POLITECNICO DI TORINO
Repository ISTITUZIONALE

Microplastics contamination in Isinuka springs and CO2- seeping freshwater systems in South Africa: a baseline study

Original
Microplastics contamination in Isinuka springs and CO2- seeping freshwater systems in South Africa: a baseline study / Mutshekwa, T., Mlambo, M.C., Odume, O.N., Madikizela, P., Owowenu, E.K., Mulaudzi, F., Balestra, V., Mofu, L., Naidoo, T., Motitsoe, S.N.. - In: AFRICAN JOURNAL OF AQUATIC SCIENCES. - ISSN 1608-5914. - (2026), pp. 1-8. [10.2989/16085914.2026.2701109]

Availability:
This version is available at: 11583/3016414 since: 2026-10-07T19:38:01Z

Publisher:
Taylor & Francis

Published
DOI:10.2989/16085914.2026.2701109

Terms of use:

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

Publisher copyright

(Article begins on next page)

27 November 2026



African Journal of Aquatic Science

ISSN: 1608-5914 (Print) 1727-9364 (Online) Journal homepage: www.tandfonline.com/journals/taas20

Microplastics contamination in Isinuka springs and CO_2-seeping freshwater systems in South Africa: a baseline study

Thendo Mutshekwa , Musa C Mlambo , Oghenekaro N Odume , Phindile Madikizela , Enahoro K Owowenu , Fulufhelo Mulaudzi , Valentina Balestra , Lubabalo Mofu , Trishan Naidoo & Samuel N Motitsoe

To cite this article: Thendo Mutshekwa , Musa C Mlambo , Oghenekaro N Odume , Phindile Madikizela , Enahoro K Owowenu , Fulufhelo Mulaudzi , Valentina Balestra , Lubabalo Mofu , Trishan Naidoo & Samuel N Motitsoe (04 Aug 2026): Microplastics contamination in Isinuka springs and CO_2-seeping freshwater systems in South Africa: a baseline study, African Journal of Aquatic Science, DOI: 10.2989/16085914.2026.2701109

To link to this article: https://doi.org/10.2989/16085914.2026.2701109

© 2026 The Author(s). Co-published by NISC Pty (Ltd) and Informa UK Limited, trading as Taylor & Francis Group

View supplementary material

Published online: 04 Aug 2026.

Submit your article to this journal

Article views: 9

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=taas20

African Journal of Aquatic Science, 2026
Printed in South Africa — All rights reserved
Open Access article distributed in terms of the
Creative Commons Attribution License [CC BY 4.0]
(http://creativecommons.org/licenses/by/4.0)
This is the final version of the article that is published ahead of the print and online issue

Copyright © 2026 The Author(s)
AFRICAN JOURNAL OF AQUATIC SCIENCE
ISSN 1608-5914 EISSN 1727-9364
https://doi.org/10.2989/16085914.2026.2701109

Short Communication

Microplastics contamination in Isinuka springs and CO_2-seeping freshwater systems in South Africa: a baseline study

Thendo Mutshekwa[1,2,3]*, Musa C Mlambo[1,4,5†], Oghenekaro N Odume[2], Phindile Madikizela[2], Enahoro K Owowenu[2], Fulufhelo Mulaudzi[4,6], Valentina Balestra[5], Lubabalo Mofu[6,7], Trishan Naidoo[4,6] and Samuel N Motitsoe[3,6]

[1] *Department of Freshwater Invertebrates, Albany Museum, Makhanda (Grahamstown), South Africa*
[2] *Institute for Water Research, Rhodes University, Makhanda (Grahamstown), South Africa*
[3] *School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[4] *Department of Zoology and Entomology, Rhodes University, Makhanda (Grahamstown), South Africa*
[5] *Department of Agricultural, Forest, and Food Sciences, University of Turin, Grugliasco, Italy*
[6] *National Research Foundation-South African Institute for Aquatic Biodiversity, Makhanda (Grahamstown), South Africa*
[7] *Department of Ichthyology and Fisheries Science, Rhodes University, Makhanda (Grahamstown), South Africa*
[†] *Deceased*
* *Correspondence: thendomutshekwa@gmail.com*

Microplastic (MP) pollution is increasingly being reported in freshwater ecosystems, yet culturally significant and geochemically unique systems remain poorly studied. This study provides baseline evidence of MP contamination in South Africa's sacred freshwater systems, specifically Isinuka springs and CO_2-seeping freshwater systems along the Bongwana Fault. Surface water samples were collected from nine sites, including Isinuka springs (four sites; *n* = 4) and CO_2-seeping freshwater systems (five sites; *n* = 5). Microplastics were found at all sites, with abundances ranging from 5 to 48 particles l^{-1} (average ≈ 20.4 particles l^{-1}). Transparent MP particles were the most common colour (38.6%), fibres/filaments were the dominant shape (69.6%), particles ≥ 20 µm accounted for the highest proportion by size (51.1%), and polypropylene (PP) was the most frequently identified polymer (38%). A variety of MP patterns were observed, with the highest number of MP particles found at the most intensively used sacred site (Isinuka spring; Site 1). These findings highlight that sacred freshwater systems, often regarded as pristine due to their cultural and traditional significance, are vulnerable to MP contamination, with potential ecological, cultural and public health implications for communities that rely on these waters for drinking, healing and spiritual practices. This study provides essential baseline data and underscores the need for wider MP monitoring in South African sacred freshwater systems, including sediments and biota, while also emphasising the importance of combining scientific approaches with cultural management and community awareness to protect these sacred waters from MP pollution.

