



Analysis of potentially hazardous metals and health risk assessment to safeguard shellfish-dependent livelihoods for food security

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ABSTRACT

Shellfish are an important source of proteins, essential minerals, and vitamins vital for many coastal communities in Africa, however, consuming metal contaminated shellfish can pose health risks to humans. Using filter feeders and grazers as bioindicators, we investigated the trophic transfer of metals along the food web, their bio-magnification/biodilution, non-carcinogenic and carcinogenic health risks associated with the consumption of shellfish at urbanised and non-urbanised (natural) sites along South Africa's southeast coast. Filter feeders bioaccumulated/biomagnified elevated Cd, Co, Ni, As, Cu and Zn at urbanised sites in higher doses than grazers. Comparably, $\delta^{15}\text{N}$ was enriched in samples from urban sites with the highest $\delta^{15}\text{N}$ values being recorded in filter feeder and grazers. Ni, Pb, Cu and Zn showed significant trophic magnification ($\text{TMS} > 1$, $p < 0.05$), with Al, Cr, and As showing weak magnification, while Cd showed biodilution ($\text{TMS} < 1$). Considering elevated risk quotients (EDI, EWI, THQ, HI > 1) for Cr, Cd, Cu, and target carcinogenic risk ($\text{TCR} > 10^{-4}$) for As, Cd in filter feeders and grazers, this poses health concerns for the consumption of edible shellfish (e.g., mussels, oysters, and limpets) along this coastline. This study underscores the critical need to avoid consumption of metal contaminated shellfish to safeguard seafood security and protect the livelihoods of recreational and subsistence gatherers, particularly in the Global South.

1. Introduction

Globally, over 110 million people rely on subsistence fishing to serve their daily nutrient needs (FAO Food and Agriculture Organization Fisheries, 2024). Coastal systems provide sustenance and livelihood for humans, with seafood being a significant food security for the world's growing population (Okafor-Yarwood et al., 2022). Metal contaminants accumulated in seafood, however, threaten food safety associated with shellfish consumption (Dey et al., 2024). African livelihoods are dependent on shellfish subsistence for food security and livelihoods, especially in South Africa, where the proportion of subsistence gatherers is notably high (Virdin et al., 2023). Thus, assessing the link between the risk of metal contamination and subsistence fisheries is crucial for providing awareness and reducing the exposure of livelihoods dependent on subsistence gathering in the Global South.

Research on the rising levels of toxic contamination in coastal ecosystems has primarily aimed to determine metal concentration levels, assess ecological impacts, understand the bioaccumulation ability of coastal species, and identify associated health risks (Ansari et al., 2004; Yap et al., 2016; Kumar et al., 2019; Birch et al., 2020; Aboal et al., 2023). Environmental contaminants such as metals accidentally or deliberately enter the aquatic environment through anthropogenic sources and natural means (Gu et al., 2022), including coastal development (Birch et al., 2020). Although coastal development globally provides multiple economic benefits by supporting fisheries and aquaculture industries, the maritime, mineral, and tourism sectors, coastal regions still face metal pollution challenges that worsen the state of water quality (Gu et al., 2022).

Metals and risk assessment-based studies have addressed the impacts of metal pollution worldwide (Whyte et al., 2009; Olmeldo et al., 2013;

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Yap et al., 2016; 2021; 2023; Liu et al., 2018; 2019; Yang et al., 2021; Barchiesi et al., 2020; Garcia-Rico & Tejeda-Valenzuela, 2020), with little focus on Africa (Kouali et al., 2022; Fourie et al., 2022; Nekhoroshkov et al., 2021; 2024). Archaeological evidence proves, prehistoric African human societies heavily relied on shellfish for protein (Habu et al., 2011; Szabó, Amesbury, 2011; Parkington, 2003; Agbekpomu et al., 2021), a tradition that is maintained to today, as large groups still harvest seafood for recreational and subsistence purposes (Agbekpomu et al., 2021). Given this historic dependence on shellfish, this regional gap in knowledge is of concern, as resources are increasingly accumulating pollutants. South Africa places significant importance on seafood consumption for maintaining good health both presently (Barendse et al., 2018; Duggan et al., 2020), and historically, as evidenced by prehistoric shell middens (Habu et al., 2011; Szabó, Amesbury, 2011; Parkington, 2003). In addition to being a primary source of protein for many rural residents along the southeast coast of the country (Lasiak, 1993), shellfish provide essential minerals and vitamins crucial for human health (Agostini et al., 2024). The consumption of shellfish to obtain necessary nutrients may, however, reflect an increased ingestion of metal contamination (Sioen et al., 2007). Consequently, the benefits are outweighed by the negative health risks posed by metal uptake, since ingested toxic metals can cause kidney damage, gastrointestinal distress, immune dysfunction, and carcinogenic risks as outlined by the Food and Agricultural Organisation/World Health Organisation (FAO/WHO, 2011; 2019).

The coastal and marine environment of South Africa supports the national economy through various industries, including tourism, small-scale and commercial fishing, shipping, and operations of offshore exploration for oil and minerals (Turpie, Wilson, 2011). Although the exploration of natural resources in industries provides economic benefits, these activities have direct repercussions on water quality and can lead to the presence of various metals in the coastal environment (Turpie, Wilson, 2011; Krug et al., 2024). Moreover, inland activities, like illegal artisanal mining of toxic elements and agricultural wastewater discharge, have a detrimental impact on water quality (Dube et al., 2024), especially in coastal waters, as metals from inland aquatic bodies end up in the coast. The pollutants deposited into coastal systems can be bioaccumulated by organisms (Rainbow, 2002). In a food chain, metals can biomagnify or biodilute (Sun et al., 2020), with biomagnification being the process of a gradual accumulation of toxic substances, including metals, in higher levels of the food web; while biodilution is a gradual decrease in accumulation of metals in higher levels of the food web (Borga et al., 2011). Along urban coastal areas of South Africa, recent research has indicated increasing metal contamination within urban ecosystems (Wepener & Degger, 2020; Fourie et al., 2022; Sparks, Awe, 2022; Mbandzi-Phorego et al., 2024), however, information regarding links of metal pollution to bioaccumulation and trophic transfer is still largely lacking (Erasmus et al., 2020; Mbandzi et al., 2022).

