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Research paper

Ostracoda and Foraminifera as bioindicators of (aquatic) pollution in the protected area of uMlalazi estuary, South Africa
Ostracodes et foraminifères comme bioindicateurs de la pollution (aquatique) dans la zone protégée de l'estuaire d'uMlalazi, Afrique du Sud

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ABSTRACT

To mitigate ecological and health risks, implementing a comprehensive multidisciplinary monitoring strategy is imperative. This approach aims to effectively identify and record potential declines in water quality and ecological conditions. Utilizing cost-effective and efficient monitoring tools is crucial, especially for developing nations. Despite the previously reported uMlalazi River's pristine status within a protected natural reserve at South Africa's eastern coast, our findings challenge the assumption of its cleanliness, emphasizing the need for ongoing proactive monitoring. Here we reassess the pollution levels and ecological status of aquatic life of the river, and use this to enhance the indicator value of microfauna in South Africa. We analysed 25 surface sediment samples from the uMlalazi estuary, covering a salinity range from oligohaline to euhaline, with a focus on marginal marine Ostracoda and Foraminifera as potential indicators. All samples contained Ostracoda and Foraminifera, with the exception of two. Among the identified ostracod species, there were 17 species belonging to 14 genera. Typical taxa are the brackish water species *Perissocytheridea estuaria, Sulcostocythere knysnaenis,* and *Australoloxoconcha favornamentata*. We identified 19 Foraminifera species from 16 genera, with dominant taxa such as *Ammonia* sp., *Quinqueloculina* sp., and *Miliolinella* sp. Three distinct assemblages were observed: A) *Ammonia* sp. and *Quinqueloculina* sp., with very low diversity and abundances in general, located along the river course at stations exceeding Pollution Load Index (PLI), which indicates deterioration of sites quality; B) *Ammonia* sp., *Quinqueloculina* sp., and *Sulcostocythere knysnaenis* associated with higher salinity and lower PLI; C) *Ammonia* sp., *Quinqueloculina agglutinans*, and *Cribroelphidium articulatum* located in mudflats with minimal PLI. Our findings align with the commonly observed diversity trend, which indicates reduced species diversity corresponding to elevated pollution levels. Notably, the examined samples revealed a range of Foraminiferal Abnormality Index (FAI) up to 23%, exhibiting anomalies such as multiple tests, changes in coiling, and abnormal chamber shapes. Geochemical analysis indicates that the catchment is subjected to substantial anthropogenic pressure, as evidenced by elevated concentrations of heavy metals, sulphur, and microplastic. Sugarcane farming, urban sewage, titanium mining, and fish farming are the primary sources of pollution in the catchment area. Ongoing investigations in South African estuaries are expanding our dataset and will contribute to a better understanding of the species-specific responses of Ostracoda and Foraminifera to anthropogenic pressure.

Résumé: Pour atténuer les risques écologiques et sanitaires, la mise en œuvre d'une stratégie globale de surveillance multidisciplinaire est impérative. Cette approche vise à identifier et enregistrer efficacement les déclins potentiels de la qualité de l'eau et des conditions écologiques. L'utilisation d'outils de surveillance rentables et efficaces est cruciale, en particulier pour les pays en développement. Malgré le statut pur de la rivière uMlalazi au sein d'une réserve naturelle protégée le long de la côte est de l'Afrique du Sud, nos résultats remettent

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en question l'hypothèse de sa propreté, soulignant la nécessité d'une surveillance proactive continue. Ici, nous réévaluons les niveaux de pollution et l'état écologique de la vie aquatique de la rivière, et utilisons cela pour améliorer la valeur indicatrice de la microfaune en Afrique du Sud. Nous avons analysé 25 échantillons de sédiments de surface de l'estuaire d'uMlalazi, couvrant une gamme de salinité allant de l'oligohaline à l'euhaline, en mettant l'accent sur les foraminifères marins marginaux et les ostracodes comme indicateurs potentiels. Tous les échantillons contenaient des ostracodes et des foraminifères, à l'exception de deux. Parmi les espèces d'ostracodes identifiées, il y avait 17 espèces appartenant à 14 genres. Les taxons typiques sont les espèces d'eau saumâtre *Perissocytheridea estuaria, Sulcostocythere knysnaensis* et *Australoloxoconcha favornamentata*. Nous avons identifié 19 espèces de foraminifères appartenant à 16 genres, avec des taxons dominants tels que *Ammonia* sp., *Quinqueloculina* sp. et *Miliolinella* sp. Trois assemblages distincts ont été observés: A) *Ammonia* sp. et *Quinqueloculina* sp., avec une diversité et des abondances en général très faibles, situées le long du cours de la rivière dans des stations dépassant l'indice de charge de pollution (PLI), ce qui indique une détérioration de la qualité des sites; B) *Ammonia* sp., *Quinqueloculina* sp. et *Sulcostocythere knysnaensis* associés à une salinité plus élevée et à un PLI inférieur; C) *Ammonia* sp., *Quinqueloculina agglutinans* et *Cribroelphidium articulatum* situés dans des vasières avec un PLI minimal. Nos résultats s'alignent sur la tendance de diversité couramment observée, qui indique une diversité réduite des espèces correspondant à des niveaux de pollution élevés. Notamment, les échantillons examinés ont révélé une gamme d'indice d'anomalie des foraminifères (FAI) allant jusqu'à 23%, présentant des anomalies telles que des tests multiples, des changements dans l'enroulement et des formes anormales de chambre. L'analyse géochimique indique que le bassin versant est soumis à une pression anthropique importante, comme en témoignent les concentrations élevées de métaux lourds, de soufre, de microplastique. La présente étude a identifié la culture de la canne à sucre, les eaux usées urbaines, l'exploitation minière du titane, la pisciculture et l'impact anthropique des colonies voisines comme les principales sources de pollution dans le bassin versant. Il est important de souligner que les organismes vivants servent d'indicateurs de l'environnement actuel, ce qui est précieux à des fins de surveillance. En revanche, les marqueurs géochimiques de pollution trouvés dans les sédiments de surface refléteront une période de temps nettement plus longue, même si la durée exacte reste incertaine. Les enquêtes en cours dans les estuaires sud-africains élargissent notre ensemble de données et contribueront à une meilleure compréhension des réponses spécifiques aux espèces d'ostracodes et de foraminifères à la pression anthropique.

1. Introduction

Water is an invaluable natural resource, playing a role in the metabolic processes that form the basis of life. For South Africa, clean water availability is restricted by insufficient precipitation, with an average annual rainfall of 464 mm falling well below the world average of 860 mm (World Bank Climate Change Knowledge Portal, 2023). This situation is exacerbated by both natural (prolonged droughts) and anthropogenic (social and industrial) demands on a dwindling fragile natural resource. Decades of uncontrolled economic development and urbanization have taken an environmental toll, and in the province of KwaZulu-Natal, informal and unregulated settlements pose a significant threat to coastal areas (Mkhize et al., 2023). As a water-scarce country, South Africa is projected to face a general water shortage by 2025, driven by growing demands from diverse user groups and current consumption trends (Department of Water and Sanitation, 2019). Critical is water quality, which is negatively impacted upon by anthropogenic activities, which include discharges and adjustments to flow regimes (Bunn and Arthington, 2002).

To manage ecological and health threats it is necessary to carry out an appropriate monitoring strategy using a multidisciplinary approach to highlight and document the potential deterioration of the water quality and ecological status of the river systems. Furthermore, appropriate monitoring tools are required to obtain rapid reliable results at low cost, in particular within developing countries.

Bioindicators are versatile tools of water quality monitoring (Sagova-Mareckova et al., 2021), with several taxonomic groups used for monitoring in aquatic ecosystems, each offering unique insights into environmental conditions. Notable among these are foraminifera, and diatoms, which have gained prominence for their role as bioindicators (e.g., Alve, 1995; Boltovskoy and Wright, 1976; Sagova-Mareckova et al., 2021; Yanko et al., 1994). These groups are well studied as tracers of past climatic variations and for their potential for conservational (palaeo)biology. They are not only indicative of contemporary ecological conditions but can also be applied in palaeo-reconstructions and modern analogues techniques.

Foraminifera, wide-spread in marine and outer estuarine settings, are sensitive to environmental changes, and their assemblage composition and diversity can reveal information about water quality and pollution levels (e.g., Alve et al., 2009; Armynot du Châtelet and Debenay, 2010; Bouchet et al., 2007; Jorissen et al., 2009; Nagy and Alve, 1987; Schönfeld et al., 2012). Ostracoda, a group of minute Crustacea with a double valved calcified carapace, are important index fossils and proxies in geosciences, but rarely used for water quality assessment so far. They inhabit all water conditions and complement Foraminifera and diatoms as bioindicators in estuarine systems with variable salinity.

Ostracods have been studied in the context of climate change and anthropogenic impacts on marine meiofauna (Zeppilli et al., 2015). They have been used as proxies in (geo-)archaeology to investigate paleoclimate, habitat and landscape changes, water availability and quality, land use, and other anthropogenic impacts (Quante et al., 2022). Freshwater ostracods have already been recognized as valuable environmental tracers (Ruiz et al., 2013). They can serve as indicators of water quality through the detection of population and community changes, as well as geochemical changes in their carapace (Ruiz et al., 2013). Moreover, they have also been used as sentinels of anthropogenic impacts in marine and brackish-water environments (Ruiz et al., 2005). Their presence and abundance can reflect the effects of human activities on these ecosystems (Ruiz et al., 2005). In recent decades, there are studies on ostracods as bioindicators, covering various regions worldwide (e.g., Echeverría Galindo et al., 2019; Frenzel et al., 2010; Gildeeva et al., 2021; Laut et al., 2016; Wetterich et al., 2005). As well as in the field of ecotoxicology, ostracods have been used to assess the spatial distribution of trace elements and the ecotoxicity of bottom sediments in reservoirs (Baran et al., 2016).

The aim of this study is to improve the understanding of the uMlalazi estuarine ecosystem by analyzing distribution patterns of Ostracoda and Foraminifera and the enrichment of heavy metals and microplastic. This way we provide useful information for further environmental conservation of the nature reserve and for a sustainable management and active protection of this vulnerable area.

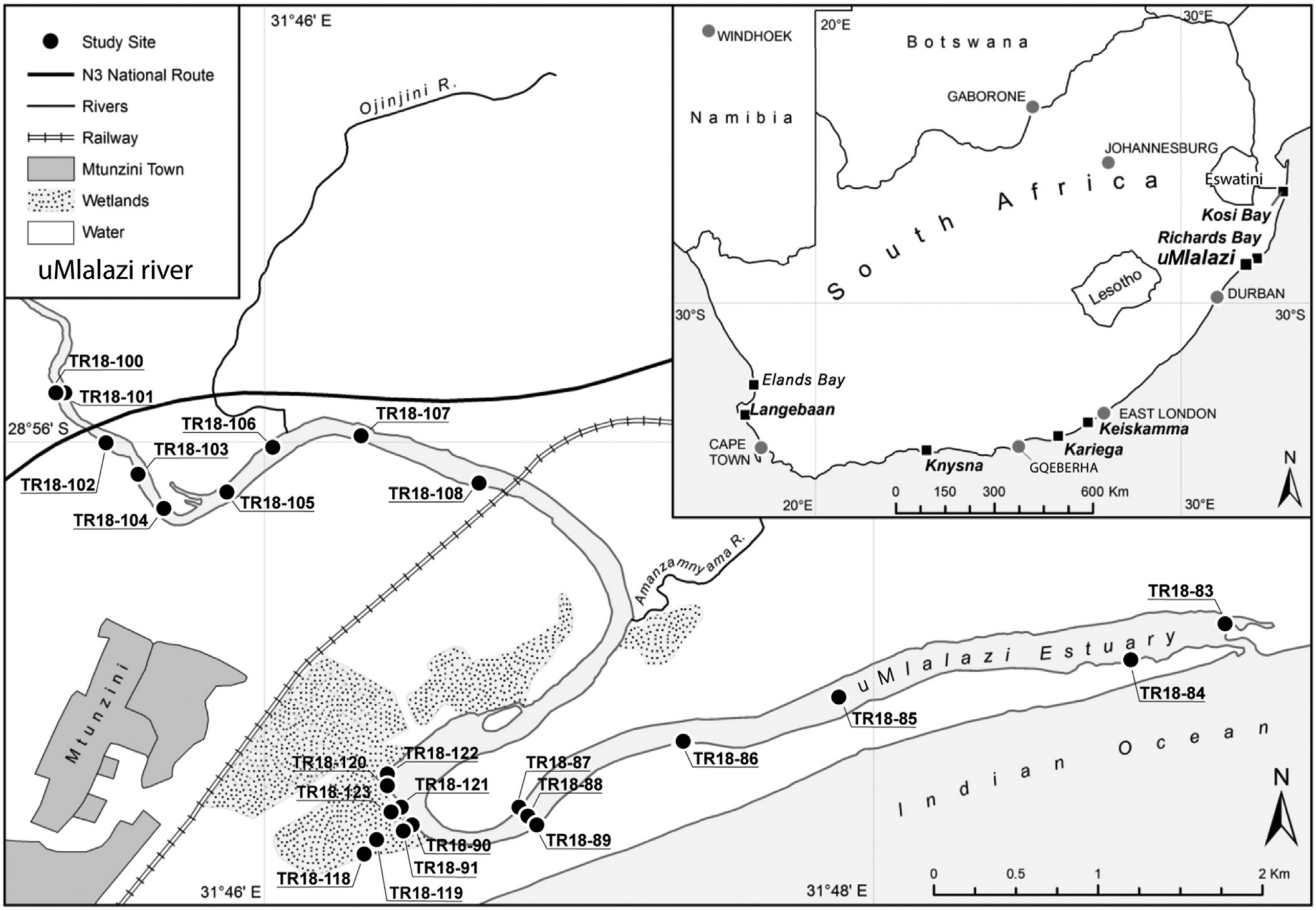

Fig. 1. The location of uMlalazi river in South Africa with the sampling stations in 2018, except TR18–98 which was collected near a small creek that ran from the river at the beach line (see Fig. 12).

2. Study area

The uMlalazi is situated on the eastern coast of South Africa within a nature reserve (Fig. 1). The river's total length spans approximately 54 km, with a catchment area covering 415 km^2 (Day, 1981). The uMlalazi river originates at an altitude of 480 m on the Ngoye ridge, near Eshowe town. Situated approximately 40 km south of Richards Bay alongside the village of Mtunzini in northern KwaZulu-Natal is the uMlalazi Nature Reserve. The uMlalazi Estuary (28°57′S; 31°48′E) runs approximately 11 km upstream of the uMlalazi River (Papadopoulos et al., 2002). This estuary has been permanently open since 1952 (Hill, 1966; Papadopoulos et al., 2002) and is characterized by the presence of sandbanks near the river mouth. Nonetheless, Kelbe and Taylor (2019) note that since the early 1800s, the catchment and estuary have experienced escalating human impacts. The significant alteration has been the recurring breaching of the estuary mouth (Kelbe and Taylor, 2019; Taylor, 2020), typically occurring within a few weeks after closure (with the earliest recorded instance dating back to the late 1890s). The microtidal nature of the study site, uMlalazi Estuary, is characterized by a maximum tidal range of 2.13 m at Mtunzini Beach (Ortega-Cisneros et al., 2011). The study site experiences high rainfall, approximately 1250 mm per year, with a peak during the austral summer (December–February) (Department of Environmental Affairs and Tourism (DEAT), 2001). The estuary length is dynamic, ranging from about 11 km in the dry season to 7 km during the rainy season as indicated by the reach of brackish water conditions (Hill, 1966).

The Mlalazi River flows through a range of different geological formations, including the Karoo Supergroup, the Table Mountain Group, and the Natal Group (Kelbe and Taylor, 2019). They consist of sedimentary rocks, including sandstone, shale, conglomerate and basalt. The soil types that are most prevalent today are loamy clay and sandy soils (Sudan, 1999). As soon as the river is reaching the cultivated areas, it increases sediment load and changes into a slowly running river. The river is known for its periodical severe floodings (Kelbe and Taylor, 2019). Mangrove swamps can be found along the lower 4 km of the estuary, stopping approximately 1 km from the mouth on the inner low-energy banks (Macnae, 1963; Ward, 1960). Interestingly, mangroves only began to appear in the uMlalazi area in the 1940s, as prior to this, sandbanks dominated the landscape (Hill, 1966). This transformation is attributed to the farming community depositing alluvium in the estuary (Hill, 1966; Macnae, 1963).

