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Distribution and risk assessment of trace metals in *Leptodius exarata*, surface water and sediments from Douglas Creek, Qua Iboe estuary

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Abstract

Five trace metals in *Leptodius exarata*, epipellic sediments and surface water from an intertidal ecosystem in Niger Delta (Nigeria) were investigated to evaluate their spatial distribution, degree of contamination, ecological and health risks. Results show Cd (cadmium), Cr (chromium), Ni (nickel), Pb (lead) and Zn (zinc) concentrations in sediment ranged between $0.550 - 1.142$, $9.57 - 15.95$, $9.15 - 13.96$, $2.00 - 8.90$ and $91.5 - 121.6$ mg/kg dw, respectively; while *L. exarata* tissue metal content vary from $0.162 - 0.931$, $3.81 - 8.62$, $4.45 - 17.15$, $1.90 - 7.35$, and $125.55 - 269.75$ mg/kg, dw, respectively. The bioconcentration factor ranking for trace metals followed the sequence Zn>Ni>Pb >Cr>Cd. High biota to sediment accumulation factor (BSAF) in *L. exarata* reveals a sentinel metal bioindicator. Sediments from most sites were uncontaminated to moderately contaminated (geoaccumulation, $I_{geo} > 0$) with Cd and Zn associated with anthropogenic intrusions. Low mean-ERM (effect range-median) and mean-PEL (probable effect level) quotients of sediments are observed, indicating low–medium degree of contamination with 30% and 21% probability of being toxic. The multi-metal potential ecological risk index (R_I) for the intertidal ecosystem indicates low–moderate risk. Health risks associated with crabs (*L. exarata*) consumption are significant in children than adults.

Keywords: Trace metals; surface water; bioindicator; sediment contamination; pollution indices; health risk assessment

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1. Introduction

The ecological integrity of most intertidal coastal aquatic ecosystems in the world has been widely threatened and degraded by unprecedented levels of trace metals and metalloids pollution arising mainly from human activities. Varying quantities of trace metals and organic pollutants have been discharged directly into coastal systems as by products of many commercial and industrial processes, land and municipal sewage runoff, agricultural and domestic wastewater, effluents, and atmospheric deposition [1-3]. Previous investigations on intertidal estuarine and associated aquatic ecosystems in this part of the world have revealed that different human-mediated activities arising from crude oil spillage [4-6] can adversely alter the ecological integrity of these fragile aquatic ecosystems, leading to bioaccumulation of pollutants by biota [5-9], and heavy metals enrichment in sediment [10, 11]. However, the transport, mobilization and pollution of trace metals in aquatic ecosystems especially intertidal coastal water bodies have become an important problem due to their toxic effects, accumulation and bioconcentration through the food chain [12, 13].

Metal toxicity mainly depends on the metal speciation and bioavailability, as well as the means of uptake, accumulation and excretion rates of the organisms [12,14-16]. Body levels of some crustacean (crab) are capable of regulating essential trace metals such as Zn, Cu, Mn, Fe and Cr at concentrations below threshold level. These metals play a vital role in many physiological processes, but have a toxic effect when present at high concentrations in the surrounding medium. On the contrary, body levels of nonessential metals such as Cd and Pb are not regulated by crustacean and are toxic even at trace concentrations [17]. This could result in adverse effects such as disruption of reproductive potential, and endocrine disruption for higher trophic level organisms [18-21].

The bioavailability/remobilisation of trace metals onto aquatic substrates such as sediment, surface water, aquatic organisms and microorganisms is dependent on their

