Environmental preferences for a particular species do not always correlate around the world but the information provided was used for comparison(Gell et al., 2002 in Australia; Potapova and Charles, 2005 in the USA, Mirzahasanlou et al., 2018 in Iran, and in South Africa Bate et al., 2002; Matlala, 2010; Holmes and Taylor, 2015, Holmes, 2022; Gell et al., 2002 in Australia; Potapova and Charles, 2005 in the USA, Mirzahasanlou et al., 2018 in Iran, and in South Africa Bate et al., 2002; Matlala, 2010; Holmes and Taylor, 2015).

To explore the influence of environmental variables on diatom assemblage composition across the Karoo study sites, a two-step constrained ordination approach was applied using Canoco 4.5 (ter Braak and Šmilauer, 2002). Firstly, a Redundancy Analysis (RDA) with forward selection was performed to identify the subset of water chemistry variables that best explained variation in the diatom community data. This step was used to reduce the dimensionality of the full environmental dataset and eliminate collinear or non-informative variables.

To assess and manage multicollinearity among the predictor variables, Variance Inflation Factors (VIFs) were calculated. Variables with VIF values exceeding 10 were considered collinear and excluded from further analysis. Maintaining low VIF values ensured that the final set of environmental variables included in the ordination model contributed independent and interpretable information. Monte Carlo Permutation tests (499 permutations) had a significance level of 0.05.

Once colinear water chemistry variables were removed, a CCA was performed to determine the measured environmental variables with the greatest impact on the diatom distribution within the Karoo study area. A comparison was made with the results from Holmes et al. (2023) in which only a portion (n = 290) of this dataset (n = 510) was analysed. The environmental variables, reduced to 33, together with the diatom species (with a relative abundance >1% to remove any influence of rare species, n = 322) were analysed.

3. Results

3.1 Water Chemistry

As previously found (Holmes et al., 2023), Karoo water is generally alkaline (pH 6.1 to 9.9) with a wide range of values within the measured chemistry variables (Table 2). For example, the lowest electrical conductivity for the combined dataset was 158 µS/cm (spring water) while the highest value was 8400 µS/cm (borehole water). The latter site (borehole and reservoir) also had the maximum concentrations for chloride of 2176 mg/L, sulphate (1556 mg/L), boron (888 µg/L) and titanium (330 µg/L). Although not the highest concentrations recorded in this project, strontium (554 µg/L) and uranium (35.7 µg/L) levels were towards the higher end at this borehole and reservoir. Two borehole sites recorded the highest concentration for iron (1760 µg/L) and calcium (1517 mg/L) respectively. These three sites are in the same geographical area as the afore mentioned site. The highest maximum concentration for oxidized nitrogen (1620 mg/L) was measured in borehole water but only once in the three interval samples. Temporal variation was observed among several variables in the surface water sample sites, including changes in electrical conductivity exceeding 200 µS/cm and fluctuations in macronutrients such as calcium, magnesium, oxidized nitrogen, and silica in one site. These findings confirm that water chemistry is highly variable over time.

Table 2: Descriptive statistics for all the water chemistry variables measured in the diatom Karoo Shale Gas Project during the period 2015 to 2017. Radioactive elements in bold.

Variable	Valid n	Median	Minimum	Maximum	Std. Dev.	Range
Physical variables						
Reservoir - pH	508	7.9	6.1	9.9	0.6	3.8
Reservoir - Electrical conductivity (EC) µS/cm	508	769	158	8400	1072	8242
Reservoir - Temperature °C	505	18.9	8.0	31.0	4.0	23.0
Dissolved oxygen (DO) mg/L	481	7.9	0.2	30.5	4.2	30.3
CO_3 Alkalinity (calculated)	494	28.6	0.0	244.5	32.9	244.5

Table 2(Cont.): Descriptive statistics for all the water chemistry variables measured in the diatom Karoo Shale Gas Project during the period 2015 to 2017. Radioactive elements in bold.

HCO_3 Alkalinity (calculated)	494	166.5	-108.9	873.1	103.7	981.9
P - Alkalinity as mg $CaCO_3$/L	494	27.1	0.0	254.6	27.7	254.6
T - Alkalinity as mg $CaCO_3$/L	496	390.6	59.0	1997.6	179.7	1938.6
Nutrients and macro elements in mg/L						
Calcium (Ca^{2+})	471	61.9	0.0	1517.0	97.8	1517.0
Chloride (Cl^-)	492	47.3	4.1	2175.8	267.7	2171.7
Fluoride (F^-)	492	0.6	0.0	11.1	1.2	11.1
Magnesium (Mg^{2+})	471	31.2	0.0	88.0	21.7	88.0
Oxidized nitrogen (NOx^-)	494	7.0	0.0	1619.6	73.5	1619.6
Phosphate (PO_4^{3-})	492	0.5	0.0	5.0	2.0	5.0
Potassium (K^+)	471	1.7	0.0	64.2	4.5	64.2
Silica (Si^{4+})	471	9.8	0.0	88.0	10.0	88.0
Sodium (Na^+)	471	50.8	0.0	88.0	28.1	88.0
Sulphate (SO_4^{2-})	490	45.8	0.5	1555.7	186.2	1555.2
Trace elements in µg/L						
Aluminium (Al^{3+})	471	0.2	0.0	142.0	14.8	142.0
Antimony (Sb^{3-})	471	0.1	0.0	35.3	2.3	35.3
Arsenic (As^{3+})	471	2.1	0.0	69.8	6.8	69.8
Boron (B)	471	176.0	18.8	880.0	184.5	861.2
Barium (Ba^{2+})	471	85.0	13.0	176.0	40.4	163.0
Beryllium (Be^{2+})	471	0.2	0.0	5.3	0.3	5.2
Cadmium (Cd^{2+})	471	0.0	0.0	8.1	0.5	8.0
Cerium (Ce^{3+})	471	3.3	1.1	21.7	1.6	20.6
Cobalt (Co^-)	471	0.0	0.0	5.4	0.4	5.4
Chromium (Cr^+)	471	0.0	0.0	32.6	2.8	32.6
Copper (Cu^{2+})	471	1.1	0.0	110.0	12.7	110.0
Iron (Fe^{2+})	471	247.0	1.4	1760.0	322.9	1758.6
Lead (Pb^{2+})	471	0.0	0.0	8.9	0.8	8.8
Lithium (Li^+)	471	17.2	0.0	514.0	61.1	514.0
Manganese (Mn^{2+})	471	4.2	0.0	589.0	60.5	589.0
Mercury (Hg^{2+})	471	0.2	0.1	3.4	0.7	3.3
Molybdenum (Mo^{2+})	471	1.0	0.1	51.5	7.3	51.4
Nickel (Ni^{2+})	471	0.8	0.0	8.3	1.1	8.3
Rhodium (Rh^{3+})	471	0.0	0.0	38.5	18.5	38.5
Rubidium (Rb^+)	471	0.0	0.0	15.4	1.1	15.3
Scandium (Sc^{3+})	471	14.1	1.6	34.0	7.6	32.5
Selenium (Se^{2-})	471	1.9	0.1	37.2	4.2	37.1
Strontium (Sr^{2+})	471	33.3	0.0	578.0	103.7	578.0
Thorium (Th^{4+})	471	6.2	6.2	24.5	1.2	18.3
Titanium (Ti^{4+})	471	66.7	0.0	330.0	53.5	330.0
Uranium (U^{2+})	471	3.8	0.0	49.6	6.9	49.6
Vanadium (V^{2+})	471	6.7	0.0	96.7	13.4	96.7
Yttrium (Y^{3+})	471	2.1	1.9	14.6	0.8	12.7
Zinc (Zn^{2+})	471	3.3	0.0	110.0	10.8	110.0

As expected, temperature had a large range (23 °C) due to the climatic extremes in the Karoo (personal observation). Dissolved oxygen levels (min 0.2 to max 30.5 mg/L) were dependent, among other factors, on the water turnover rate in the reservoirs as well as the presence of other aquatic plants in the reservoirs.