Keywords: cultural waters, emerging pollutants, freshwater contamination, mineral-rich systems, plastic debris

Supplementary material: available at https://doi.org/10.2989/16085914.2026.2701109

Microplastic (MP) pollution has become a global environmental concern, affecting a wide range of aquatic systems, including rivers (Klein et al. 2015; Dalu et al. 2021), lakes (Nava et al. 2023), wetlands (Dalvand and Hamidian 2023), groundwater (Mutshekwa et al. 2025; Mutshekwa et al. 2026), and marine and coastal environments (Andrady 2011; Naidoo and Glassom 2019). According to Hidalgo-Ruz et al. (2012), MPs are tiny plastic particles smaller than 5 mm in diameter derived from the breakdown of larger plastic waste or made directly for use in products and industrial applications. Their small size and durability allow MPs to move easily through the environment, raising concerns for ecosystems and human health (Andrady 2017). Although research on MPs has expanded rapidly since the first discovery by Carpenter and Smith (1972), over the past decade studies have largely focused on marine environments and urban or industrial freshwater systems (Thompson et al. 2024), while remote, culturally important, or geologically unique freshwater ecosystems, also known as sacred waters (Surpi et al. 2025), have been studied far less.

Sacred waters, such as natural springs and water pools, particularly in Africa, play a critical role in supporting rural livelihoods and cultural practices (Altman 2002). These sacred springs are preserved through indigenous knowledge and cultural traditions and are often used for drinking, ritual cleansing, and traditional healing (Faniran et al. 2001; Wilkens 2011; Itu et al. 2024). According to Altman (2002), sacred waters are often seen as pristine by local communities because of their spiritual importance and

Published online 04 Aug 2026

natural origins. However, growing human activity, changes in land use, and widespread pollution can threaten their environmental quality (Jumbam 2012; Ncube et al. 2020). Despite their ecological and socio-cultural importance, sacred freshwater systems remain understudied in MP research, particularly in South Africa (Dahms and Greenfield 2024), and are prone to plastic pollution due to limited regulations protecting these waters. The lack of regulations not only threatens aquatic organisms that inhabit these ecosystems but also poses potential health and livelihood risks for the communities that rely on these water systems for drinking water, rituals, and traditional practices.

South Africa contains a range of geologically distinct freshwater systems including sulphur-rich springs such as Isinuka springs and natural CO_2-seepage along major fault zones, such as the Bongwana Fault, which extends through parts of the Eastern Cape and KwaZulu-Natal provinces (Johnson et al. 2017). Many of these sulphur-rich and CO_2-seeping freshwater systems hold strong cultural and spiritual significance, linked to ancestral beliefs and traditional practices (Johnson et al. 2017). While previous studies have focused on the geological origin (e.g. Johnson et al. 2017), hydrochemistry (e.g. Faniran et al. 2001), and gas emissions (e.g. Bond et al. 2017) of South African sacred freshwater systems, MP contamination in these freshwater systems has not been investigated (Dahms and Greenfield 2024; Mokgalaka-Fleischmann et al. 2024). This study presents the baseline evidence of MP contamination in Isinuka springs and CO_2-seeping freshwater systems in South Africa. This short communication aimed to investigate the occurrence and characteristics of MPs in the study area. We hypothesised that (*i*) MPs would be found in all samples, particularly those that are intensively used or located near larger settlements such as Isinuka springs and (*ii*) MP characteristics (size, colour, shape) would vary among sites. By providing baseline data from these previously unstudied sites, this research aims to highlight that sacred freshwater systems in South Africa are vulnerable to plastic pollution and the need to include environmental monitoring in the conservation and management of culturally protected waters.

Water sampling was conducted in March 2025 across nine sacred freshwater systems (Figure 1; Table S1). Three sites (Sites 1–3) were located in the Isinuka springs (also known as Isinuka Mud Caves and Sulphur Pools) in the Isinuka Village, and one (Site 4) located 10 km from Isinuka village near Mzimvubu River (see Ncube et al. 2020 and Ogola et al. 2025 for detailed site descriptions). Five sites on CO_2-seeping freshwater systems were located along the Bongwana gas fault in the Bongwana village (n = 3; Site 5–7), and a further two sites were located at Mbizana/Nomkololwana village near Mtamvuna River in the border of Eastern Cape and KwaZulu-Natal (n = 2; Sites 8–9) (see Harris et al. 1997; De Decker 1981 for detailed site descriptions). The Isinuka springs are regarded as sacred due to their long-standing use for ritual bathing, spiritual cleansing, healing, and domestic water collection by local communities (Ncube et al. 2020). Similarly, several CO_2-rich seeping systems along the Bongwana gas fault hold cultural significance linked to ancestral beliefs and traditional practices (Bond et al. 2017).

At each site, a 1-l surface water sample was collected using newly purchased glass containers fitted with steel lids. All containers were thoroughly washed with dilute hydrochloric acid and rinsed with distilled water at least 24 hours prior to sampling to minimise contamination following the protocol of Stock et al. (2019). Surface water samples were collected by scooping water directly into the container until full. To minimise contamination, field personnel wore cotton laboratory coats and nitrile gloves, sampling containers remained closed except during sample collection, and all equipment was rinsed with distilled water before use. During transport and laboratory processing, samples were covered whenever possible (Gwinnett and Miller 2021; Munno et al. 2023).