The uMlalazi Nature Reserve attained the status of an EKZNW (Ezemvelo KwaZulu-Natal Wildlife) reserve in 1948 and is an integral component of the Siyaya Coastal Park, which also encompasses the Amatikulu Nature Reserve to the south (Ezemvelo KZN Wildlife, n.d.).

Approximately 47% of the uMlalazi catchment area is cultivated (46% agriculture and 1% urban use) and 53% natural (South African Estuary Information System, 2023). The largest part of the catchment was initially covered with grassland, until it was divided into sugar cane farms in the first decades of the 1900s. Parts of the catchment became forestry plantations whilst other parts are under traditional land use (subsistence agriculture, small-scale cane farming and woodlots) and there has been significant wooding up of non-cultivated areas (BirdLife South Africa, 2018).

Other key challenges for the ecosystem along the river include invasive plant species, soil erosion, siltation, land transformation (services through the Protected Area), illegal grazing, fish farming, sewage and wastewater discharges (Department of Water and Sanitation 2015; Kelbe and Taylor, 2019). For instance, uMlalazi river and its estuary receive nutrient-enriched wastewater from a fish farm close to the estuary's south saltmarsh channel (Kelbe and Taylor, 2019). Bulk infrastructure exists close to or across the river: bridges, one waste management site (solid waste is collected by a private company and being removed), a sewerage plant outside the reserve, and an informal helipad (Rambarath et al., 2009). Moreover, there is a large titanium mining area to the south of Mtunzini (OMS, 2013). The most notable threats include pollution by pesticides and fertilizers from sugar cane farms (Kelbe and Taylor, 2019), fish farm and mining to the northern side of the dunes (PBS NewsHour, 2019). The issue of sand mining is a global concern that has far-reaching consequences on both the environment and society. Currently, the rate of sand extraction surpasses the natural replenishment rate, rendering mined ecosystems slow to recover or, in some cases, unable to recover at all (Environment, 2023). One of the most profound impacts of sand mining is the alteration it brings to the flow of waterways, and its potential to cause flooding. This poses a huge threat to water security and lowers groundwater capacity, leading to increased water costs for local communities (Environment, 2023). Furthermore, sand mining results in habitat loss and a significant reduction in biodiversity, particularly affecting aquatic ecosystems (Environment, 2023). At present, the mine's ecological footprint primarily impacts sugarcane farmland and *Eucalyptus* plantations, rather than natural habitats (BirdLife South Africa, 2018). Nevertheless, concerns revolve around potential hydrological effects on the uMlalazi River estuary and the Siyaya River. Fairbreeze mine is planning to extend further east from the uMlalazi Nature Reserve, posing even more significant environmental challenges (Major Mines and Projects, KwaZulu-Natal (KZN) Sands Operation, 2023). This proposed expansion could intensify the existing concerns related to hydrology and potential impacts on the local ecosystems.

There are contributing studies in uMlalazi river on mangroves and bleaching effects (Kelbe and Taylor, 2019; Taylor, 2020), ecosystem functioning (Ortega-Cisneros, 2013), nutrient dynamics of estuarine invertebrates (Ortega-Cisneros and Scharler, 2015), comparison of geochemical parameters in the water, sediment and crab tissues (Adeleke et al., 2020), the surveys by ACER (Africa) Environmental Consultants (2019) and Department of Water and Sanitation, South Africa (2022), the contamination of organochlorine pesticides (Mehlhorn et al., 2023), bioavailable metals in tourist beaches (Vetrimurugan et al., 2016), and vertical zonation of foraminifera assemblages (Strachan et al., 2015; Strachan et al., 2017).

Nonetheless, surveys lack adequate information regarding the availability and quality of water, not providing data on chemical compounds, and there is currently no established monitoring scheme in place. In an extensive review conducted by Whitfield and Baliwe (2013), titled "A Century of Science in South African Estuaries: Bibliography and Review of Research Trends," the characterization of the uMlalazi River states that it is in a good condition as well as it is in good health, as indicated by a Blue (Good) assessment in terms of its NBA (National Biodiversity Assessment). However, the review also underscores the limited availability of information on the river, especially on physical and chemical parameters. To address the lack of available data and increasing environmental stress, there has to be done a comprehensive environmental impact assessment, focusing on hydrological effects and potential ecological impacts on the uMlalazi River estuary, the Siyaya River, and the surrounding ecosystems.

3. Materials and methods

Twenty-five surface sediment samples were collected with a box corer of 20 cm x 20 cm and a height (maximum penetration depth) of 35 cm. The uppermost centimetre of the sediment was taken for analysis.

The maximum water depth sampled was 3.8 m and measured using a hand-held echosounder. Shallow depths of less than 50 cm were sampled wading and water depth was estimated. Geographical coordinates within the WGS84 system were registered using a hand-held GPS device with a horizontal error of 3–6 m. Water quality parameters, salinity via specific conductivity, oxygen content, pH and water temperature were recorded at the sampling sites (Fig. 1) using a WTW 340i multiple parameter probe. Habitats were described including substrate and thickness of the oxygenized surface sediment layer.

Samples were collected from the estuary covering the river channel, river banks, the mudflats in the mangrove area and close and distant to the uMlalazi village for comparison. Surface samples were mixed with Rose Bengal dissolved in ethanol for later distinction between living and dead Foraminifera at the time of sampling, following the method by Lutze and Altenbach (1991). Before sieving, the volume of each sediment sample was measured as reference for abundance calculation. To minimize the time-consuming task of picking from sandy samples, we used the sodium polytungstate density separation technique which meets the quantitative criteria of efficiently concentrating foraminifera and ostracods without altering the faunal composition (Parent et al., 2018).

This method was necessary as the sediments were predominantly sandy, resulting in the foraminifera and ostracods being widely dispersed among a large volume of sediment grains. Parent et al. (2018) showed, that for densities greater than or equal to 2.1, the recovery rates of foraminifera were proven to be 95% or higher. After the heavy liquid separation, for quantitative foraminiferal and ostracod analysis, the samples were sieved through >200, 125–200 and 63-µm sieves and split into sub-samples using a micro-splitter. Valves and tests from subsequent splits were counted until 300 of both, Foraminifera and Ostracoda, for the >200 µm fraction and additionally >200 foraminifer tests for the >125 µm fraction were reached. Articulated ostracod carapaces were counted as two valves. The species proportions and the total abundance were calculated from these two counts. For the identification of living ostracods, the closed double valved individuals were opened and checked for remaining soft parts inside if this was not recognisable from outside. Living foraminifera were recognised by the occurrence of stained tests with remaining stained or pale-greenish germplasm in some chambers (Lutze and Altenbach, 1991; Murray and Bowser, 2000).

Malformed foraminifera were counted to calculate the Foraminiferal Abnormality Index (FAI) (Frontalini and Coccioni, 2008). The Foraminiferal Abnormality Index (FAI) is a quantitative measure used in (paleo)ecology to assess the health and environmental conditions affecting foraminiferal populations. It quantifies the frequency of abnormal or deformed foraminiferal tests (shells) in a sample, providing insights into environmental stress, pollution, or other disturbances. A higher FAI value indicates a greater prevalence of abnormalities, suggesting adverse conditions that may impact foraminiferal communities. Reworked individuals of both Foraminifera and Ostracoda were counted separately, e.g., with abraded ornamentation or largely filled with sediment inside the chambers of the test. To assess water turbulence and to check for the possible removal of thinner juvenile valves by dissolution or fragmentation, the adult/juvenile ratio was determined following Boomer et al. (2003). Identification relies predominately on Benson and Maddocks (1964), Dingle (1992), Dingle (1993), Dingle and Honigstein (1994) and Fürstenberg et al. (2017) for Ostracoda and on Schmidt-Sinns (2008) and the WoRMS - World Register of Marine Species (http://www.marinespecies.org/) for Foraminifera. Identification was performed with a low-power binocular microscope occasionally supported by a Keyence Digital Microscope.

A Hierarchical Cluster Analysis (WARD method) of the relative Ostracoda and Foraminifera abundance of all samples with at least 50 specimens was applied to determine similarities between associations as a proxy for habitat similarity. Species diversity was evaluated using Shannon's diversity (H') index (Shannon, 1948). A Mann-Whitney test was performed for "equal medians" in order to identify species specific re-

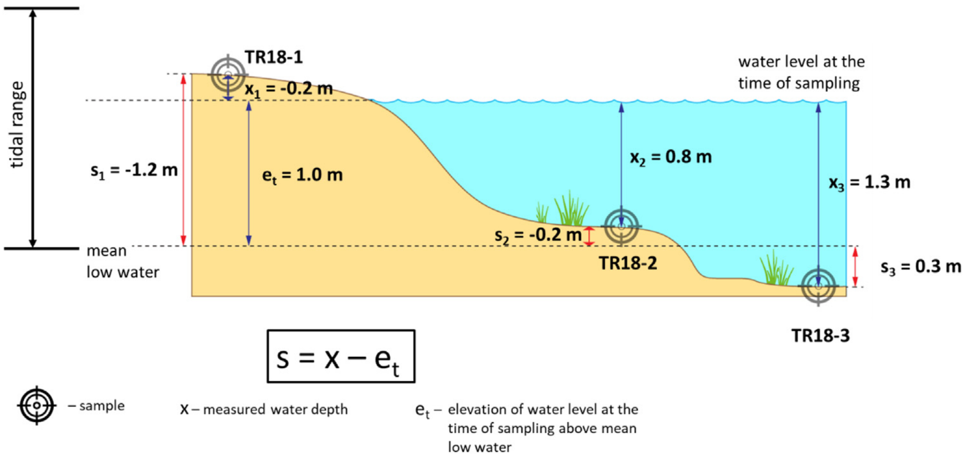

Fig. 2. Calculation of standardised water depth (s) from measured water depth (x). Standardised water depths are given as positive values of distances between the mean low-water level and the sampling point if it lays below low-water level and as negative values if above the low-water level.

sponse to pollution. All listed analyses were done using the program package PAST 4.03 (Hammer et al., 2001). Distribution maps were created with ArcMap 10.5 and the Spline with Barriers (SWB) tool was configured with a cell size 15 and 0 as a smooth factor. The interpolation shows the spatial distribution of the most influential environmental factors and spatial distribution of foraminifer and ostracod associations. The R software (R Core Team, 2017) with the package EMMAgeo v. 0.9.6. (Dietze and Dietze, 2019) was used for visualisation of grain size and grain size versus LOI data, enrichment factor (EF), pollution load index (PLI) and silt+clay versus TOC%.

3.1. Water depth standardization

Samples were taken at different times and thus with different temporary water levels depending on tidal effects. Standardizing water depth using tide chart values (Tide Times - A-Connect, 2023) and water level at the time of sampling; it is important for understanding real elevation regarding tidal range (Fig. 2). The reference level is the mean low water, which was taken from the tides chart.

3.2. Grainsize analysis

To conduct grain-size analysis, 0.5–2 g of wet sediment aliquots were utilized. The sediment was soaked in 2 ml of hydrochloric acid (HCl, 10%) and 5 ml of hydrogen peroxide (H_2O_2, 10%). The resulting residues were dispersed overnight using an overhead shaker with 5 ml of tetrasodium pyrophosphate ($Na_4P_2O_7$•$10H_2O$, 0.1 M). The Laser Diffraction Particle Size Analyzer (Fritsch Analysette 22; FRITSCH GmbH, Germany) was used to measure the samples in multiple runs until a reproducible signal was achieved (Mehlhorn et al., 2021).

3.3. Microplastic

For the semi-quantitative analysis of microplastics, freeze-dried samples were milled for 3 min at 50 revolutions per second, then diluted with IR transparent potassium bromide (KBr). After that, samples were analysed with a Bruker Vertex 70 spectrometer. Calibration models of Hahn et al. (2019) were applied for LDPE (Low Density Polyethylene) and PET (Polyethylene terephthalate), based on synthetic mixtures of sediment with a predefined and artificially created microplastic content.

Plastic fragments larger than 1 mm were counted semi-quantitatively during picking of microfossils under a low power stereo-microscope.

3.4. Loss on ignition

Carbon and carbonate contents were analysed using loss-on-ignition (LOI) at 550 °C and 1100 °C, respectively. The LOI was determined using a Nabertherm muffle furnace (L9/11) and a laboratory balance Sartorius "Quintix 6102–1x" with an accuracy of 0.001 g. An agate mortar was used to grind the sediment into powder (approximately 1.5 g per sample). The sediment powder was then dried in crucibles in the muffle furnace at 105 °C until the weight was constant. After cooling in a desiccator, the sediment was weighed into porcelain crucibles, and the LOI was determined according to DIN 18128. The organic content is represented by LOI_{550} °C. Subsequently, the carbonate content was determined as LOI_{1100} °C. Total organic carbon (TOC) was analysed by combustion of dried and homogenized samples with a CNS elemental analyser (EuroEA) as described in Mehlhorn et al. (2023).

3.5. Geochemistry

Samples were freeze-dried and then ground to a particle size of <60 μm and homogenized. A modified aqua regia treatment was used to digest subsamples in polytetrafluoroethylene (PTFE) crucible pressure bombs at 160 °C for 3 h. An Agilent 725- ES Inductively coupled plasma optical emission spectroscopy (ICP-OES) (Al, Ca Fe, K, Mg, Mn, Na, P, S, Sr, Ti) and a Thermo Fischer Scientific X-Series II inductively coupled plasma mass spectrometry (ICP-MS) (As, Cd, Co, Cr, Cu, Ni, Pb, Zn, Rb, Hg) were used to measure quantitative element concentrations. Laboratory blanks were included in each digestion batch, and calibration was performed with multi-element calibration standards. Calibration verification standards were regularly used during analysis, and calibration curves were evaluated. Samples were measured three times, and Grubbs's test was used to analyse outliers.

3.6. Shell chemistry

The chemical composition of foraminiferal tests was determined with use of ICP-MS and ICP-OES. Two abundant and ubiquitous genera of foraminifera with secreted calcareous tests - *Ammonia* Brünnich, 1771 and *Quinqueloculina* d'Orbigny, 1826, were chosen for the analysis and twenty and ten individuals, respectively, were picked from each locality, where there was enough material. For microwave digestion, 10 ml XPRESS vessels were used. Digestion was performed with 2 ml HNO_3 and topped up to 25 ml with ultrapure water at the end. Samples then were heated to 180 °C, kept at this temperature for 15 min and then cooled for 30 min.

The element contents of Ca, Mg, P, and Sr were analysed using the ICP-OES Spectrometer 725ES with a CCD area detector from Agilent. Liquid samples were fed into the device using an autosampler ASX 520 from Teledyne CETAC, and three measurements were taken on each sample. An outlier test according to Grubbs with a significance level of 90% was conducted. The mean, absolute values, and standard deviations were calculated and recorded. Similarly, the element contents

Table 1
The degree of metal enrichment based on Geo-accumulation (I_{geo}) classification.

I_{geo} value	I_{geo} Class	Designation of sediment quality
$I_{geo} < 0$	0	Unpolluted
$0 < I_{geo} < 1$	1	Unpolluted to moderately polluted
$1 < I_{geo} < 2$	2	Moderately polluted
$2 < I_{geo} < 3$	3	Moderately to strongly polluted
$3 < I_{geo} < 4$	4	Strongly polluted
$4 < I_{geo} < 5$	5	Strongly to extremely polluted
$I_{geo} > 5$	6	Extremely polluted

of Al, As, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Ti, U, V, W, and Zn were determined using the ICP-MS 8900 Triple Quadrupole from Agilent. Tungsten was excluded because of the pre-treatment of samples with sodium polytungstate. An autosampler with automatic dilution (prepFAST2DX system from Elemental Scientific) was used to send the liquid samples to the device. Internal standards of 20 μg l^{-1} Ru and 10 μg l^{-1} Re were added for drift correction. Three measurements were taken on all samples, and an outlier test according to Grubbs with a significance level of 90% was conducted. The mean, absolute values, and standard deviations were calculated and recorded. The values determined are only relative concentrations because the mass of the samples was too small to weigh them, and they had to be standardized to Ca.