3.2 Diatoms

A total of 495 taxa were recorded in 510 samples. A total of 466 cells could not be identified to genus level and were allocated to ZZZZ. The overall percentage of deformed cells was 1.2%. Taxa considered common were those contributing over 5% within a sample. A total of 116 species met this criterion with three species scoring the highest percentage over all the samples, namely *Achnanthidium minutissimum* (17.9%), *Denticula kuetzingii* (10.8%) and *Encyonopsis krammeri* (6.2%). The genus with the highest proportion of diatom cells was *Encyonopsis* (18.3%) followed by *Achnanthidium* (17%).

3.2.1 Diatoms – correlations

The diatom indices, used as a proxy for environmental conditions were the Biological Diatom Index (BDI, uses 14 water chemistry parameters in calculation including biological oxygen demand), Indice polluosensibilité spécifique (SPI, measures organic pollution, salinity and eutrophication), The Generic Diatom Index (GDI) and the Trophic Diatom Index Pollution Tolerant (TDI %PTv, which uses tolerance to nutrients, mainly phosphates and nitrates) show most correlations below $r_s < 0.2$ (positive or negative) over the full dataset. Three of the four indices (SPI, GDI, and TDI %PTv) show significant correlations over the whole dataset, with BDI being the exception (Table 3). Both the SPI and GDI correlate negatively with T-Alkalinity (r_s = -0.24 and -0.22, respectively) and magnesium (r_s = -0.24 and -0.25, respectively; Table 3).

Table 3: Spearman Rank Order Correlations (r_s) between selected diatom indices and water chemistry variables. Radioactive variables are in bold. Only those with $r_s \geq 0.2$ (positive or negative) are included.

Variable	BDI 2014	SPI	GDI	TDI %PT
T-Alkalinity		-0.24	-0.22	
Magnesium (Mg^{2+})		-0.24	-0.25	
Sulphate (SO_4^{2-})		-0.21		
Lithium (Li^+)				-0.25
Uranium (U^{2+})		-0.20		
Vanadium (V^{2+})				0.20

The correlation analysis yielded relationships between 291 species and environmental variables (Table 4) with most of them being very low (r_s < 0.2). Only eight species had correlations with a r_s > 0.3 (positive or negative, Table 4). *Amphora* sp0, previously misidentified as *Halamphora coffeaeformis* (in Holmes et al., 2023) once again has several correlations (positive, Table 4). Correlations confirm that *Amphora* sp0, *Seminavis pusilla* and *Diploneis* sp0 (previously misidentified as *D. elliptica*) have a positive association with electrical conductivity (r_s = 0.46; 0.45 and 0.47 respectively) and chloride (r_s = 0.41; 0.42 and 0.42 respectively, Table 4). *Epithemia gibba*, which has an endosymbiont for nitrogen fixation, showed a negative correlation with *oxidized nitrogen* (r_s = -0.41, Table 4)

Table 4: Spearman Rank Order correlation coefficients (r_s) for diatom species abundance (those >1 % in a sample) and water chemistry variables. The threshold *p* was taken to be <= 0.05. Only significant correlations (p < 0.05) where r_s > 0.3 (positive or negative) are shown with r_s > 0.4 (positive or negative) in bold. Variables with no significant correlations omitted. Radioactive elements in bold and italics.

Variable	*Adlafia bryophila*	*Amphora* sp0	*Stephanocyclus meneghiniana*	*Denticula kuetzingii*	*Diploneis* sp0	*Epithemia adnata*	*Navicymbula pusilla*
Physical variables							
Electrical conductivity (EC)		**0.44**	0.34				**0.43**
Nutrients and macro elements							
Calcium (Ca^{2+})		0.36			**0.40**		
Chloride (Cl^-)		0.36			0.36		0.37
Flouride (F^-)							0.30
Oxidized nitrogen (NOx^-)				**0.40**		-0.33	
Sodium (Na^+)		0.30			0.30		0.35
Sulphate (SO_4^{2-})	-0.30	0.38	0.30				0.39
Trace elements							
Boron (B)		0.36			0.32		0.37
Cobalt (Co^-)					0.31		
Lithium (Li^+)		0.37			0.38		0.39
Selenium (Se^{2-})		0.34					0.33
Strontium (Sr^{2+})		**0.43**					0.38
Uranium (U^{2+})		0.32					

3.2.2 Diatoms - Multivariate Analysis

A Canonical Correspondence Analysis of the diatom species-environmental relationship explained by the first two axes of 12.7% and 21.9% (Table 5). VIFs that were above ten were boron (14.1), barium (14, 6), cadmium (30, 5), antimony (11.7) and thorium (17.6) and p = 0.002. This result suggests that collinearity may exist with these environmental variables and care should be taken with any inference using them.

Table 5: Summary of the Monte Carlo permutations (n = 499) in the CCA of diatom species in the Karoo (2015 - 2017) with a relative abundance > 1% in at least one sample and reduced water chemistry variables. The significance value was set at p <0.05.

Axes	1	2	Total inertia
Eigenvalues	0.41	0.30	26.03
Species-environment correlations:	0.80	0.76	
Cumulative percentage variance			
of species data	1.6	2.7	
of species-environment relation	12.7	21.9	
Sum of all eigenvalues			26.03
Sum of all canonical eigenvalues			3.22

Most of the species are found at median levels of the measured water quality variables (Fig. 3).

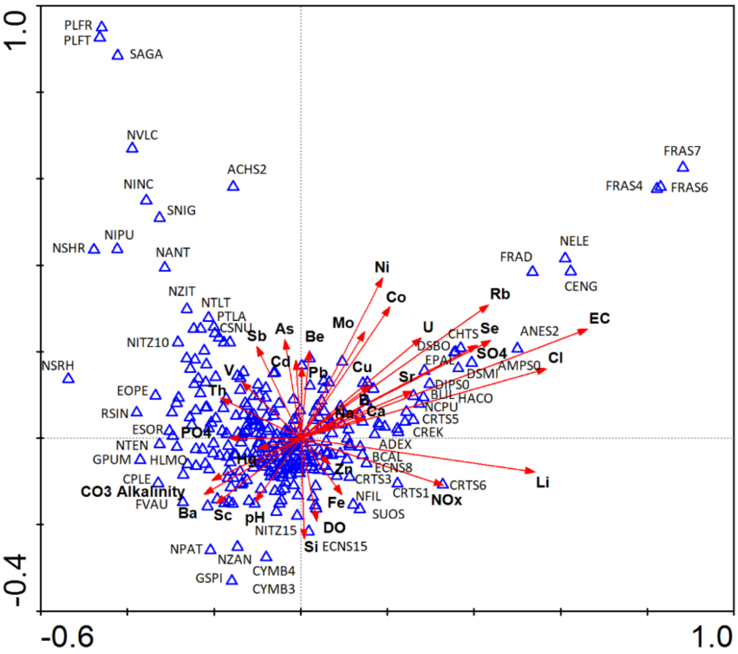

Figure 3: Canonical Correspondence Analysis of the diatom species (relative abundance of >1% in at least one sample) and water chemistry variables (n = 33). The triangles indicate species, and the arrows indicate water chemistry variables. Species codes follow those used in Omnidia.