In the laboratory, water samples were processed using a filtration approach (Le et al. 2023). Each sample was filtered through cellulose membrane filters representing particle size fractions of 1.7–10 µm, >10–20 µm, and >20 µm. To minimise contamination during filtration, all glassware and filtration equipment were rinsed with distilled water before use, laboratory surfaces were cleaned prior to processing, personnel wore cotton laboratory coats, and procedural blanks were analysed alongside samples following recommendations by Munno et al. (2023). Once dried, filters were examined under stereomicroscope (Olympus SZX7, Olympus Optical, Japan) equipped with a KL 1600 LED light source and an integrated Olympus Cam-SC50 camera, operated using Stream Essential 2.3 software and MPs were identified based on shape and colour (Qiu et al. 2016). Laboratory quality control procedures were implemented throughout sample processing as described by Mutshekwa et al. (2025). For polymer identification, a random subsample (~60%) of the particles of >20 µm in size was analysed using attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy (Bruker, Germany) and compared against reference libraries to determine polymer composition (see Mutshekwa et al. 2023 for detailed method). Particles <20 µm were not subjected to polymer confirmation due to analytical constraints associated with the ATR-FTIR instrument used. Therefore, the reported polymer composition reflects only the >20 µm MP fraction. Polymer identifications were accepted only when the spectral match exceeded 70%. Spectra with lower similarity scores or ambiguous matches were classified as “other” and were not assigned to a specific polymer type.

As replicate samples were not collected at each site due to restrictions by the local communities at some sites, inferential statistical analyses were limited to variables that allowed comparison across sites (Allua and Thompson 2009). Microplastic abundance data were analysed using a generalized linear model (GLM) with a Poisson error distribution to assess differences among particle size classes while accounting for spatial variation among sites. Site was included in the model to account for heterogeneity in MP abundance across sampling locations. Microplastic colour and shape data were analysed descriptively to explore spatial patterns across sites (Frere et al. 2017). Furthermore, Principal component analysis (PCA) was conducted to assess differences in MP shape and colour composition across sites using PAST Ver 4.03.



Figure 1: Images of selected sampled sacred freshwater systems: (a) Isinuka Spring (also known as Isinuka Healing Spa/the naturally pink spring; Site 1); (b) Sulphur-rich spring located ~10 km from Isinuka Spring (Site 4); (c) and (d) CO_2-seeping systems at Nomkololwana village in Mbizana. Photographs by Thendo Mutshekwa

Contamination was not found in controls (i.e. controls in the laminar flow and bench top). As procedural blanks showed no MP contamination, a conservative observational reporting threshold of 5 particles l^{-1} was adopted, corresponding to the lowest non-zero concentration detected among samples. A total of 184 MPs were found across all 9 sampled sites, ranging from 5 to 48 particles l^{-1} and an average of 20.4 (± 4.3 SD) particles l^{-1}. The highest abundance of MP particles, 48 particles l^{-1}, was found in Site 1 (Isinuka spring), and the lowest abundance was found in site 5 (CO_2-seeping) (Figure 2a). The most common MP colours found were transparent (38.6%), white (19.0%), and blue (15.8%) (Figure 2b), while fibres/filaments (69.6%) and fragments (21.7%) dominated in shape, with foam (3.8%), film (3.3%), and pellets (1.6%) being less frequent (Figure 2c). Microplastic particles were also found across different sizes, with particles >20 µm contributing to the largest proportion of MP particles (51.1%) followed by 1.7 µm (29.9%), and 10 µm (19.0%) (Figure 2d).

The generalised linear model (Poisson error distribution) revealed a significant effect of microplastic size class on microplastic abundance ($\chi^2 = 57.42$, df = 2, $p < 0.001$). From the observed MP particles, a random subsample (~60%) of suspected particles underwent FTIR/ATR-FTIR analysis, resulting in six polymer types: 38.0% polypropylene (PP), 30.0% polystyrene (PS), 15.0% polyethylene terephthalate (PET), 12.0% polyethylene (PE), 4.0% polydimethylsiloxane (PDMS), and 1.0% polyvinyl chloride (PVC).

Principal Component Analysis (PCA) revealed distinct site-level differences in MP shape and colour composition (Figures 3 and 4). The first principal component (PC1) represented MP abundance, with Sites 1, 8, and 9 characterised by higher counts of fibres/filaments, fragments (Figure 3), and transparent particles (Figure 4). The second principal component (PC2) also reflected differences in MP shape and colour.