3.7. Assessment of contamination

To assess the contamination level and ecological health status of uMlalazi river, we applied a range of pollution indicators; geoaccumulation index (I_{geo}), enrichment factor (EF), and pollution load index (PLI). The geoaccumulation index (I_{geo}) by Müller (1969) was used to compare the current concentration of heavy metals and metalloid elements in sediments with its pre-industrial levels. This index is used worldwide to estimate the contamination status of sediments (Alves et al., 2018; Shafie et al., 2013). The index is calculated based on the equation:

$$I_{geo} = \log_2 \frac{C_n}{1.5 \times B_n}$$

where:

C_n is the measured concentration of the element in the sediment (mg kg^{-1}), B_n is the geochemical background value in soil, reference value or pre-industrial concentration. We used the continental crust values by Turekian and Wedepohl (1961). Factor 1.5 is constant and always used to consider a possible fluctuation of the element in the background value. Based on the output of I_{geo} indices, further classification lies in seven classes, which is shown in Table 1.

The enrichment factor (EF) measures metal and metalloid enrichment in sediment. This index is widely used to study soils, marine sediments, and freshwater sediments (Moghtaderi et al., 2020; Shafie et al., 2013; Shirani et al., 2020). The EF indicates whether heavy metals and metalloids in sediments originated from anthropogenic sources. Typically, Fe, Sn, Mg or Al are used for normalisation (Jahan and Strezov, 2018; Shafie et al., 2013). Here, Fe was chosen as the standard element because of its even distribution in nature, its fourth place among major elements in the Earth's crust, its frequent occurrence in fine surface sediments, and because of its geochemistry that is similar to that of numerous trace elements (Haris and Aris, 2013).

The factor is calculated based on the equation:

$$EF_n = \frac{C_n/C_{Fe}}{B_n/B_{Fe}}$$

where:

C_n is the measured concentration of heavy metals or metalloids in the sediment (mg·kg^{-1}), C_{Fe} — the concentration of iron, B_n — the reference geochemical background concentration (Turekian and Wedepohl, 1961) and B_{Fe} — the reference geochemical background value of iron (Fe).

Table 2
The degree of sediment contamination based on Sutherland (2000) EF (Enrichment Factor) classification.

EF classification	EF sediment quality
EF < 2	Deficiency to minimal enrichment
2 ≤ EF < 5	Moderate enrichment
5 ≤ EF < 20	Significant enrichment
20 ≤ EF < 40	Very high enrichment
EF > 40	Extremely high enrichment

Table 3
The degree of sediment contamination based on Tomlinson et al. (1980) Pollution Load Index (PLI) classification.

PLI value	PLI sediment quality
PLI < 1	free from contamination
PLI = 1	base line level of pollution
PLI ≥ 1	deterioration of site quality

The value of the enrichment factor, close to or less than 1, typically indicates that the primary source of trace elements is from a natural origin, such as crustal or marine environments. However, if the enrichment factor is greater than 1, this usually shows that anthropogenic activities are the primary source. Based on that EF there is a classification into five classes, as shown in Table 2.

To assess contamination levels, the pollution load index (PLI) was calculated (Tomlinson et al., 1980). The PLI provides a summary indication of the level of toxic metals in a particular sample based on how often the metal content in a sample exceeds the average natural background concentration. Interpretation of sediment quality based on the output of the PLI can be found in Table 3.

The PLI is calculated based on the equation:

$$PLI = (CF_1 \times CF_2 \times CF_3 \times \ \ldots\ldots \times CF_n)^{1/n}$$

where:

n is the number of metals ($n = 8$ in this study).

The Contamination Factor (CF) stands for individual impact of every single metal on the sediment. To obtain CF, the following equation was used:

$$CF = \frac{C_n}{B_n}$$

where:

C_n corresponds to metal concentration from a sediment sample and B_n corresponds to the geochemical background concentrations (Turekian and Wedepohl, 1961).

4. Results

4.1. Environmental data

Summarised statistics for water and sediment collected along the uMlalazi river are presented in the Supplementary Table A. According to the Venice System (The Venice System for the Classification of Marine Waters According to Salinity, 1958), salinity data represents oligohaline, mesohaline, polyhaline and euhaline zones (3.3 – 33.2) with a coefficient of variation (CV) of 54.9 (see Supplementary Tab. A), which indicates a high variability.

Sandy sediment and sandy fine grained greyish to brownish sediment with common shells and living snails dominate the samples. An exception is the mangrove area and two samples upstream in the river meanders, where mostly black mud occurs. The LOI 550 ºC of the surface sediment shows considerable variation (0.1 – 10.7%). The highest organic carbon contents were observed in TR18–91, TR18–107 and TR18–118. Overall organic content significantly correlates with the grain size,

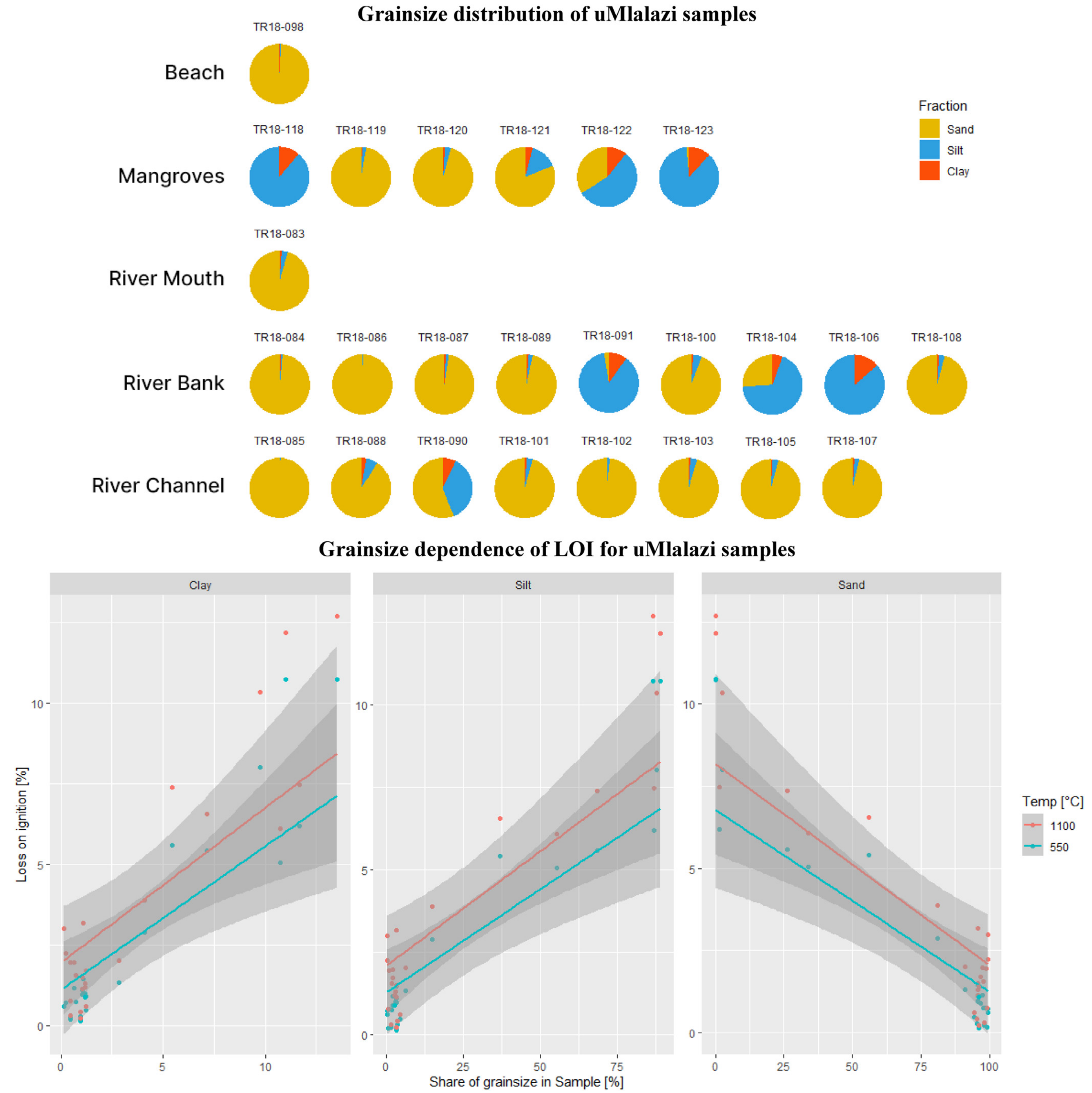

Fig. 3. Grainsize distribution and grainsize-dependence of LOI of uMlalazi samples.

with the lowest values in sandy sediments and highest in silt and clay (Fig. 3). The heavy metal and metalloids data show that the concentrations of As, Co, Cu, Ni, Sr, and to lesser degree Pb and Zn, in the surface sediments of the uMlalazi natural reserve are anomalously high. The mean concentrations of the heavy metals descended in the order of Sr (58 mg kg^{-1}) > Cr (44 mg kg^{-1}) > Zn (37 mg kg^{-1}) > Ni (14 mg kg^{-1}) > Cu (12 mg kg^{-1}) > Pb (8 mg kg^{-1}) > Co (7 mg kg^{-1}) > As (5 mg kg^{-1}). High to very high concentrations of cobalt were observed at all sampling sites.

Sulphur showed extremely high concentrations, ranging from 75 to 15,115 mg/kg. The other metals did not show high values, such as Ca, Na, Fe, Mn, Mg, K, Ti, and only iron showed a few peak concentrations.

Geoaccumulation index (I_{geo}), enrichment factor (EF) and Pollution Load Index (PLI) were applied to evaluate the severity of contamination of the sediments as well as to identify if there is an anthropogenic enrichment (Fig. 4). A comparison between the three indices shows a high correlation, clearly pointing to an anthropogenic pollution in the river. Based on I_{geo} assessment, all samples are polluted. I_{geo} index revealed that 4% the of stations in uMlalazi are moderately polluted, 16% are moderately to strongly polluted, 36% are strongly polluted, 28% are strongly to extremely polluted and another 16% are extremely polluted.

The EF shows that all the sampling stations are classified as significantly enriched with cobalt, with values ranging from 11 to 17, and all

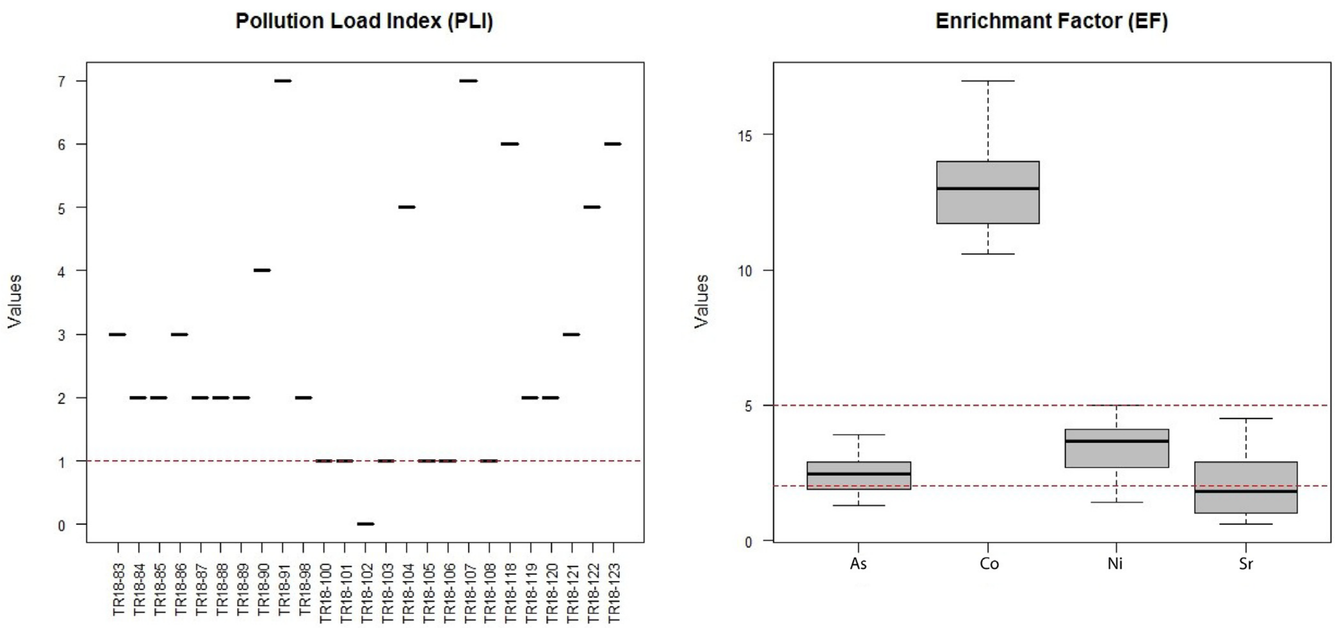

Fig. 4. Pollution Load Index (left) and Enrichment Factor (right) based on EFs of As, Co, Ni and Sr. The tick line inside the boxes is the median of the dataset. The red dashed lines are the thresholds (see Tables 2, 3). The shown heavy metals displayed the highest values.

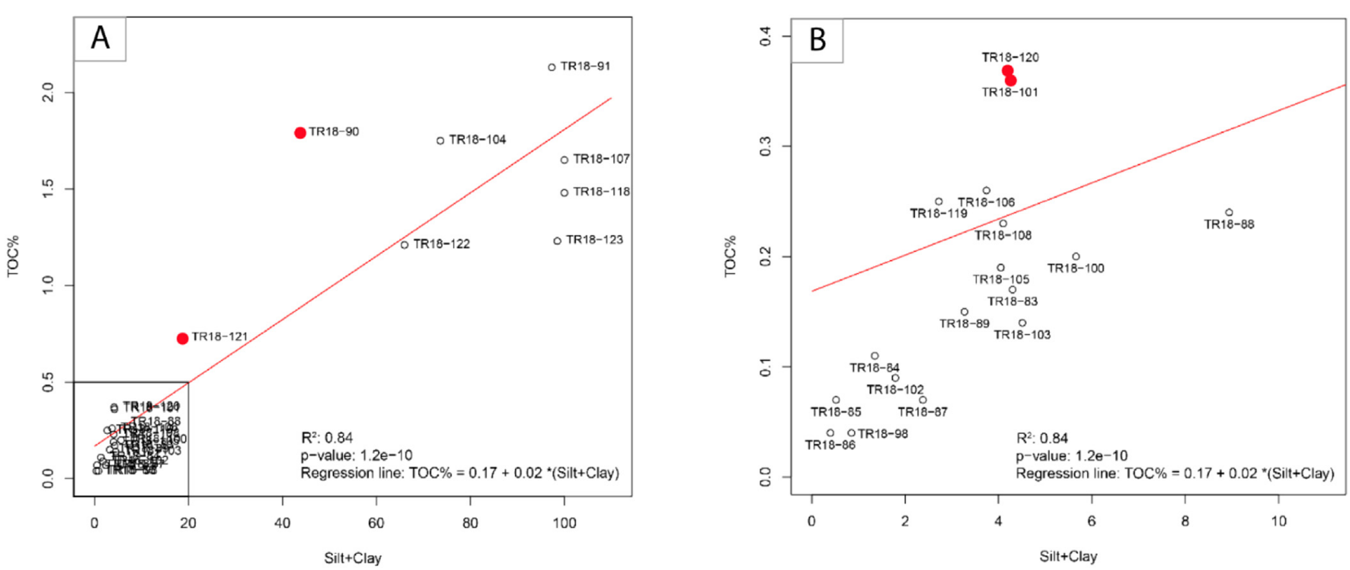

Fig. 5. a) fine grain material silt+clay against the percentage of TOC. b) zoomed in distribution. Samples marked in red are enriched in organic carbon.

samples are moderately enriched with nickel. Moreover, 96% of stations showed moderate enrichment of arsenic, with only one station TR18–103 lying in deficiency to minimal enrichment range. TR18–105 showed an extremely high copper enrichment of 53, coupled with significant enrichment of zinc and moderate enrichments of sulphur, lead, strontium, nickel, and arsenic. Sulphur EF values ranged from moderate enrichment to deficiency to minimal enrichment, with sample TR18–104 being a clear outlier with a value of 17. Some of the sites, which showed EF<2, can reflect a natural or anthropogenic enrichment, depending on the location.