Species that are less tolerant to higher concentrations of sulphate, selenium, oxidized nitrogen, lithium and chloride but more tolerant of alkaline conditions, include *Gomphonema spiculoides* (GSPI), *Nitzschia palea* var. *tenuirostris* (NPAT), *Navicula zanoni* (NZAN) and two species of *Cymbella* (bottom left quadrant, Fig. 3).

Many species, including *Nitzschia* (*N. pusilla* – NIPU; *N. valdecostata* - NVLC; *N. inconspicua* – NINC;) and *Navicula* (*N. schroeteri* – NSHR; *N. antonii* – NANT; *Navicula subrhynchocephala* - NSRH) as well as *Stephanodiscus agassizensis* (SAGA), *Planothidium frequentissimum* (PLFR and PLFT), *Sellaphora nigri* (SNIG) and an *Achnanthidium* species tolerate higher levels of rarely tested for metals while exhibiting a lower tolerance for oxidized nitrogen and lithium (Fig. 3, top left quadrant).

Species preferring the environmental gradient of higher concentrations of dissolved oxygen, silica, iron, lithium, and oxidized nitrogen, and lower levels of phosphate, arsenic, and antimony include species of *Nitzschia* (NITZ15; *Nitzschia filiformis* – NFIL), *Encyonopsis* (ECNS8; ECNS13; ECNS15), and *Craticula* (CRTS1; CRTS3; CRTS6) species. (Fig. 3, bottom right quadrant).

The species displaying a negative relationship with silica but tolerant of elevated levels of electrical conductivity, chloride, sulphate, and selenium include three species from the *Fragilaria* genus (FRAS4, FRAS6, FRAS7, Fig. 3) are well-adapted to highly mineralized waters, though their Spearman rank correlations with these variables were low ($r_s < 0.2$). The other species exhibiting these environmental preferences were *Cocconeis engelbrechtii* (CENG), *Nitzschia elegantula* (NELE), *Fragilaria gracilis* (FGRA), *Amphora* sp. 0 (AMPS0), *Entomoneis paludosa* (EPAL), *Aneumastus* sp. 2 (ANES2), *Chaetoceros* (CHTS) and *Diploneis* species (DIPS0, *Diploneis subovalis* – DSBO; *Diploneis smithii* – DSMI) (Fig. 3, top right quadrant). The correlations between *Amphora* sp. 0 and sulphate (r_s = 0.35), chloride (r_s = 0.41), electrical conductivity (r_s = 0.46), selenium (r_s = 0.36) and lithium (r_s = 0.38) (Table 4) are confirmed in Figure 3.

The high number of species in Figure 3 complicates the interpretation of environmental preferences for several abundant species. To clarify these influences, non-target species were removed from the biplot in Figure 4. This reduction in species in the CCA output provides a clearer view of the preferred ecological conditions for the selected indicator species (Fig. 4).

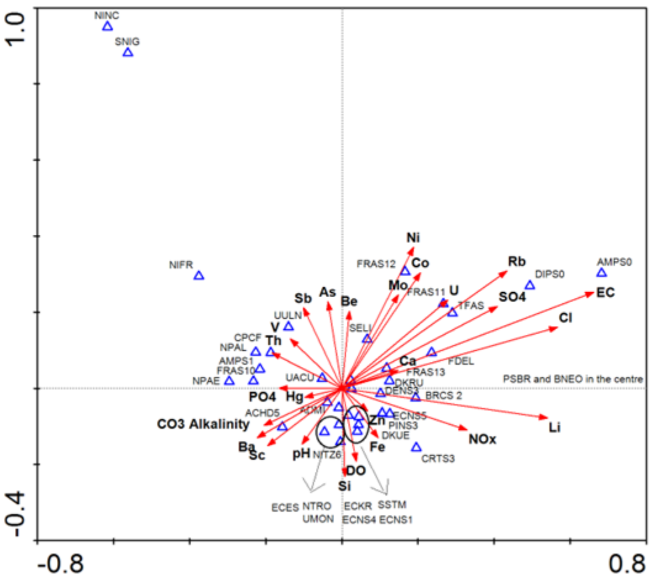

Figure 4: Canonical Correspondence Analysis of selected diatom species and water chemistry variables. The triangles indicate species, and the arrows indicate water chemistry variables. Species codes follow those used in Omnidia.

Taxa within the genera *Brachysira*, *Craticula*, *Fragilaria* and *Encyonopsis* were distinguished based on subtle morphological variations. The ordination of these morphotypes reveals distinct environmental preferences among them (Fig. 4). The species *Brachysira* sp. 2 (BRCS2) is similar in morphology to *B. microcephla*. This taxon showed tolerance for higher levels of lithium when compared to *Brachysira* aff *neoexilis* (BNEO). The distinct placement of *Fragilaria* taxa (FRAS10, FRAS11, FRAS12, FRAS13) suggests that these groupings represent separate taxa (Fig. 4). *Achnanthidium minutissimum* (ADMI) and *Ulnaria acus* (UACU) are positioned around the median levels of the environmental variables (Fig. 4). The *Ulnaria monodii* (UMON) complex, positioned in Figure 4 may not fully reflect its ecological preferences.

4. Discussion

4.1 Water Chemistry

The combined water chemistry dataset from the Karoo illustrates the region's characteristically variable and often highly mineralised nature, particularly underground water (Table 2). These waters are mostly alkaline (pH 6.1 to 9.9) with surface water less mineralised (lower electrical conductivity) than the subsurface water. Notably, groundwater chemistry exhibited considerable spatial and temporal variability, even within individual sites. Variables responsible for eutrophication are nitrogen and phosphate (Dallas and Day, 2004; Dalu & Froneman, 2016) and are particularly concerning. Oxidized nitrogen has a very high degree of variability reaching 1620 mg/L in a borehole in the Jansenville district (site KB001) at the first sampling (October 2015). This site is not near any irrigated pastures and is 0.5 km from an ephemeral river (Sundays River) and 4.9 km from the town. There was no significant rainfall event in this area during the sampling period. One year later, the oxidized nitrogen was sample value was 0.5 mg/L and 18 months after the first sample it was at 3.9 mg/L. This suggests the possibility of contamination of the aquifer from other sources. This example highlights the limited understanding of subsurface water flow, which is known to be highly variable. Without established baselines and long-term biomonitoring, it is challenging to assign responsibility for contamination accurately (Leonard-Roche, 2015; Pietersen et al., 2016).

4.2 Diatoms

A total of 495 diatom taxa were recorded, with an additional 48 taxa groupings included from ZZZZ. While there were many taxa known to be cosmopolitan, there were several that could not be identified to species level and may be new to science. Surprisingly, statistical correlations between diatom indices and individual water chemistry parameters were generally low across the full dataset. This may be due to the complex interplay of multiple water chemistry variables, particularly the wide variation in ionic composition across the Karoo.