One of the key techniques used to understand metal transfer is tracking the nitrogen stable isotopes ($\delta^{15}\text{N}$) which can indicate biomagnification/biodilution of metals in the food chains (Liu et al., 2018; Sun et al., 2020; Gu et al., 2022). $\delta^{15}\text{N}$ estimates an organism's trophic level and serves as a natural biomarker of the flow of energy in trophic webs (Peterson & Fry, 1987). Coastal filter feeders, which sieve particulate matter when feeding, and grazers, which feed on algae, are good biomarkers for environmental biomonitoring as these organisms are largely sedentary, abundant, readily sampled, can accumulate metals/metalloids (e.g., aluminium-Al, arsenic-As, cadmium-Cd, copper-Cu, cobalt-Co, chromium-Cr, nickel-Ni, lead-Pb and Zn), and some of them are regularly harvested for human consumption (Rainbow & Phillips, 1993; Reguera et al., 2018).

Along the South African coastline, several studies show that metal concentrations in coastal biota are site-specific, with elevated levels recorded near ports, industrial discharges, and urban runoff (Sparks et al., 2018; Nekhoroshkov et al., 2021; Fourie et al., 2022; Puccinelli

et al., 2022; Mbandzi-Phorego et al., 2024), suggesting localised contamination hotspots. Although some South African coastal sites comply with seafood safety limits (Sparks et al., 2018; Firth et al., 2023), Cd, Pb, Cr, Fe, Ni, Zn, As, Se concentrations have been reported along the south east coast and west coast to exceed the safety thresholds (Fourie et al., 2022; Mbandzi et al., 2022; Nekhoroshkov et al., 2024), posing potential hazardous health concerns. Metal assessments in mussels and other seafood generally show THQs < 1 for Mn, Fe, Co, Ni, Se, Sr, Sb, and U (Nekhoroshkov et al., 2021; 2024) and for Pb, Hg, and Cd (Fourie et al., 2022), indicating low non-carcinogenic risk for consumers. Coastal communities that rely on small-scale or subsistence harvesting can be disproportionately affected in areas where local contamination occurs, and where seafood safety will be reduced, leading to long-term health concerns (Dey et al., 2024). Spatial monitoring remains uneven and temporally inconsistent and limited species-specific transfer studies make it difficult to assess chronic exposure for the coastal communities in Africa. In South Africa, only a few studies have focused on the transfer and bioaccumulation patterns of specific metals/metalloid such as Cd, As, Ni, and Pb in filter-feeding and herbivorous shellfish (Erasmus et al., 2020; Mbandzi et al., 2022). This gap limits our understanding of potential human health risks associated with shellfish consumption, particularly from species that play a key role in metal transfer within coastal food webs. Addressing this knowledge gap enhances the novelty and relevance of the present study in contributing to national seafood safety assessments and marine pollution monitoring frameworks.

There is limited work on the transfer of metal in the food web in South Africa, as such this study aimed to provide insight into the extent of metal pollution at urbanised and natural coastal sites by tracking the trophic transfer of metals within a food web and assessing potential health risks through contaminated shellfish consumption. Due to different feeding behaviours of the target shellfish (filtering and grazing), we expected filter feeders to exhibit higher biomagnification of metals than grazers due to the more direct exposure to contaminated water. As the main source of human exposure to toxic metals is through the consumption of contaminated seafood (Yap et al., 2016), we expected toxic indices of metals to indicate human health risks linked to shellfish ingestion at urbanised sites.

2. Methodology

2.1. Study sites

Sampling took place between March and April 2023 at six stations along the southeast coast of South Africa, including four urban sites and two natural ones. The four urban sites included two large ports located inside Algoa Bay, Port of Ngqura and Port of Port Elizabeth, and two small ports/marinas, the Port of St Francis and the Port Alfred Marina. Kini Bay and Cannon Rocks, were chosen as natural sites (Fig. 1). The urban sites were selected based on their proximity to the industrial, agricultural and anthropogenic activities. The two major ports, Port of Ngqura and Port of Port Elizabeth receive contamination inputs from the urbanised Swartkops and Bakaens river, both influenced by industrial and peri-urban activities (Emmerson et al., 1983; Newman & Watling, 2007; Adams et al., 2019). Port Alfred Marina is affected by the Kowie estuary, linked to nearby agricultural and recreational activities (Watling, Watling, 1983). The Port of St Francis is influenced by the Kromme estuary, which delivers metal-rich sediments to the Bay (Watling & Watling, 1981). In contrast, no industrial activities occur at the close proximity of the natural sites.

2.2. Sample collection

Samples of particulate organic matter (POM), algae, filter feeders, and grazers were collected in triplicate from each site to assess metal concentrations and trophic transfer of metals. Seawater samples (300 mL), food source of filter feeders, were filtered on-site through