The PLI provides an overall sediment quality value per site based on overall levels of heavy metals and metalloids. In most studied stations, except for TR18–102, the PLI values are equal or greater than 1 (Fig. 4), with the highest value of 7 for site TR18–91 and TR18–107. Thus, PLI points to deterioration of sites quality.

4.2. Organic enrichment

For 19 out of the 25 samples, sand is the dominant grain size while coarse silt prevails in the remaining six samples. We plotted the proportion of grains < 63 µm against %TOC, which shows a significant positive correlation (r^2 = 0.84, p-value = 12e-10) with a range of values (Fig. 5). Loosely based on the method suggested by Briz Parent (2019), we compared the measured%TOC value with the value corresponding to the station's grain size. Based on the regression equation, we can calculate

Table 4
Comparison of the sediment quality guidelines proposed by South Africa with various international empirical methods. Values with Probable Effect Concentrations are printed in bold. Threshold values from Burton (2002).

Element	As	Cr	Cu	Pb	Ni	Zn
TR18–83	5	***29***	7	5	14	28
TR18–84	3	17	4	3	6	12
TR18–85	3	23	5	3	8	27
TR18–86	3	***43***	6	3	10	17
TR18–87	2	***27***	6	3	10	13
TR18–88	4	25	6	4	9	33
TR18–89	5	***27***	6	5	9	21
TR18–90	***7***	***90***	12	10	***20***	40
TR18–91	***13***	***117***	27	21	***41***	80
TR18–98	4	***35***	4	4	8	47
TR18–100	1	11	1	3	2	37
TR18–101	1	14	2	4	4	16
TR18–102	1	9	0	3	1	5
TR18–103	1	9	2	4	2	20
TR18–104	***10***	***94***	15	13	***29***	55
TR18–105	1	8	***97***	7	2	100
TR18–106	2	14	2	4	3	30
TR18–107	***14***	***130***	26	23	***45***	79
TR18–108	2	13	1	3	3	22
TR18–118	***9***	***101***	26	20	***39***	89
TR18–119	3	17	6	3	8	11
TR18–120	2	***39***	5	5	9	20
TR18–121	5	***48***	10	7	***16***	26
TR18–122	***9***	***78***	16	13	***29***	65
TR18–123	11	***98***	20	18	***38***	52
Min	1	8	0	3	1	5
Max	14	130	97	23	45	100
Mean	5	44	12	8	14	37
Median	3	27	6	4	9	27
St. Dev	4	37	19	6	13	26
TEC	6	70	48	28	67	110
LEL	6.0	26.0	16.0	31.0	16.0	120.0
TEL	5.9	37.3	35.7	35.0	18	123
NOAA ERL	8.2	81.0	34.0	46.7	20.9	150.0
ANZECC ERL	20	81.0	34.0	47.0	21.0	200.0
SQAV TEL-HA28	11	36.0	28.0	37.0	20.0	98.0
SQO Netherlands Target	2.9	–	36.0	85.0	–	140.0
Hong Kong ISQG-low	8.2	80.0	65	75	40.0	200.0
PEL	17	90	197	91.3	36	315
TET	17	100	86	170	61	540
PEC	23	280	190	110	270	430

TEC, threshold effect concentration; LEL, lowest effect level; TEL, threshold effect level; NOAA, National Oceanic and Atmospheric Administration; ANZECC, Australian and New Zealand Environment and Conservation Council; ISQG, Interim Sediment Quality Guidelines; SQAV, Sediment Quality Advisory Value; SQO, Sediment Quality Objective; PEL, Probable Effects Level; TET, Toxic Effect Threshold; PEC, Probable Effect Concentration;.

an enrichment factor of organic matter for each station (Fig. 5) (find calculations in the Supplementary Table B).

A high proportion of samples display a natural variability in TOC content based on sediment grain size. However, we observed that samples such as TR18–101 near the bridge, TR18–90 close to the mangroves, and TR18–120 and TR18–121 directly within the mangroves, were all affected by organic enrichment.

4.3. Sediment quality guidelines

The primary objective of sediment quality guidelines (SQGs) are to protect the ecosystem and its aquatic organisms from the adverse and toxic impacts associated with sediment-borne pollutants, and therefore it is a valuable tool for this study. The assessment of heavy metal contamination in river sediments was conducted by comparing the sediment quality guidelines proposed for South Africa with various international empirical methods Table 4.

These methods rely on the co-occurrence of benthic macroinvertebrate effects and total sediment concentrations and are based on field and laboratory data that show negative impact on benthic organisms when exposed to contaminated sediments. Although these methods differ in the way they establish thresholds, the informative value is similar. These approaches include the Effects Range Approach (Ingersoll et al., 1996; Long and Morgan, 1991), Effects Level Approach (Ingersoll et al., 1996; Smith et al., 1996), Apparent Effects Threshold Approach (Cubbage et al., 1997), and Screening Level Concentration Approach (Persaud et al., 1993). Typically, these approaches establish two threshold levels: one below where effects seldom occur (such as Lowest Effect Level (LEL), Threshold Effect Level (TEL), Effects Range Low (ERL), and Threshold Effect Concentration (TEC)), and one above which effects are probable (Probable Effects Level (PEL), Toxic Effect Threshold (TET), and Probable Effect Concentration (PEC)) (Burton, 2002).

The South African National Guideline on Sediment Quality sets out limits for heavy metals, including As, Cd, Cr, Cu, Pb, Hg, Ni, and Zn. Like in other international approaches, the advice values for heavy metals are given in two categories: Threshold Effect Concentrations (TECs) and Probable Effect Concentrations (PECs).

The direct comparison of the heavy metal concentrations with Sediment Quality Objective (SQO) Netherlands Target guideless values indicate that all the sampling sites exceed threshold limits at least of one heavy metal. Overall, As, Cr and Cu concentrations exceed TECs, which represents a limit for lowest concentration of a contaminant that may cause an adverse effect. Samples TR18–100, TR18–101, TR18–102, TR18–103, TR18–106 and TR18–108 did not have any concentration above thresholds. However, it should be noted that only As, Cr, Cu, Pb, Ni, and Zn were compared, and the concentration of other heavy metals, such as Co, was not available for comparison due to the lack of threshold data.

4.4. Shell chemistry

Upon closer examination of the heavy metal concentrations in the shells of *Quinqueloculina* sp. and *Ammonia* sp., it was evident that significant variations existed for each metal tested, including Al, Fe, and Mn, as illustrated in Fig. 6. The results obtained from the analysis of sample TR18–84, specifically *Quinqueloculina* sp., demonstrated remarkably elevated levels of Fe at 20.7, a concentration notably higher than that observed in *Ammonia* sp. Furthermore, the concentrations of Al in *Quinqueloculina* sp. was found to be double that of *Ammonia* sp. in all tested samples.

4.5. Microplastics

The distribution patterns of PET and LDPE in the sediment show discrete differences, as illustrated in Fig. 7. PET was found in most samples, with the highest abundances upstream close to the bridge and near settlements (TR18–100 to TR18–106) and decreasing in concentration towards the river mouth, whilst LDPE was only found in TR18–107.

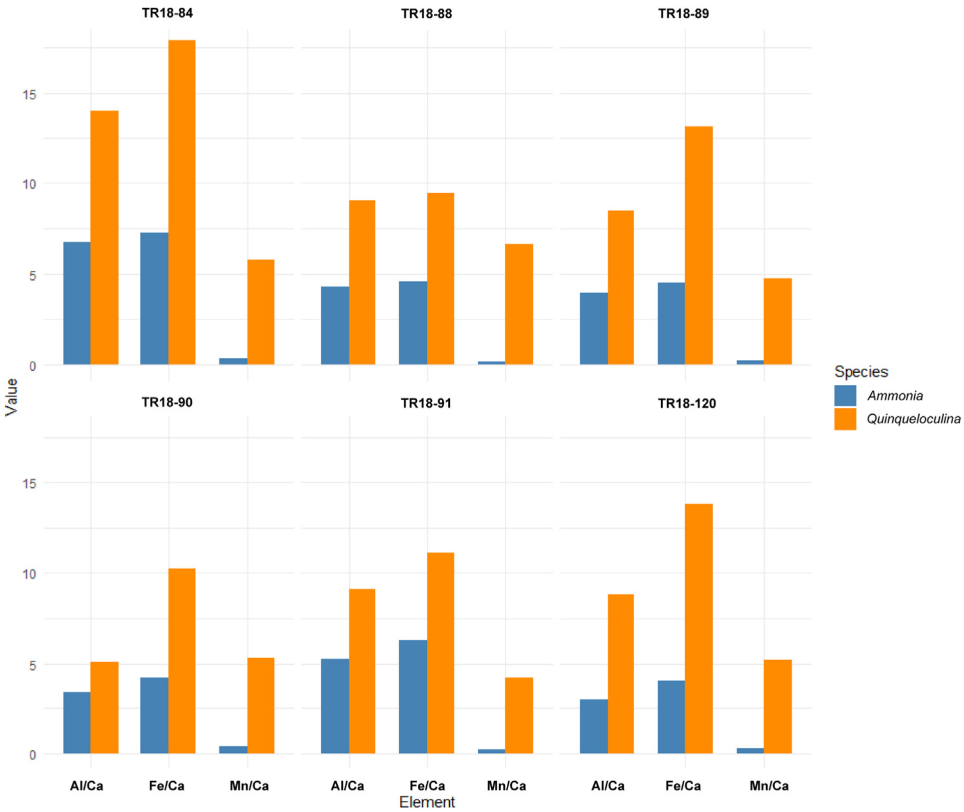

Fig. 6. Al/Ca, Fe/Ca and Mn/Ca ratios of *Quinqueloculina* sp. and *Ammonia* sp.

(a)

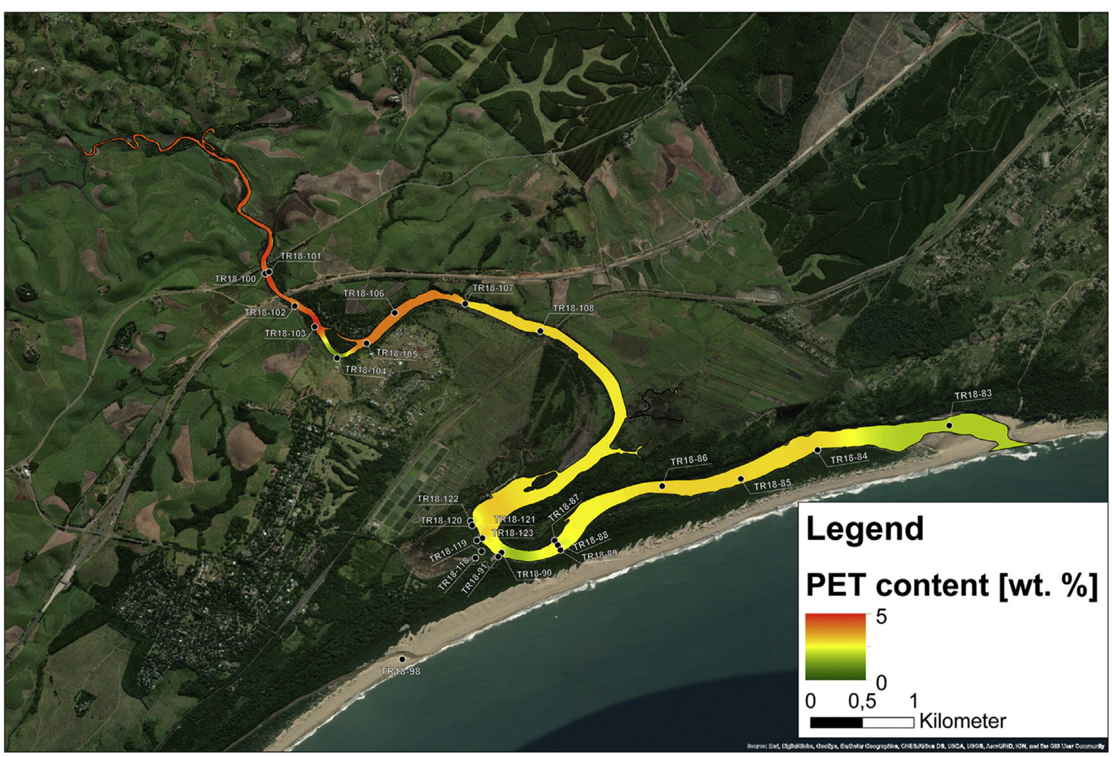

Fig. 7. Distribution of a) PET and b) of LDPE within the sediment samples.

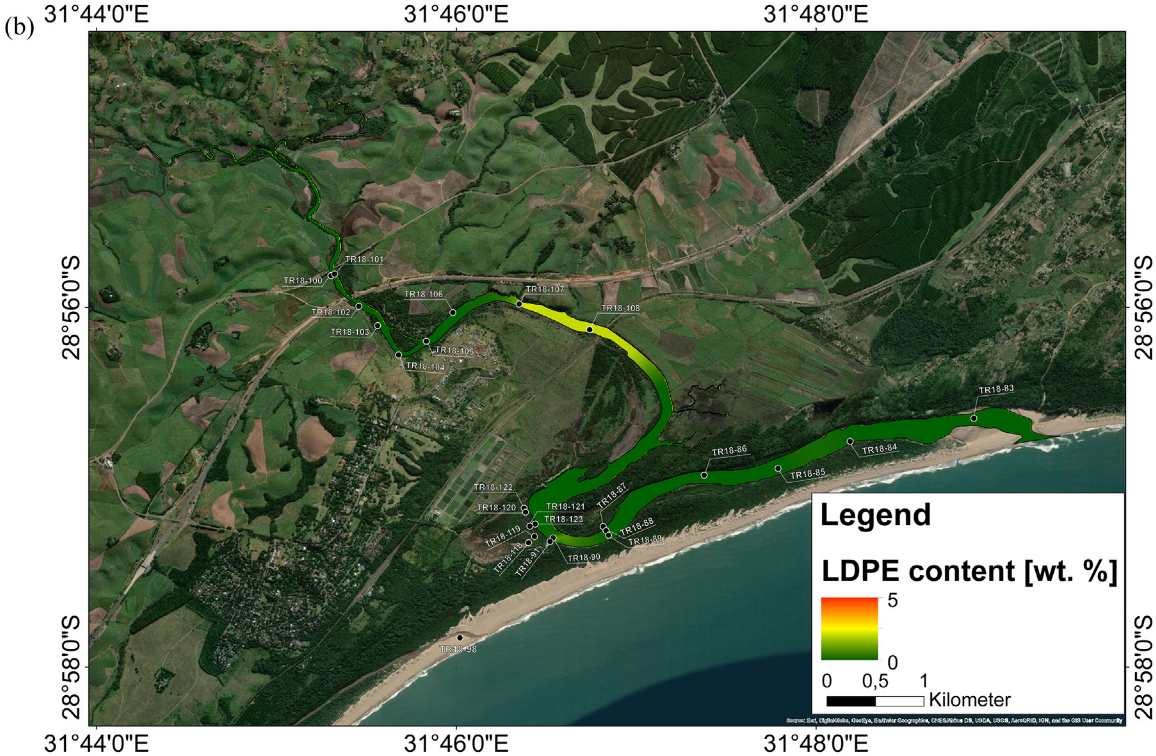

Fig. 7. Continued

4.6. Ostracods and foraminifers

In total, 17 ostracod species belonging to 14 genera were found. Foraminifera were present with 19 species belonging to 16 genera. They are documented with the following taxonomic reference list and on Figs. 8 and 9. Systematics are based on the World Register of Marine Species (WoRMS - World Register of Marine Species, 2023). The asterisks (*) represent living specimens.