This study provides baseline evidence of MP contamination in Isinuka springs and CO_2-seeping freshwater systems in South Africa. In the current study, MPs were found at all sampled sacred freshwater sites, highlighting that geologically unique and spiritually significant freshwater systems are also prone to MP

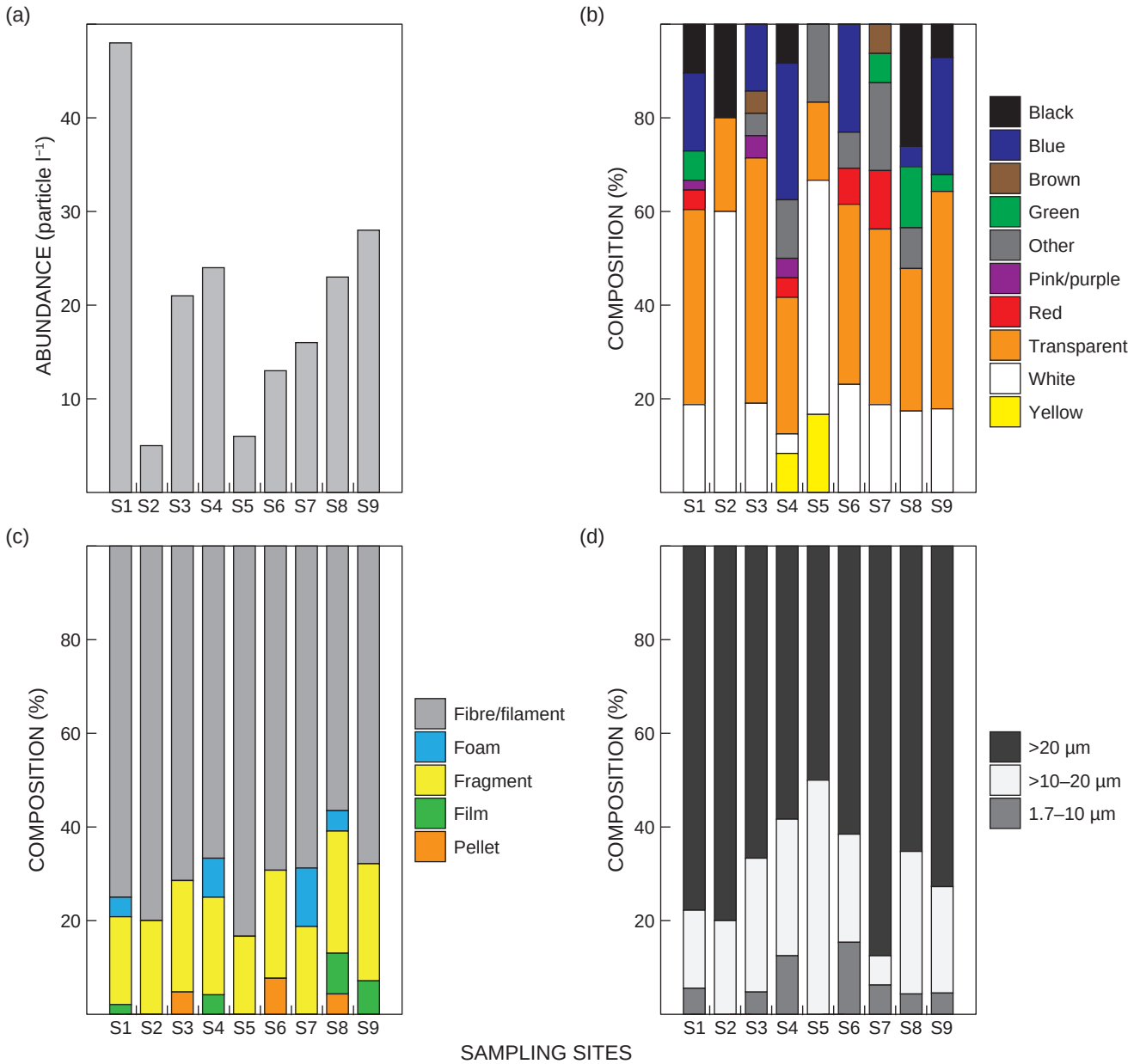

Figure 2: Microplastic characteristics in sacred freshwater systems: (a) abundance, (b) colour, (c) shape, and (d) size in Isinuka springs (sites 1–4) and CO_2-seeping faults (sites 5–9) in the Eastern Cape and KwaZulu-Natal provinces, South Africa. S1–S9 denote sites 1 to 9

pollution. Observed MP particles ranged from 5 to 48 particles l^{-1}. These particles occurred in a range of colours, shapes, and sizes comparable to those reported in other freshwater systems such as rivers, lakes, wetlands, and groundwater both in South Africa (Dahms and Greenfield 2024; Mokgalaka-Fleischmann et al. 2024) and elsewhere in Africa (Nyaga et al. 2024). However, the MP abundances observed in this study were notably higher than those reported in South African natural springs (2–38 particles l^{-1}) (Mutshekwa et al. 2026). Unlike many freshwater systems previously studied that are mainly influenced by urbanisation, wastewater discharge, or industrial activities, the sacred freshwater sites in this study are culturally significant and used for ritual and domestic purposes. The presence of MPs in these systems shows that even culturally perceived pristine waters are not exempt from plastic pollution, highlighting the need to include such sites in future monitoring and conservation programmes.