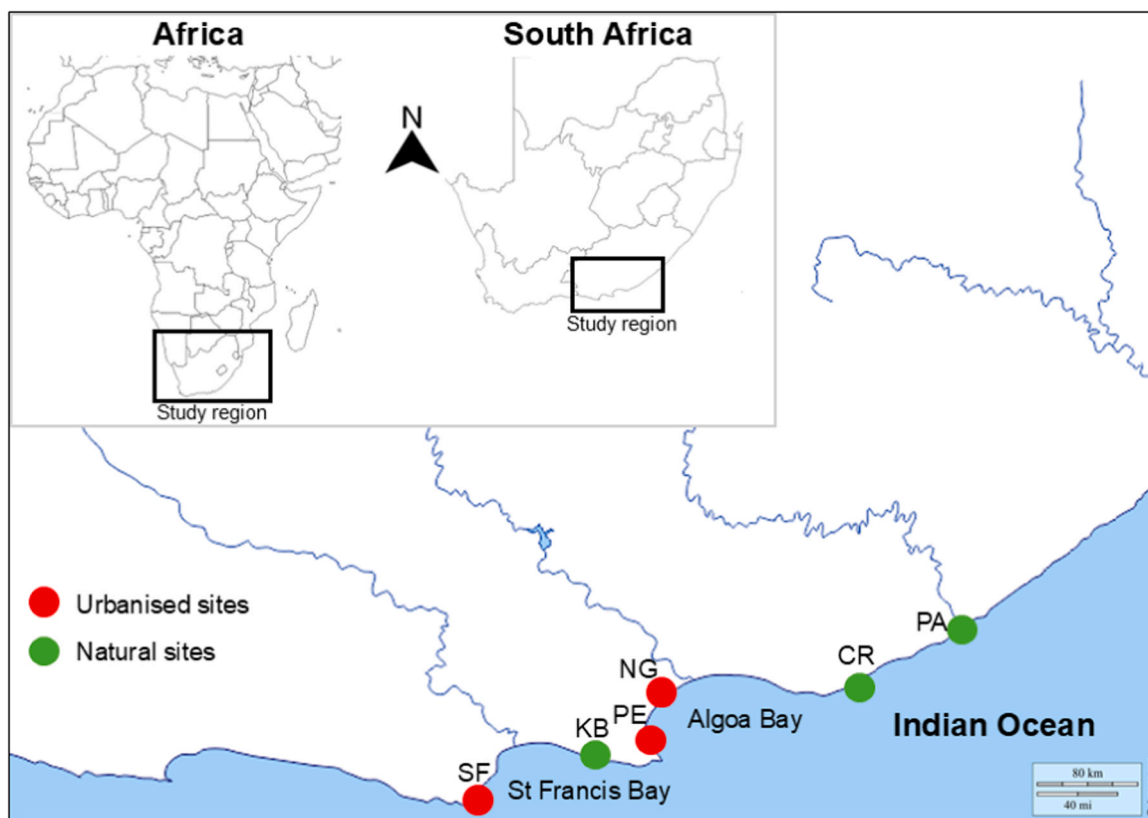


Fig. 1. Study area located within the south east coast of South Africa, including the six sampled sites, four urbanised (red) Port Alfred Marina (PA), Port of Ngqura (NG), Port of Port Elizabeth (PE), Port of St Francis (SF) and two natural sites (green), Cannon Rocks (CR) and Kini Bay (KB).

ashed (450 °C for 5 hr) GF/F filters to capture POM, and stored in a foil envelope at −20 °C pending further analysis. Samples of algae, including *Ulva lactuca* (Linnaeus 1753), *Gelidium pristoides* (Turner) Kützinger 1843, *Pseudoralfsia verrucosa* (Areschoug) Parente, Fletcher & G.W. Saunders, 2020, formally known as *Ralfsia verrucosa* were collected using nitric acid-soaked and cleaned stainless-steel scrapers. POM and algae represented the food sources for filter feeders and grazers, respectively. Algal samples were not always present at all sites: *U. lactuca* and *G. pristoides* could be collected at natural sites only (Cannon Rocks, Kini Bay), while *P. verrucosa* was collected at urbanised sites only (Port of Ngqura, Port of Port Elizabeth). The targeted adult invertebrate species for this study included filter feeders: mussel *Perna perna* (Linnaeus 1758), barnacle *Octomeris angulosa* (Sowerby 1825), and oyster *Magallana gigas* (Thunberg 1793), formally known as *Crassostrea gigas*; and grazers: limpets *Scutellastra longicosta* (Lamarck 1819), *Scutellastra granularis* (Linnaeus 1758), *Cymbula oculus* (Born 1778) and *Siphonaria serrata* (Fischer von Waldheim 1807). All species are broadly distributed along the south coast of South Africa (Branch et al., 2016). Samples were cleaned of epibionts with site seawater and individually placed inside labelled polyethylene plastic bags at collection. Subsequently, samples were transported in ice to the South African Institute for Aquatic Biodiversity (NRF-SAIAB), Makhanda, South Africa, where they were prepared for storage at −20 °C until further analysis.

To prepare samples for metal and stable isotope analyses, samples were defrosted, and soft tissues of invertebrates (filter feeders and grazers) were removed from their shells. Subsequently, the filter feeders and grazer's soft tissues were rinsed with distilled water and oven-dried at 60 °C for 48 h, while algal samples were dried for 24 h. Once dried, samples were crushed to a fine powder using a mortar and pestle. Thereafter, subsamples (~0.2 g dry weight) were stored in a desiccator pending metal and stable isotope analyses.

2.3. Metal analysis

Approximately 0.2 g of solid samples (algae and invertebrates) were weighed using a Mettler Toledo XP205 analytical balance and digested in 10 mL aqua regia using a Mars6 (CEM) microwave system at 200 °C for 35 min (USEPA United States Environmental Protection Agency, 2011; Mbandzi-Phorego et al., 2024). Subsequently, digestion samples were filtered, transferred to 50 mL tubes, and diluted 10x with 1 % HNO₃. Solid samples were diluted 10-fold by pipetting 1000 µL into a 15-mL tube, and then further diluted to 10 mL with 1 % HNO₃ to ensure that the % of total dissolved solids did not affect plasma or sample nebulization. Seawater samples were diluted 20x with 1 % HNO₃. Concentrations of Al, As, Cu, Co, Cr, Cd, Ni, Pb, and Zn were analysed using a Perkin Elmer NexION 300 ICP-MS, with Sc, Rh, and Ir as internal standards. Analytical accuracy was validated using certified reference material, NIST2976, and ERM-CE278 (European Institute for Reference, Materials and Measurements), with recoveries ranging from 81 to 90 % for invertebrates and 63–111 % for algae. Results are reported in mg/kg.

2.4. Particulate organic matter (POM) samples

Filters were oven-dried at 40 °C for 24 h, trimmed to remove unused perimeter, and pelletised in tin capsules (Puccinelli et al., 2022). Samples were analysed for δ¹⁵N using a Flash Elemental Analyser 1112 Series coupled to a Delta V Plus Isotope Ratio Mass Spectrophotometry (IRMS) housed at Stable Light Isotope Laboratory, University of Cape Town. Stable isotope ratios are expressed in conventional (δ) notation in per mil (‰) relative to the standard:

$$\delta X_{\text{sample}} \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \times 1000$$

where X represents ¹⁵N and R is the corresponding ¹⁵N/¹⁴N ratios

(Paterson & Fry, 1987). A laboratory running standard (Merck Gel: $\delta^{15}\text{N} = 6.8\text{‰}$, $\text{N} \% = 14.64$) and blanks (pre-combusted unused GF/F filters), were run after every 10–20 samples. All results are referenced to N_2 in air. Analytical precision was $< 0.1\text{‰}$.

2.5. Algae and Shellfish

Approximately 1 mg of algae, filter feeders and grazers samples were weighed using a Cubis® Micro Balance MSE6.6S-OCE-DM. Subsequently, samples were encapsulated in the Elemental Microanalysis D1008, $5 \times 9\text{ mm}$ ultrapure tin capsules. $\delta^{15}\text{N}$ in algae samples was analysed following the same protocol as the POM samples above.