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physicochemical forms [15]. Several studies have indicated that phytoplankton and other aquatic organisms can remove, bioaccumulate and transfer bioconcentrated trace metals from lower to higher trophic levels in food webs [22-23]. These biological systems could be used in environmental studies as bioindicators or biomonitors [24-26]. To date, there are scarce data pertaining to bioaccumulation and integrated risk assessment of trace metals in aquatic substrates of this important estuary. Despite the increasing environmental and health concerns posed crude oil pollution to aquatic ecosystems in Niger Delta, the state or degree of contamination by heavy metals and associated human health risk through dietary exposure have not been duly assessed to date. Most previous studies on the occurrence of trace metals were mainly focused on quantifying hydrocarbons and heavy metals levels in water, zoobenthos, and sediment [4, 5, 6, 7]. Studies have indicated enhanced levels of trace metals in soil, surface water, sediments and biota from aquatic ecosystems in the area [5, 6, 7, 10, 67]. Many tropical ecosystems in the Niger Delta serve as primary recipients of petroleum exploration-exploitation wastes, domestic and industrial wastes generated by multinational oil companies that are found in the region. Little information is available outlining the utilization of multivariable pollution and risk assessment tools to evaluate the pollution status and potential ecological and human health risks of heavy metals in this region. Hence, the present study has been initiated with the following objectives: (1) to determine the levels of trace metals accumulation and distribution in surface water, intertidal sediment and crab (*L. exarata*) from Douglas Creek, (2) to assess its potential ecological environmental and health risks, thus contributing to the knowledge and management of this region in future. (3) to evaluate the sediment quality and environmental risks of investigated trace metals by comparison with sediment quality guidelines (SQGs); and (4) to identify the possible sources of trace metal pollution and assess their ecotoxicological significance. Our results would provide a baseline against which future anthropogenic effects can be evaluated.

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2. Materials and methods

2.1 Study area

Douglas Creek is a major tributary of Qua Iboe Estuary (Fig. 1). The estuary is characterized by shallow intertidal mudflats that are surrounded by mangroves and is perennially subjected to sediment deposition from Qua Iboe River and marine sand from the Atlantic Ocean. It is located close to several coastline settlements within an oil producing area in Southeastern Nigeria. The Qua Iboe Estuary and Douglas Creek lie within latitude 4° 30' to 4° 45'N and longitude 7° 30' to 8° 00'E. It serves as the receiving water body for residential, agricultural and petrochemical wastes generated from multinational oil companies located in the oil producing communities. Fine sandy beaches fringed with mangrove swamps and tidal mud flats on which Nypa palm vegetation dominates are distinctive features of the marginal shore of the Estuary. The area is characterized by a humid tropical climate with an annual rainfall of about 4021 mm, average humidity of 80% and means minimum and maximum temperatures of 22^oC and 30^oC respectively. Tidal currents are strong especially during the wet seasons along estuary upper reaches and creek, and this plays an important role in biota distribution, trace metal laden, waste transportation, industrial and domestic waste transportation.

2.2 Sampling

Five sampling stations (DC-A, DC-B, DC-C, DC-D, and DC-E) were established along the upstream, midstream and downstream stretch of Douglas Creek extending into Qua Iboe estuary for the collection of surface water samples. Similarly, locations DC-V, DC-W, DC-X, DC-Y and DC-Z were also mapped out as sampling locations for collection of intertidal (epipellic) sediments and crabs samples. At each sampling station, one (1) surface

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water sample and three (3) each of the intertidal sediments and crab samples were obtained from different locations, and were carefully transferred into clean polyethylene glass containers, polythene bags and preserved in ice-cooled boxes. All water samples were collected from the surface (10–25 cm) in sterile polyethylene glass bottles fitted with airtight stoppers. The containers were opened to fill and closed below the water. All containers were prewashed with 20% analytical grade nitric acid and rigorously rinsed with distilled deionized water. Prior to sampling, the containers were further rinsed at least three times with the water being sampled before collection. Preservation of collected water samples was done by acidifying with 5 mL of analytical grade nitric acid to give pH < 2, in order to minimize precipitation and sorption losses to the container walls. A short core sampler was used to collect the intertidal sediment with undisturbed sediment-water interfaces and intertidal sediment samples were collected from the top layer 1 to 5 cm, homogenized and the subsamples were stored in a black polythene bag with proper labelling. The crab (*Leptodius exarata*) was also handpicked along the tidal shores of Douglas Creek and thoroughly cleaned with fresh water followed by distilled water in order to get rid of soil and sediment before transferring them into labelled aluminium foil. Therefore, a total of 35 samples, comprising 5 samples of surface water and 15 each of intertidal sediment and crab samples were collected from the study area. After collection, all the samples were stored in ice-packed boxes and transported to the laboratory. They were further refrigerated in the laboratory at $4^{o}C$ to inactivate microbes and preserve the integrity of the samples prior to analysis.