The abundance of MPs varied across sites; however, Isinuka Spring (a naturally pink spring; Site 1) had the highest MP abundance. The relatively higher MP abundance observed here may be associated with its greater accessibility and the variety of human activities reported at the site, including ritual bathing, spiritual cleansing, drinking, tourism, and commercial activities related to the sale of spring water and sediments than at other sites (Faniran et al. 2001; Jumbam 2012). The

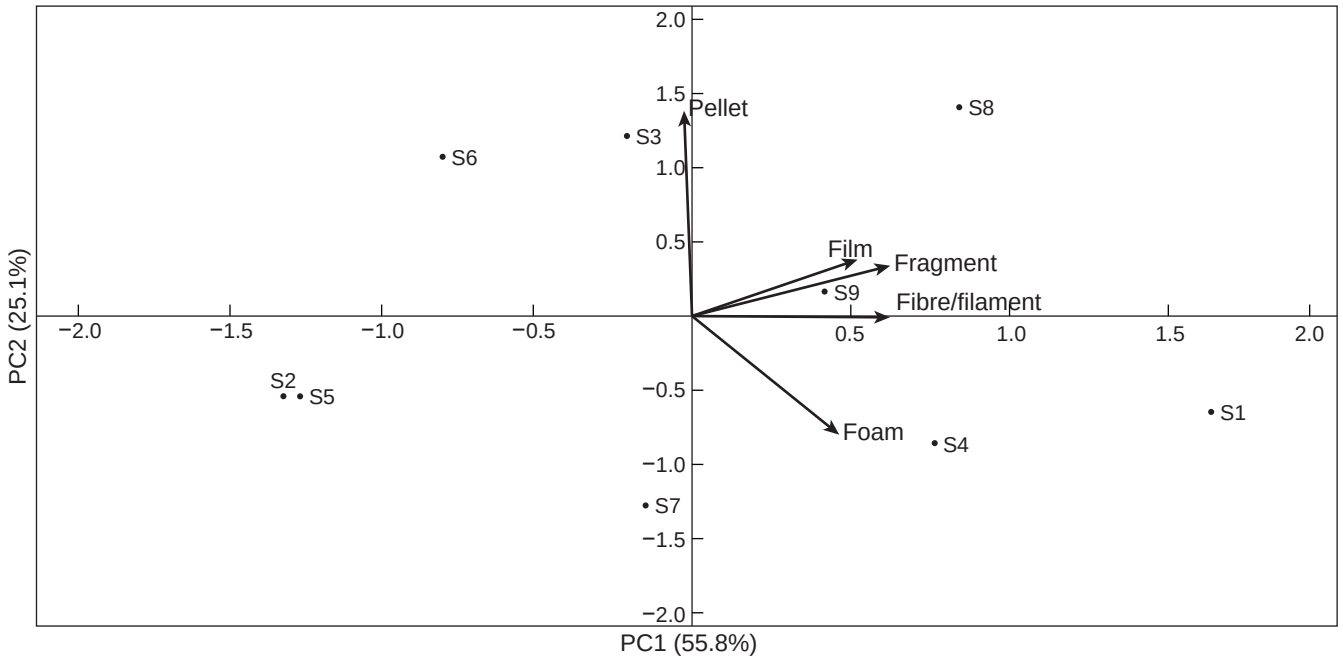

Figure 3: Principal Component Analysis (PCA) of MP shapes between sampled sites in the Eastern Cape and KwaZulu Natal, South Africa. S1–S9 denote sites 1 to 9

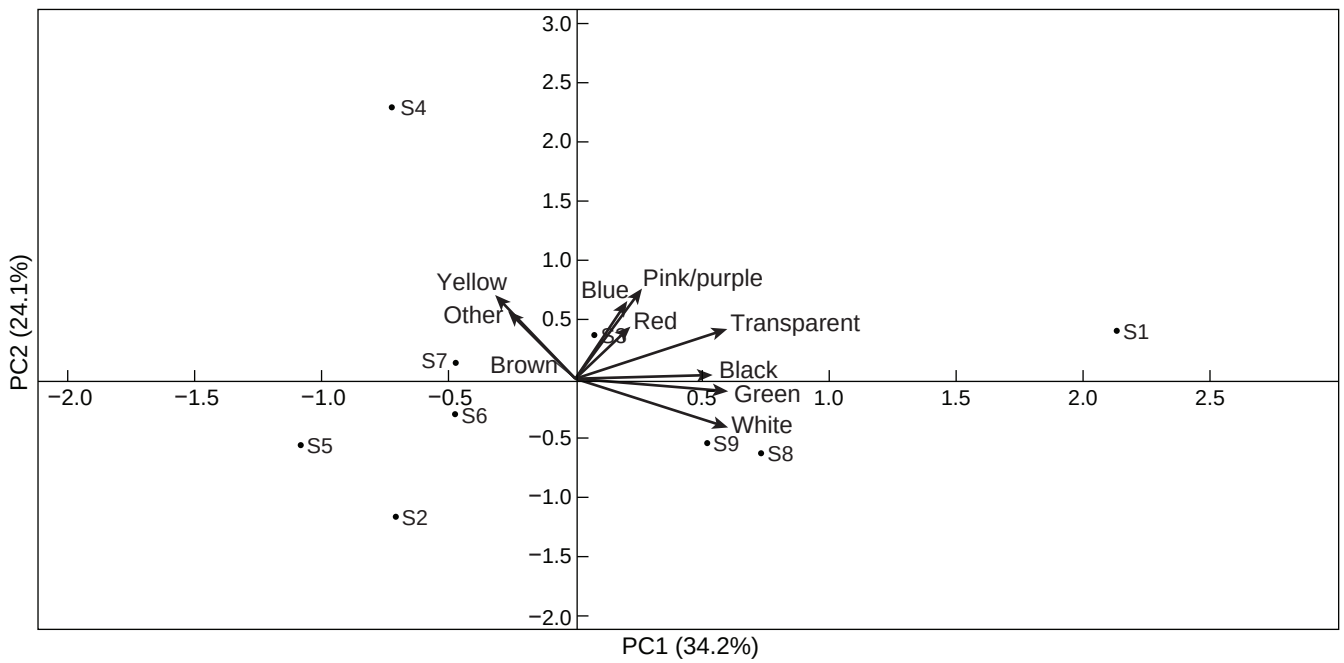

Figure 4: Principal Component Analysis (PCA) of MP colours between sampled sites in the Eastern Cape and KwaZulu Natal, South Africa. S1–S9 denote sites 1 to 9