To examine the individual prey–predatory relationship of metal concentration, biomagnification factor (BMF) was determined based on the $\delta^{15}\text{N}$ composition as follows:

$$\text{BMF} = \frac{M_{\text{predator}}/M_{\text{prey}}}{\delta^{15}\text{N}_{\text{predator}}/\delta^{15}\text{N}_{\text{prey}}} \quad (1)$$

where M_{predator} and M_{prey} are the concentrations of metals and $\delta^{15}\text{N}_{\text{predator}}/\delta^{15}\text{N}_{\text{prey}}$ are the $\delta^{15}\text{N}$ (‰) of the predator and prey, respectively (Jara-marini et al., 2009). The $\text{BMF} > 1$ indicates biomagnification and if $\text{BMF} < 1$ indicates biodilution of metals in filter feeders and grazers (Jara-marini et al., 2009). Filter feeders continuously sift particulate matter, including algal residuals, from the water column, making algae a primary prey source for both filter feeders and grazers (Puccinelli et al., 2022); therefore, due to the lack of metal data for POM, algae were used as the diet base for BMF calculations for both grazers and filter feeders.

To investigate biomagnification/biodilution of metals, each metal measured in organisms was regressed against the $\delta^{15}\text{N}$, following (Hu et al., 2024; Saidon et al., 2024):

$$\text{Log}(M) = a + b \times \delta^{15}\text{N} \quad (2)$$

where (a) is the intercept and (b) is the slope of the regression. The slope (b) was used to determine the trophic magnification slope (TMS) of each metal, following:

$$\text{TMS} = 10^b \quad (3)$$

TMS is assumed to represent the average biomagnification in a food web, with $\text{TMS} > 1$ indicating biomagnification and if $\text{TMS} < 1$ biodilution (Wang et al., 2024).

2.6. Non carcinogenic human health risk assessment

Health risk indexes, which included the estimated daily intake (EDI), estimated weekly intake (EWI), target hazard quotient (THQ) and hazard index (HI), were calculated to assess risks for humans associated with shellfish consumption, following method in Yap et al. (2016) and Nekhoroshkov et al. (2021):

$$\text{EDI} = \frac{C \times \text{CRd}}{\text{BW}} \quad (4)$$

$$\text{EWI} = \frac{C \times \text{CRw}}{\text{BW}} \quad (5)$$

where C represents the concentration of metal, CRd indicates daily consumption rate, CRw indicates weekly consumption rate, and BW indicates body weight.

THQ was calculated as follows:

$$\text{Non carcinogenic THQ} = \frac{\text{EF} \times \text{ED} \times \text{CR} \times \text{MC}}{(\text{RfD} \times \text{BW} \times \text{AT})} \times 10^3 \quad (6)$$

where EF is the exposure time-frequency (365 days). This EF value of 365 does not assume that all individuals consume mussels daily; rather, it is a standard conservative risk assessment used to estimate long-term

exposure under high-exposure conditions. ED is the exposure duration based on average age life expectancy (70 years), CR is the consumption rate of a given food estimated to 28.6 g/day, MC is the metal concentration in organisms, RfD is the reference dose represented by the daily exposure of humans to each metal (Nekhoroshkov et al., 2021, NDOH 2019). For the RfD of each metal, the regulatory limits for Cr (0.05 mg/kg), Ni (8.9 mg/kg), and Cu (30 mg/kg) were based on the guidelines provided by the WHO (2011), while regulatory limits for As (3.0 mg/kg), Cd (3.0 mg/kg), and Pb (4.0 mg/kg) were based on the guidelines provided by the RSA Republic of South Africa, (2004). BW is the body weight, and in this study, 70 kg was used following Nekhoroshkov et al. (2021) and NDOH (2019). The averaging time (AT) is the period over which the exposure is averaged for non-carcinogens or carcinogens ($\text{ED} \times 365 \text{ days/year}$) (Uren et al., 2020; USEPA, 2023; Nekhoroshkov et al., 2024).

Hazard Index (HI), indicating the total hazard quotients of metals (n) for each station, was calculated following (Yap et al., 2016) as:

$$\text{HI} = \sum_{i=1}^n \text{THQi} \quad (7)$$

The calculations of the human health risk assessment are based on the four edible species that are consumed by the residents along the southeast coast of South Africa, comprising the mussel *P. perna*, the oyster *M. gigas* and the limpets *S. longicosta* and *C. oculus* (Lasiak, 1993). Due to the lack of recent available regulatory limits for Cu, Cr, Co, Zn, Ni in shellfish, set for human consumption in South Africa, human health indices were based on guidelines from the Food and Agricultural Organisation/World Health Organisation (FAO/WHO). The THQ calculations for Cr (0.05 mg/kg), Ni (8.9 mg/kg), and Cu (30 mg/kg) were based on FAO/WHO regulatory limits, while As and Cd (3.0 mg/kg for both) were based on the Department of Health of the Republic of South Africa RSA-DOH.

2.7. Carcinogenic human health risk assessment

Carcinogenic Risk

Target cancer risk (TCR), an index of cancer-causing risk (Dey et al., 2024), was calculated using

$$\text{TCR} = \frac{\text{MC} \times \text{EF} \times \text{ED} \times \text{IR} \times \text{CsF}}{(\text{BW} \times \text{AT})} \times 10^3 \quad (8)$$

CsF indicates the carcinogenic slope factor; MC, EF, ED, IR, BW, and AT are reported in Eq. 6. The CsF values for Ni, Cd, Cr, Pb, and As are 1.7, 6.3, 0.5, 0.009 and 1.5 mg/kg/day, respectively. The standard and acceptable risk limit range from 10^{-6} ($\sim 1\text{E-}06$) to 10^{-4} ($\sim 1\text{E-}04$) and $\text{TCR} > 10^{-4}$ indicates carcinogenic risk effects (USEPA, 2023).

2.8. Statistical analyses

Prior to statistical analyses of metals and $\delta^{15}\text{N}$, data normality and homogeneity were tested using Shapiro-Wilk normality test and Levene's test, respectively. Depending on the normality of data, differences in metal concentrations and $\delta^{15}\text{N}$ among sites and species were tested using two-way-ANOVA and Kruskal Wallis. Specifically, we investigated the effect of two factors, sites and species: (factor-site, fixed $n = 4-6$; factor-species, $n = 3-4$). Additionally, the $\delta^{15}\text{N}$ difference between functional feeding groups (filter feeders and grazers) was determined. Where significant differences existed, Tukey's honest post-hoc tests were performed. Using R package lme4, linear regression models were used to investigate whether the metal concentrations in algae, filter feeders, and grazers depended on the $\delta^{15}\text{N}$ composition. For each model, $\delta^{15}\text{N}$ was treated as predictor variable and metals as response variables. The statistical analyses were conducted in R-studio. To investigate different questions, subset of metal concentrations data in algae, filter feeders, and grazers published by Mbandzi-Phorego et al. (2024), was used to complement the evaluation of trophic transfer of metals, supported by

stable isotope analysis, non-target cancer risk quotients, target cancer risk indices, and human health risk assessment.