The CCA (Fig. 3) illustrates the main environmental drivers are electrical conductivity (EC), chloride, rubidium and lithium, with oxidized nitrogen and sulphate having a reduced influence. Interestingly, selenium and nickel, elements not previously highlighted as influential in this region (Holmes et al., 2023), also contributed to species differentiation (Fig. 3).

The most commonly occurring species in the Karoo project was the pioneer species *Achnanthidium minutissimum* (ADMI, Dalu et al., 2014) and was found in 273 samples and dominant (RA > 5%) in 272 samples. This species was found, overall, to tolerate at most the median levels of all the variables (Figs. 3 & 4) and had significant negative correlations with EC, chloride and sulphate ($r_s = -0.23$, $r_s = -0.25$ and $r_s = -0.22$ respectively). Its widespread tolerance may reflect a preference for disturbed environments or early successional biofilms (Ács et al., 2004; Martin & Fernandez, 2012), particularly relevant given the dynamic hydrology and seasonal fluctuations in Karoo reservoirs.

Denticula kuetzingii (DKUE), typically associated with clean, moderately mineralised water and often from carbonate substrates (Taylor et al., 2007a; Matlala et al., 2005; Lange Bertalot et al., 2017), showed a strong positive correlation with oxidised nitrogen ($r_s = 0.37$), and moderate associations with calcium, lithium, and chloride (Fig. 4). These findings align with reports from semi-arid Mediterranean systems (Ros et al., 2009), suggesting this species may be more ecologically flexible than previously thought.

Encyonopsis krammeri (ECKR), generally considered an indicator of low-nutrient, slightly acidic water, demonstrated tolerance for alkaline, mineral-rich conditions in the Karoo. The species showed significant positive correlations with oxidised nitrogen, iron, dissolved oxygen, and silica, and appeared to favour higher pH values (Fig. 4). These traits suggest adaptation to region-specific conditions and support observations by Lange-Bertalot et al. (2017) regarding carbonate-rich preferences.

Fragilaria gracilis (FGRA) preferred highly mineralised conditions in this study (Fig. 4), deviating from literature that suggests moderate electrolyte preferences (Bey & Ector, 2013). This discrepancy may indicate the presence of morphologically similar but ecologically distinct taxa. Several unidentified Fragilaria morphotypes (e.g., FRAS4, FRAS6, FRAS7) also exhibited unique environmental preferences, highlighting the need for further taxonomic resolution.

The *Ulnaria* species grouped within the *Ulnaria monodii* complex in this study ordinated towards the median range of environmental conditions showing a slight preference for more mineralised conditions (lithium, oxidized nitrogen, iron, Fig. 4) and higher oxygen levels. However, this ecological niche is atypical for *U. monodii*. In this study, several morphological variations (including size differences to type, were identified that could be placed in several species (*Ulnaria monodii* / *Ulnaria biceps* / *Ulnaria acuscypriacus*) (Cantonati et al., 2018). Given that these species are understudied in South Africa, they are included here in one complex. In South Africa these species are often mistakenly placed within the *Ulnaria biceps* taxon (Cantonati et al., 2018). Another group of species that requires investigation to establish correct identification in South Africa are taxa form the genera *Pseudostaurosira* and *Staurosira*. Genera that are morphologically similar under light microscopty are *Nanofrustulum* (brackish and freshwater, Morales et al. 2023) and the small celled species of *Staurosirella* (Beauger et al., 2023) also need detailed examination under light microscopy and SEM.

A potential limitation of the reported chemical analysis was that each ion was analysed independently. This does not account for the specific species or forms in which these ions may exist *in situ*. This leaves some uncertainty regarding their actual ecological effects, as different ionic forms could interact differently with the environment. The fact that both lithium and chloride had a significant influence on diatom communities suggest the possibility that they were present in the water as lithium chloride (LiCl), known to be found in saline environments (Cubillos et al., 2018). Lithium correlated positively with chloride ($r_s = 0.57$). The same applies to the toxicity (dependent on the form in which it is found and pH) of chloride and selenium (Läuchli, 1993; Holmes, 2022). Selenium, which shares similar chemical properties to sulphur (Läuchli, 1993), is thought to enter the cell using the sulphate transporters (Kumar et al., 2022). While required for algal growth, marine diatoms did not exhibit negative effects until exposed to high concentrations of selenate or selenite (Läuchli, 1993).

While generally considered non-toxic little is known about the biological role of scandium (Horovitz, 2000; Iain and Chassé, 2016). However, it has been found to negatively impact the uptake of essential nutrients in wheat seedlings (Shtangeeva, Ayrault and Jain, 2004). The study reported that scandium not only mimics but also interferes with the metabolism of nutrients such as calcium and magnesium (Horovitz, 2000).

The mechanism by which lithium enters into the diatom cell is not understood and remains to be elucidated. In brown algae the adsorption of lithium reduced the uptake of magnesium into the cell. This process involves the carboxylic acid groups of alginates and the phosphonate groups of the plasma lipid membrane (Park et al., 2019). A positive correlation between lithium and magnesium ($r_s = 0.23$) was observed in this study.

These findings highlight the complexity of water chemistry within ecological systems. For example, a species preferring low phosphate levels in South Africa may, in fact, be more tolerant of higher levels of nitrogen or to the toxicity of rarely measured variables such as lithium. Chloride has long been used to distinguish diatom preferences (fresh, brackish, salt water; Patrick, 1948). Elevated chloride levels can damage cell pigments (chlorophyll *a*, carotenoids), inhibit photosynthesis and respiration induce toxin production in the cell and affect diatom cell size and mobility (Mitra et al., 2012; DWAF, 1996; Vinitha et al., 2010; Lelong et al., 2012). Diatom species can adapt to changes in salinity, with some being more permanent (evolutionary change; Orr, 2005) while others are temporary (osmo-acclimatisation), but responses are species specific (Clavero et al. 2000; Stenger-Kovács et al., 2023). Given the wide range of chloride concentrations (2171.7 mg/L) measured in this study it is, therefore, not surprising that this variable would have a considerable influence on diatom species distribution in the Karoo. It is important to note that several of the taxa tolerating the higher chloride concentrations were not identified to species. *Encyonopsis cesatii*, typically found in poorly mineralised calcareous bicarbonate rich water was found to prefer slightly higher than median levels of alkalinity (median 28.6, range 244.5), pH and silica (median 9.8 mg/L, range 88 mg/L; Fig. 4) (Bahls, 2013; Bey and Ector 2013; Lange Bertalot et al., 2017). Although *E. cesatii* ordinated on the lower levels of calcium (Fig. 4), it must be considered that the median levels were 61.90 mg/L with a range of 1517 mg/L.

5. Conclusions And Recommendations

This study provides the most comprehensive dataset to date on diatom biodiversity and water chemistry in the Karoo, offering an essential baseline for future ecological monitoring in the region. The identification of 495 diatom taxa, many with poorly understood or novel environmental tolerances, highlights both the richness and complexity of the region's aquatic ecosystems. The inclusion of rarely measured elements such as lithium, selenium, scandium, and rubidium add considerable value, allowing for a nuanced assessment of chemical influences on diatom assemblages. These variables, often overlooked in routine water quality assessments, were shown to exert significant effects on diatom distribution and community structure.