pinkish water and sediments in Site 1 are believed to possess healing and protective powers by the local communities due to their odour, caused mainly by hydrogen sulphide gas (H_2S) (Ogola et al. 2025). This has resulted in a small business hub near the spring where locals sell the pinkish-coloured water and sediments to tourists and motorists. Other Isinuka springs were also contaminated with MPs and are used for similar purposes; however, ritual bathing and spiritual cleansing are limited at these sites due to their small size and the absence of pinkish-coloured water. In contrast, the CO_2-seeping sites along the Bongwana fault are more isolated, some

being located in private property (Site 5, 6 and 7) or only accessed through permission from the locals, which may partially explain their lower MP abundances when compared to Isinuka spring (Site 1). Nonetheless, the CO_2-seeping freshwater systems at Nomkololwana village in Mbizana (sites 8 and 9) are also used for the same traditional beliefs and ancestral communications (Bond et al. 2017), which explains the increased MP contamination when compared to other CO_2-seeping sites. Additionally, sampling was conducted during the rainy season, a period known to enhance MP transport through surface runoff, erosion, and the mobilisation of land-based sources into freshwater systems (Xia et al. 2021). This seasonal effect may have further contributed to elevated MP inputs, particularly at sites experiencing higher human activity and disturbance as at the Isinuka healing spa site.

Microplastics across sites were characterised by a variety of shapes, colours, sizes and polymer types. Fibres/filaments, transparent MP particles, >20 µm sized particles and polypropylene (PP) were the most common. This pattern is consistent with studies from within South Africa (e.g. Dalu et al. 2021; Mutshekwa et al. 2025) and globally (Han et al. 2020; Jiang et al. 2023), and is not surprising as sources of MPs at the study sites may originate from a variety of human activities, run-off during heavy rains, atmospheric deposition and tourist activity (T Mutshekwa, pers. observ.). Clear differences in MP colour and shape between sites, shown by the PCA, suggest that local water conditions, land use, and accessibility can influence MP characteristics. The distinct geochemical characteristics of sulphur-rich and CO_2-seeping freshwater systems represent an important area for future research. However, the present study did not measure physicochemical factors controlling MP transport, degradation, or retention, and therefore no mechanistic conclusions can be drawn regarding their influence on MP behaviour. Although this study did not directly test these effects, the results highlight the need for future research on how factors such as extreme pH, gas seepage, and mineral build-up influence MP behaviour in these environments.

Overall, the presence of MPs in the Isinuka and nearby CO_2-seeping freshwater systems of South Africa has important ecological, cultural, and public health implications, especially for communities that use these waters for drinking, healing, and spiritual practices. This study was based on a single surface-water sample per site collected during the rainy season, with no replicate samples collected (Lusher et al. 2015); therefore, the findings should be interpreted as baseline evidence of MP occurrence during the rainy season only and do not represent seasonal variability or annual contamination patterns within these freshwater systems. In addition, the size classification used in this study was constrained by the filtration system, with the largest size category reported as >20 µm. Since MPs are defined as particles up to 5 mm in size, larger particles may not have been fully characterised, limiting the interpretation of microplastic size distributions. Despite these limitations, the occurrence of MPs highlights the vulnerability of sacred freshwater systems to plastic pollution in South Africa. Future research should incorporate a wider range of size classes, replicated and seasonal sampling, sediment and biota analyses, and investigations of interactions between MPs and the distinctive geochemical conditions of these freshwater systems.

Declarations

Acknowledgements — We would like to thank Takalani Maravha for assisting with fieldwork. We also thank the communities of Mbizana and Baker Farm in Bongwana Village, KwaZulu-Natal, for granting permission to collect samples and guiding us to the sampling sites.

Funding — The work described in this paper was financially supported by the Water Research Commission (Project: C2025/2026-01859) awarded to Thendo Mutshekwa, Rhodes University Research Council, Albany Museum.

Ethics approval and consent to participate — Not applicable.

Availability of data and material — Data will be made available on reasonable request.

Competing interests — The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions — Thendo Mutshekwa: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, validation, visualisation, writing—original draft, writing—review and editing. Musa C. Mlambo: Conceptualisation, formal analysis, investigation, supervision, methodology, project administration, funding acquisition, resources, validation, visualisation. Oghenekaro N. Odume: writing—review and editing. Phindile Madikizela: Investigation, writing—review and editing. Enahoro K. Owowenu: Investigation, writing—review and editing. Fulufhelo Mulaudzi: Data curation, software, writing—review and editing. Valentina Balestra: writing—review and editing. Lubabalo Mofu: writing—review and editing. Trishan Naidoo: Data curation, formal analysis, writing—review and editing. Samuel N. Motitsoe: Conceptualisation, investigation, supervision, methodology, project administration, resources, validation, visualization, writing—review and editing.