2.3 Analytical procedures for sample pre-treatment and chemical analysis

The surface water samples collected were preconcentrated following a standard procedure as described by Ramesh et al. [27] and Essien et al. [6]. Five polyethylene bottles with snap caps were first treated with dilute HNO_3 and subsequently washed with a warm

organic detergent dissolved in distilled water. Thereafter, about 100 mL of water samples from each location ((DC-A, DC-B, DC-C, DC-D, and DC-E) were filtered using a 0.2 μm filter to remove any suspended matter and stored in prepared polyethylene bottles. After filtration, the filtrates were treated separately with concentrated HNO_3 to adjust their pH value to 4.00±0.05 before they were buffered with 2 mL of 0.1M potassium hydrogen phthalate solution (pH 4). Precisely, 2 mL of 1% (w/v) methanolic solution of sodium dibenzyl dithiocarbamate was later added to each filtrate and the solutions were stirred intermittently for about 18 - 20 minutes. Each solution was thereafter filtered under vacuum through a 0.45 μm pore membrane filter. The sorbed trace metals on the filters were subsequently eluted with 4M HNO_3 and the acid eluates were kept for metal analysis.

The intertidal sediment samples collected were air-dried by exposure to ambient air for 48 hours. Stones, sticks, organic matter and shells were manually removed from air-dried samples, while sediments with large aggregate grains were further pulverized using porcelain pestle and mortar, and sieved through a 2 mm mesh, which was hand shaken for 5-10 minutes. The sieved samples were subsequently transferred into labelled polythene bags and stored for subsequent treatment. Care was taken to avoid mixing and cross contamination of samples. Later, sieved intertidal sediment samples were separately placed in a pre-combusted glass jar, freeze dried, homogenized and sieved to collect less than 63 μm grain sizes and stored at -20^oC until further analysis. Thereafter, each intertidal sediment sample was digested as described by Ho et al. [28]. About 2.0 g of each sample was digested with a solution of concentrated HCl (6.0 mL) and HNO_3 (0.3 mL) to near dryness and allowed to cool before 20 mL of 5.0 M (1 M = 1 mol dm^{-3}) HNO_3 solution was added. Each digested intertidal sediment sample solution was allowed to stay for about 12 hours before they were filtered. The filtrates were subsequently transferred into 100 mL volumetric flask and made up to the mark with 0.5M HNO_3 prior to elemental analysis. A reagent blank was also prepared using a

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mixture of HCl and HNO_3 following the stepwise analytical procedure described for the sample preparation.

Samples of *Leptodius exarata* were dissected with sterilized scissors to collect tissue samples. Each tissue samples were oven dried at 105ºC for 1 hour and allowed to cool at room temperature, and then ground to powder using porcelain pestle and mortar. Precisely, 2.0 g of each sample was digested with a combination of concentrated HNO_3 (2.5 mL) and H_2SO_4 (2.5 mL) as described by Hosseini et al. [29]. The digested sediment and crab sample solution, acid eluates desorbed from the filter, and the blank were analyzed for the concentrations of trace metals (cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb), Zinc (Zn)) using an Atomic Absorption Spectrophotometer (S Series S4 AA System – Thermo Electron Corporation). Blanks were used for zeroing the instrument before each analysis to avoid matrix interference. The analysis was duplicated to verify the precision of the method of digestion.

2.4 Quality assurance / quality control

Buffalo River Sediment Reference Material (SRM 8704), sourced from National Institute of Standards and Technology (US), intended primarily for use in the analysis of sediments, soils, or materials of a similar matrix was analysed with the sediment samples for quality assurance purposes. Reference values and the analytical results for the concentrations of five trace metals are given in Table 1. The recoveries of the AAS analytical results for Cd, Cr, Ni, Pb and Zn ranged between 97.67 – 104.23%. The concentrations of certified materials SRM 8704 indicated results within the range of the reference values. Therefore, the method employed for this work is reliable and reproducible. Blanks were also monitored throughout the analyses and blank subtractions were employed to correct metal concentrations obtained.

2.5 Statistical analysis

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The data were analysed using the XLSTAT-Pro software (AddinSoft, Inc., NY, USA). Pearson's correlation analysis, Factors analysis (FA) and Principal component analysis (PCA) were employed to explore the interrelationships among trace metals in epipellic sediment samples and identify their probable origin. The various statistical methods were performed with a 95% confidence interval (significance p<0.05).