Phylum **Foraminifera** d'Orbigny, 1826
Class **Globothalamea** Pawlowski, Holzmann, Tyszka, 2013
Order **Lituolida** Lankester, 1885
Superfamily **Verneuilinoidea** Cushman, 1911
Family **Prolixoplectidae** Loeblich & Tappan, 1985
***Eggerelloides* sp.**
Order **Textulariida (**Delage & Hérouard, 1896)
Superfamily **Textulariidea** Ehrenberg, 1838
Family **Textulariidea** Ehrenberg, 1838
Subfamily **Textulariidae** Ehrenberg, 1838
Textularia* sp.
Order **Rotaliida** Delage & Hérouard, 1896
Superfamily **Rotalioidea** Ehrenberg, 1839
Family **Rotaliidae** Ehrenberg, 1839
Subfamily **Ammoniinae** Saidova, 1981
***Ammonia* sp.** [probably a new species]*
Subfamily **Turrilinoidea** Cushman, 1927
Pseudouvigerina plummerae Cushman, 1927
Family **Elphidiidae** Galloway, 1933
Subfamily **Elphidiinae** Galloway, 1933
Cribroelphidium articulatum (d'Orbigny, 1839)*
Elphidium crispum Linnaeus, 1758*
***Elphidium* sp.**
Superfamily **Planorbulinoidea** Schwager, 1877
Family **Cibicididae** Cushman, 1927
Subfamily **Cibicidinae** Cushman, 1927
***Cibicides* sp.**
Superfamily **Asterigerinoidea** d'Orbigny, 1839
Family **Amphisteginidae** Cushman, 1927
***Amphistegina* sp.**
Superfamily **Buliminoidea** Jones, 1875
Family **Siphogenerinoididae** Saidova, 1981
Subfamily **Tubulogenerininae** Saidova, 1981
Rectuvigerina (Rectuvigerina)* cf. *nicoli Mathews, 1945
Superfamily **Globigerinoidea** Carpenter et al., 1862
Family **Globigerinidae** Carpenter et al., 1862
Subfamily **Globigerininae** Carpenter et al., 1862
Globigerina* sp.
Subfamily **Globorotaliidae** Cushman, 1927
Globorotalia menardii d'Orbigny in Parker, Jones & Brady, 1865
Superfamily **Nonionoidea** Schultze, 1854
Family **Nonionidae** Schultze, 1854
Subfamily **Nonioninae** Schultze, 1854
***Nonion* sp.**
Superfamily **Calcarinoidea** Schwager, 1876
Family **Calcarinidae** d'Orbigny, 1826
Subfamily **Pararotaliinae** Reiss, 1963
***Pararotalia* sp.**
Order **Lituolida** Lankester, 1885
Superfamily **Trochamminoidea** Schwager, 1877
Family **Trochamminidae** Schwager, 1877
Portatrochammina murrayi Brönnimann & Zaninetti, 1984*
Superfamily **Lituoloidea** Blainville, 1827
Family **Lituolidea** Blainville, 1827
Subfamily **Ammomarginulinae** Podobina, 1978
Ammotium morenoi (Acosta, 1940)
Subfamily **Trochammininae** Schwager, 1877
Trochammina inflata (Montagu, 1808)*
Class **Tubothalamea** Pawlowski, Holzman, Tyszka, 2013
Order **Miliolida** Delage and Hérouard, 1896

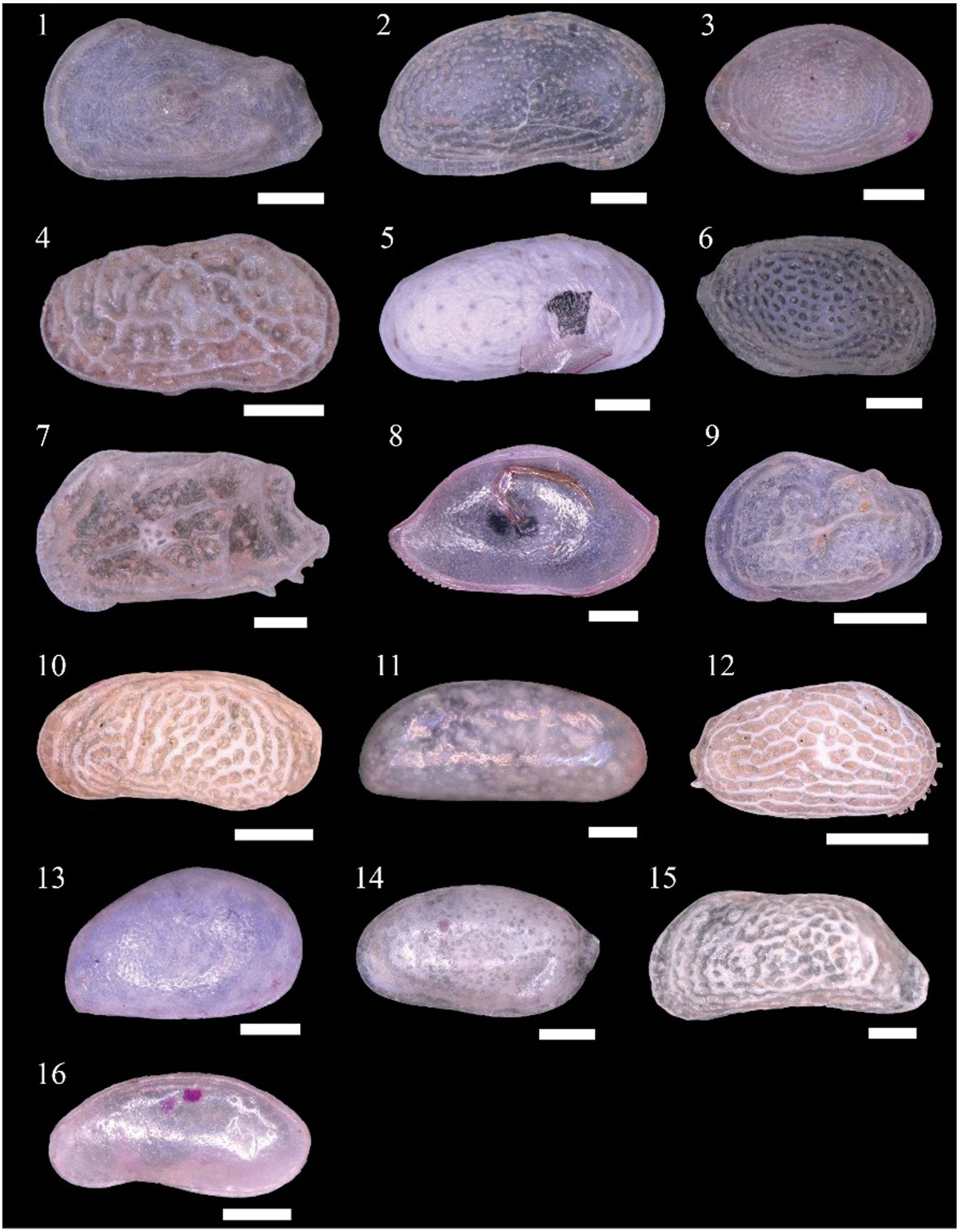

Fig. 8. Ostracod taxa of uMlalazi Estuary. All pictures are giving external views if not stated otherwise; all of them are light microscope photos. Scale bar length = 100 µm. (1) *Ambostracon* sp., LV, sample TR18–83; (2) *Aurila kliei* (Hartmann, 1974), RV, TR18–83; (3) *Australoloxoconcha favornamentata* (Hartmann, 1974), RV, TR18–90; (4) *Callistocythere* cf. *eulitoralis* (Hartmann, 1974), RV, TR18–83; (5) *Cytheromorpha ?milleri* (Dingle and Honigstein, 1994), RV, TR18–84; (6) *Loxoconcha* sp., RV, TR18–83; (7) *Mutilus bensonmaddocksorum* (Hartmann, 1974), LV, TR18–83; (8) *Neonesidea* sp., LV, internal view, TR18–83; (9) *Sulcostocythere knysnaensis* (Benson and Maddocks, 1964), LV, juvenile, TR18–84; (10) *Tanella africana* (Hartmann, 1974), RV, TR18–89; (11) *Argilloecia* sp., RV, TR18–83; (12) *Ruggieria* sp., RV, juvenile, TR18–83; (13) *Cyprideis* sp., RV; juvenile, TR18–84 (14) *Paradoxostoma griseum* (Klie, 1940), LV, TR18–83; (15); *Perissocytheridea estuaria* (Benson and Maddocks, 1964), LV, TR18–85; (16) *Sclerochilus* sp., RV, TR18–83;.

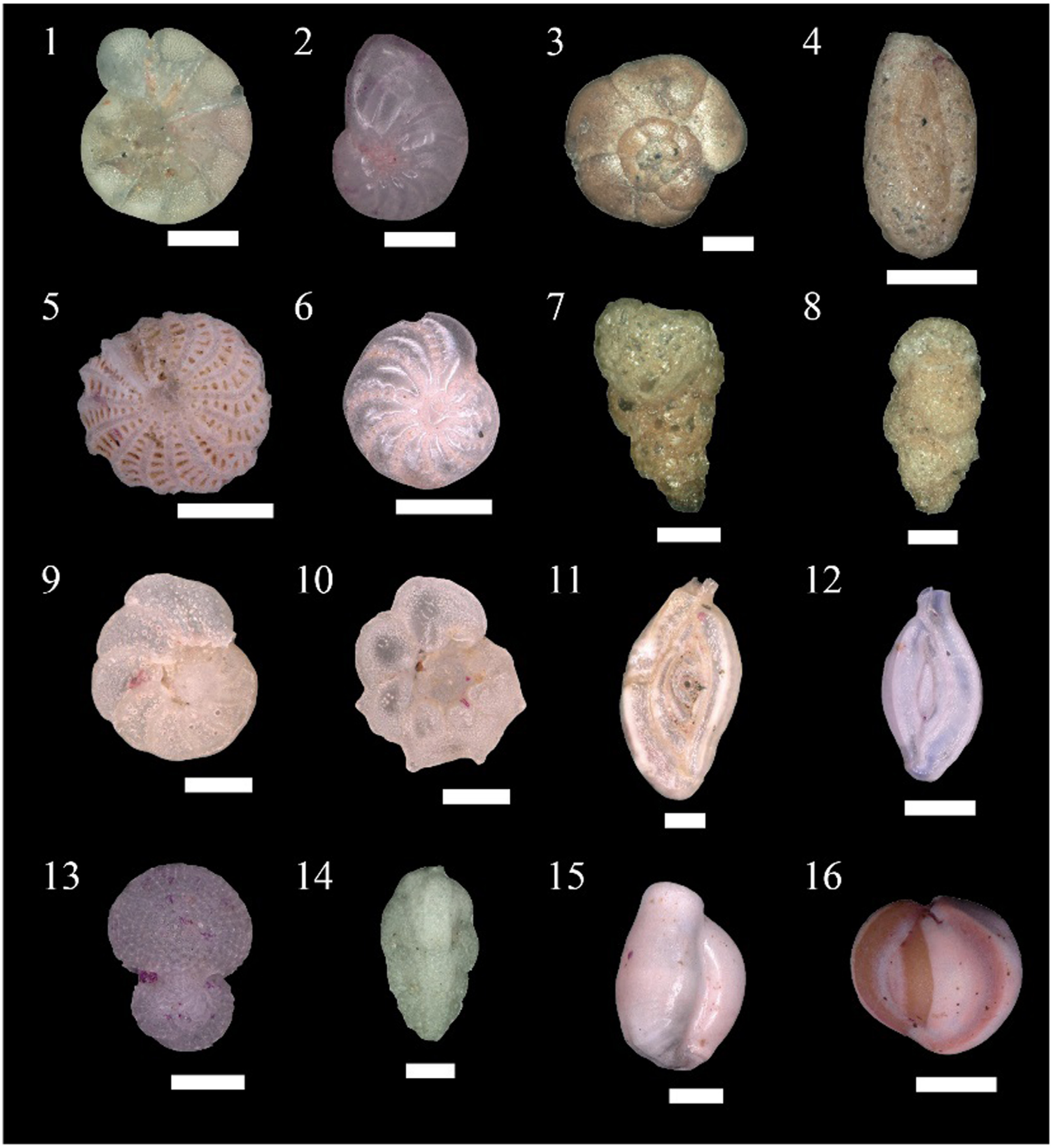

Fig. 9. Foraminifer taxa of uMlalazi Estuary. All photos were obtained with a light microscope. Scale bar length = 100 µm. (1) *Ammonia* sp., side view, TR18–90; (2) *Nonion* sp., ventral view, TR18–83; (3) *Trochammina inflata* (Montagu, 1808), dorsal view, TR18–121; (4) *Quinqueloculina agglutinans* (d'Orbigny, 1839), front view, TR18–107; (5) *Cribroelphidium* sp., dorsal view;, TR18–89; (6) *Cribroelphidium articulatum* (d'Orbigny, 1839), dorsal view, TR18–84; (7) *Eggerelloides* sp., side view, TR18–98; (8) *Textularia* sp., side view, TR18–100; (9–10) *Pararotalia* sp., both dorsal view, TR18–83; (11) *Sigmoilina* sp., dorsal view, TR18–83 (12) *Spiroloculina* sp., dorsal view, TR18–83 (13) *Globigerina* sp., side view; TR18–83; (14) *Pseudouvigerina plummerae* (Cushman, 1927), side view; TR18–100; (15) *Quinqueloculina* sp., ventral view, TR18–89; (16) *Miliolinella* sp., dorsal view, TR18–84.

Superfamily **Milioloidea** Ehrenberg, 1839
Family **Hauerinidae** Schwager, 1876
Subfamily **Sigmoilinitinae** Łuczkowska, 1974
***Sigmoilina* sp.**
Subfamily **Haueribinae** Schwager, 1876
Quinqueloculina* spp.
Quinqueloculina agglutinans d'Orbigny, 1839*
Miliolinella* spp.
Family **Spiroloculinidae** Wiesner, 1920
***Spiroloculina* sp.**
Phylum **Arthropoda** von Siebold, 1848
Class **Ostracoda** Latreille, 1806
Subclass **Podocopa** G.W. Müller, 1894
Order **Podocopida** Sars, 1866
Superfamily **Cytheroidea** Baird, 1850
Family **Cytherideidae** Sars, 1925
Sulcostocythere knysnaensis (Benson and Maddocks, 1964)*
Perissocytheridea aestuaria (Benson and Maddocks, 1964)*

Family **Trachyleberididae** Sylvester-Bradley, 1948
Ruggieria* sp.
Superfamily **Pontocypridoidea** Müller, 1894
Family **Pontocyprididae** Müller, 1894
Argilloecia* sp.
Superfamily **Cytheroidea** Baird, 1850
Family **Loxoconchidae** Sars, 1925
Australoloxoconcha favornamentata (Hartmann, 1974)*
Cytheromorpha*? *milleri (Dingle and Honigstein, 1994)*
***Elofsonia* sp.**
***Loxoconcha* sp.**
Family **Cytherideidae** Sars, 1925
Cyprideis remanei Klie, 1940*
Family **Leptocytheridae** Hanai, 1957
Tanella africana (Hartmann, 1974)
Family **Bythocytheridae** Sars, 1866
***Sclerochilus* sp.**
Family **Xestoleberididae** Sars, 1928
***Xestoleberis* sp.**
Family **Paradoxostomatidae** Brady & Norman, 1889
Paradoxostoma griseum Klie, 1940
Family **Hemicytheridae** Puri, 1953
***Ambostracon* sp.**
Family **Leptocytheridae** Hanai, 1957
Callistocythere* cf. *eulitoralis (Hartmann, 1974)
Family **Trachyleberididae** Sylvester-Bradley, 1948
Mutilus bensonmaddocksorum (Hartmann, 1974)
Family **Hemicytheridae** Puri, 1953
Aurila kliei (Hartmann, 1974)
Superfamily **Bairdioidea** Sars, 1865
Family **Bairdiidae** Sars, 1865
***Neonesidea* sp.**

4.7. Distribution of Foraminifera and Ostracoda

Using a data matrix composed of 23 sites and 37 species of ostracods and foraminifera (with counts of at least 100 specimens and taxa with a relative abundance greater than 3%), a cluster analysis was conducted. The results revealed three distinct groups of sites labelled A, B and C with the subclusters that were characterized by different benthic foraminiferal and ostracod associations (Fig. 10). The dominant traits of each of the three clusters and the subclusters are presented in Table 5. Samples TR18–104 and TR18–122 were not included in the analysis due to poor preservation of the shell material, possibly due to transport processes.