Continued biomonitoring in the Karoo could yield more, as yet unidentified species and identifying them accurately will be important for future studies. As with any new species, the possibility exists that they may be beneficial for other applications (biofuel, medicinal etc). Continued monitoring would also allow for rapid identification of allochthonous species which could indicate negative changes (as with any invasive species) within the aquatic system. As selenium can be released into the environment through the mining of metal ore (sulphur containing) deposits, it may be useful to include this in future water analyses in those industries.

Future monitoring should prioritise:

- Establishing long-term baselines for both water chemistry and diatom assemblages to better track changes and assign potential sources of contamination.
- Refining species-level identifications, especially within problematic genera (e.g., *Ulnaria, Fragilaria, Pseudostaurosira, Achnanthidium, Brachysira, Craticula*), through detailed morphological and molecular work.
- Incorporating this dataset into the South African Diatom Index to improve its geographic and chemical representativeness.
- Developing a national diatom database, accessible to researchers and practitioners, to collate species occurrences, trait data, and ecological preferences. This should include historic diatom occurrence information but would face huge challenges including the harmonization of identifications.
- Biomonitoring through DNA analysis using diatoms is its infancy in South Africa, primarily due to cost and technology access. By expanding DNA initiatives, using the species-environment relationships from this study to build reference libraries that support rapid, non-invasive biomonitoring.

Acknowledgements

Mr. R.C. Holmes is acknowledged for his financial and logistical support during these projects. Gratitude is extended to the landowners for granting unrestricted access to the study sites and to SANParks for permitting access to the Mountain Zebra National Park and Camdeboo National Park (Permit reference HOLM1332). The 2016/2017 project was funded by Iphakade (reference UID 9475) and was conducted as part of the AEON Karoo Shale Gas Project (KSGP) baseline study. The AEON publication number is 221. Special recognition is given to the late Prof. Maarten de Wit, whose leadership of the KSGP was instrumental in facilitating this research.

Data Statement

The raw chemistry data and the relative abundance data of the diatom species with their Omnidia species codes has been published on the Mendeley repository. DOI 10.17632/9fhrht6k6y.1

References

Archer, E., Du Toit, J., Engelbrecht, C., Hoffman, M. T., Landman, W., Malherbe, J., and Stern, M., 2022. The 2015-19 multi year drought in the Eastern Cape, South Africa: it's evolution and impacts on agriculture. Journal of Arid Environments, 196, Pp. 104630.

Bahls, L., 2013. Encyonopsis cesatii. In Diatoms of North America. Retrieved October 26, 2023, from https://diatoms.org/species/encyonopsis_cesatii .

Barinova, S., 2017. Essential and practical bioindication methods and systems for the water quality assessment. Int. J. Environ. Sci. Nat. Resour, 2(3), Pp. 0079-089. http:// dx.doi.org/10. 19080/IJESNR.2017.02.555588

Bate, G. C., Adams, J. B., and Van der Molen, J. S., 2002. Diatoms as indicators of water quality in South African river systems. Pretoria: Water Research Commission.

B-Béres, V., Stenger-Kovács, C., Buczkó, K., Padisák, J., Selmeczy, G. B., Lengyel, E., and Tapolczai, K., 2023. Ecosystem services provided by freshwater and marine diatoms. Hy drobiologia, 850(12), Pp. 2707-2733. https://doi.org/10.1007/s10750-022-04984-9

Beauger, A., Allain, E., Voldoire, O., Blavignac, C., Caillon, G., Vijver, B. V. D., and Wetzel, C. E., 2023. A new species of Staurosirella (Bacillariophyta) observed in a spring of the catchment of the Regional Natural Reserve of Jolan and Gazelle Peatlands, French Massif Central, France. Nova Hedwigia. https://doi.org/10.1127/nova_hedwigia/2023/0860

Bere, T., and Tundisi, J. G., 2010. Biological monitoring of lotic ecosystems: the role of diatoms. Brazilian Journal of Biology, 70, Pp.493-502. https://doi.org/10.1590/S1519-6984 2010005000009

Bey, M.Y., and Ector, L., 2013. Atlas des diatomées des cours d'eau de la région Rhône-Alpes. 1 Centriques, Monoraphidées; 2 Araphidées, Brachyraphidées; 3 Naviculacées, Naviculoidées; 4 Naviculacées, Naviculoidées; 5 Naviculacées Cymbelloidées, Gomphonématoidées; 6 Bacillariacées, Rhopalodiacées, Surirellacées; Direction régionale de l'Environnement, de l'Aménagement et du Logement Rhône-Alpes. https://www.auvergne-rhone-alpes.deve loppe ment-durable.gouv.fr/atlas-des-diatomees-a3480.html

Blanco, S., 2024. What do diatom indices indicate? Modeling the specific pollution sensitivity index. Environmental Science and Pollution Research, 31(20), Pp. 29449-29459.https://doi.org/10.1007/s11356-024-33115-1

Bornette, G., and Puijalon, S., 2011. Response of aquatic plants to abiotic factors: a review. Aquatic sciences, 73, Pp. 1-14. https://doi.org/10.1007/s00027-010-0162-7

Braune, E., and Xu, Y., 2008. Groundwater management issues in Southern Africa–An IWRM perspective. Water SA, 34(6), Pp. 699-706. On-line version ISSN 1816-7950

Cantonati, M., Armanini, D. G., Demartini, D., Papatheodoulou, A., Bilous, O. P., Colombo, F., Angeli, R.S., Dörflinger, G. and Manoylov, K. M., 2024. Has climate change over the last ten years caused a banalisation of diatom communities in Cypriot streams?. Science of the Total Environment, 947, Pp.174495. https://doi.org/10.1016/j.scitotenv.2024.174495

Cantonati, M., Bilous, O., Spitale, D., Angeli, N., Segadelli, S., Bernabè, D., Lichtenwöhrer, K., Gerecke, R., and Saber, A. A., 2022. Diatoms from the spring ecosystems selected for the long-term monitoring of climate-change effects in the Berchtesgaden National Park (Germany). Water, 14(3), Pp.381. https://doi.org/10.3390/w14030381

Cantonati, M., Lange-Bertalot, H., Kelly, M. G., and Angeli, N., 2018. Taxonomic and ecological characterization of two Ulnaria species (Bacillariophyta) from streams in Cyprus. Phytotaxa, 346(1), Pp. 78-92. https://doi.org/10.11646/phytotaxa.346.1.4

Clavero, E., Hernández-Mariné, M., Grimalt, J. O., and Garcia-Pichel, F., 2000. Salinity tolerance of diatoms from thalassic hypersaline environments. Journal of Phycology, 36(6), Pp. 1021-1034. https://doi.org/10.1046/j.1529-8817.2000.99177.x

Conradie, B., Piesse, J., and Stephens, J., 2019. The changing environment: Efficiency, vulnerability and changes in land use in the South African Karoo, 2012–2014. Environmental Development, 32, Pp.100453. https://doi.org/10.1016/j.envdev.2019.07.003

Costa, A. P. T., and Schneck, F., 2022. Diatoms as indicators in running waters: trends of studies on biological assessment and monitoring. Environmental Monitoring and Assessment, 194(10), 695. https://doi.org/10.1007/s10661-022-10383-3