Consent for publication — All authors agreed to the publication of this manuscript.

References

Allua S, Thompson CB. 2009. Inferential statistics. *Air Medical Journal* 28:168–171. https://doi.org/10.1016/j.amj.2009.04.013.

Altman N. 2002. *Sacred water: The spiritual source of life*. Mahwah: Paulist Press.

Andrady AL. 2011. Microplastics in the marine environment. *Marine Pollution Bulletin* 62: 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Andrady AL. 2017. The plastic in microplastics: A review. *Marine Pollution Bulletin* 119: 12–22. https://doi.org/10.1016/j.marpolbul.2017.01.082.

Bond CE, Kremer Y, Johnson G, Hicks N, Lister R, Jones DG, Haszeldine RS, Saunders I, Gilfillan SM, Shipton ZK, Pearce J. 2017. The physical characteristics of a CO2-seeping fault: the implications of fracture permeability for carbon capture and storage integrity. *International Journal of Greenhouse Gas Control* 61: 49–60. https://doi.org/10.1016/j.ijggc.2017.01.015.

Carpenter EJ, Smith Jr KL. 1972. Plastics on the Sargasso Sea surface. *Science* 175: 1240–1241. https://doi.org/10.1126/science.175.4027.1240.

Dahms HT, Greenfield R. 2024. A review of the environments, biota, and methods used in microplastics research in South Africa. *South African Journal of Science* 120: 16669. https://doi.org/10.17159/sajs.2024/16669.

Dalu T, Banda T, Mutshekwa T, Munyai LF, Cuthbert RN. 2021. Effects of urbanisation and a wastewater treatment plant on microplastic densities along a subtropical river system. *Environmental Science and Pollution Research* 28: 36102–36111. https://doi.org/10.1007/s11356-021-13185-1.

Dalvand M, Hamidian AH. 2023. Occurrence and distribution of microplastics in wetlands. *Science of the Total Environment* 862: 160740. https://doi.org/10.1016/j.scitotenv.2022.160740.

De Decker RH. 1981. Geology of the Kokstad Area. Explanation Sheet 3028. Department of Mineral and Energy Affairs, Pretoria, South Africa.

Faniran JA, Ngceba FS, Bhat RB, Oche CY. 2001. An assessment of the water quality of the Isinuka springs in the Transkei region of the Eastern Cape, Republic of South Africa. *Water SA* 27: 241–250.

Frère L, Paul-Pont I, Rinnert E, Petton S, Jaffré J, Bihannic I, Soudant P, Lambert C, Huvet A. 2017. Influence of environmental and anthropogenic factors on the composition, concentration and spatial distribution of microplastics: a case study of the Bay of Brest (Brittany, France). *Environmental Pollution* 225: 211–222. https://doi.org/10.1016/j.envpol.2017.03.023.

Gwinnett C, Miller RZ. 2021. Are we contaminating our samples? A preliminary study to investigate procedural contamination during field sampling and processing for microplastic and anthropogenic microparticles. *Marine Pollution Bulletin* 173: 113095. https://doi.org/10.1016/j.marpolbul.2021.113095.

Han M, Niu X, Tang M, Zhang BT, Wang G, Yue W, Kong X, Zhu J. 2020. Distribution of microplastics in surface water of the lower Yellow River near estuary. *Science of the Total Environment* 707: 135601. https://doi.org/10.1016/j.scitotenv.2019.135601.

Harris C, Stock WD, Lanham J. 1997. Stable isotope constraints on the origin of CO_2 gas exhalations at Bongwan, Natal. *South African Journal of Geology* 100: 261–266.

Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M. 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environmental Science & Technology* 46: 3060–3075. https://doi.org/10.1021/es2031505.

Itu PCO, Uquetan IU, Udousoro IL, Joe B, Amah JE, Ekpo OO, Ozoh SI. 2024. Water spirits and sacred rituals: the role of African traditional juju in protecting blue ecosystems in Cross River State. *Socialscientia: Journal of Social Sciences and Humanities* 9: 50–59.

Jiang JJ, Hanun JN, Chen KY, Hassan F, Liu KT, Hung YH, Chang TW. 2023. Current levels and composition profiles of microplastics in irrigation water. *Environmental Pollution* 318: 120858. https://doi.org/10.1016/j.envpol.2022.120858.

Johnson G, Hicks N, Bond CE, Gilfillan SMV, Jones D, Kremer Y, Lister R, Nkwane M, Maupa T, Munyangane P, Robey K. 2017. Detection and understanding of natural CO_2 releases in KwaZulu-Natal, South Africa. *Energy Procedia* 114: 3757–3763. https://doi.org/10.1016/j.egypro.2017.03.1505.

Jumbam ND. 2012. Demographic characteristics associated with Isinuka traditional spa near Port St Johns in the Eastern Cape Province of South Africa. *Indilinga African Journal of Indigenous Knowledge Systems* 11: 20–31.

Klein S, Worch E, Knepper TP. 2015. Occurrence and spatial distribution of microplastics in river shore sediments of the Rhine-Main area in Germany. *Environmental Science & Technology* 49: 6070–6076. https://doi.org/10.1021/acs.est.5b00492.

