Heliyon 6 (2020) e04725

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Comparative analysis of trace metal levels in the crab *Dotilla fenestrata*, sediments and water in Durban Bay harbour, Richards Bay harbour and Mlalazi estuary, Kwazulu-Natal, South Africa

Babatunde Adeleke *, Deborah Robertson-Andersson, Gan Moodley

Marine Biology, School of Life Sciences, University of KwaZulu-Natal, Westville, Durban, KZN, South Africa

ARTICLE INFO

Keywords:
Marine biology
Fauna
Environmental analysis
Environmental pollution
Water chemistry
Environmental toxicology
Trace metals
Dotilla fenestrata
Water
Sediments
Estuary

ABSTRACT

Durban Bay and Richards Bay Harbours are the largest and most economically active shipping harbours in South Africa supporting a diversity of ecosystems and biota of ecological importance. This study assessed and compared levels of metals in selected tissues of the sand bubbler crab (*Dotilla fenestrata*), water and sediments from anthropogenically impacted Durban and Richards Bay Harbours with those of Mlalazi estuary, a considered pristine site due to its sheltered catchments. Metal concentrations (Cd, Cu, Pb and Zn) were investigated using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Metals concentrations in crab tissues were as follows; exoskeleton > gill > digestive gland, metal concentrations in tissues followed the order Cu > Zn > Pb > Cd. Metal levels in crabs from Durban Harbour (Cd 0.42, Cu 83.8, Pb 2.43and Zn 6.4 μg/g) were significantly higher than Richards Bay (Cd 0.22, Cu 27.7, Pb 1.23 and Zn 9.54 μg/g) and Mlalazi estuary (Cd 0.17, Cu 18.7, Pb 3.53 and Zn 6.91 μg/g). Metal levels in sediments followed the order Zn > Cu > Pb > Cd. Mlalazi had significantly elevated metal levels in sediment (Cd 6.83, Cu 35.63, Pb 33.43 and Zn 56.27 μg/g) compared to Durban Harbour (Cd 2.73, Cu 16.07, Pb 12.20 and Zn 38.70 μg/g) and Richards Bay (Cd 3.10, Cu 16.00, Pb 11.43 and Zn 26.07 μg/g). Metal concentrations in water were Cu > Zn > Pb > Cd save Mlalazi estuary with significantly higher Zn levels. Mlalazi estuary, the considered pristine site had significantly elevated metal levels in the sediments and water compared with the impacted sites; however, had significantly lower metal levels in the crabs' tissues due to lower metal bioavailability. Factors such as high natural metal concentrations, metal speciation, sediment grain and organic matter content could possibly account for high metal concentrations without corresponding bio-accumulation and magnification in crabs from Mlalazi estuary.

1. Introduction

Estuarine ecosystems globally are being altered and threatened due to rising impacts of growing urban development, industrialization, mining activities, tourism and GCC (Solomon et al., 2007; Vivier, 2010). Growing human pressures, including climate change, are exerting profound and diverse consequences on marine ecosystems (Doney et al., 2012). As a result, complex mixtures of particulate material, nutrients and contaminants result in over-enrichment and bioaccumulation of toxic waste within these systems (Vivier, 2010).

Metals are common pollutants in marine environments that accumulate in high levels in marine and estuarine water and sediments (Ivanina and Sokolova, 2015). Metals are introduced into the marine environment via many routes, which are either a combination of natural or anthropogenic means (Ali et al., 2019). These routes include riverine and atmospheric inputs, ore-bearing rock, windblown dust, volcanic activity, forest fires, industrial dumping, shipping and harbour activities, sewage sludge and dredging spoils (Gautam et al., 2016). For many metals, the inputs into marine systems exceed the outputs resulting in elevated metal concentrations over time (Clark et al., 1997). Emerging technologies such as microbial electrochemical technologies (MET) however are currently being trialled for the removal and recovery of metals from industrial effluents by targeting different pathways such as anodic removal, cathodic electrochemical reduction and metal uptake by biocathode microbes (Das et al., 2019). Metals in marine systems originate from both natural and anthropogenic sources, however, for metals such as cadmium, copper, lead, mercury, nickel and zinc, the anthropogenic inputs are significantly higher than the natural inputs (1–3 orders

* Corresponding author.
E-mail address: tundeadeleke@gmail.com (B. Adeleke).

https://doi.org/10.1016/j.heliyon.2020.e04725
Received 4 May 2020; Received in revised form 14 July 2020; Accepted 11 August 2020

of magnitude) (Schindler, 1991; Masindi and Muedi, 2018). Some metals (e.g. Cu and Zn) are essential cofactors in many biochemical processes, while others (Cd, Hg and Pb) have no known biological functions in animals; though, all metals are toxic at elevated concentrations (Ivanina and Sokolova, 2015).

Metals are potentially dangerous in marine and coastal ecosystems as they tend to bioaccumulate (Chiarelli and Roccheri, 2014) and their concentrations in these systems are usually monitored by measuring their concentrations in water, sediment and biota (Camusso et al., 1995). Metals are of serious concern in marine and estuarine systems, as they cause many biological alterations from molecular to the tissue level, depending on their concentrations and duration of exposure (Figueira et al., 2012). The levels of trace metals accumulated by marine organisms are not only dependent on water quality, but also seasonal factors, temperature, salinity, diet, spawning and individual variation, among other factors (Rajeshkumar and Li, 2018).

Cadmium and Pb are highly toxic trace metals even at very low concentrations and are common inorganic contaminants of marine and coastal sediments (Sokolova et al., 2004; Ivanina et al., 2008, 2010). Cadmium and Pb can enter the food chain through the air, water, soil and biota and are derived from industrial effluent, agricultural runoff, mining and mineral processing, stormwater runoff, geological weathering, petroleum, fossil fuel combustion, chemicals, non-ferrous metal works and atmospheric transport (Gautam et al., 2016) which are typical of activities around Durban and Richards Bay Harbours. Cadmium and Pb have no known physiological function and cannot be regulated by the normal physiological processes of crustaceans. However, some decapod crustaceans can regulate body levels of essential metals such as Cu, Cr, Mn, Zn and Ni at concentrations below the threshold level (Rainbow, 2007). Both Cd and Pb are capable of substituting essential cations ($Zn^{2+}$and Cu^{2+}), which serve as cofactors in some enzymes (Nassiri et al., 1997).

Crabs are capable of taking up and accumulating trace metals in their tissues and are, therefore a suitable bioindicator for environmental contamination assessment (Kumar et al., 2000; Bastami et al., 2012). *Dotilla fenestrata* (Hilgendorf, 1869), the sand-bubbler crab, is a small species and is about 1cm across the carapace (Dray and Paula, 1998; Gherardi et al., 2002; Flores et al., 2005). They belong to the Ocypodidae family of brachyuran crabs and are widely distributed along the East African coast from Kenya to South Africa and also found in Madagascar and The Comoro Islands (Hartnoll, 1973; Bulcao and Hodgson, 2012). They are burrowing decapod crustaceans and occur abundantly on soft sediment shores in tropical and sub-tropical climates (Maitland, 1986; Bulcao and Hodgson, 2012). Sand bubbler crabs are distributed mainly in the north of Durban, South Africa (29° 52′ S; 31° 04′E), although small numbers are found in warm temperate regions as far south of the Breede River estuary (Day, 1974, 1981; Rius et al., 2010). *Dotilla fenestrata* plays important ecological roles like other burrowing crustaceans as a deposit feeder and bioturbator within its habitat (Flores et al., 2005). Its bioturbation function, i.e. the process that is responsible for a rapid rate of sediment turnover that results in a change in the physical, chemical and biological characteristics of the sediment (Branch and Pringle, 1987; Dray and Paula, 1998; Flores et al., 2005) has been shown to affect the productivity of sandy shores and changing of meiofaunal communities (Flores et al., 2005). This study aims to determine field concentrations of Cd, Cu, Pb and Zn in sediment, water and selected tissues (gills, digestive gland and exoskeleton) of *Dotilla fenestrata*, in Durban Harbour, Richards Bay Harbour and Mlalazi Estuary, and to determine the most suitable tissue compartment to sample for metal concentrations in this crab when constrained with time and resources. It is hypothesized that metal concentrations in crab tissues, sediments and water from the impacted study sites (Durban Harbour and Richards Bay Harbour) will be significantly elevated compared to those of Mlalazi estuary, the considered pristine site. The study also hypothesized that metal concentrations vary in the selected crab tissues from the study sites.