The Cluster analysis of the microfauna identified three main clusters, of which the A group was divided into three subclusters, whereas B and C were divided into two subclusters respectively.

Cluster A is distinctly subdivided into its constituent sub-clusters, namely A1, A2, and A3. It represents a collection of environmental samples from diverse locations (river mouth, river course and mangroves), exhibiting varying levels of species diversity, sediment composition and contamination or enrichment of heavy metals.

Three samples (TR18–118, TR18–107, and TR18–123) located in the mangrove mudflats and the river channel were included in subcluster A1. They have low species diversity and abundance, no living Ostracoda, with a relatively high number of living Foraminifera in TR18–118 (30%) and TR18–123 (27%), without characteristic taxa, covering a broad variation in salinity (15–31). The FAI showed high variation in test malformations, with no abnormalities in TR18–118 and TR18–123 and reaching 23% in TR18–107. The mean LOI content was (9.2%), with sediments containing high silt content (87–89.5%) and lowest sand content (0.01–1.5%) (Fig. 3). These particulate samples showed extremely high PLI values, being highly enriched with Co, Ni and As.

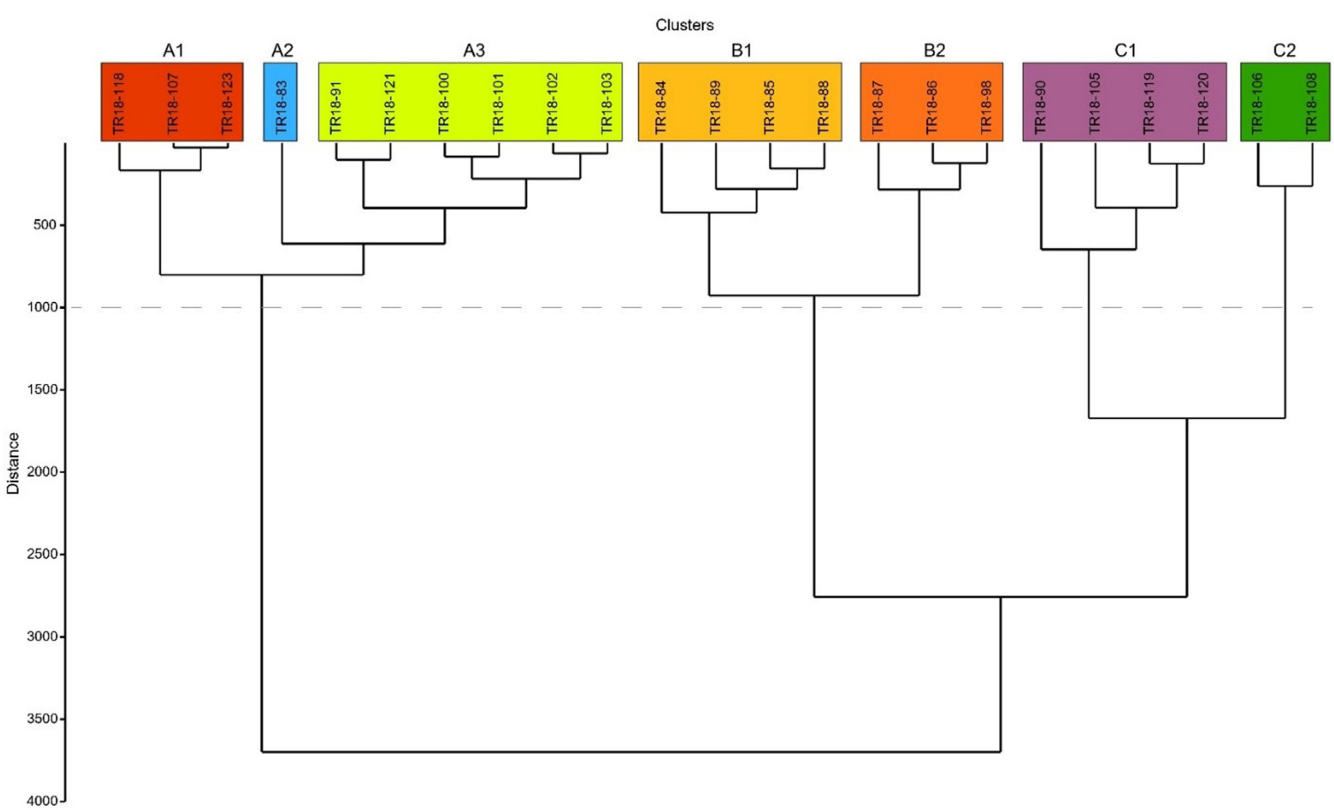

Fig. 10. The dendrogram of a cluster analysis of microfossil distribution.

Table 5
Cluster descriptions based on ostracod and foraminifer taxa in uMlalazi river.

Cluster	Sample	Shannon diversity index	FAI [%]	Pollution Load Index	Enrichment Factor	Taxa & Characteristics
A1	TR18–118 TR18–107 TR18–123	0.9–1.2	0–23	6–7	Co>Ni>As	No characteristic taxa. Very low diversity and abundances
A2	TR18–83	2.8	2.8	3	Co>Ni>Sr >As	Very diverse brackish-marine taxa: *Ammonia, Miliolinella, Sigmoilina, Cibicides, Pararotalia,* Globigerininae, *Cytheromorpha milleri, Sulcostocythere knysnaensis, Perissocytheridea estuaria*
A3	TR18–91 TR18–121 TR18–100 TR18–101 TR18–102 TR18–103	0.4–1.6	0–20.5	0–7	Co>Ni>Zn >As	*Ammonia, Quinqueloculina, Perissocytheridea estuaria*
B1	TR18–84 TR18–85 TR18–88 TR18–89	0.5–2.0	2.8–10.7	2	Co>Ni>Sr >As	*Ammonia, Quinqueloculina, Sulcostocythere knysnaensis*
B2	TR18–86 TR18–87 TR18–98	0.6–1.3	0.8–2.5	2–3	Co>Ni>Sr >As	*Ammonia, Quinqueloculina, Miliolinella, Sulcostocythere knysnaensis*
C1	TR18–90 TR18–119 TR18–120 TR18–105	0.9–1.9	8.2–12.4	1–4	Cu>Co>Zn >Ni>Sr>As	*Ammonia, Trochamina inflata, Sulcostocythere knysnaensis, Perissocytheridea estuaria, Australoloxoconcha favornamentata*
C2	TR18–106 TR18–108	1.1–1.4	4.4–4.5	1	Co>Zn>As >Ni	*Ammonia, Quinqueloculina agglutinans, Cribroelphidium articulatum*

With a distance of 1075 m to the river mouth and a salinity of 31.1, A2 (TR18–83) is the subcluster closest to the Indian Ocean. A2 is characterized by high number of living Foraminifera (24.5%), the highest diversity of marine taxa and allochthonous material. Considered allochthonous are planktonic foraminifera and strongly abraded tests or valves, often filled with consolidated sediment. The Fisher Alpha index was highest here (5.7), however with low evenness (0.6). The FAI was 2.8% with notable malformations of *Quinqueloculina, Sigmoilina* and *Cribroelphidium articulatum*. The LOI content did not show high values (1.5%), with sediments containing high sand content (96%) (Fig. 3). TR18–83 had a PLI of 3, pointing to a contamination of a low degree, however with high EF of Co, Ni, Sr and As.

A3 consists of samples from a mosaic of sites (TR18–91, TR18–121, TR18–100, TR18–101, TR18–102 and TR18–103), without a common salinity range, but with low diversities and high abundances of *Ammonia* sp., *Quinqueloculina* sp. and *Perissocytheridea estuaria*. TR18–101 yields no living Foraminifer and Ostracoda, but TR18–100, TR18–102, TR18–103 have 32%, 20% and 20% of living ostracods respectively, when TR18–91 and TR18–121 has 7% and 9% of living foraminifers. The mean Fisher Alpha (1.1) and evenness (0.6) were low. The FAI displayed a very high variation in test malformations, with abnormalities discovered only in TR18–91 (21%) and TR18–100 (8%). The LOI content showed some variation (0.5–8%), with one sample containing mainly silt (TR18–91) and the rest made up of fine sand (TR18–121, TR18–100, TR18–101, TR18–102 and TR18–103) (Fig. 3). PLI showed high variability reaching 7 in TR18–91 and descending in values in the remaining subcluster samples (0–3), nonetheless EF had high values of Co, Ni, Zn and As among all these sampling points.

Cluster B comprises of two subclusters, B1 and B2, both characterized by their location in the river channel, polyhaline salinity range, and dominant foraminifera species: *Ammonia* sp., *Quinqueloculina* sp. Both subclusters exhibit signs of deteriorating site quality and enrichment of certain elements, with B1 displaying higher species richness and a wider range of test malformations compared to B2.

B1 subcluster is located in the river channel (TR18–84, TR18–85, TR18–88 and TR18–89) and is characterized by a salinity of the polyhaline range. *Ammonia* sp., *Quinqueloculina* sp. and *Perissocytheridea estuaria* are dominant and overall, these samples have high species richness. All samples contained living foraminifers TR18–84 (11%), TR18–85 (15%), TR18–88 (9.5%) and TR18–89 (7.5%), and living ostracods were found in TR18–84 (7%), TR18–88 (32%) and TR18–89 (16%), however, TR18–85 contained no living ostracods. The mean Fisher Alpha showed higher values (1.6), than subcluster A3, but again low evenness (0.5). Test malformation was observed in all samples, however with a high range of FAI (2.8–10.7%). The LOI content showed very low values (0.7–1.3%), with all samples containing mainly sand (91–99.6%) (Fig. 3), with a notably thick oxidized layer (>30 mm). PLI for all localities showed a deterioration of the sites' quality, however with comparably low value (2) and high EF values for Co, Ni, Sr and As.

Subcluster B2 (TR18–86, TR18–87 and TR18–98) is very similar to B1 considering the salinity range, however diversity and abundances are a little lower. *Ammonia* sp., *Quinqueloculina* sp., *Miliolinella* sp. and *Sulcostocythere knysnaensis* are the characteristic taxa for theses samples. All samples contained living Foraminifera with TR18–86 (6%), TR18–87 (9%) and TR18–98 (2%), with rather low numbers of living ostracods TR18–87 (3%), TR18–98 (12%) and no living ostracods in TR18–86. The mean Fisher Alpha is a bit lower than in subcluster B1 (1.2), with the same low evenness (0.5). The proportion of tests malformations is lower, than in subcluster B1 covering a small range of FAI (0–2.5%). The LOI content showed the lowest values (0.2–0.8%), with all samples containing mainly sand (97.6–99.6%) (Fig. 3). Showing the same pattern as subcluster B1, the PLI values for all localities revealed a deterioration of the sites' quality, with a bit more elevated values (2–3) and exactly as high EF values for Co, Ni, Sr and As.

Cluster C displays a clear division into two subclusters, C1 and C2, with varying salinity ranges and specific dominant foraminifera species. Both subclusters exhibit unique patterns of species diversity, sediment composition, and contamination or enrichment.

Subcluster C1 (TR18–90, TR18–119, TR18–120 and TR18–105) covers the mesohaline range and is characterised by highest diversity of ostracods with high proportion of agglutinated foraminifera. *Ammonia* sp., *Trochammina inflata, Sulcostocythere knysnaensis, Perissocytheridea estuaria* and *Australoloxoconcha favornamentata* are the most dominant taxa. All samples contained living ostracods TR18–90 (15%), TR18–119

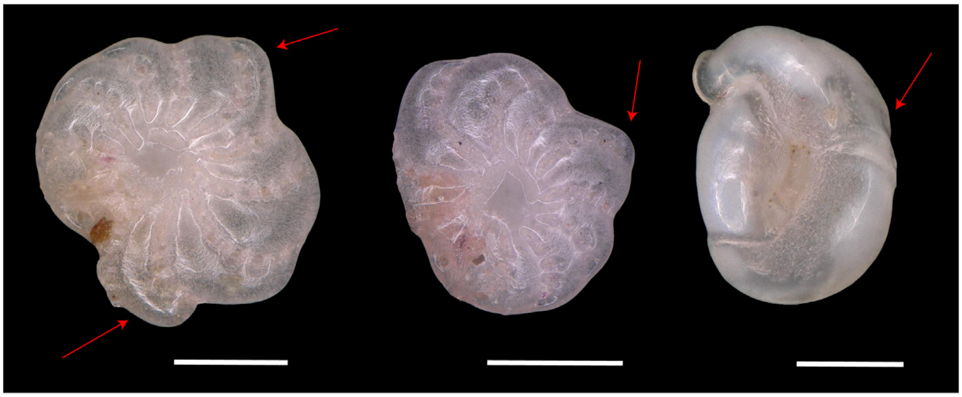

Fig. 11. Side views of malformed tests from sample TR18–107. The arrows point to malformation features such as dwarf chambers or an accessory chamber. Scale bar = 200 μm.

(12%), TR18–120 (17%) and TR18–105 (10%), and living foraminifers TR18–90 (7%), TR18–119 (41%), TR18–120 (10%) and no livings in TR18–105. The mean Fisher alpha is higher (1.8) and the evenness (0.4) is lower, compared to C2 subcluster. The FAI% is quite high in this subcluster (8–12%). The LOI content showed a high range (0.1–5%) for these sand-dominated samples (Fig. 3). The PLI values are variable (1–4) and with very high EF values for Cu, Co, Zn, Ni, Sr and As, and extreme peaks of Cu and Zn in TR18–105.

Subcluster C2 covers the mesohaline range, with two samples (TR18–106 and TR18–108) located upstream in the river channel. The cluster is characterized by high abundances of the secondarily agglutinated foraminifer *Quinqueloculina agglutinans* as well as *Ammonia* sp. and *Cribroelphidium articulatum*. This subcluster has no living foraminifers and only TR18–108 contains living ostracods (18%). The mean Fisher Alpha is lower, than in C1 (0.6), however with higher evenness (0.8). The FAI showed a lower and similar signal in both samples (c 4.5%). The LOI content is low (c 1%), matching the clear dominance of sand (c 96%) (Fig. 3). The PLI values are stable for both sampling sites (1) and with high EF values for Co, Zn, As and Ni.

4.8. Test abnormalities

Most of the studied samples contained a high number of foraminifera exhibiting abnormal test morphology with exception of TR18–101, TR18–102, TR18–103, TR18–118, TR18–121 and TR18–123. The FAI varied from a minimum of 0.8% to a maximum of 23% (TR18–107), and with higher numbers in sampling sites upstream, which are close to Mtunzini town. At the same time, some samples along the river and in the mudflats showed a low abnormality index.

Various types of test abnormalities have been reported in the literature (Geslin et al., 2000; Mancin and Darling, 2015; Polovodova and Schönfeld, 2008) and we recognized several of them: abnormal addition of a chamber, abnormally protruding chambers, reduced chamber size, distorted chamber arrangement, twisted tests, and complex forms (Fig. 11). Out of the total assemblage, five species exhibited some form of test abnormality. While species such as *Ammonia* sp., *Elphidium* sp., and *Cribroelphidium articulatum* displayed multiple types of test abnormalities, *Quinqueloculina* sp. and *Miliolinella* spp. exhibited only shape modifications with twists of the tests. *Ammonia* sp. was the species with the highest frequency of malformation, with a mean abnormality of 56%, followed by *Quinqueloculina* sp. (26%), *Cribroelphidium articulatum* (12%), *Miliolinella* spp. (5%), *Sigmoilina* sp. (2%), and *Elphidium* sp. (0.4%).

5. Discussion

5.1. Sediment characteristics

The composition of sediment in a river is influenced by a variety of factors, including the geology of the surrounding area, the slope of the riverbed, the current velocity of the water, and the amount of sediment supply. Sand is the most common sediment type found in the river (Fig. 3, Supplementary Tab. B), as it can be transported over long distances. Silt and clay particles are present in areas of low flow velocity such as the inner bends of the river or in areas close to mangroves, which are additionally trapping fine sediments through their root systems.