3. Results

3.1. Metal concentration

Results of metal concentration (mg/kg), mean $\pm$ standard deviation in grazers, filter feeders, and algae are illustrated in Table S1. Among studied algae species, *P. verrucosa* concentrated the highest levels of Al (761.00 ± 541.66), followed by As, and Zn at Port of Port Elizabeth. Though not significantly high, Cd (2.38 ± 0.52) concentrations were recorded for *G. pristoides* at both natural sites than in other algae. Ni (2.98 ± 1.43) bioaccumulated slightly more in *U. lactuca* at Kini Bay when compared to the other sites and species. Filter feeders had higher metal concentrations at urbanised sites as compared to natural sites and had greater bioaccumulation affinity than grazers. In filter feeders, *O. angulosa* accumulated elevated concentrations of Al (499.86 ± 375.34), followed by As, Cr, Cd, than in other species; whereas *M. gigas* attained high concentrations of Zn (3307.24 ± 1560.18) followed by Cu, while high Pb was recorded in *P. perna* from Port of Port Elizabeth. At the natural site Kini Bay, *P. perna* reached the highest concentrations of Ni and Cd. Likewise, grazers *S. longicosta* accumulated high concentrations of Cd (16.21 ± 15.76) at Kini Bay. *S. serrata* accumulated higher Al, Cr, Cu, Ni, and Zn at urbanised sites than the other grazers.

3.2. The stable nitrogen isotope

Overall, $\delta^{15}\text{N}$ composition in primary producer POM was generally low across all study sites and averaged between 1.8 ± 0.1 ‰ at Cannon Rocks to 3.3 ± 1.9 ‰ at Port of St Francis (Fig. 2). $\delta^{15}\text{N}$ values for algae were higher than POM, with the highest records at Port of Port Elizabeth, for *P. verrucosa*. The two-way ANOVA (Table S2), however, showed no significant differences in $\delta^{15}\text{N}$ in primary producers across sites ($F = 1.91$; $P = 0.12$).

The $\delta^{15}\text{N}$ composition between feeding functional groups (filter feeders and grazers) differed significantly ($F = 32.77$; $P < 0.01$) across sites. Comparably, among ports, samples of grazers at Port of Port

Elizabeth were enriched in $\delta^{15}\text{N}$ than in other ports, while, among natural sites, samples from Cannon Rocks were slightly enriched than Kini Bay. This was depicted by higher $\delta^{15}\text{N}$ values in *S. granularis* at Port of Port Elizabeth (13.0 ± 0.8 ‰), and Cannon Rocks (9.7 ± 1.1 ‰; Fig. 2). Overall, across species, the average $\delta^{15}\text{N}$ values were (9.1 ± 1.4 ‰) for filter feeders and (9.4 ± 1.9 ‰) for grazers (Fig. 2). The results of two-way-ANOVA performed on $\delta^{15}\text{N}$ values for filter feeders and grazers (Table S3) indicated that functional groups differed significantly among species of filter feeders and grazers ($P < 0.001$). Tukey's honest post-hoc test indicated that *O. angulosa* exhibited higher $\delta^{15}\text{N}$ than other species and significantly contributed to the interspecific differences among filter feeders ($P < 0.05$), while *S. serrata* contributed to the differences observed among grazers.

3.3. Biomagnification/Bio-dilution of metals

Biomagnification (BMF) and biodilution results were presented for two natural (Cannon Rocks, Kini Bay) and two urbanised sites (Port of Port Elizabeth, Port of Ngqura) where prey (algae) and predators (filter feeders/grazers) co-occurred. Filter feeders generally exhibited higher BMFs than grazers (Tables S3–S4). High BMFs of Cd, Zn, and Cu were observed in filter feeders at Port of Port Elizabeth, while As and Ni showed moderate biomagnification at natural sites. Conversely, Pb, Cr, and Al were consistently biodiluted across sites and species. Cd showed the highest BMF = 41.40 in *O. angulosa* at Port of Port Elizabeth, likely due to feeding on *P. verrucosa*, and in *P. perna* at Kini Bay via *U. lactuca* (BMF = 28.07). *M. gigas* exhibited higher Zn (BMF = 71.49) and Cu (BMF = 132.39) biomagnification at Port of Ngqura. Grazers showed slight Pb biomagnification at natural sites (BMF >1), associated with *G. pristoides*, which also contributed to Al biomagnification in *C. oculus* and *S. serrata* at Cannon Rocks. The highest BMF (45.88) was recorded for Cd in the *S. longicosta*–*U. lactuca* relationship at Kini Bay.

3.4. Trophic magnification/dilution with increasing trophic level

By modelling $\delta^{15}\text{N}$ as predictor variable and metals/metalloid as response variables, results reveal that $\delta^{15}\text{N}$ had no correlation with Al and Cd; a weak correlation with As, Cr, Ni and Pb; and a moderate correlation with Zn and Cu (Fig. S1). The slope of linear regressions was