2. Materials and methods

2.1. Study area

Durban Bay (29° 52′ S; 31° 04′E) (Figure 1)and Richards Bay Harbours (28° 51′ S; 32° 03′E) (Figure 2) are subjected heavily to anthropogenic activities such as industrial effluent discharges resulting in contaminants entering the harbour (Borja et al., 2008). These two systems have their catchment areas situated within populated coastal cities and drain into the Indian ocean. The Mlalazi estuary (28° 57′ S; 31° 49′E), situated in a coastal nature reserve, south of Richards Bay, KwaZulu-Natal drains into the Indian Ocean (Figure 3).

2.2. Sampling

Ethical approval to handle fish were approved (protocol reference number: AREC/105/015M) by the animal research ethics committee of the University of KwaZulu-Natal. *Dotilla fenestrata* (7 ± 1 mm carapace width) were collected at Durban Harbour, Richards Bay Harbour and Mlalazi Estuary, cleaned with filtered seawater and subsequently with distilled water to remove debris and their morphometrics recorded. Crabs were stored in a freezer at – 20 °C until trace metal analysis. Sediment samples were collected randomly from the three study sites at a depth of about 10–15 cm (i.e. average depth of crab burrow) using acid pre-washed plastic bags and hand shovel, packed in acid pre-washed plastic bags, preserved and transported to the laboratory for analysis. Also, water samples from the three locations were collected in plastic bottles, preserved by adding a few drops of concentrated nitric acid and stored in a freezer for metal analysis. All equipment, glassware and plastic containers were pre-washed and soaked in 10 % nitric acid and rinsed thoroughly in double-distilled and deionized water before use, to avoid contamination.

2.3. Sample preparation and analysis

Crabs were thawed and dissected for their tissues (exoskeleton, digestive gland and gills). Dissected tissues were weighed, and oven-dried at 50 °C to constant weight for at least 48 h. 0.5 g of pooled dried tissues from the crabs were replicated thrice and digested in 20 ml concentrated nitric acid for 24 h. Subsequently, the digested samples were mixed with 10 ml of concentrated nitric and perchloric acid (4:1) and heated on a hot plate at 120 °C until complete evaporation of acid mixture and dryness. Residues were made up to 20 ml solution by adding 20 ml solution of Milli-Q water with 20 % nitric acid and filtered with Whatman filter paper (Sudharsan et al., 2012). Trace metal concentrations in tissues were determined with Inductively Plasma Optical Emission Spectrometry (ICP-OES, Perkin Elmer).

Sediments were weighed and oven-dried at 60 °C to constant weight for at least 48 h. Dried sediments were ground into a powdery form using an electronic ball shaker, sieved with a 75 μm mesh. One gram of homogenized sediment was digested with acid in triplicates in line with the methods of Sudharsan et al. (2012). Water samples were thawed and filtered with Whatman filter paper. Five ml of water samples were digested in 10 ml AR grade nitric acid, made up to 50 ml with deionized water and analyzed for metal concentrations with ICP-OES.

2.4. Quality assurance and quality control (QA/QC)

Crab tissues, sediment and waters samples were analyzed for Cd, Cu, Pb and Zn. The QA/QC of the analytical methods of metal detection in the crab tissues, sediments and water were conducted using Crab paste (LGC 7164), Toronto Harbour Sediment (TH-2) and Estuarine Water (LGC 6016) certified reference materials to test for analytical efficiency of Cd, Cu, Pb and Zn. The result of the QA/QC is highlighted in Table 1.

Figure 1. Aerial photo of Durban Bay Harbour showing sampling area. (Image source; Google Earth Maxar Technologies).

Figure 2. Aerial photo of Richards Bay Harbour showing the sampling area. (Image source; Google Earth Maxar Technologies).

2.5. Statistical analysis

The null hypothesis (H_0) for this study, states that there is no statistical difference in the metal concentrations in the crab tissues, sediments and water from the impacted (Durban Harbour and Richards Bay Harbour) and pristine (Mlalazi) study sites statistically tested using a one-way analysis of variance (ANOVA) after testing for equality of variance and normality ($p > 0.05$). Pairwise comparison analysis using Tukey's HSD test was used to determine the significant difference in mean concentrations at 95 %, and 99 % confidence limits. Pearson's correlation coefficient was used to determine the relationship between metal concentrations in the crab tissues, sediment and water. Statistical analysis was done using Statistica 13.0 software program.

3. Results

3.1. Trace metals in crab tissues

Mean concentrations of metals in the crab tissues from Durban Bay, Richards Bay and Mlalazi estuary occurred in the following order; Cu > Zn > Pb > Cd, tissue accumulation of metal occurred in the order of exoskeleton > gill > digestive gland, while metal accumulations in crab tissues with regards to study sites were in the order of Durban Bay > Richards Bay > Mlalazi estuary (Table 2 and Figure 4). Mean concentrations ± standard deviation of Cd (0.42 ± 0.00 μg/g) in the exoskeleton and Cd (0.41 ± 0.02 μg/g) in the digestive gland was significantly higher (ANOVA HSD: df 6; $p < 0.01$) than Cd concentrations in the gills of crabs sampled from Durban Harbour. Mean concentrations Cu and Zn in the exoskeleton (Cu 83.84 ± 1.56 and Zn 26.41 ± 0.32) and gill (Cu 78.47 ± 3.30 and Zn 27.60 ± 3.72) were significantly higher (ANOVA HSD: df 6; $p < 0.01$) than those of the digestive gland in the crabs from Durban Harbour. The mean concentrations of Cd, Cu and Zn in the exoskeleton (Cd 0.22 ± 0.02; Cu 27.71 ± 1.07 and Zn 9.54 ± 0.35) and gill (Cd 00.15 ± 0.00; Cu 52.46 ± 4.86 and Zn 13.41 ± 1.40) were significantly higher (ANOVA HSD: df 6; $p < 0.01$) than those in the digestive gland of crabs from Richards Bay Harbour (Table 2). Concentrations of Cu and Zn in the exoskeleton (Cu 18.72 ± 0.26 and Zn 6.91 ± 0.12) and gill (Cu 23.82 ± 1.09 and Zn 6.71 ± 0.64) were significantly higher (ANOVA HSD: df 6; $p < 0.01$) than those of the digestive gland in the crabs from Mlalazi estuary (Table 2).

Figure 3. Aerial photo of Mlalazi Estuary showing sampling area. (Image source; Google Earth Maxar Technologies.)

Table 1. Measured and certified values of trace metal concentrations in μg/g certified reference materials.