2.6 Assessment of bioaccumulation factors

Bioaccumulation factors (BAFs) are multipliers used to estimate concentrations of chemicals that can accumulate in tissues through any route of exposure [30]. It is refereed to as bioconcentration factor (BCF) for aquatic invertebrates. The BCF and biota to sediment accumulation factor (BSAF) of trace metals from sediment or surface water to animal tissues can be determined in different samples using the following equations:

$$BCF = \frac{concentration\ of\ heavy\ metal\ in\ animal\ tissue}{concentration\ of\ heavy\ metal\ in\ water\ sample} \quad (1)$$

$$BSAF = \frac{concentration\ of\ heavy\ metal\ in\ animal\ tissue}{concentration\ of\ heavy\ metal\ in\ sediment\ sample} \quad (2)$$

2.7 Contamination factor (CF) and Pollution load index (PLI)

The integrity of this intertidal ecosystem vis-à-vis the degree of anthropogenic trace metal accumulation in aquatic sediments was evaluated using Tomlinson's pollution load index (PLI) [31,32]. Generally, the PLI presents a generic signature of the trace metal toxicity status of a specific sediment sample of interest while expressing the number of times by which the trace metal level could actually exceeds the average natural background concentration. Thus, PLI can be employed to determine and evaluate the integrated pollution status of combined trace metals at sampling sites [3]. This parameter is expressed as a contamination factor (CF) of individual trace metal with reference to the natural background

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concentrations of each metal in sediment. The PLI is usually expressed as the nth root of the product of the n CF as:

$$CF = \frac{\overline{C}_{metal}}{\overline{C}_{background}} \qquad (3)$$

$$PLI = \sqrt[n]{CF_1 \ x \ CF_2 \ x \ CF_3 \ x........... \ CF_n} \qquad (4)$$

where, CF is the contamination factor, n = number of metals (five in the present study)

$$\overline{C}_{metal} = \text{the mean metal concentration in polluted sample,}$$

$$\overline{C}_{background} = \text{the mean natural background value of that metal}$$

For this work, the "background concentration" simply refers to predetermined concentrations of trace metals in pristine sediment devoid of any anthropogenic contamination. However, considering the unprecedented contamination of marine and coastal ecosystems in the Niger Delta region of Nigeria associated with crude oil spillages, industrial effluents, municipal sewage runoff, untreated waste dumping, urbanization and deforestation, the attribution of "background concentration" to sediment from these aquatic systems could be misleading and inappropriate. Thus, the concept of "background concentration" used here refers to the pre-anthropogenic (preindustrial) concentrations of trace elements in shale sediments as reported by Loska et al. [33] and Turekian and Wedepohl [34].

2.8 Modified degree of contamination (mCd) and Geoaccumulation index (Igeo)

The modified degree of contamination commonly denoted as *mCd* is an empirical and generalized form of the Håkanson [35] equation introduced by Abrahim [36] for the calculation of the overall degree of contamination at a given sampling site. It is expressed as follows:

$$mCd = \frac{\sum_{i=1}^{n} CF_i}{n} \qquad (5)$$

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The following gradations was proposed for the classification and description of the modified degree of contamination in sediments: mCd < 1.5 refers to nil to very low degree of contamination; $1.5 \leq mCd < 2$ indicates low degree of contamination; $2 \leq mCd < 4$ implies moderate degree of contamination; $4 \leq mCd < 8$ indicates high degree of contamination; $8 \leq mCd < 16$ means very high degree of contamination; $16 \leq mCd < 32$ implies extremely high degree of contamination and $mCd \geq 32$ refers to ultra high degree of contamination.

The *Igeo* values for the studied trace metals were calculated using the following equation developed by Müller [37,38]:

$$I_{geo} = \log_2\left(\frac{C_n}{1.5 B_n}\right) \qquad (6)$$

where, C_n is the measured concentration of selected element (n) in the sediment sample and B_n is the natural background concentration of metal n. The *Igeo* consists of seven grades along with associated sediment quality according to the degree of metal pollution (Table 5).