Coste, M., Boutry, S., Tison-Rosebery, J., and Delmas, F., 2009. Improvements of the Biological Diatom Index (BDI): description and efficiency of the new version (BDI-2006). Ecological indicators, 9(4), Pp. 621-650. https://doi.org/10.1016/j.ecolind.2008.06.003

Cubillos, C. F., Aguilar, P., Grágeda, M., and Dorador, C., 2018. Microbial communities from the world's largest lithium reserve, Salar de Atacama, Chile: Life at high LiCl concentrations. Journal of Geophysical Research: Biogeosciences, 123(12), Pp. 3668-3681. https://doi. org/10. 1029/2018JG004621

Dallas, H. F., and Day, J. A., 2004. The effect of water quality variables on aquatic ecosystems: a review. WRC Report TT-244-04. Pretoria: Water Research Commission. https://www.wrc. org.za/wp-content/uploads/mdocs/TT-244-04.pdf

Dalu, T., and Froneman, P. W., 2016. Diatom-based water quality monitoring in southern Africa: challenges and future prospects. Water SA, 42(4), Pp.551-559. http://dx. doi.org/ 10. 4314/wsa.v42i4.05

Dalu, T., Froneman, P. W., and Bere, T., 2016. Assessment of water quality based on diatom indices in a small temperate river system, Kowie River, South Africa. Water SA, 42(2), Pp. 183-193. http://dx.doi.org/10.4314/wsa.v42i2.02

Dalu, T., Froneman, P. W., Chari, L. D., and Richoux, N. B., 2014. Colonisation and community structure of benthic diatoms on artificial substrates following a major flood event: A case of the Kowie River (Eastern Cape, South Africa). Water SA, 40(3), Pp. 471-480. https:// doi.org/10.4314/wsa.v40i3.10

DWAF, Department of Water Affairs and Forestry. 1996. South African water quality guidelines. Volume 7: aquatic ecosystems. Pretoria: DWAF. https://www. dws.gov.za /iwqs/ wq_guide/edited/Pol_saWQguideFRESH_vol7_Aquaticecosystems. pdf

Garg, A., Yadav, B. K., Das, D. B., and Wood, P. J., 2022. Improving the assessment of polluted sites using an integrated bio-physico-chemical monitoring framework. Chem osphere, 290, Pp. 133344. https://doi.org/10.1016/j.chemosphere.2021.133344

Gell, P. A., Sluiter, I. R., and Fluin, J., 2002. Seasonal and interannual variations in diatom assemblages in Murray River connected wetlands in north-west Victoria, Australia. Marine and freshwater research, 53(6), Pp. 981-992. https://doi.org/10.1071/MF01021

Geris, J., Comte, J. C., Franchi, F., Petros, A. K., Tirivarombo, S., Selepeng, A. T., and Villholth, K. G., 2022. Surface water-groundwater interactions and local land use control water quality impacts of extreme rainfall and flooding in a vulnerable semi-arid region of Sub-Saharan Africa. Journal of Hydrology, 609, 127834. https://doi.org/10.1016/j.jhydrol.2022.12 7834

Gholizadeh, M., 2021. Effects of floods on macroinvertebrate communities in the Zarin Gol River of northern Iran: implications for water quality monitoring and biological assessment. Ecological Processes, 10(1), Pp.46. https://doi.org/10.1186/s13717-021-00318-0

Gnjato, S., Narancic, B., Antoniades, D., Pienitz, R., Alibert, M., and Gnjato, O., 2025. Recent environmental changes in alpine Lake Kotlaničko as revealed by sedimentary diatom assemblages (Dinaric Alps, Bosnia and Herzegovina). https://doi.org/10.21203/rs.3.rs-6234543/v1

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: Report to the Water Research Commission. WRC Report No. 1707/1/11. Water Research Commission.

Harkness, J. S., Swana, K., Eymold, W. K., Miller, J., Murray, R., Talma, S., Whyte, C.J., Moore, M.T., Maletic, E.L., Vengosh, A., and Darrah., T.H., 2018. Pre-drill groundwater geochemistry in the Karoo Basin, South Africa. Groundwater, 56(2), Pp. 187-203. https:// doi.org/10.1111/gwat.12635

Hill, M.O., and Šmilauer, P., 2005. TWINSPAN for Windows Version 2.3. Centre for Ecology and Hydrology & University of South Bohemia, Huntingdon, UK and Ceske Budejovice.

Hobbs, P., Day, E., Rosewarne, P., Esterhuyse, S., Schulze, R., Day, J., Ewart-Smith, J., Kemp, M., Rivers-Moore, N., Coetzee, H., Hohne, D., Maherry, A., and Mosetsho, M., 2016. Water Resources. In Scholes, R., Lochner, P., Schreiner, G., Snyman-Van der Walt, L. and de Jager, M. (eds.). 2016. Shale Gas Development in the Central Karoo: A Scientific Assessment of the Opportunities and Risks. CSIR/IU/021MH/EXP/2016/003/A, ISBN 978-0-7988-5631-7, Pretoria: CSIR. Https://Seasgd.Csir.Co.Za/Wp-Content/Uploads/2016/12/SGD-Scientific-Assessment-Binder1_Low-Res_Incl-Addenda_21Nov2016.Pdf

Holmes, M., 2022. Can diatoms be used as a biomonitoring tool for surface and groundwater? Towards a baseline for Karoo water. Doctoral thesis, Nelson Mandela University, Port Elizabeth.

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), Pp.321-337. https://doi.org/10.2989/16085914.2015.1086722

Holmes, M., Campbell, E. E., de Wit, M., and Taylor, J. C., 2022. The impact of drought in the Karoo-revisiting diatoms as water quality indicators in the upper reaches of the Great Fish River, Eastern Cape, South Africa. South African Journal of Botany, 149, Pp. 502-510. https://doi.org/10.1016/j.sajb.2022.06.046

Holmes, M., Campbell, E. E., de Wit, M., and Taylor, J. C., 2023. Can diatoms be used as a biomonitoring tool for surface and groundwater? Towards a baseline for Karoo water. South African Journal of Botany, 161, Pp. 211-221. https://doi.org/10.1016/j.sajb.2023.08.013

Horovitz, C. T., 2000. Biochemistry and Physiology of Scandium and Yttrium. In Biochemistry of Scandium and Yttrium, Part 2: Biochemistry and Applications, Pp. 39-163. Boston, MA: Springer US.