5.2. Geochemistry

The geochemical indices show high levels of heavy metal pollution in the natural reserve, which is likely due to anthropogenic activities. The enrichment factor, geo-accumulation index, and contamination factor showed high values for Co, Cd, and Ni, while other heavy metals had values less than 1, indicating natural sources. Further information was derived from the PLI with the highest values observed in the upstream stations and close to the mudflats, which are closer to residential buildings, a recreational area, sugarcane farms and other human activities (Fig. 12).

Vetrimurugan et al. (2016) researched bioavailable metals in tourist beaches located in Kwazulu-Natal, South Africa. Among investigated beaches was Port Durnford Beach, which lays directly at the uMlalazi river mouth. The study included a comparison of enrichment factors for various elements, revealing minor enrichment for Cu, Ni, Pb, and Co, moderate enrichment for As, moderately to severely enriched for Zn, and severely enriched for Cd. This partly aligns with our findings, as we also discovered minor enrichment of Cu and Pb (except TR18–105), moderate enrichment of As and minor to severely enrichment for Zn. However, in our case, Ni falls into moderate enrichment and Co is significantly enriched. Unfortunately, we do not have data on Cd for a direct comparison. Moreover, Vetrimurugan et al. (2016) calculated I_{geo}, classifying Port Durnford Beach as moderately polluted. Taking into account, that our sampling stations fall across all five classes, with only 4% of them being moderately polluted and the rest showing much higher I_{geo} values, together with dilution effect of the ocean water, the uMlalazi river could be a possible source of the descibed pollution of Port Durnford Beach.

Furthermore, Mehlhorn et al. (2023) investigated the contamination of organochlorine pesticides in sediments within the uMlalazi Nature Reserve. Two distinct sampling sites were chosen, one located at the border of the Mangroves and the other within the uMlalazi River near a slipway. The cumulative concentration of organochlorine pesticides (ΣOCP) in the uMlalazi Nature Reserve was found to be relatively low at 160 ± 25 ng/g. Additionally, chlorinated compound levels were detected at an average of 20 ng/g in the nature reserve. The study concluded that the contamination of organochlorine pesticides in sediments within the uMlalazi Nature Reserve is low. Based on these findings, we assume that the pesticides pollution of the uMlalazi river plays a minor role compared to eutrophication and heavy metal contamination affecting the meiofauna.

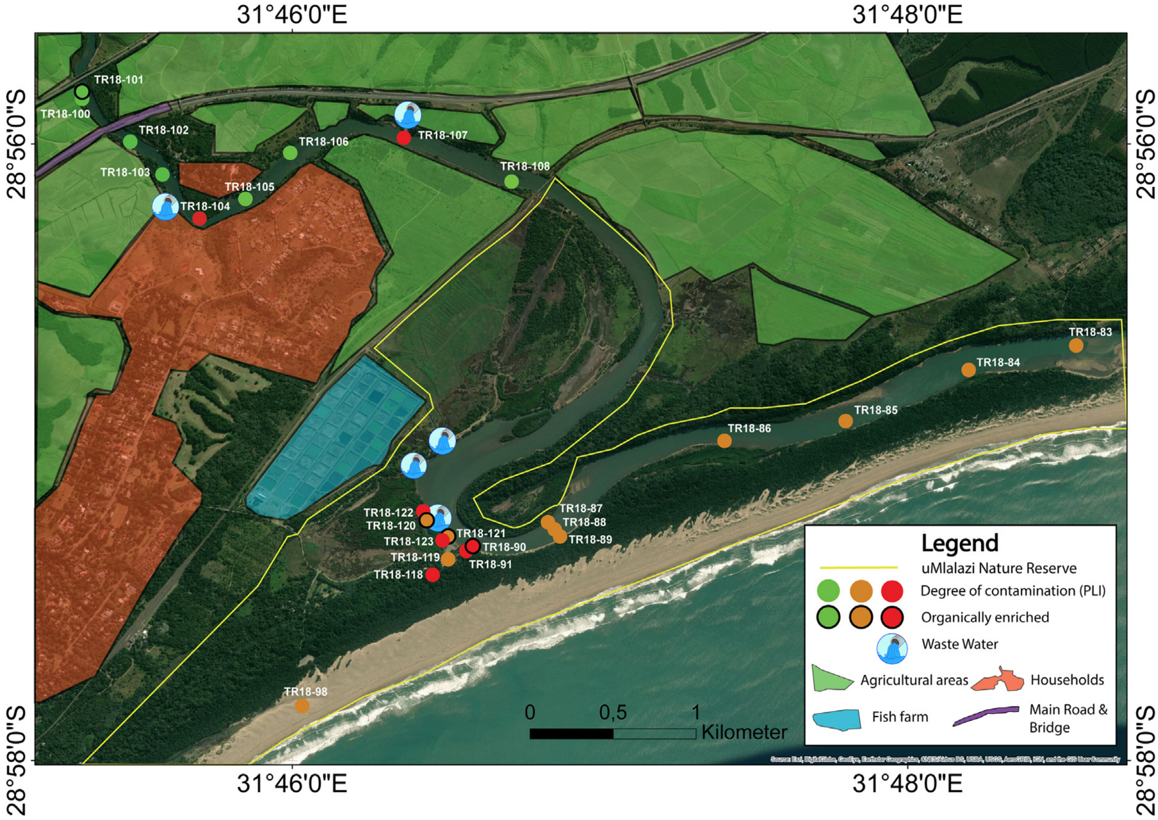

Fig. 12. uMlalazi river with sampling sites showing level of PLI and organic enrichment. Polygons are showing the primary pollution sources, based on ACER (Africa) Environmental Consultants (2019): households, waste water pipes, agriculture, aquaculture and a bridge.

We used the data from ACER (Africa) Environmental Consultants (2019) to map sewage points, which they subsequently sampled in 2019 for *Escherichia coli*, total coliforms, orthophosphate as P, ammonia as N, and nitrate as N. The findings revealed that, at all sampling stations in close proximity to sewage points, the *E. coli* counts exceeded four times the general limit, rendering the water unsuitable for recreational and drinking purposes according to water quality guidelines. Similarly, the total coliforms counts surpassed four times the general limit, indicating a significant degradation in water quality that is unfit for both recreational activities and drinking. Investigations in 2019 by ACER (Africa) Environmental Consultants (2019) revealed that the existing wastewater treatment works in Mtunzini, established in the early 1990s with a design capacity of 240 m^3/day, were overloaded and in an unacceptable operating condition. This resulted in the discharge of substandard treated sewage into the nearby the uMlalazi Nature Reserve. Emergency refurbishments and upgrades were undertaken to address these issues, increasing the plant's capacity to 480 m^3/day. However, these upgrades were considered interim and unsustainable, and the plant is currently operating at maximum capacity, depositing wastewater into the estuarine system (Mtunzini Sanitation Project – MBB, 2023)

Heavy metals concentrations in our samples exceeded threshold limits for South African and many other countries. However, there was a lack of available data from surveys on geochemistry for a direct comparison. A study conducted by Adeleke et al. (2020) examined the concentrations of Cd, Cu, Pb, and Zn in sediments from the uMlalazi estuary in the Mangroves area. The results revealed significantly elevated levels for (Cd 6.83 ± 0.06, Cu 35.63 ± 0.35, Pb 33.43 ± 1.31, and Zn 56.27 ± 1.39 µg/g) roughly doubled, compared to sediments from Durban Bay (Cd 2.73 ± 0.06, Cu 16.07 ± 0.91, Pb 12.20 ± 0.66, and Zn 38.70 ± 0.80 µg/g) and Richards Bay Harbour (Cd 3.10 ± 0.10, Cu 16.00 ± 0.44, Pb 11.43 ± 1.83, and Zn 26.07 ± 26.07 µg/g). Additionally, concentrations of Pb, Zn and Cu in water were high, especially with Zn (162.93 ± 11.23 µg/g) being significantly higher compared to both Durban Bay and Richards Bay Harbour.

The river and protected natural reserve are clearly under anthropogenic pressure from the nearby village, agriculture, deforestation, and fish farming. The mining of Titanium expected to expand further and overall anthropogenic stress may include additional sources that we may not have yet identified. This critical finding underscores the urgent need for authorities to take swift action in implementing effective quality control strategies and monitoring programs. Failure to do so on time could result in detrimental impacts on aquatic life within the protected nature reserve.

5.3. Microplastic

The distribution pattern of PET and LDPE do not match. However, TR18–104 and TR18–105, situated near the town, exhibited elevated levels of both PET and LDPE, suggesting a possible common source for these plastic types within the sediment. The density of PET is higher than of LDPE, which may explain why the particles sink to the sediment and can be retrieved in the sediment samples, while LDPE may be more dispersed and harder to detect. The higher density of PET can be explained by generation from the breakdown of larger plastic debris, as well as from sources such as microbeads in personal care products, synthetic fibres from clothing, and plastic pellets used in manufacturing. These microplastics can enter the river system through direct discharge of wastewater or through heavy rain runoff. The distinct distribution patterns of PET and LDPE in the sediment are indicative of their varying densities, underscoring different transport dynamics. This observation not only points to contrasting origins but also suggests divergent pathways through which they enter the river system.

Until now there was no evidence of direct influence of microplastics on ostracod distribution published. However, high densities of microplastic, like we discovered, can also have indirect effects ostracods by altering the physical and chemical properties of sediments, affecting food availability and habitat quality. On the other hand, for foraminifera there are already several studies published.

Despite the critical role of foraminifera in marine ecosystems, Bouchet et al. (2023) identified only four studies addressing the impact of microplastics (MPs) and nanoparticles (NPs) on these organisms. Nonetheless, those studies showed that plastics can significantly alter foraminiferal feeding behaviour, inducing oxidative stress. The study by Ciacci et al. (2019) revealed that exposure to polystyrene nanoparticles induced physiological stress in *Ammonia parkinsoniana*, leading to the accumulation of neutral lipids and increased production of reactive oxygen species. Joppien et al. (2022a) observed that *Amphistegina gibbosa*, a coral-reef species, fed indifferently on seawater-soaked polyethylene microplastics and *Artemia* sp. Nauplii, altering its heterotrophic behaviour. Birarda et al. (2021) reported the colonization of plastic bags, likely polyethylene, by foraminifera species, with observed protein beta-sheet accumulation suggesting oxidative stress cytotoxicity. Laboratory experiments by the same authors demonstrated the incorporation of bis-(2-ethylhexyl) phthalate in the cytoplasm of *Rosalina globularis*. However, Langlet et al. (2020) found no change in motion behaviour or respiration rate in *Haynesina germanica* exposed to leachates from virgin polypropylene pellets.

Additionally, Joppien et al. (2022b) conducted a laboratory experiment exploring the impact of the global marine plastic pollution on larger benthic foraminifera (LBF). In contrast to previous research focused on physiological responses in specific organism groups, this study revealed a novel observation: polymer nanoparticles incorporating into the calcite skeleton of LBF. More specifically, LBF demonstrated rapid nanoplastic ingestion and incomplete egestion, with microalgae presence enhancing initial feeding responses. Despite 40% of ingesting LBF expelling nanoplastics, residues attached to test surfaces were encrusted by calcite. This underscores the need for further investigation into plastic pollution's broader impacts on calcifying organisms like LBF, emphasizing their potential role as plastic sinks and the consequential effects on sediment production.

Bouchet et al. (2023) noted a challenge in synthesizing findings, because of the methodological heterogeneity among the studies, impeding direct comparisons. To overcome this limitation and comprehensively address the potential hazard of plastic on foraminifera, they propose the development of a roadmap for future research in this area. Such a roadmap could provide a standardized framework for investigating the multifaceted interactions between foraminifera and plastic pollutants, contributing to a more cohesive understanding of the microplastic impact. Overall, Bouchet et al. (2023) underscore the various pathways through which foraminifera are exposed to plastic, including inclusion in the test, ingestion, and exposure to plastic leachates.

5.4. Microfauna

Main natural factors influencing the distribution of Ostracoda and Foraminifera in estuarine systems with a strong tidal impact are salinity and elevation of intertidal microhabitats in relation to low- and high-water levels. Sudden changes in abundance and species diversity can be correlated with environmental changes such as current velocity, salinity, temperature, or pollution (Francescangeli et al., 2018; Martins et al., 2016). It should be noted, however, our sampling was conducted during a dry season at the end of August, which falls outside the heavy rainfall period. Thus, we sampled under more stable environmental conditions with less erosion and transport and higher salinities along the estuary than during rainy season.

It is essential to note the salinity-related distribution patterns of foraminifer and ostracod species within the river ecosystem. Upstream areas of the river host brackish species like *Perissocytheridea aestuaria* and *Australoloxoconcha favornamentata*, whereas planktonic foraminifera such as Globigerininae have been observed at the river mouth, suggesting a clear transition from mixohaline waters to open marine influence within the course of the estuary.

Generally, the intertidal areas are covered by water only periodically. However, sites higher within the intertidal range are covered for shorter periods than sites lower within the intertidal range which affects the abundance of foraminifer associations along normalised water depth (Fig. 13). Based on this observation, Strachan et al. (2017) reported a zonation for intertidal and supratidal foraminifera abundances for several South African estuaries which therefore has the potential to be used for the reconstruction of past sea levels from fossil foraminifer associations. Ostracods occur within the intertidal zone as well but prefer tidal ponds because of their lower tolerance against air exposure (Scheder et al., 2019). Furthermore, strong mixing of autochthonous and allochthonous faunal elements must be considered in the intertidal zone.

Clear elevation-bound distribution patterns are recognisable for many species (Fig. 13). Taxa as *Cribroelphidium articulatum*, higher numbers of *Nonion* sp. and *Sulcostocythere knysnaensis* are restricted to subtidal environments whereas *Aurila kliei, Mutilus bensonmaddocksorum, Trochammina inflata* and *Portatrochammina murrayi* could be found in the upper intertidal range only. These observations are in good agreement with results by Strachan et al. (2017) for foraminifers of the uMlalazi estuary. Our results demonstrate the high potential of foraminifera and ostracods as palaeo-sea level indicator. Their future application needs, however, more regional distribution data along the elevation gradient.

Among ostracods, the dominant species include *Perissocytheridea estuaria, Sulcostocythere knysnaenis,* and *Australoloxoconcha favornamentata. Perissocytheridea estuaria* is an estuarine species known to prefer high water tide levels (Benson and Maddocks, 1964). *Sulcostocythere knysnaenis* is an extremely adaptable estuarine brackish water species that thrives predominantly at low water neap tide levels. Interestingly, Hartmann (1974) observed that Sulcostocythere *knysnaenis* occupies the ecological niche previously associated with the *Cyprideis* genus in the Cape. *Australoloxoconcha favornamentata*, on the other hand, demonstrates a tolerance for salinity fluctuations in polyhaline-marine areas and is typically found in the lower parts of the mangrove-covered upper tidal flats according to Hartmann (1974), which does not align with our observations.

Overall, our results show significant variations in live/dead ratios, species abundances, and habitat structures among samples. Higher live/dead ratios suggest destruction or washing away of empty foraminifer tests and ostracod valves, while differences in counts and percentages indicate species-specific responses to salinity variation and heavy metals enrichment. Lower density and species diversity are known to be found in water bodies of strong currents such as fast flowing rivers or tidal zones with wave activity due to mechanical abrasion of smaller and less resistant foraminiferal specimens (Armynot du Châtelet et al., 2009; Francescangeli et al., 2018). This effect was observed in most of the studies carried out in polluted aquatic environments (Bodergat and Ikeya, 1988; Gildeeva et al., 2021; Irizuki et al., 2015; Schafer, 1970). For the effects of pollution onto microfauna composition see the following chapter 5.5.