3. Results and discussion

3.1 Trace metal content

Trace metal levels (mg/L) in water samples were generally low with mean concentrations of Cd ($0.0018\pm4.5\times10^{-1}$), Zn ($0.0022\pm1.3\times10^{-3}$), Pb ($0.0024\pm8.9\times10^{-4}$), Ni ($0.0024\pm1.9\times10^{-3}$), and Cr ($0.020\pm6.0\times10^{-3}$) (Table 2). The values were within the WHO limit (mg/L) for these trace metals (Table 2), except at locations DC-Z where Cd and Pb levels exceeded the WHO water quality criteria [39]. As can be seen from Table 2, the concentrations of trace metals studied in the surface water were relatively low when compared with those obtained from the sediment and *Leptodius exarata* tissues.

Sediments are important host for toxic metals. Due to their static nature, they tend to accumulate more toxicant than water, which may undergo relatively rapid self-purification.

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Results (Table 2) revealed that the three sampling locations recorded the highest mean levels of trace metals, i.e, DC-X: Zn (161.4 mg/kg) and Ni (13.96 mg/kg), DC-Z: Pb (8.90 mg/kg) and Cd (1.142 mg/kg) and DC-V: Cr (15.95 mg/kg). A comparison with USEPA sediment quality guideline indicated that epipellic sediment of the investigated ecosystem was moderately polluted by Zn (Fig. 2). However, the enhanced levels of Cd, Cr, Ni, Pb and Zn in the coastal sediments indicated possible human-induced pollution such oil spillage, agricultural runoff (fertilizers, herbicides, pesticides), discharge of untreated industrial effluents and sewage containing metals into water bodies [21], and less of natural enrichment through geological weathering in the study area.

Of all trace metals examined in *Leptodius exarata* tissues (Table 2), Zn was found to be the most abundant metal. However, Cd levels observed at DC-Y (0.550 mg/kg) and DC-Z (0.931 mg/kg) and Pb levels (1.90 to 7.35 mg/kg) at all sampling locations exceeded the FAO/WHO permissible level of Cd (0.5 mg/kg) and Pb (0.3 mg/kg) (FAO/WHO [40], indicating that *Leptodius exarata* is unsuitable for human consumption. This finding emphasises the need for enforcement of stricter methods of wastes/effluents disposal, regular monitoring of metal status in this fragile ecosystem for effective management and conservation of the estuary to ensure the safety of the environment and safeguard public health. The order of trace metals accumulation potential in *L. exarata* tissues followed the sequence: Zn > Ni > Cr > Pb > Cd.

3.2 Trace metal accumulation using bioaccumulation factors

The bioaccumulation factors (BAFs), bioconcentration factor (BCF) and biota to sediment accumulation factor (BSAF) computed for *Leptodius exarata* are presented in Table 3. Results revealed a significant increase in levels of all the trace metals in *L. exarata* than in the surface water samples. The BCF values for trace metals in *L. exarata* followed the order:

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Zn>Ni>Pb >Cr>Cd. Relatively high concentration factors were observed for Zn (269,750) at DC-Y; Ni (11,650) and Pb (3300) at DC-W; Cr (478.89) and Cd (465.5) at DC-Z. The enhanced bioconcentration factors for these trace metals in tissues of *L. exarata* showed that the organism might serve as a good bioindicator for monitoring metals in polluted aquatic ecosystems. However, the BCF values for Cr and Cd were comparatively lower than Zn, Ni and Pb factors. This might suggest that *Leptodius exarata* has low retention of Cr and Cd when compared with other aquatic organisms such as oyster and mussels, which had been reported to accumulate Cd in their tissues at levels up to 100,000 times the levels observed in background water where they live [41-43]. The trend of trace metal accumulation in organisms used in the present study agrees with reports by other authors on different species of freshwater organisms [44-46]. Therefore our results indicate that *L. exarata* is a sentinel organism for biomonitoring of Zn, Ni and Pb in freshwater ecosystem.