Metal	Cadmium (Cd) μg/g			Copper (Cu) μg/g			Lead (Pb) μg/g			Zinc (Zn) μg/g		
Samples	Measured	Certified	% recovery	Measured	Certified	% recovery	Measured	Certified	% recovery	Measured	Certified	% recovery
Crab	8.98 ± 0.08	9.20 ± 0.48	97.6	23.8 ± 0.33	20.1 ± 2.40	118	0.49 ± 1.39	0.47 ± 0.01	104	66.0 ± 0.43	56.8 ± 5.50	110
Sediment	7.29 ± 1.87	5.52 ± 0.750	132	109 ± 2.57	116 ± 22.0	94.0	172 ± 3.82	187 ± 22.0	92.0	907 ± 55.7	861 ± 165	105
Water	105 ± 23.7	101 ± 2.00	103	180 ± 45.6	190 ± 4.00	94.7	203 ± 32.9	196 ± 3.00	104	50.2 ± 17.7	55.0	94.6

Table 2. Metal (Cd, Cu, Pb and Zn) concentrations in the crab tissues between Durban Bay, Richards Bay and Mlalazi Estuary. n = 27 (Mean value (μg/g) ± SD). (*) denotes significant difference ($p < 0.05$), (**) denotes high significant difference ($p < 0.01$).

Metals	Site	Cd df = 6	Cu df = 6	Pb df = 6	Zn df = 6
Tissues					
Exoskeleton	Durban Harbour	0.42** ± 0.00	83.8** ± 1.56	2.43** ± 0.10	26.4** ± 0.32
	Richards Bay Harbour	0.22* ± 0.02	27.7** ± 1.07	1.23 ± 0.13	9.54** ± 0.35
	Mlalazi Estuary	0.17 ± 0.02	18.7 ± 0.26	3.53** ± 0.27	6.91 ± 0.12
Digestive Gland	Durban Harbour	0.41** ± 0.02	63.7** ± 1.01	4.71** ± 0.20	19.4** ± 0.57
	Richards Bay Harbour	0.13 ± 0.03	20.4** ± 0.14	1.58 ± 0.10	6.78* ± 0.16
	Mlalazi Estuary	0.18 ± 0.00	15.0 ± 0.17	1.72 ± 0.23	4.69 ± 0.11
Gill	Durban Harbour	0.27** ± 0.00	78.5** ± 3.30	3.17** ± 0.30	27.6** ± 3.72
	Richards Bay Harbour	0.15 ± 0.00	52.5** ± 4.86	1.54 ± 0.10	13.4* ± 1.40
	Mlalazi Estuary	0.18 ± 0.03	23.8 ± 1.09	1.68 ± 0.03	6.71 ± 0.64

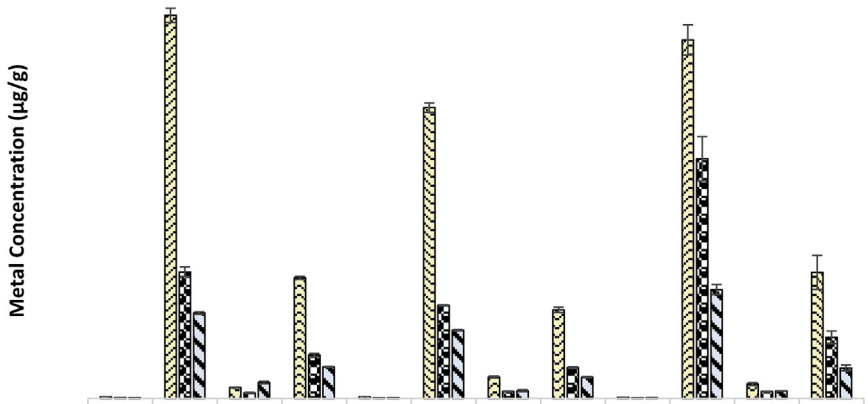
The concentration of Cd, Cu and Zn (Cd 0.42 ± 0.00, Cu 83.84 ± 1.56 and Zn 26.41 ± 0.32) in the exoskeleton of crabs from Durban Harbour were significantly higher (ANOVA HSD: df 6; $p < 0.01$) compared to those of Richards Bay Harbour and Mlalazi estuary, the concentration of Cd, Cu, and Zn (Cd 0.22 ± 0.02; Cu 27.71 ± 1.07 and Zn 9.54 ± 0.32) in the exoskeleton of crabs from Richards Bay Harbour were significantly higher (ANOVA HSD: df 6; $p < 0.01$) compared to those of Mlalazi estuary, while the concentration of Pb (3.53 ± 0.27) in the exoskeleton of crabs in Mlalazi estuary were significantly higher (ANOVA HSD: df 6; $p < 0.01$) compared to those from Durban Harbour and Richards Bay Harbour (Table 2 and Figure 4).

Bioconcentration of Cd, Cu, Pb and Zn in the digestive gland (Cd 0.41 ± 0.02, Cu 63.67 ± 1.01, Pb 4.71 ± 0.20 and Zn 19.41 ± 0.57) and gill (Cd .27 ± 0.00, Cu 78.47 ± 3.30, Pb 3.17 ± 0.30 and Zn 27.60 ± 3.72) of crabs from Durban Bay were significantly higher (ANOVA HSD: df 6; $p < 0.01$) compared to those from Richards Bay Harbour and Mlalazi estuary, Cu and Zn concentrations in the digestive gland (Cu 20.39 ± 0.14 and Zn 6.78 ± 0.16) and gill (Cu 52.46 ± 4.86 and Zn 13.41 ± 1.40) of crabs from Richards Bay Harbour were significantly higher (ANOVA HSD: df 6; $p < 0.01$) compared to crabs from Mlalazi estuary, while concentrations of Cd and Pb in the digestive gland and gill were not significantly different (ANOVA HSD: df 6; $p > 0.05$) in crabs from Richards Bay Harbour and Mlalazi estuary (Table 2 and Figure 4).

The Pearson's Correlation coefficient between the trace metals (Cd, Cu, Pb and Zn) concentrations the crab tissues, sediment and water measured in the Durban Bay Harbour, Richards Bay Harbour and Mlalazi estuary were not significantly correlated ($p > 0.05$) (Table 3).

3.2. Trace metals in sediment

Mean metal concentrations (Cd, Cu, Pb and Zn) in sediments from Durban Bay Harbour, Richards Bay Harbour and Mlalazi estuary



	Exoskeleton				Digestive Gland				Gill			
	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
Durban Bay Harbour	0.4	83.8	2.4	26.4	0.4	63.7	4.7	19.4	0.3	78.5	3.2	27.6
Richards Bay Harbour	0.2	27.7	1.2	9.5	0.1	20.4	1.6	6.8	0.2	52.5	1.5	13.4
Mlalazi Estuary	0.2	18.7	3.5	6.9	0.2	15.0	1.7	4.7	0.2	23.8	1.7	6.7

Figure 4. Mean metal concentrations (µg/g ± SD) in crab tissues from Durban Bay, Richards Bay and Mlalazi Estuary.

Table 3. Pearson's Correlation Coefficient (R) between Cd, Cu, Pb and Zn concentrations in the crab tissue, sediment and water measured in the Durban Bay, Richards Bay and Mlalazi Estuary. *n = 12 (*) denotes significant correlation (p < 0.05), (**) denotes high significant correlation (p < 0.01).*

Site	Crab Tissue	Sediment	Water
Durban Harbour	0.76	0.82	0.74
Richards Bay Harbour	0.70	0.44	0.46
Mlalazi Estuary	0.43	0.58	0.46

occurred in the following order; Zn > Cu > Pb > Cd. Concentrations of Cd, Cu, Pb and Zn in the sediments from Mlalazi estuary (Cd 6.83 ± 0.06, Cu 35.63 ± 0.35, Pb 33.43 ± 1.31 and Zn 56.27 ± 1.39 µg/g) were significantly higher (ANOVA HSD: df 6; $p < 0.01$) compared to those of 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), Cd (3.10 ± 0.10) concentrations in the sediments from Richards Bay Harbour were significantly higher ($p < 0.01$) compared to Durban Harbour, Zn (38.70 ± 0.80) levels in the sediments from Durban Bay were significantly higher (ANOVA HSD: df 6; $p < 0.01$) compared to Richards Bay Harbour (Table 4 and Figure 5).