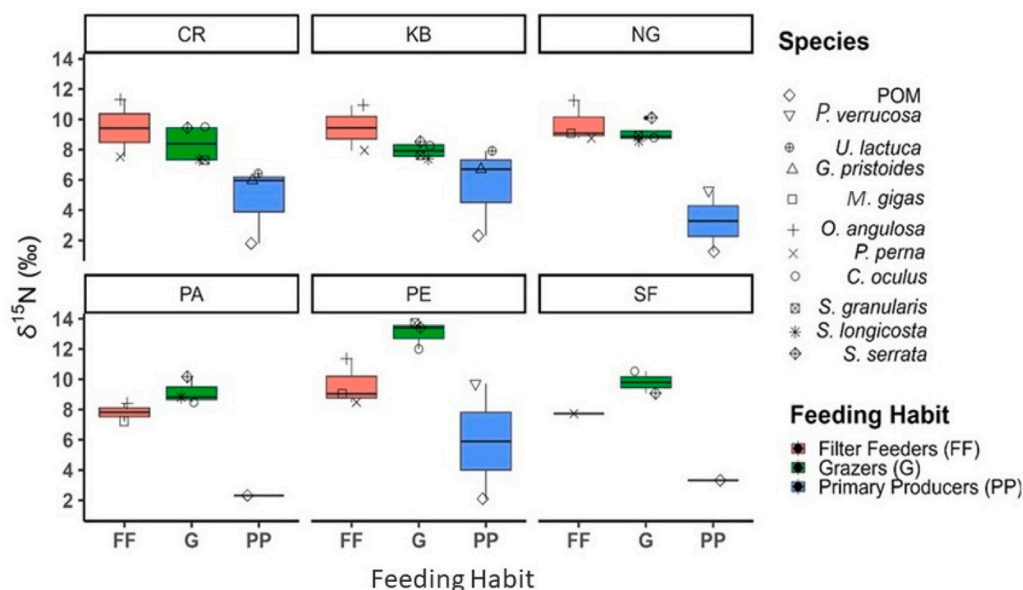


Fig. 2. The $\delta^{15}\text{N}$ composition (mean $\pm$ standard error) of primary producers (blue colour), particulate organic matter (POM) and algae (*PseudoRalfsia verrucosa*, *Ulva lactuca*, *Gelidium pristoides*); and primary consumer filter feeders (red colour) (*Magallana gigas*, *Octomeris angulosa*, *Perna perna*) and grazers (green colour) (*Cymbula oculus*, *Scutellastra granularis*, *S. longicosta*, *Siphonaria serrata*). The sites sampled are indicated on the top x-axis: Port of St Francis (SF), Kini Bay (KB), Port of Port Elizabeth (PE), Cannon Rocks (CR) and Port Alfred Marina (PA).

used to calculate TMS and results indicated that Ni, Cu, Zn, Pb, showed $TMS > 1$ ($P < 0.05$), indicating trophic magnification through the food web. Nickel showed the highest magnification through the food web ($TMS = 3.82$), while Al, Cr, and As indicated weak trophic magnification (Table 1). Cd biodiluted through the trophic web ($TMS < 1$).

3.5. Risk quotient based on estimated daily and weekly intakes

The estimated daily intake (EDI) of metals for filter feeders ranged between 0.0 – 0.1 mg/kg for Cr and Ni and 0.1 – 92.2 mg/kg for Cu at Port of Port Elizabeth, indicating potential high risk by ingestion of Cu (Table S5), as this element exceeded EDI for oyster *M. gigas*. Ni estimated weekly intake (EWI) through *P. perna* consumption from Cannon Rocks was 5x higher than the other sites (Table S5). The EDI of metals through limpets were 2.2 – 5.6 mg/kg for As; 0.1 – 2.8 mg/kg for Cu; 0.1 – 2.7 mg/kg for Cd; 0.1 – 1.0 mg/kg for Pb; 0.0 – 0.2 mg/kg for Al; 0.0 – 0.1 mg/kg for Ni. Cr showed elevated EWI through *S. longicosta* consumption from Port Alfred Marina (1.0 mg/kg).

3.6. Target hazard quotients (THQ) and hazard index (HI)

Target hazard quotients ($THQ < 1$) for Ni, As, Pb through the consumption of filter feeders and grazers indicated no potential risk of non-carcinogenic risk to human health (Table S5). However, ($HI > 1$) non-carcinogenic risk to human health via consumption of *M. gigas*, *P. perna* at Port of Port Elizabeth and Port of St Francis. Moreover, $HI > 1$ for grazer *O. oculus* at Cannon Rock, Port Alfred Marina and *S. longicosta* at Port of Port Elizabeth indicated non-carcinogenic risk to human health. The THQ (2.41 mg/kg) for Cr in the mussel *P. perna* from Port of Port Elizabeth and Port of St Francis, respectively, however, exceeded the THQ value of 1.0 mg/kg FAO/WHO regulatory limits. *M. gigas* had $THQ > 1$ for Cu only at Port of Port Elizabeth and $THQ < 1$ at all the other sites. THQ for As, Pb and Cd were < 1 , indicating values within the regulatory limit of RSA-DOH. Contrarily, Cr showed $THQ > 1$ (2.43 mg/kg), for the limpet *C. oculus* from Port of Port Elizabeth and *S. longicosta* at Port Alfred Marina, respectively.

Table 1

Trophic Magnification Slope (TMS) determined from the slope of $\log(\text{metals})$ and $\delta^{15}\text{N}$ of producers, grazer and filter feeders. TMS are assumed to represent the average biomagnification in a food web. Filter feeders, continuously sift particulate matter, including algal residuals, from the water column, as such algae were considered as prey for both filters and grazers.

Log (metals & $\delta^{15}\text{N}$)	intercept	Slope	TMS	P-value	Interpretation
Al	4.65	0.06	1.15	0.303	Not significant; no evidence of trophic magnification of aluminium
Cr	-0.41	0.14	1.37	0.005	Significant trophic magnification of chromium
Ni	-1.15	0.58	3.82	0.008	Strong and significant biomagnification of nickel. with a high TMS
Cu	-0.83	0.35	2.26	< 0.001	Significant trophic magnification of copper
Zn	1.41	0.34	2.18	< 0.001	Significant biomagnification of Zn
As	2.01	0.05	1.13	0.010	Weak but significant biomagnification of arsenic
Cd	1.88	-0.05	0.89	0.318	Not significant, indicating biodilution of cadmium
Pb	-0.92	0.20	1.57	0.038	Significant biomagnification of Pb

3.7. Target Cancer Risk (TCR)

The target cancer risk (TCR) ranges were in order of Cd (5.45×10^{-1} to 1.17×10^2), Cr (0.00×10^0 – 1.81×10^{-1}), As (5.01×10^{-1} to 1.41×10^0), Ni (0.00×10^0 – 3.72×10^0), Pb (0.00×10^0 – 3.28×10^2) (Table S5). TRC values for As and Cd in all species exceeded (10^{-4} and 10^{-6}), indicating a high cancer risk, especially in the case of *P. perna* Cd ($TCR = 3.43 \times 10^1$) and *M. gigas* from Kini Bay and Port of Port Elizabeth (Table S5). Cd ($TCR = 1.17 \times 10^2$) and As ($TCR = 1.66 \times 10^0$) indicated a high cancer risk through consumption of *S. longicosta*, with a higher risk at Kini Bay than the other sites (Table S5). Ni and Cr showed a moderate risk of cancer to humans through the consumption of filter feeders and grazers, while Pb posed no cancer risk associated with mussels and oysters ($TRC < 10^{-4}$).