Hwang, E. A., Cho, I. H., Kim, H. K., Yi, C., and Kim, B. H., 2023. The relationship between rainfall pattern and epilithic diatoms in four streams of Central-Western Korea for three years (2013–2015). International Journal of Environmental Research and Public Health, 20(5), 4099. https://doi.org/10.3390/ijerph20054099

Iain, S.M., and Chassé, M., 2016. Scandium. William M. White. Encyclopedia of Geochemistry, Springer International Publishing Switzerland, Pp.1-4, 2016, ff10.1007/978-3-319-39193-9_281-1ff. ffhal01420736f

Jyrkänkallio-Mikkola, J., Meier, S., Heino, J., Laamanen, T., Pajunen, V., Kimmo, T., Tolonen, K. T., Tolkkinen, M., and Soininen, J., 2017. Disentangling multi-scale environmental effects on stream microbial communities. Journal of Biogeography, 44(7), Pp. 1512-1523. https://doi. org/10.1111/jbi.13002

Kumar, C. V., Karthick, V., Inbakandan, D., Kumar, V. G., Rene, E. R., Dhas, T. S., Ravi, M., Sowmiya, P., and Das, C. A., 2022. Effect of selenium nanoparticles induced toxicity on the marine diatom Chaetoceros gracilis. Process Safety and Environmental Protection, 163, Pp. 200-209. https://doi.org/10.1016/j.psep.2022.05.021

Lange-Bertalot, H., Hofmann, G., Werum, M., and Cantonati, M., 2017. Freshwater benthic diatoms of Central Europe: Over 800 common species used in ecological assessment Vol. 942. M. G. Kelly (Ed.). Schmitten-Oberreifenberg: Koeltz Botanical Books.

Lavoie, I., Campeau, S., Grenier, M., and Dillon, P. J., 2006. A diatom-based index for the biological assessment of eastern Canadian rivers: an application of correspondence analysis (CA). Canadian Journal of Fisheries and Aquatic Sciences, 63(8), Pp. 1793-1811. https://doi.org/10.1139/f06-084

Le Maitre, D., Colvin, C., Maherry, A., 2009. Water resources in the Klein Karoo: the challenge of sustainable development in a water-scarce area. South African Journal of Science, 105(1), Pp. 39-48. https://journals.co.za/doi/pdf/10.10520/EJC96877

Lecointe, C., Coste, M., and Prygiel, J., 1993. Omnidia : software for taxonomy, calculation of diatom indices and inventories management. Hydrobiologia 269(270): Pp.509–513. https://doi.org/10.1007/BF00028048

Lecointe, C.M., Coste, M., Prygiel, J., and Ector, L., 1999. Le logiciel OMNIDIA version 3, une puissante base de données pour les inventaires de diatomées et pour le calcul des indices diatomiques européens. Cryptogamie Algologie 20 (2): Pp. 1320,134.

Lecointe, M. M. L., Coste, M., and Ector, L., 2016. Logiciel OMNIDIA version 6: refonte complète. In 35ème Colloque de l'ADLaF , Pp. 38-38.

Lelong, A., Hégaret, H., Soudant P., and Bates, S. S., 2012. Pseudo-nitzschia (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms. Phycologia 51: Pp.168–216

Leonard-Roche, A., 2015. Legal Causation: Hydraulic Fracturing and Groundwater Contamination. SMU Science and Technology Law Review, 18, 279. https://doi.org/10.33 90%2Ftoxins14010060

Ludes, B. P., Chambre, A., and Delabarde, T., 2025. The diatom test in the field of forensic medicine: a review of a long-standing question. International Journal of Legal Medicine, 139(2), Pp. 597-605. https://doi.org/10.1007/s00414-024-03370-5

Mangadze, T., Taylor, J. C., Froneman, W. P., and Dalu, T., 2019. Water quality assessment in a small austral temperate river system (Bloukrans River system, South Africa): Application of multivariate analysis and diatom indices. South African Journal of Botany, 125, Pp. 353-359. https://doi.org/10.1016/j.sajb.2019.08.008

Martín, G., and Fernandez, M. R., 2012. Diatoms as indicators of water quality and ecological status: Sampling, analysis and some ecological remarks. Ecological Water Quality - Water Treatment and Reuse*,* 9, Pp. 183-204. https://doi.org/10.5772/33831

Masouras, A., Karaouzas, I., Dimitriou, E., Tsirtsis, G., and Smeti, E., 2021. Benthic diatoms in river biomonitoring—present and future perspectives within the water framework directive. Water, 13(4), Pp. 478. https://doi.org/10.3390/w13040478

Masouras, A., Karaouzas, I., Dimitriou, E., Tsirtsis, G., and Smeti, E., 2021. Benthic diatoms in river biomonitoring—present and future perspectives within the water framework directive. *Water*, 13(4), Pp.478. https://doi.org/10.3390/w13040478

Mathivha, F. I., Mabala, L., Matimolane, S., and Mbatha, N., 2024. El Niño-induced drought impacts on reservoir water resources in South Africa. Atmosphere, 15(3), Pp. 249. https://doi.org/10.3390/atmos15030249

Matlala, M. D., 2010. The use of diatoms to indicate water quality in wetlands: A South African perspective. Doctoral dissertation, North-West University, Potchefstroom. http://hdl.handle.net/10394/4410

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. Pretoria: Water Research Commission. https://www.wrc.org.za/wp-content/uploads/mdocs/KV%20270-11.pdf

Matlala, M., Taylor, J. C., and van Rensburg, L., 2008. The use of diatoms to indicate the quality of water in wetlands, a South African perspective. Water Institute of South Africa, Pp. 145.

Mertens, A., van der Wal, J., Verweij, G., Pex, B., van Dulmen, A., and van Dam, H., 2025. A revised list of diatom ecological indicator values in The Netherlands. Ecological Indicators, 172, Pp. 113219. https://doi.org/10.1016/j.ecolind.2025.113219

Mirzahasanlou, J. P., Nejadsattari, T., Ramezanpour, Z., Namin, J. I., and Asri, Y., 2018. The epilithic and epipelic diatom flora of the Balikhli River, Northwest Iran. Turkish Journal of Botany, 42(4), Pp. 518-532. DOI 10.3906/bot-1711-46

Mitra, A., Zaman, S., Ray, S. K., Sinha, S., and Banerjee, K., 2012. Inter-relationship between phytoplankton cell volume and aquatic salinity in Indian Sundarbans. National Academy Science Letters 35: Pp. 485–491. https://doi.org/10.1007/s40009-012-0083-1

Morales, E. A., Novais, M. H., García, M. L., Maidana, N. I., and Morais, M. M., 2023. Two new species of Nanofrustulum (Bacillariophyta) from temporary rivers in the Alentejo region, southern Portugal. Nova Hedwigia. https://doi.org/10.1127/nova_hedwigia/2023/0833

Ncube, L., 2021. A Diatom-based Palaeoclimatic Reconstruction for the Border of Leribe-Makhotlong-Butha-Buthe Districts, Eastern Lesotho Highlands, Master's thesis, University of the Witwatersrand, Johannesburg, South Africa.

Niinemets, Ü., Kahru, A., Mander, Ü., Nõges, P., Nõges, T., Tuvikene, A., and Vasemägi, A., 2017. Interacting environmental and chemical stresses under global change in temperate aquatic ecosystems: stress responses, adaptation, and scaling. *Regional Environmental Change*, 17, Pp.2061-2077. https://doi.org/10.1007/s10113-017-1196-3

Nyawo, B. L., 2017. Groundwater and surface water interaction in the Uitenhage Artesian Basin, Eastern Cape, South Africa: case study of the Swartkops and Coega aquifer. University of the Witwatersrand, Johannesburg (South Africa).