Le LT, Bui XB, Tran CS, Chiemchaisri C, Pandey A. 2023. Membrane and filtration processes for microplastic removal. In: *Current Developments in Biotechnology and Bioengineering*. Amsterdam: Elsevier. pp. 203–220. https://doi.org/10.1016/B978-0-443-19180-0.00019-5.

Lusher AL, Tirelli V, O'Connor I, Officer R. 2015. Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. *Scientific Reports* 5: 14947. https://doi.org/10.1038/srep14947.

Mokgalaka-Fleischmann NS, Melato FA, Netshiongolwe K, Izevbekhai OU, Lepule SP, Motsepe K, Edokpayi JN. 2024. Microplastic occurrence and fate in the South African environment: a review. *Environmental Systems Research* 13: 59. https://doi.org/10.1186/s40068-024-00389-w.

Munno K, Lusher AL, Minor EC, Gray A, Ho K, Hankett J, Lee CFT, Primpke S, McNeish RE, Wong CS, Rochman C. 2023. Patterns of microparticles in blank samples: A study to inform best practices for microplastic analysis. *Chemosphere* 333: 138883. https://doi.org/10.1016/j.chemosphere.2023.138883.

Mutshekwa T, Munyai LF, Mugwedi L, Cuthbert RN, Dondofema F, Dalu T. 2023. Seasonal occurrence of microplastics in sediment of two South African recreational reservoirs. *Water Biology and Security* 2: 100185. https://doi.org/10.1016/j.watbs.2023.100185.

Mutshekwa T, Motitsoe SN, Naidoo T, Majingo Z, Mlambo MC. 2025. Plastics underground: Microplastic pollution in South African freshwater caves and associated biota. *Hydrobiologia*. https://doi.org/10.1007/s10750-025-05800-w.

Mutshekwa T, Mlambo MC, Naidoo T, Kolisi S, Odume ON, Mudzielwana R, Motitsoe SN. 2026. Plastic Particles in Pristine Waters? Investigating Microplastic Contamination in Natural Springs. *Water, Air, & Soil Pollution* 237: 486. https://doi.org/10.1007/s11270-026-09081-4.

Naidoo T, Glassom D. 2019. Sea-surface microplastic concentrations along the coastal shelf of KwaZulu–Natal, South Africa. *Marine Pollution Bulletin* 149: 110514. https://doi.org/10.1016/j.marpolbul.2019.110514.

Nava V, Chandra S, Aherne J, Alfonso MB, Antão-Geraldes AM, Attermeyer K, Bao R, Bartrons M, Berger SA, Biernaczyk M, Bissen R. 2023. Plastic debris in lakes and reservoirs. *Nature* 619: 317–322. https://doi.org/10.1038/s41586-023-06168-4.

Ncube S, Mlunguza NY, Dube S, Ramganesh S, Ogola HJO, Nindi MM, Chimuka L, Madikizela LM, 2020. Physicochemical characterization of the pelotherapeutic and balneotherapeutic clayey soils and natural spring water at Isinuka traditional healing spa in the Eastern Cape Province of South Africa. *Science of the Total Environment* 717: 137284. https://doi.org/10.1016/j.scitotenv.2020.137284.

Nyaga MP, Shabaka S, Oh S, Osman DM, Yuan W, Zhang W, Yang Y. 2024. Microplastics in aquatic ecosystems of Africa: A comprehensive review and meta-analysis. *Environmental Research* 248: 118307. https://doi.org/10.1016/j.envres.2024.118307.

Ogola HJO, Selvarajan R, Ncube S, Madikizela L. 2025. Thiocapsa, Lutimaribacter, and Delftia are major bacterial taxa facilitating the coupling of sulfur oxidation and nutrient recycling in the sulfide-rich Isinuka spring in South Africa. *Biology (Basel)* 14: 503. https://doi.org/10.3390/biology14050503.

Qiu Q, Tan Z, Wang J, Peng J, Li M, Zhan Z. 2016. Extraction, enumeration and identification methods for monitoring microplastics in the environment. *Estuarine, Coastal and Shelf Science* 176: 102–109. https://doi.org/10.1016/j.ecss.2016.04.012.

Stock F, Kochleus C, Bänsch-Baltruschat B, Brennholt N, Reifferscheid G. 2019. Sampling techniques and preparation methods for microplastic analyses in the aquatic environment–A review. *TrAC Trends in Analytical Chemistry* 113: 84–92. https://doi.org/10.1016/j.trac.2019.01.014.

Surpi NK, Seriadi SLN , Jemiwi Jero NW. 2025. Integrating traditional ecological knowledge and environmental science for sustainable lake conservation: Danu Kerthi in Bali. *Journal of Environmental Science and Sustainable Development* 8: 467–489.

Thompson RC, Courtene-Jones W, Boucher J, Pahl S, Raubenheimer K, Koelmans AA, 2024. Twenty years of microplastic pollution research—what have we learned? *Science* 386: eadl2746. https://doi.org/10.1126/science.adl2746.

Wilkens K. 2011. *Holy water and evil spirits: Religious healing in East Africa* (Vol. 47). Münster: LIT Verlag.

Xia F, Yao Q, Zhang J, Wang D. 2021. Effects of seasonal variation and resuspension on microplastics in river sediments. *Environmental Pollution* 286: 117403. https://doi.org/10.1016/j.envpol.2021.117403.

Manuscript received: 2 February 2026, revised: 2 July 2026, accepted: 3 July 2026
Associate Editor: H Ewerts