4. Discussion

This study investigated the role of filter feeders and grazers in accumulating metals at urban and natural coastal sites in South Africa, followed by an assessment of human health risk posed by consumption of these species. We found that filter feeders and grazers accumulated considerably high concentrations of metals, providing early warning of toxic metals, as indicated by WHO (2011, 2019), and the long-term bioavailability of contaminants in their tissues. Comparably, filter feeders had higher metal concentrations at urbanised than natural sites and greater bioaccumulation affinity than grazers. Samples from urban sites were more enriched in $\delta^{15}\text{N}$ composition than those in natural sites. Overall, Ni, Cu, Zn, and Pb showed significant trophic magnification ($TMS > 1$, $p < 0.05$), with Ni exhibiting the highest magnification ($TMS = 3.82$). Considering the elevated risk quotients (EDI, EWI, THQ, $HI > 1$) for Cr, Cd and Cu at some of the sites (including a natural one), and the carcinogenic risk associated to As, Cd, Ni, Cr in edible filter feeders and grazers, our results indicated health concerns for the consumption of mussels, oysters, and limpets along South African coastline. According to the International Agency for Research on Cancer, high exposure to potentially hazardous metalloid such as As has been associated with a high risk of lung, skin, and bladder cancer, while prolonged exposure to Cd, Cr has been linked to lungs, kidneys and liver cancer, whereas exposure to Ni causes hypernickelaemia (Chai et al., 2021; Dey et al., 2024; Nekhoroskov et al., 2024), threatening shellfish-dependant livelihood.

In this study the urbanised sites within Algoa Bay, including Port of Ngqura is located near a permanently open Sundays estuary and Port of Port Elizabeth, situated near Bakeens Swartkops rivers discharging significant amounts of inorganic nutrients into the bay (Emmerson et al., 1983; Lemley et al., 2022; Adams et al., 2019), which could have led to the elevated $\delta^{15}\text{N}$ recorded. Municipal wastewater discharge in South African coastal regions has increased over the past years due to an increasing human population residing near the coast (Sink et al., 2012; Claassens et al., 2022), consequently favouring anthropogenic nutrient inputs along the coast (Hill et al., 2006), which could have explained the “urban” pattern observed. Additionally, differences in $\delta^{15}\text{N}$ composition could be attributed to several factors, including geospatial variations in metabolism (Caut et al., 2009), and multiple anthropogenic sources such as raw sewage, wastewater effluents, nutrient-rich soil erosion from agriculture, industrial effluents, and atmospheric nitrogen deposition (Puccinelli et al., 2016; Irandoost et al., 2021; Birch et al., 2020).

Overall, the $\delta^{15}\text{N}$ -based trophic magnification slope results showed that Ni, Cu, Zn, Pb were transferred through the food web. Magnification links between Ni, Cu, Pb, As, and $\delta^{15}\text{N}$ composition were weak, while those of Zn and Cr were moderate. This trend may result from different accumulation pathways, as environmental factors, including nitrogen sources (anthropogenic rather than dietary), can influence the $\delta^{15}\text{N}$ composition independently of metal (Irandoost et al., 2021). The EDI and EWI > 1 for Ni and Cu at urban sites showed high potential for metal ingestion through mussel and oyster consumption. Though Ni showed

high uptake, the THQ < 1 indicated non-carcinogenic potential risk, a pattern confirmed at all sites. On the contrary, THQ < 1 for Cr, and Cu pose health risks when ingested through shellfish consumption. The HI > 1 indicated non-carcinogenic risk to human health via consumption of filter feeders and grazers at Cannon Rock, Port Alfred Marina, and Port of Port Elizabeth. While we recognised that our sampling was conducted only once, and it might not be representative of conditions throughout a year, to safeguard human health, continuous monitoring of metal contaminants is needed for healthy seafood consumption.

In general, the urbanised sites of Port of Ngqura and Port of Port Elizabeth depicted bioaccumulation of Al, Pb, Cr, and metalloid As in invertebrates. The anthropogenic source of these metals/metalloid is attributed to port activities, dredging and the use of metal-containing products that wear off to coastal water (Watling & Watling, 1983; Wepener & Degger, 2020; Nekhoroshkov et al., 2021; Fourie et al., 2022; Mbandzi-Phorego et al., 2024). Globally, elevated metal concentrations in organism's tissues from urban sites have been associated with sewage discharges, port activities (marine traffic), and stormwater outlets (Walker & MacAskill, 2014; Ragi et al., 2017), suggesting a high negative impact posed by these activities. Natural sites had different bioaccumulation patterns, with Kini Bay depicting higher bioaccumulation of metals by invertebrates than Cannon Rocks. This effect can be attributed to Kini Bay being situated close to a residential village, with possible sewage spillage from drainage outlets entering the beach, which can lead to an increase of metal at this coastal site. Stormwater inflows, and upstream industrial or agricultural discharges were not explicitly quantified, but are likely to have influenced the observed metal distributions. Considering the stormy, heavy rain received in February–March 2023, as reported by the South African weather service (South African Weather Service SAWS, 2023), before our sampling period in March–April 2023, stormwater outlet containing metals could have also contributed to the recorded high metal concentrations at this natural site.

Once in the coastal system, metals can be directly assimilated into animal's tissue, as indicated by the biosediment-accumulation factor and biowater accumulation factor [BSAF/BWAF > 1] (Gu et al., 2022; Mbandzi-Phorego et al., 2024), but our results showed that dietary uptake (e.g., POM and algae) is an additional pathway for metal transfer in marine species along the South African south coast. In general, bioaccumulation and biomagnification of metals were species-site dependent. Elevated BMFs resulted from increased bioaccumulation of these metals in the *M. gigas* tissues, is a pattern consistent with previous research (Szefer et al., 1997; Shi et al., 2019; Lu et al., 2021). As filter feeders, oysters process large volumes of water containing particulate matter essential for their growth/survival (Widmeyer, Bendell-Young, 2008), a process through which metals can gradually accumulate. Similarly, Cu and Zn tend to accumulate in consumer's tissue, particularly in invertebrates, due to the absence of alternative detoxification and excretion mechanisms (Won et al., 2018; Gao et al., 2021). *P. perna* exhibited BMF > 1 for Cd and Zn at urban sites; meanwhile, Ni, and As biomagnified in *P. perna* from natural sites, suggesting exposure of these mussels to toxic metals/metalloid in these environments. Although Cd and $\delta^{15}\text{N}$ showed no significant effect, the prey-predatory relationship indicated by BMF > 1 suggests that the source of Cd in filter feeders was through diet.