3.3. Trace metals in water

Mean metal (Cd, Cu, Pb and Zn) concentrations in the water column from Durban Bay, Richards Bay Harbour and Mlalazi estuary occurred in the following order: Zn > Cu > Pb > Cd similar to those of the sediments. Concentrations of Zn (162.93 ± 11.23 µg/g) in the water from Mlalazi estuary were significantly higher ($p < 0.01$) compared to Durban Bay and Richards Bay Harbour. The mean concentrations of Cd, Cu and Pb, were not significantly different ($p > 0.05$) in water from the three study sites (Table 6 and Figure 6). The background water quality parameters of the three study sites are highlighted in Table 5.

4. Discussion

Trace metals are of primary concern particularly in many developed nations due to pollution of sediments, water resources and organisms, and because of their toxicity, persistence and bioaccumulative nature (Ikem et al., 2003). Trace metal concentrations in estuaries can be influenced strongly by hydrology, point source pollution and sediment type (Orr, 2007). Therefore, metal concentrations in the tissues of the crabs are likely to reflect a high degree of both spatial and temporal variability.

4.1. Metal bioconcentration in crab tissues

Trace metal contamination levels in marine fauna, particularly the potential to accumulate in the different organs of crustaceans, are of particular interest due to potential transfer and biomagnification along the food chain (Bastami et al., 2012). The uptake of metals from the environment by crustaceans is mainly dependent on metal speciation and bioavailability (Bastami et al., 2012). All marine invertebrates accumulate metals in their body tissues, irrespective of the essentiality of these metals to metabolism (Rainbow, 2002). Invertebrates accumulate metals in varying concentrations in their tissues, organs and bodies (Rainbow, 2002; Mistri et al., 2020), which is dependent on membrane permeability and enzymatic reactions in different species (Bastami et al., 2012).

Table 4. Metal (Cd, Cu, Pb and Zn) concentrations in the sediments from Durban Bay, Richards Bay and Mlalazi Estuary. n = 9 (Mean value (µg/g) ± SD). (*) denotes significant difference ($p < 0.05$), (**) denotes high significant difference ($p < 0.01$).

Metals	Cd df = 6	Cu df = 6	Pb df = 6	Zn df = 6
Sites				
Durban Bay	2.73 ± 0.06	16.1 ± 0.91	12.2 ± 0.66	38.7** ± 0.80
Richards Bay	3.10** ± 0.10	16.0 ± 0.44	11.4 ± 1.83	26.07 ± 26.1
Mlalazi	6.83** ± 0.06	35.6** ± 0.35	33.4** ± 1.31	56.3** ± 1.39

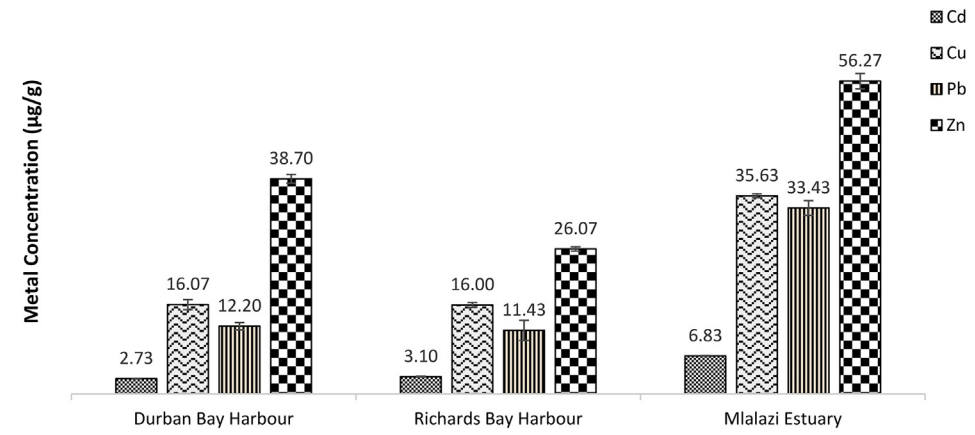


Figure 5. Mean metal concentrations (µg/g ± SD) in sediment from Durban Bay, Richards Bay and Mlalazi Estuary.

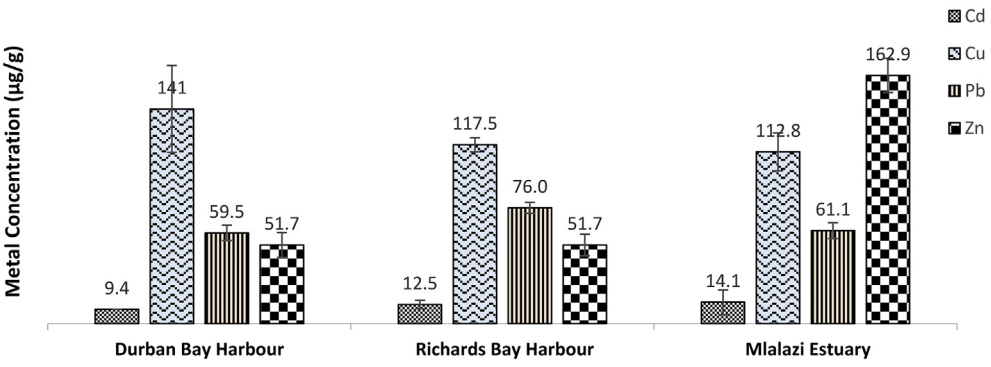


Figure 6. Mean metal concentrations (µg/g ± SD) in water from Durban Bay, Richards Bay and Mlalazi Estuary.

Table 5. Background physicochemical properties of the water in Durban Bay Harbour, Richards Bay Harbour and Mlalazi Estuary (± SD). n = 45.

Sites	Durban Bay Harbour	Richards Bay Harbour	Mlalazi Estuary
Water Parameters			
Temperature (°C)	26.4 ± 0.26	21.1 ± 0.34	27.5 ± 0.31
Salinity (psu)	35.3 ± 0.23	39.2 ± 0.43	32.8 ± 0.37
pH	8.10 ± 0.08	7.78 ± 0.10	7.90 ± 0.05
Dissolved Oxygen (mg/l)	3.90 ± 0.42	4.66 ± 0.37	3.81 ± 0.29

Table 6. Metal (Cd, Cu, Pb and Zn) concentrations in the water from Durban Bay, Richards Bay and Mlalazi Estuary. n = 9 (Mean value (µg/g) ± SD). (*) denotes significant difference ($p < 0.05$), (**) denotes highly significant difference ($p < 0.01$).