5.5. Effects of pollution

Our findings indicate a positive correlation between higher organic matter and increased heavy metal concentrations. Specifically, sampling sites located closer to villages, within the bends of river meanders where the current velocity is lower, and in the river estuary and mouth influenced by tidal flow, exhibit higher LOI values due to enhanced deposition. The proximity to villages and the decreased velocity within river bends can contribute to the retention of pollutants and organic matter, resulting in elevated LOI values. In the sediment sample TR18–104, we detected a remarkably elevated sulfur concentration of 60 ppm, and no meiofauna was observed within. Moreover, three out of four

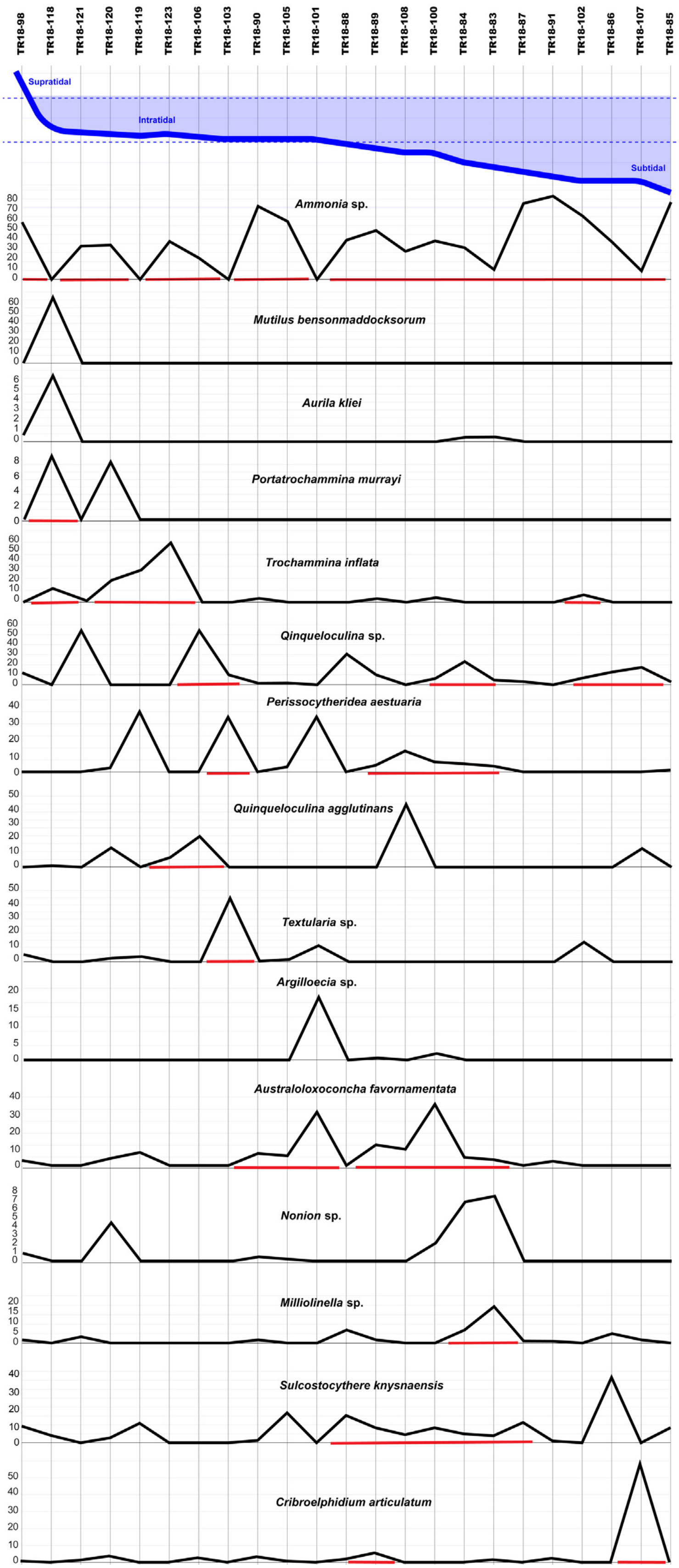

Fig. 13. Elevation-bound distribution patterns of Foraminifera and Ostracoda. Red lines indicate occurrences of living individuals. Y axis shows relative abundances.

most organically enriched samples could be connected to the wastewater from the aquacultures. However, we believe that the enrichment factors (Fig. 6) do not sufficiently describe the relative extent of anthropogenic organic enrichment for all samples due to the high abundance of sandy sediment, which makes it hard to evaluate the actual situation in the uMlalazi Nature Reserve (for more details see Supplementary Table A).

When the source of pollution is severe and well identified (sewage outfall and industrial waste), a barren zone with very low diversity occurs around it. In our study, both abundance and diversity increase repeatedly away from the village and mudflats, with highest richness closer to the river mouth. Similar results were found from different parts of the world (Armynot du Châtelet et al., 2004; Barut et al., 2015; Debenay et al., 2001; Ruiz et al., 2005; Tan et al., 2021). Thus, a lowering in diversity and abundance of foraminifers can be considered as pollution indicator if adverse taphonomical effects can be excluded.

Ortega-Cisneros and Scharler (2015) studied the nutrient dynamics of estuarine invertebrates in the uMlalazi River. Their investigation involved analyzing elemental content (C, N), stoichiometry (C:N), and diet composition, considering seasonal variations in river inflow and rainfall within the permanently open uMlalazi Estuary. The findings highlighted a significant impact of river inflow on suspended organic matter and, to a much lesser extent, on sediment organic matter, which backups our findings and confirms a low disturbance of meiofauna.

Many studies discovered a correlation between heavy metal pollution and abundance of benthic Foraminifera (e.g., Cong et al., 2022; El-Kahawy et al., 2018; Frontalini and Coccioni, 2011; Li et al., 2015) and of Ostracoda (Gildeeva et al., 2021; Ruiz et al., 2004; Ruiz et al., 2006; Schornikov, 2000; Tan et al., 2021; Zenina, 2009). According to these studies, an increase in the concentration of certain heavy metals, including Cu, Cd, Pb, and Zn, is typically associated with a decrease in species richness and abundance of both groups. In areas that are heavily polluted, benthic foraminifera may disappear and more tolerant species will take over the microhabitats, then they can exhibit morphological deformities or vanish as well. For ostracods there are no morphological changes known so far, but the same tolerant species adaptation scheme, migration, or a complete vanishing are visible. Studies have shown that exposure to high levels of heavy metals can affect the growth, reproduction, and survival of benthic foraminifera and ostracods (Cong et al., 2022; Barut et al., 2015). For example, Youssef et al. (2021) showed that high concentrations of heavy metals in sediment were associated with reduced species diversity and abundance of benthic foraminifera in the Gulf of Aqaba, Red Sea. Further, Shuhaimi-Othman et al. (2011) found that exposure to high levels of cadmium led to reduced survival and reproduction of ostracods in laboratory experiments. In alignment with previous studies, our investigation in the uMlalazi River revealed an absence of living Foraminifera and Ostracoda in heavily polluted sites adjacent to two upstream sewage points (TR18–104 and TR18–107). Furthermore, sample TR18–104 had no meiofauna within.

The analysis of the SQGs is consistent, showing high values for the same sites where also other indices are high and therefore imply adverse effects on aquatic organisms such as benthic foraminifera and ostracods. In areas with elevated heavy metal concentrations, we noticed decreased abundances, lower diversity and decreasing proportions of living Ostracoda, sometimes leading to samples devoid of fossilisable meiofauna. Conversely, in samples with lower heavy metal levels, we found greater diversity and higher abundances in both studied meiofaunal groups.

The contaminated samples were primarily dominated by foraminifera species such as *Quinqueloculina* spp., *Ammonia* sp., and *Miliolinella* sp. Species of these tree genera tend to exhibit greater resilience to anthropogenic pressures (Elshanawany et al., 2011; Gildeeva et al., 2021). Slightly less prevalent was *Cribroelphidium articulatum*, which is also recognized for its tolerance, as smaller elphidiid species with a rounded periphery possess the ability to adapt to changes in food availability and environmental conditions as well as the ability to change from epifaunal to infaunal habitats (Debenay et al., 2001). Ostracods were less abundant in these polluted environments, explaining the prevalence of foraminifera in such conditions. Based on our results, Ostracods seem to be more sensitive to organic pollution and heavy metals, compared to Foraminifera.

A Mann-Whitney test of species distributions between the highly and lowly polluted stations underlined a species-specific correlation with polluted environments. Because of low sample numbers, however, the significance of the discrimination was low: *Australoloxoconcha favornamentata* is less tolerant to pollution ($p = 0.22$) and *Perissocytheridea estuaria* is more sensitive to pollution ($p = 0.06$).

The exact mechanisms by which heavy metals affect these organisms are not well understood, but it is assumed that they can interfere with cellular processes such as enzyme activity and DNA repair, as well as affect the functioning of organs such as the gills and digestive system (Le Cadre and Debenay, 2006).

5.6. Shell chemistry

Heavy metals showed higher values in *Quinqueloculina* sp. tests, than in *Ammonia* sp. Both species are benthic and have calcite tests, but *Quinqueloculina* is a miliolid genus with a high-Mg shell whereas *Ammonia* belongs to the rotaliids possessing a hyaline shell with low Mg-content. The different concentrations of heavy metals with generally higher values in *Quinqueloculina* may be due to difference in biomineralization processes during shell formation, which can affect the incorporation of metals. Rotaliida, to which *Ammonia* belongs, synthesize calcium carbonate by modifying water chemistry and pH by physiological processes involving vacuoles, endo- and exocytosis, ion pumps and membranes (De Nooijer et al., 2014; Weiner and Dove, 2003). On the other hand, the exact biomineralization path of Milioliida, to which *Quinqueloculina* belongs, is still not understood. The significant difference is that Rotaliida synthesize and build up their calcite crystals and their shells extracellularly, while Milioliida synthesize calcite needles in vacuoles, and these are transported extracellularly (Robbins et al., 2017).

Calcareous shell-bearing foraminifera have the capacity to absorb heavy metals from their surrounding environment, although with notable variations in their uptake rates as demonstrated in the study by Khokhlova et al. (2022). Nevertheless, recent research indicates that while the chemical composition of foraminiferal shells provides valuable insights, their soft tissues offer a more accurate representation of the aquatic environment's status, as highlighted by Montroni et al. (2021).

The comparison of heavy metal concentrations in foraminiferal shells with sediment values did not show a clear correlation, despite both displaying elevated heavy metal levels. This discrepancy may be attributed to factors such as limited heavy metal bioavailability in the sediments, variable transport mechanisms affecting metal exposure, and asynchronous deposition processes. Further research is required to understand the specific influences of these factors and better understand the observed discrepancies in heavy metal concentrations between foraminiferal shells and sediment samples.

6. Conclusions

This study provides the first integrated analysis of estuarine Ostracoda and Foraminifera in the KwaZulu-Natal region of South Africa and contributes new data to the sparse knowledge on the distribution and ecology of these taxonomic groups in Africa.

Comparing the faunal composition between different associations, we observe distinct patterns that can be attributed to factors such as productivity, hydrographic regime, transport of allochthonous material, and pollution. These drivers play a significant role in shaping the faunistic patterns observed in the study area. Furthermore, the influence of tide in the river estuary affects sediment deposition, which could also lead to higher organic matter contents. These areas serve as depositional zones for allochthonous material transported by the tide, which can contribute to increased faunal productivity and altered faunistic patterns.

Each species of Ostracoda and Foraminifera has its unique ability to tolerate different environmental parameters and types of pollution. In anthropogenically impacted areas, we often observe the dominance of resilient taxa such as *Quinqueloculina* and *Ammonia* (e.g., Sgarrella and Moncharmont Zei, 1993). *Quinqueloculina* is known for its ability to thrive in stressed environments (Alve, 1995; Debenay et al., 2001) and its tolerance to various pollutants (Setty, 1982; Yanko et al., 1994). It has also been associated with high numbers of test abnormalities in studies, indicating either anthropogenic pressure or significant salinity variation (e.g., Yanko et al., 1994; Geslin et al., 2000; Geslin et al., 2002).

The high FAI values obtained in our study indicate severe disturbances in the river ecosystem. It is well-established that FAI values are known to be influenced by high heavy metal concentrations and variable salinity, as confirmed by Geslin et al. (2002). In our case, the elevated FAI values were strongly linked to high concentrations of heavy metals and variable salinity, implying that anthropogenic activities played a role as contributing factor.

In our observations, *Australoloxoconcha favornamentata* exhibited lower tolerance to pollution, as indicated by its distribution in less polluted areas ($p = 0.22$). *Perissocytheridea estuaria* showed sensitivity to pollution, with a p-value of 0.06, suggesting potential significance.

Consistent with the common trend observed in highly polluted environments, the results of our study show lower species diversity associated with sites of higher heavy metal concentrations or complete mortality where no living fauna was observed. The highest species richness and abundances were found near the river mouth, where marine salinity prevails. These sites reflect the accumulation of allochthonous specimens originating from the open sea. However, while we consider tides as hydrological characteristics, the setup of our study cannot further distinguish between the effects of pollution and elevation on diversity. To mitigate potential confounding factors, we considered establishing reference sites at various elevations for future studies. These sites would serve as benchmarks for minimal pollution conditions, enabling a clearer comparison. Furthermore, we believe that these combined strategies will contribute to a more comprehensive understanding of the nuanced interplay between pollution and elevation effects on biodiversity.

According to the latest report from the Department of Water and Sanitation (2022), the uMlalazi River is facing significant fishing pressure and moderate levels of flow and habitat degradation. The system also experiences some pollution pressure, particularly from agriculture and aquaculture activities. Despite being considered ecologically highly important and forming part of a protected area, the river is undergoing notable stress. In their overall assessment of the uMlalazi estuary, the Department rates the pollution level as low, indicating that the river is fully protected.

Our findings clearly show that the state of the uMlalazi river has drastically changed compared to results of Ortega-Cisneros (2013), until when the river maintained relatively unaltered conditions compared to its pristine state. Based on one among a few available reports on the water quality state of uMlalazi estuary, the total coliforms count was four times higher than the General Limit in 2019 (ACER (Africa) Environmental Consultants, 2019). The report states that based on water quality guidelines, the water in uMlalazi river is not suitable for recreational activities or drinking purpose. The study by Adeleke et al. (2020) compared Durban Harbour, Richards Bay Harbour and uMlalazi Nature Reserve and strikingly found that the nature reserve has highest concentrations of heavy metals in the sediment and water samples among the studied areas. On the other hand, Adeleke et al. (2020) found lower concentrations of heavy metals in crab tissues, compared to the other two harbours, which indicates lower bioavailability of metals in the uMlalazi estuary. It is clear, that there is an urgent need for water quality monitoring and renaturation strategies to be in place. Therefore, the conclusion of the Department of Water and Sanitation (2022), raises significant concerns, as it appears to be highly questionable given the reported pressures and clear environmental impact.

The concentrations of heavy metals in the uMlalazi river exceed the TECs established by the South African National Guideline on Sediment Quality. As, Cr and Cu are among the heavy metals that are above these limits. In addition, Cr and Cu exceed the TET based on the screening level concentration approach for freshwater sediments (Persaud et al., 1993). These elevated concentrations not only indicate severe sediment contamination, but also raise concerns regarding the bioaccumulation of pollutants in sediment-dwelling organisms and the potential for biomagnification up the food chain, suggesting a broader ecological impact.

Our study identified indications of other pollutants, such as extremely high sulfurconcentration and the presence of PET particles in the river. While the direct negative effects of PET particles on Ostracoda are yet to be established, their abundance raises concerns about the potential influence on aquatic life.

The combined use of Ostracoda and Foraminifera in our study has revealed complex patterns in relation to anthropogenic impacts and other environmental factors, confirming findings from previous studies on these taxa mentioned above. Our study therefore could prove that Ostracoda and Foraminifera have great potential as sentinel organisms for detecting and assessing environmental stress, in line with various studies (e.g., Gildeeva et al., 2021; Ruiz et al., 2005; Schornikov and Zenina, 2014).

In order to implement Foraminifera and Ostracoda in water quality monitoring management there is more information needed. A clearer correlation between meiofaunal associations and the effects of pollution must be established, further observations are necessary. Continued seasonal monitoring in the study area will improve our ability to draw explicit conclusions and to better understand the relationship between meiofaunal communities and the impact of pollution in estuarine ecosystems of South Africa.

Declaration of competing interest

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.

Data availability

The micropalaeontological material will be transferred to the collection of the South African Museum in Cape Town. Data are available on PANGAEA (https://www.pangaea.de/).

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Supplementary materials

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