Grazers, especially *S. longicosta*, had BMF > 1 for Al, Cd at natural sites, confirming previous evidence of this gastropod serving as a good Cd biomarker (Mbandzi et al., 2022; Mbandzi-Phorego et al., 2024). Moreover, grazers showed slight biomagnification of Pb, indicating a potential geological weathering of this metal (Webb et al., 2020), contributing to Pb-enrichment. The interspecific differences within a functional group regarding the metal transfer can also be driven by the spatial distribution of these species along the rocky shores (i.e., high shore vs low shore). Some species of grazers (*C. oculus*, *S. granularis*, *S. serrata*) are distributed in the upper shore (Branch et al., 2016), while most of the filter feeders considered in this study, including mussels and

oysters, are generally distributed on the low rocky shore, leading to differences in the time of water submersion (McQuaid & Branch, 1984). As such, filter feeders might have been exposed for longer time to metal contamination than grazers, which would have resulted in the observed high concentrations of Pb in our samples. Ultimately, habitat, feeding type/dietary uptake, and metal storage mechanisms are the drivers influencing metal bioaccumulation and biomagnification (Rainbow, 2002).

In this study, Ni, As, Pb of filter feeders exhibited THQ < 1, indicating no potential of non-carcinogenic risk associated with bioaccumulation and/or biomagnification of these metals in seafood following dietary transfer to humans. In contrast, our results indicated the potential risk for consumption of *P. perna*, *M. gigas*, *S. longicosta* and *C. oculus* in Port of Port Elizabeth, where Cr and Cu THQ were > 1 (Bosch et al., 2016). Although shellfish collection is restricted at this Port, there are nearby rock outcrops where humans can harvest shellfish. As such, seafood safety remains a concern for households that consume shellfish from these urban surroundings. Based on the mussel consumption rate limit (0.5 kg/day; Guendouzi et al., 2020), Cr is considered a limiting toxic metal for mussel consumption (Guéguen et al., 2011; this study). The EWI > 1 for Cd in *P. perna* and *S. longicosta* suggested a potential high human exposure to Cd contamination. Kini Bay is freely accessible for subsistence and recreational collection, and consuming *P. perna* meat poses a potential health risk (Fourie et al., 2022). The high exposure of *Perna* spp. to Cd has been previously reported (e.g., Whyte et al., 2009), with Jović et al. (2012) estimating that the intake of approximately 1.2 kg of freshly cultivated mussels meat leads to long-term human uptake of Cd.

The trophic magnification observed for Ni, Zn, Cu, Pb, As, Cr, Al in this study raises significant concerns regarding ecological and human health risks. Contrary to THQ < 1 of Ni, TMS = 3.82 showed that Ni exhibited the highest trophic magnification in this study, flagging health implications as Ni is classified as a possible human carcinogen also known to cause respiratory problems (e.g. bronchitis and asthma; Guéguen et al., 2011; Bosch et al., 2016). While potentially hazardous, As has also been linked to long-term health effects, including cancers of the lung, skin, and bladder (Ali and Khan, 2019; Bosch et al., 2016). Cr, which also showed positive trophic transfer in this study, is associated with respiratory cancers and tumor development (Bosch et al., 2016). Even at low concentrations, Pb exposure can impair neurological development in children and cause cardiovascular, hematological, and reproductive disorders in adults (Bosch et al., 2016). Considering the recorded TMS < 1, Cd was not easily accumulated from the prey, which could have reflected in an overall limited bioavailability in primary consumers. Cadmium however, remains a toxic metal of concern due to its well-documented cumulative effects in biological systems (Jović et al., 2012; Barchiesi et al., 2020). While Zn, and Cu are essential micronutrients involved in physiological processes, excessive accumulation through dietary exposure has been linked with liver and kidney damage, gastrointestinal distress, and immune dysfunction (Yap et al., 2016). The significant biomagnification of metals observed in this study suggests a risk of exposure, particularly for top predators and human consumers in contaminated areas.

Although regulatory limits of Cd, As, and Pb have been set for human consumption, such limits are outdated in South Africa (RSA Republic of South Africa, 2004) and do not account for more recent urban development undertaken along the coast. A need to update and regulate these standards is urgent, and further in-depth monitoring of metal health risks is needed, especially considering that TRC > 10^{-4} for the metalloid As, and metals Cd, and Cr, indicating a high cancer risk to humans through the consumption of filter feeders and grazers, at natural rather than urban sites. Prolonged exposure of humans to these metals through seafood consumption has been highlighted globally (Chai et al., 2021; Dey et al., 2024), suggesting that more awareness is needed to safeguard seafood-dependent communities, mostly in Africa.

5. Conclusions

This study ultimately exposes the increased vulnerability of populations in the Global South to health risks associated with subsistence and recreational consumption of metal-contaminated shellfish. Because sampling was conducted once at six sites, the findings provide a snapshot, which partly limits the ability to capture broader spatio-temporal variations in metal concentrations and transfer dynamics. Consequently, this constrains the full interpretation of long-term exposure trends and inter-annual variability. Nevertheless, key contributing factors include dependence on coastal resources, inadequate regulatory oversight, and limited public awareness of contamination risks. To effectively mitigate these exposures, region-specific policy responses are urgently needed, including enhanced monitoring of metal bioavailability and speciation, the incorporation of cancer risk modelling into dietary risk assessments, and the development of tailored dietary advisories for maximum allowable intake limits. This study further encourages a strong control for industrial effluent discharges and coastal water management, and public awareness, which can promote the prevention of metal contamination at the source. Unless proactive measures are taken, marine pollution will continue to endanger biodiversity, food security, and the livelihood of people who depend on coastal fisheries. Establishing community-based monitoring programmes could empower local fishers and residents to participate in early detection and reporting of pollution events. Public awareness and education campaigns are essential to inform coastal communities about safe harvesting, and presence of species with higher contamination potential. Also, integrating metal contamination data into local seafood safety advisories and coastal management frameworks would help guide consumption practices and promote sustainable use of coastal seafood. Such measures would not only strengthen environmental stewardship, but also enhance the socio-ecological resilience of coastal communities that depend on shellfish as a food source.

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CRediT authorship contribution statement

Petrus Philippus Pieterse: Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Eleonora Puccinelli:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Investigation, Conceptualization. **Nokubonga Mbandzi-Phorego:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Francesca Porri:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Jabulani Ndaba:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jfca.2025.108637](https://doi.org/10.1016/j.jfca.2025.108637).

Data availability

Data will be made available on request.

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