Metals	Cd df = 6	Cu df = 6	Pb df = 6	Zn df = 6
Sites				
Durban Bay	9.40 ± 0	141. ± 28.6	59.5 ± 5.10	51.7 ± 8.14
Richards Bay	12.5 ± 2.71	117 ± 4.70	39.2 ± 3.51	51.7 ± 7.12
Mlalazi	14.1 ± 8.14	113 ± 12.4	61.1 ± 5.21	163** ± 11.2

Therefore, marine invertebrates even at species level inhabiting the same environment could differ in body concentrations of trace metals (Jakimska et al., 2011). Results from this study indicated statistically significant differences in tissue (exoskeleton, digestive gland and gill) concentrations of metals (Cd, Cu, Pb and Zn) (Table 2) as hypothesized, therefore, the hypothesis of varying metal concentrations in the selected crab tissues was accepted. Reinecke et al. (2003) also reported significant differences in tissue (carapace, digestive gland, gills, gonads and muscle) accumulation of Cd and Pb in freshwater crab *Potamonautes perlatus*. Chaiyara et al. (2013) reported significant differences in tissue accumulation of Cd, Cu, Pb and Zn in the mangrove crab *Sesarma mederi*. As observed in this study, the exoskeleton of the crab *Dotilla* showed the highest significant levels of mental concentration when compared to the gill and digestive gland (Table 2 and Figure 4).

The exoskeleton of this crab is, therefore, recommended as the most suitable tissue compartment to sample when constrained by resources and time. Many studies have shown that metal uptake and concentration in crabs varies amongst the body tissues, with the most elevated proportion of body concentrations occurring in the exoskeleton, gills and digestive gland (Khan et al., 2011). Cadmium and Ca, the main

constituent of the exoskeleton has similar chemical properties like charge number, ion number and diameter hence, Cd accumulates first in the exoskeleton of crabs and is capable of replacing Ca entering the body through the exoskeleton (Jennings et al., 1979; Kang et al., 2012b). The crab *Carcinus maenas* exposed to 10 ppm Cd stock in seawater showed the highest levels of Cd in the exoskeleton (Jennings et al., 1979). This result was also consistent with the findings of Silvestre et al. (2005) (Silvestre et al., 2005) where *Sinopotamon yangtsekiense* exposed to acute concentrations of Cd showed the highest accumulation of Cd in the exoskeleton. The uptake and accumulation of metals by crabs are dependent on environmental conditions as an increase in bioavailable metal concentrations result in elevated metal accumulation (Ali et al., 2019; Kang et al., 2012a). *Dotilla spp*., a burrowing and submerging crab which emerges at low tide to feed on detritus by browsing on sediments and leaving behind pellets of processed sand, therefore, are more likely to take up and accumulate more metals in their exoskeleton compared to surface feeders because of their feeding, burrowing and submerging habits; thus they potentially take up metals from both water and sediments. Bioconcentrations of Cu and Zn in crabs from all the study locations were more elevated than Cd and Pb (Table 2 and Figure 4) suggesting influences of industrial and other anthropogenic activities within their catchments, especially those of Durban Bay and Richards Bay (Table 2 and Figure 4).

The levels (low or high) of metal concentrations in aquatic invertebrates cannot be measured on an absolute scale, as the accumulation significance depends on the specific tissues and invertebrate involved. Therefore, body concentration of the metal considered abnormally high for one species could be considered too low for another (Rainbow, 2002). The metal accumulation threshold for the *Dotilla spp* could not be validated as there was no prior metal level study on this species. Hence in a single taxon, metal accumulation in crustacean tissues and whole body vary greatly, even in the absence of anthropogenic metal pollution (Rainbow, 1998). Marine invertebrates take up metals either from surrounding water medium or food with the relative proportion of uptake from each route differing with invertebrate species and relative metal bio-availabilities in water and diet (Rainbow, 2002; Van Den Brink et al., 2019). The consequent fate of the trace metal is dependent on the specific physiology of the crustacean, i.e. if the metal is utilized for an essential metabolic function, excreted, or stored in the body. The exoskeleton is the most significant storage tissue for crustaceans as it accounts for the largest body mass of crabs (De Kock, 2001). Moulting is a primary excretory mechanism utilized by crustaceans and accounts for a massive loss of bioaccumulated metal due to moulting of the carapace (Bergey and Weis, 2007). Therefore, it is pertinent to study metal accumulation in crabs during their moulting cycles to precisely assessing changes in metal concentrations during ecdysis process.

Elevated concentrations of essential metals (Cu and Zn) in *D. fenestrata* as shown in this study is a common feature of aquatic invertebrates due to their capability of storing essential metals in a detoxified form (Rainbow and Luoma, 2011). The method of metal accumulation in marine crustaceans which is a net metal accumulator is to detoxify and store the excess metabolically available metals in a detoxified form, without upper limits of concentration. Hence a metal is available metabolically upon entry, not until the physiological function of the invertebrate acts to excrete or bind it to a specific molecule of high affinity to prevent the metal from escaping; thus the metal is detoxified (Mason and Jenkins, 1995), by often binding to proteins such as metallothioneins or insoluble metalliferous granules (Rainbow, 2002). Similarly, the accumulation pattern without excretion for nonessential metals is to detoxify the bioaccumulated metals, and then toxic effects are instigated when metal uptake exceeds detoxification. This pattern is generally applicable to crustaceans when Cd and Pb are taken up from food or aquatic medium (Rainbow and Luoma, 2011). The result of this study shows that the crab *Dotilla fenestrata* can be used as a suitable bioindicator due to its tendency to accumulate high concentrations of metals in its exoskeleton. Therefore, due to their submerging, burrowing and deposit-feeding habits, they are potential bioindicators for assessing metal contaminants in estuarine systems where they are distributed widely.

4.2. Trace metals in sediment

Metal concentration in sediments from Durban Bay Harbour, Richards Bay Harbour and Mlalazi estuary were generally comparable to those from the Mhlathuze estuary (Mzimela et al., 2003), Richards Bay Harbour (Wepener and Vermeulen, 2005) and sediments in aquatic ecosystems in the eThekwini area of KwaZulu-Natal (Newman et al., 2015). Trace metal concentrations in sediments from the three study sites were in the order of Zn > Cu > Pb > Cd (Table 4 and Figure 5). A striking observation from this study, however, is the significantly high concentration of metals in the sediments from Mlalazi estuary (Cd 6.83 ± 0.06, Cu 35.63 ± 0.35, Pb 33.43 ± 1.31 and Zn 56.27 ± 1.39 μg/g) a considered pristine site due to its watershed being located in a nature reserve, compared to anthropogenically impacted 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 (Cd 3.10 ± 0.10, Cu 16.00 ± 0.44, Pb 11.43 ± 1.83 and Zn 26.07 ± 26.07 μg/g) whose catchments are situated within densely populated urban settlements and industrial areas (Table 4 and Figure 5). Therefore, the hypothesis of elevated metal concentrations in the sediments of the impacted sites (Durban Harbour and Richards Bay Harbour) compared to Mlalazi estuary is rejected.

South Africa currently does not have sediment quality guidelines for coastal waters; however, metal concentrations were considerably lower than the Effects Range Low (ERL) values for Australian estuaries, except for Cd concentrations from the three study sites (Vivier, 2010). The lower metal concentrations compared to the Australian reference guidelines was indicative of a relatively uncontaminated estuary; therefore, metal concentrations are not expected to affect benthic organisms in these systems adversely. The concentrations of Cd in the sediments from Durban Harbour (Cd 2.73 ± 0.06), Richards Bay Harbour (Cd 3.10 ± 0.10) and Mlalazi estuary (Cd 6.83 ± 0.06) were above the Australian sediment quality guideline value (Effects-Range-Low – ERL) for Cd (1.2 μg/g) (Simpson et al., 2007). Concentrations of Cu in the sediments from Durban Harbour (Cu 16.07 ± 0.91), Richards Bay Harbour (Cu 16.00 ± 0.44) were below the Australian sediment quality guideline value (Effects-Range-Low – ERL) for Cu (34.0 μg/g); however, Cu (Cu 35.63 ± 0.35) concentrations in the sediments from Mlalazi estuary was slightly higher (Simpson et al., 2007). Concentrations of Pb and Zn in the sediments from Durban Harbour (Pb 12.20 ± 0.66 and Zn 38.70 ± 0.80 μg/g), Richards Bay Harbour (Pb 11.43 ± 1.83 and Zn 26.07 ± 26.07 μg/g) and Mlalazi estuary (Pb 33.43 ± 1.31 and Zn 56.27 ± 1.39 μg/g) were above the Australian sediment quality guideline value (Effects--Range-Low – ERL) for Pb (46.0 μg/g) and Zn (150.0 μg/g) (Simpson et al., 2007).