Orr, H. A., 2005. The genetic theory of adaptation: a brief history. Nature Reviews Genetics, 6(2), Pp.119-127. https://doi.org/10.1038/nrg1523

Pandey, L. K., Bergey, E. A., Lyu, J., Park, J., Choi, S., Lee, H., Depuydt, S., Young-Tae, O., Lee, S., and Han, T., 2017. The use of diatoms in ecotoxicology and bioassessment: Insights, advances and challenges. Water Research, 118, Pp. 39-58. https://doi.org/10.1016/j.watres. 2017.01.062

Park, S. H., Kim, K., Lim, J. H., and Lee, S. J., 2019. Selective lithium and magnesium adsorption by phosphonate metal-organic framework-incorporated alginate hydrogel inspired from lithium adsorption characteristics of brown algae. Separation and Purification Technology, 212, Pp. 611-618. https://doi.org/ 10.1016/ j.seppur. 2018. 11.067

Patrick, R., 1948. Factors effecting the distribution of diatoms. The Botanical Review, 14, Pp. 473-524.

Pietersen, K., Kanyerere, T., Levine, A., Matshini, A., and Beekman, H. E., 2016. An analysis of the challenges for groundwater governance during shale gas development in South Africa. Water SA, 42(3), Pp. 421-431. https://doi.org/10.4314/wsa.v42i3.07

Potapova, M., and Charles, D. F., 2005. Choice of substrate in algae-based water-quality assessment. Journal of the North American Benthological Society, 24(2), Pp. 415-427. https://doi.org/10.1899/03-111.1

Ros, M. D., Marín-Murcia, J. P., and Aboal, M., 2009. Biodiversity of diatom assemblages in a Mediterranean semiarid stream: implications for conservation. Marine and Freshwater Research, 60(1), Pp. 14-24. https://doi.org/10.1071/MF07231

Serôdio, J., and Lavaud, J., 2022. Diatoms and their ecological importance. In Life below water, Pp. 304-312. Cham: Springer International Publishing.

Shtangeeva, I., Ayrault, S., and Jain, J., 2004. Scandium bioaccumulation and its effect on uptake of macro-and trace elements during initial phases of plant growth. Soil science and plant nutrition, 50(6), Pp. 877-883. https://doi.org/10.1080/00380768.2004.10408549

Soininen, J., Jamoneau, A., Rosebery, J., and Passy, S. I., 2016. Global patterns and drivers of species and trait composition in diatoms. Global ecology and biogeography, 25(8), Pp. 940-950. https://doi.org/10.1111/geb.12452

Soininen, J., Tupola, V., Voutilainen, I., Cantonati, M., and Teittinen, A., 2025. Diatom biogeography in freshwaters–new insights from between-region comparisons and the role of unmeasured environmental factors. Diatom Research, 40(1), 73-80. https://doi.org/10.1080/ 0269249X.2021.1999859

Stenger-Kovács, C., Béres, V. B., Buczkó, K., Al-Imari, J. T., Lázár, D., Padisák, J., and Lengyel, E., 2023. Review of phenotypic response of diatoms to salinization with biotechnological relevance. Hydrobiologia, Pp. 1-24. https://doi.org/10.1007/s10750-023-05194-7

Stroebel, D. H., Thiart, C. and de Wit, M., 2019. Towards defining a baseline status of scarce groundwater resources in anticipation of hydraulic fracturing in the Eastern Cape Karoo, South Africa: salinity, aquifer yields and groundwater levels. Geological Society, London, Special Publications, 479(1), Pp. 129-145. https://doi.org/10.1144/SP479.

Szczepocka, E., and Żelazna-Wieczorek, J., 2018. Diatom biomonitoring–scientific foundations, commonly discussed issues and frequently made errors. Oceanological and Hydrobiological Studies, 47(3), Pp. 313-325. https://doi.org/10.1515/ohs-2018-0030

Taylor, J. C., 2004. The application of diatom-based pollution indices in the Vaal catchment. Doctoral dissertation, North-West University, Potchefstroom. http://hdl. handle. net/10394/66

Taylor, J. C., Harding, W. R., and Archibald, C. G. M., 2007b. A methods manual for the collection, preparation and analysis of diatom samples. (Report TT281/07). Pretoria, South Africa: Water Research Commission. https://docs.niwa. co.nz/library/ public/1770054839.pdf

Taylor, J. C., Harding, W. R., and Archibald, C. G. M., 2007a. An illustrated guide to some common diatom species from South Africa (No. TT282/07). Pretoria, South Africa: Water Research Commission. https://www.wrc.org.za/wp-content/uploads/ mdocs / TT282-07.pdf

Taylor, J. C., Prygiel, J., Vosloo, A., de la Rey, P. A., and 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, Pp. 455-464. http://dx.doi.org/10.4314/wsa.v42i4.05

Ter Braak, C. J. F., and Šmilauer, P., 2002. CANOCO 4.5: software for canonical community ordination. Ithaca, NY: Microcomputer Power.

TIBCO Software. 2022. Statistica version 13.3. On licence from Nelson Mandela University

Timm Hoffman, M., Cowling, R. M., Petersen, H., and Walker, C., 2021. Karoo research update: Progress, gaps and threats. South African Journal of Science, 117(1-2), Pp. 1-3. https://doi.org/10.17159/sajs.2021/8695

Van Dam, H., Mertens, A., and Sinkeldam, J., 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Netherlands Journal of Aquatic Ecology. *28*: Pp. 117-133. https://doi.org/10.1007/BF02334251

Verma, K., 2013. Role of diatoms in the world of forensic science. Journal of Forensic Research, 4(2), Pp. 181-18. DOI: 10.4172/2157-7145.1000181

Vinitha, E., Veeramani, P., and Venugopalan, V. P., 2010. Chlorination for power plant biofouling control: potential impact on entrained phytoplankton. International journal of environmental studies, 67(4), Pp. 515-530. https://doi.org/ 10.1016/ j.aquatox.2021.105954

Von Stosch, H. V., 1974. Pleurax, seine Synthese und seine Verwendung zur Einbettung und Darstellung det Zellwande von Diatomeen, Peridineen und anderen Algen, sowie fur eine neue Methode zur Electivfarbung von Dinoflagellaten-Penzern. Archiv für Protistenkunde, 116, Pp. 132-141.

Wolska, L., Sagajdakow, A., Kuczyńska, A., and Namieśnik, J., 2007. Application of ecotoxicological studies in integrated environmental monitoring: possibilities and problems. *TrAC* Trends in Analytical Chemistry, 26(4), Pp. 332-344. doi:10.1016 /j.trac. 2006. 11.012

Zalat, A. A., Derda, T., Welc, F., and Gwiazda, M., 2025. Environmental history of Lake Mariout at the 'Marea'/Philoxenite archeological site, northern Egypt, during the Hellenistic–early Islamic periods as seen by fossil diatoms. Journal of Quaternary Science. https://doi.org/10.1002/jqs.3686

Zhou, Y., Cao, Y., Huang, J., Deng, K., Ma, K., Zhang, T., Chen, L., Zhang, J. and Huang, P., 2020. Research advances in forensic diatom testing. Forensic sciences research, 5(2), Pp. 98-105. https://doi.org/10.1080/20961790.2020.1718901

Ziervogel, G., Lennard, C., Midgley, G., New, M., Simpson, N. P., Trisos, C. H., and Zvobgo, L., 2022. Climate change in South Africa: Risks and opportunities for climate-resilient development in the IPCC Sixth Assessment WGII Report. South African Journal of Science, 118(9-10), Pp. 11-15. https://doi.org/10.17159/sajs.2022/14492