Although Mlalazi estuary recorded significantly elevated metal concentrations in its sediments, the uptake and bioaccumulation of these metals in the crab tissues from Mlalazi estuary were significantly lower compared to those of Durban Bay and Richards Bay Harbours (Table 2 and Figure 4). The hypothesis of lower concentrations of metals in crab tissues from Mlalazi estuary is therefore accepted. Particulate matter of sediments is made up of many diverse constituents and phases which include hydrous metal oxides, carbonates, crystalline minerals and organic materials thus metals could be bound to a particulate material via a range of mechanisms. Hence the distribution of trace metals in different phases defines their mobility, bioavailability and toxicity (Usero et al., 1998; Rauret et al., 1999). The total concentration of metals in aquatic sediments do not inherently, therefore, reflect bioavailability, and elevated metal concentrations do not infer high toxicity. Instead, it is pertinent to study metal speciation than total metal concentrations to comprehend better trace metal bioavailability and potential toxicity to marine organisms (Tessier et al., 1979).

Accumulation, bioavailability and toxicity of sediment metals are influenced by many complex factors such as natural background concentration of metals, metal speciation, salinity, sediment granulometry, pH, organic content, organism physiology and feeding mechanisms (Magnusson et al., 1996; Rainbow, 2007; Simpson and Batley, 2007). These factors, therefore, needed to be taken into consideration in assessing the concentration of metals in the sediment of a particular location. The inverse relationship between the elevated metal concentrations in sediments and considerably low metal concentrations in the crab tissues from Mlalazi estuary compared to those from Durban Bay and Richards Bay could indicate the geological factors. They were thus resulting in naturally high background levels of metals and metal speciation in the estuary which could strongly affect the bioavailability of the metals, as the free ionic forms are typically the most bioavailable (Paquin et al., 2000).

Salinity is a critical controlling factor that affects the demarcation of contaminants between the water column and sediments in estuaries, as free ionic metals, i.e. bioavailable fraction usually increases with decreasing salinity (Chapman and Wang, 2001). Sediments have different capacities of adsorbing pollutants, the concentration of metals increases with decreasing grain size in uncontaminated sediments, due to the surface area of sediments being dependent on grain size. Thus, smaller particles offer a greater surface area for metal adsorption (Summers et al., 1996). Sediments with fine grain size such as silt and mud contain higher metal concentrations naturally compared to sediments with coarse grain. As a result, there is usually a strong inverse relationship between the grain size of sediments and metal concentrations. The degree of refinement of sediments sampled from Mlalazi, therefore, could also be responsible for the elevated natural concentration of metals. Begg (1978) reported extensive dredging of Mlalazi estuary along the channel of the system in 1960, which resulted in dredging spoil being deposited in the *Phragmites* swamp area of the estuary. Although the estuary appears to have recovered to a large extent from this anthropogenic event (Maro, 2012), the resultant effects of this event could have contributed to the elevated metal levels in the system.

Estuaries regularly have high levels of natural background metals in some locations, therefore, determining anthropogenic enrichment of estuarine sediment could be complex as levels of metals in unpolluted sediment can fluctuate by orders of magnitude within small spatial scales as a result of natural mineralogy and sediment granulometry (Rainbow, 2002; Newman and Watling, 2007). Therefore, elevated concentrations of metals in sediments do not inevitably indicate contamination but possibly show natural background concentrations (Orr, 2007).

4.3. Trace metals in water

This study provides the first account of Cd, Cu, Pb and Zn concentrations in Durban Bay, Richards Bay Harbour and Mlalazi estuary as prior information on trace metal concentrations in the water column for these three study sites were unavailable at the time of reporting this study. Concentrations of metals in water samples from the three study stations were similar to those of sediments and were in descending order of Zn > Cu > Pb > Cd. Mean concentration of Cd, Cu, Pb and Zn (Table 6) from the three study sites were above the South African marine water quality guideline target (Cd 4.0 μg/g, Cu 5.0 μg/g, Pb 12.0 μg/g and Zn 25.0) (MacKay et al., 1995).

Mean metal concentrations from Durban Bay, Richards Bay harbour and Mlalazi estuary compared to Mhlathuze estuary (Mzimela et al., 2003) and Nhlabane Estuary (Vivier, 2010) revealed that metal concentrations in the three study sites were significantly higher compared to these two systems. Metal concentrations in water from Mlalazi estuary were not significantly different from the other two study sites. Water quality surveys conducted in the past few decades on South African coastal waters and estuaries in northern KwaZulu-Natal indicated higher concentrations of Cd, Cu, Pb and Zn in St Lucia estuary. The relatively high concentrations, recorded at St Lucia, were observed as indicative of naturally high background concentrations of metals due to geological factors (Vivier, 2010). The elevated metal concentrations in Mlalazi estuary could, therefore, be due to high natural background concentrations of metals. Evidence of anthropogenic event of 1960, which resulted in the deposition of dredging spoil in the system and consequent desorption of metals from sediments into the water column could also be another factor responsible for high levels of metal concentrations. Modifications in estuary water chemistry due to pH fluctuations can affect metal solubility, speciation and distribution in water and sediments (Ivanina and Sokolova, 2015). Concentrations of metals in sediment usually exceed dissolved metal concentrations by some orders of magnitude (Hackney et al., 1998), making sediment an essential sink for metals. However, decreasing pH increases metal solubility and desorption from both sediments and organic ligands resulting in the higher influx of metals into the water column (De Orte et al., 2014a; De Orte et al., 2014b).

5. Conclusion

Metal levels in the sediments from the three study sites (Durban Harbour, Richards Bay Harbour and Mlalazi estuary) were relatively lower than the Australian sediment quality guideline value thus indicating no adverse effects to benthic organisms in these systems. Concentrations of Cd, Cu, Pb and Zn in the water column from the three study sites were above the South African marine water quality guideline target. The Mlalazi estuary, i.e. the considered the pristine site, recorded significantly higher concentrations of metals in its sediments and water compared to Durban Harbour and Richards Bay Harbour. Metal levels in the crab tissues from Mlalazi estuary, however, indicated lower bioconcentrations of metals compared to Durban Harbour and Richards Bay Harbour, which is indicative of lower bioavailability of metals in the Mlalazi estuary. The crab *Dotilla fenestrata* shows good bioindicator potential due to its ecological and physiological roles as well as the capacity to take up and bioaccumulate very high concentrations of metals in its tissues from sediments, water and organic matter present in the environment. The exoskeleton of the crab appears to be the most suitable tissue compartment to sample metal levels due to its capacity to accumulate higher metal concentrations compared to other body tissues. The exoskeleton is the most pronounced tissue of this crab thus reduces the number of crabs required to perform metal analysis. The interactions of complex factors, mainly metal speciation, salinity, pH and physiological functions of organisms largely determine metal uptake and bioavailability.

Declarations

Author contribution statement

Babatunde Adeleke: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Deborah Robertson-Andersson, Gan Moodley: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Funding statement

This work was supported by NRF South Africa and Gay Langmuir.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Ali, H., Khan, E., Ilahi, I., 2019. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. J. Chem. 6730305, 2019.

Bastami, A., Khoei, J., Esmailian, M., 2012. Bioaccumulation of heavy metals in sediment and crab, Portunus pelagicus from Persian Gulf, Iran. Middle East J. Sci. Res. 12 (6), 886–892.

Begg, G., 1978. The Estuaries of Natal: A Resource Inventory Report to the Natal Town and Regional Planning Commission Conducted under Auspices of the Oceanographic Research Institute. Oceanographic Research Institute, Durban.

Bergey, L.L., Weis, J.S., 2007. Molting as a mechanism of depuration of metals in the fiddler crab, Uca pugnax. Mar. Environ. Res. 64 (5), 556–562.

Borja, A., Bricker, S.B., Dauer, D.M., Demetriades, N.T., Ferreira, J.G., Forbes, A.T., Marques, J.C., 2008. Overview of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems worldwide. Mar. Pollut. Bull. 56 (9), 1519–1537.

Branch, G., Pringle, A., 1987. The impact of the sand prawn Callianassa kraussi Stebbing on sediment turnover and on bacteria, meiofauna, and benthic microflora. J. Exp. Mar. Biol. Ecol. 107 (3), 219–235.

Bulcao, C., Hodgson, A., 2012. Activity and feeding of Dotilla fenestrata (Brachyura: Ocypodidae) in a warm, temperate South African estuary. Afr. J. Aquat. Sci. 37 (3), 333–338.

Camusso, M., Vigano, L., Balestrini, R., 1995. Bioconcentration of trace metals in rainbow trout: a field study. Ecotoxicol. Environ. Saf. 31 (2), 133–141.

Chaiyara, R., Ngoendee, M., Kruatrachue, M., 2013. Accumulation of Cd, Cu, Pb, and Zn in water, sediments, and mangrove crabs (Sesarma mederi) in the upper Gulf of Thailand. Sci. Asia 39 (4), 376–383.

Chapman, P.M., Wang, F., 2001. Assessing sediment contamination in estuaries. Environ. Toxicol. Chem.: Int. J. 20 (1), 3–22.

Chiarelli, R., Roccheri, M., 2014. Marine invertebrates as bioindicators of heavy metal pollution. Open J. Met. 4, 93–106.

Clark, R., Frid, C., Attrill, M., 1997. Marine Pollution, fourth ed., 1197. Oxford University Press, Oxford, pp. 61–79. Chapter Metals.

Das, I., Das, S., Chakraborty, I., Ghangrekar, M., 2019. Bio-refractory pollutant removal using microbial electrochemical technologies: a short review. J. Indian Chem. Soc. 96 (4), 493–497.

Day, J., 1974. The ecology of Morrumbene estuary, Moçambique. Trans. Roy. Soc. S. Afr. 41 (1), 43–97.

Day, J., 1981. Summaries of current knowledge of 43 estuaries in southern Africa. In: Estuarine Ecology with Particular Reference to Southern Africa, pp. 251–329.

De Kock, E., 2001. Aspects of the General Biology and Bioaccumulation of Metals in Fresh Water Crab, Potamonautes warren. Master of Science thesis, University of Johannesburg.

De Orte, M., Lombardi, A., Sarmiento, A., Basallote, M.D., Rodriguez-Romero, A., Riba, I., Del Valls, A., 2014a. Metal mobility and toxicity to microalgae associated with acidification of sediments: CO2 and acid comparison. Mar. Environ. Res. 96, 136–144.

De Orte, M.R., Sarmiento, A.M., Basallote, M.D., Rodríguez-Romero, A., Riba, I., 2014b. Effects on the mobility of metals from acidification caused by possible CO2 leakage from sub-seabed geological formations. Sci. Total Environ. 470, 356–363.

Doney, S.C., Ruckelshaus, M., Duffy, J.E., Barry, J.P., Chan, F., English, C.A., Talley, L.D., 2012. Climate change impacts on marine ecosystems. Ann. Rev. Marine Sci. 4 (1), 11–37.

Dray, T., Paula, J., 1998. Ecological aspects of the populations of the crab Dotilla fenestrata (Hilgendorf, 1869)(Brachyura: Ocypodidae), in the tidal flats of Inhaca Island (Mozambique). J. Nat. Hist. 32 (10-11), 1525–1534.

Figueira, E., Cardoso, P., Freitas, R., 2012. Ruditapes decussatus and Ruditapes philippinarum exposed to cadmium: toxicological effects and bioaccumulation patterns. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 156 (2), 80–86.

Flores, A.A., Abrantes, K.G., Paula, J., 2005. Estimating abundance and spatial distribution patterns of the bubble crab Dotilla fenestrata (Crustacea: Brachyura). Austral Ecol. 30 (1), 14–23.

Gautam, P.K., Gautam, R.K., Banerjee, S., Chattopadhyaya, M., Pandey, J., 2016. Heavy Metals in the Environment: Fate, Transport, Toxicity and Remediation Technologies, 60. Nova Sci Publishers, pp. 101–130.

Gherardi, F., Russo, S., Lazzara, L., 2002. The function of wandering in the sand-bubbler crab, Dotilla fenestrata. J. Crustac Biol. 22 (3), 521–531.

Hackney, C.T., Grimley, J., Posey, M., Alphin, T., Hyland, J., 1998. Sediment Contamination in North Carolina's Estuaries, 198. Publication, pp. 2–59.

Hartnoll, R., 1973. Factors affecting the distribution and behaviour of the crab Dotilla fenestrata on East African shores. Estuar. Coast Mar. Sci. 1 (2), 137–152.

Ikem, A., Egiebor, N., Nyavor, K., 2003. Trace elements in water, fish and sediment from Tuskegee Lake, Southeastern USA. Water Air Soil Pollut. 149 (1-4), 51–75.

Ivanina, A., Eilers, S., Kurochkin, I., Chung, J., Techa, S., Piontkivska, H., Sokolova, I., 2010. Effects of cadmium exposure and intermittent anoxia on nitric oxide metabolism in eastern oysters, Crassostrea virginica. J. Exp. Biol. 213 (3), 433–444.

Ivanina, A.V., Cherkasov, A.S., Sokolova, I.M., 2008. Effects of cadmium on cellular protein and glutathione synthesis and expression of stress proteins in eastern oysters, Crassostrea virginica Gmelin. J. Exp. Biol. 211 (4), 577–586.

Ivanina, A.V., Sokolova, I.M., 2015. Interactive effects of metal pollution and ocean acidification on physiology of marine organisms. Current Zoo. 61 (4), 653–668.

Jakimska, A., Konieczka, P., Skóra, K., Namieśnik, J., 2011. Bioaccumulation of metals in tissues of marine animals, Part II: metal concentrations in animal tissues. Pol. J. Environ. Stud. 20 (5).

Jennings, J., Rainbow, P., Scott, A., 1979. Studies on the uptake of cadmium by the crab Carcinus maenas in the laboratory. II. Preliminary investigation of cadmium-binding proteins. Mar. Biol. 50 (2), 141–149.

Kang, X., Mu, S., Li, W., Zhao, N., 2012a. Toxic effect of cadmium on crabs and shrimps. Toxic Drugs Test 4, 221–236.

Kang, X., Mu, S., Li, W., Zhao, N., 2012b. Toxic effects of cadmium on crabs and shrimps. In: Toxicity And Drug Testing: IntechOpen.

Khan, M.F., Umarajeswari, S., Wesley, S.G., 2011. Biomonitoring 210Po and 210Pb in marine brachyuran crabs collected along the coast of Kudankulam, Gulf of Mannar (GOM), India. J. Environ. Monit. 13 (3), 553–562.

Kumar, M., Ferguson, G., Xiao, Y., Hooper, G., Venema, S., 2000. Studies on reproductive biology and distribution of blue swimmer crab (Portunus pelagicus) in South Australian Waters. Sardi Res. Rep. Ser. 47, 1324–2083.

MacKay, H., Roux, D., Ashton, P., Van Vliet, H., Jooste, S., 1995. The development of South African water quality guidelines for the natural aquatic environment. Water Sci. Technol. 32 (5-6), 293–299.

Magnusson, K., Ekelund, R., Dave, G., Granmo, A., Förlin, L., Wennberg, L., Brorström-Lundén, E., 1996. Contamination and correlation with toxicity of sediment samples from the Skagerrak and Kattegat. J. Sea Res. 35 (1-3), 223–234.

Maitland, D.P., 1986. Crabs that breathe air with their legs-Scopimera and Dotilla. Nature 319 (6053), 493.

Maro, A.Z., 2012. Modelling Hydrological Responses to Land Use and Climate Change: the Mfolozi Catchment.

Masindi, V., Muedi, K.L., 2018. Environmental contamination by heavy metals. Heavy Metals 19, 2019.

Mason, A., Jenkins, K., 1995. Metal detoxification in aquatic organisms. Metal Speciation Bioavail.Aquatic Sys. 3, 479–578.

Mistri, M., Munari, C., Pagnoni, A., Chenet, T., Pasti, L., Cavazzini, A., 2020. Accumulation of trace metals in crayfish tissues: is Procambarus clarkii a vector of pollutants in Po Delta inland waters? European Zoo. J. 87 (1), 46–57.

Mzimela, H., Wepener, V., Cyrus, D., 2003. Seasonal variation of selected metals in sediments, water and tissues of the groovy mullet, Liza dumerelii (Mugilidae) from the Mhlathuze Estuary, South Africa. Mar. Pollut. Bull. 46 (5), 659–664.

Nassiri, Y., Wery, J., Mansot, J., Ginsburger-Vogel, T., 1997. Cadmium bioaccumulation in Tetraselmis suecica: an electron energy loss spectroscopy (EELS) study. Arch. Environ. Contam. Toxicol. 33 (2), 156–161.

Newman, B., Arabi, S., Weerts, S., Pieters, R., Vogt, N., 2015. Prevalence and significance of organic contaminants and metals in aquatic ecosystems in the eThekwini area of Kwazulu-Natal. WRC Report.

Newman, B.K., Watling, R.J., 2007. Definition of baseline metal concentrations for assessing metal enrichment of sediment from the south-eastern Cape coastline of South Africa. Water 33 (5).

Orr, K.K., 2007. Spatial and Temporal Variations in Metals in the Sediment and Water of Selected Eastern Cape Estuaries, South Africa.

Paquin, P.R., Santore, R.C., Wu, K.B., Kavvadas, C.D., Di Toro, D.M., 2000. The biotic ligand model: a model of the acute toxicity of metals to aquatic life. Environ. Sci. Pol. 3, 175–182.

Rainbow, P.S., 1998. Phylogeny of trace metal accumulation in crustaceans. Metal Metabolism in Aquatic Environments. Springer, pp. 285–319.

Rainbow, P.S., 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environ. Pollut. 120 (3), 497–507.

Rainbow, P.S., 2007. Trace metal bioaccumulation: models, metabolic availability and toxicity. Environ. Int. 33 (4), 576–582.

Rainbow, P.S., Luoma, S.N., 2011. Trace metals in aquatic invertebrates. In: Environmental Contaminants in Biota: Interpreting Tissue Concentrations. Taylor and Francis Books, Boca Raton, FL, USA, pp. 231–252.

Rajeshkumar, S., Li, X., 2018. Bioaccumulation of heavy metals in fish species from the meiliang Bay, taihu lake, China. Toxicol. Rep. 5, 288–295.

Rauret, G., Lopez-Sanchez, J., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A., Quevauviller, P., 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. J. Environ. Monit. 1 (1), 57–61.

Rius, M., Branch, G.M., Griffiths, C.L., Turon, X., 2010. Larval settlement behaviour in six gregarious ascidians in relation to adult distribution. Mar. Ecol. Prog. Ser. 418, 151–163.

Reinecke, A., Snyman, R., Nel, J., 2003. Uptake and distribution of lead (Pb) and cadmium (Cd) in the freshwater crab, Potamonautes perlatus (Crustacea) in the Eerste River, South Africa. Water Air Soil Pollut. 145 (1-4), 395–408.

Schindler, P.W., 1991. The regulation of heavy metal concentrations in natural aquatic systems. Heavy Metals Environ. 1, 95–123.

Silvestre, F., Trausch, G., Devos, P., 2005. Hyper-osmoregulatory capacity of the Chinese mitten crab (Eriocheir sinensis) exposed to cadmium; acclimation during chronic exposure. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 140 (1), 29–37.

Simpson, S., Batley, G., Chariton, A., 2007. Revision of the ANZECC/ARMCANZ Sediment Quality Guidelines.

Simpson, S.L., Batley, G.E., 2007. Predicting metal toxicity in sediments: a critique of current approaches. Integrated Environ. Assess. Manag.: Int. J. 3 (1), 18–31.

Sokolova, I., Evans, S., Hughes, F., 2004. Cadmium-induced apoptosis in oyster hemocytes involves disturbance of cellular energy balance but no mitochondrial permeability transition. J. Exp. Biol. 207 (19), 3369–3380.

Solomon, S., Qin, D., Manning, M., Averyt, K., Marquis, M., Tignor, M.M., 2007. Climate Change 2007-the Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the IPCC, 4. Cambridge university press.

Sudharsan, S., Seedevi, P., Ramasamy, P., Subhapradha, N., Vairamani, S., Shanmugam, A., 2012. Heavy metal accumulation in seaweeds and sea grasses along southeast coast of India. J. Chem. Pharmaceut. Res. 4 (9), 4240–4244.

Summers, J.K., Wade, T.L., Engle, V.D., Malaeb, Z.A., 1996. Normalization of metal concentrations in estuarine sediments from the Gulf of Mexico. Estuaries 19 (3), 581–594.

Tessier, A., Campbell, P.G., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51 (7), 844–851.

Usero, J., Gamero, M., Morillo, J., Gracia, I., 1998. Comparative study of three sequential extraction procedures for metals in marine sediments. Environ. Int. 24 (4), 487–496.

Van Den Brink, N.W., Kokalj, A.J., Silva, P.V., Lahive, E., Norrfors, K., Baccaro, M., Cornelis, G., 2019. Tools and rules for modelling uptake and bioaccumulation of nanomaterials in invertebrate organisms. Environ. Sci.: Nano 6 (7), 1985–2001.

Vivier, L., 2010. Macrobenthic community and ecotoxicological status of the Nhlabane estuary vivier, L.. In: Macrobenthic Community and Ecotoxicological Status of the Nhlabane Estuary (Doctoral Dissertation) (2010).

Wepener, V., Vermeulen, L., 2005. A note on the concentrations and bioavailability of selected metals in sediments of Richards Bay Harbour, South Africa. WaterSA 31 (4), 589–596.