BSAF explores the rate of metal uptake from sediment and accumulation by the crabs (*Leptodius exarata*). In the present study, high BSAF values for trace metals were recorded for Zn (2.95), Pb (1.56) and Ni (1.42) at DC-Y; Cd (0.82) at DC-Z and Cr (0.74) at DC-X; while low BSAF values were calculated for Pb (0.21) at DC-Z; Cd (0.27) at DC-W; Cr (0.32) and Ni (0.49) at DC-V; and Zn (0.98) at DC-X. From the result obtained, the metal enrichment sequence in tissues of *L. exarata* followed the order: Zn>Pb>Ni>Cd>Cr. Zinc and lead contamination levels were found to be higher in the crabs than in the sediments, suggesting a higher rate of accumulation of Zn and Pb by *Leptodius exarata*. Also our results showed higher contamination levels in the crabs than sediments in the following trace metals: Zn (DC-V, DC-W and DC-Z), Ni (DC-Y and DC-Z) and Pb (DC-V, DC-W, DC-X and DC-Y). However, Cd and Cr concentrations were generally lower in the crab tissues than in the sediments, suggesting that the levels of contamination of these metals in the estuary were within the capacity of the crabs to regulate. The observed differences in tissue metal

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concentration in crabs is similar to reports by other authors [24, 29] and this might be attributed to variations in body size, age, growth, weight, feeding habit, reproductive condition, and metabolic rate.

3.3 Sediment pollution indices

Pollution indices are used to assess and classify metal contamination in sediment. In this study, pollution load index (PLI), contamination factor (CF), modified contamination degree (*mCd*), and geoaccumulation index (*Igeo*) have been applied to assess trace metals (Zn, Pb, Cd, Ni and Cr) contamination in the sediment samples of the study area (Table 4, 5).

3.3.1 Contamination factor (CF) and Pollution load index (PLI)

In order to understand the contamination state of epipelagic sediment and surface water of the investigated ecosystem, Tomlinson's contamination factors and pollution load index were calculated. Results of CF and PLI computed for Douglas Creek of Qua Iboe estuary are given in Tables 5. According to Håkanson classification [35] (Table 4), there are locational differences to the degree of sediment metal contamination of the studied ecosystem. The status of integrated sediment contamination based on the CF values indicated that the epipelagic sediments showed low contamination (CF<1) for Pb, Ni and Cr (at all locations) and Zn (DC-Y only); moderate degree of contamination ($1 \leq CF < 3$) for Cd (DC-V, DC-W and DC-X) and Zn (DC-V, DC-W, DC-X and DC-Z); and considerable degree of contamination ($3 \leq CF < 6$) for Cd at DC-Y and DC-Z locations. It is imperative that regular monitoring and assessment should be carried out at the sampling locations, where Zn and Cd indicated categories of moderate and considerable contamination. This is necessary since sediments are major repositories of metals, and long term environmental inputs of these toxic trace metals could eventually lead to enhanced degree of ecosystem sedimentary

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contamination as well as attendant biotransfer and bioaccumulation in plants and animals of economic importance, which may ultimately get biomagnified up the food web [47-50].

PLI was also used to further probe the degree of pollution of the intertidal sediments collected from our study area. Based on our results, the PLI values ranged between 0.42 and 0.56 (Table 4). The generally low values of PLI (< 1) at all studied locations implied that the intertidal ecosystem might not have been critically impacted by anthropogenic contamination. However, the bioavailability of anthropogenic trace metals load into similar ecosystem has been reported as remarkably enhanced in concentrations compared to their natural background sources [29,51,52].

3.3.2 Assessment of modified degree of contamination and geoaccumulation index

The range of *mCd* values (0.74 − 1.17) for the analysed metals in this study suggests a very low degree of contamination in all sites. Based on the *Igeo* data and Müller geoaccumulation index, the contamination level with respect to each metal is shown in Table 5 (*Igeo* class). The geoaccumulation index values (*Igeo*) showed very low values (<0) for Pb, Ni and Cr and Zn (except at DC-X), implying that there is no contamination by these trace metals in the Douglas creek sediments. The *Igeo* values (> 0) for Cd at DC-V, DC-W and DC-X sites and Zn (DC-X only) indicated that sediments from investigated locations are uncontaminated to moderately contaminated with Cd and Zn as a result of anthropogenic activities. However, the *Igeo* values (> 1) for Cd at DC-Y and DC-Z indicated that the epipellic sediments from these two locations were moderately contaminated with Cd.

3.4 Potential ecological risks

In order to assess the characteristics and the extent of potential ecological hazards posed by metals in sediments, a quantitative method proposed by Håkanson [35] was adopted. The potential ecological risk index (PERI) primarily assesses the probable degree of trace metal

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contamination in coastal sediments, taking into perspective the relative toxicity of the overall metals and the short- to long-term response of the environment. The risk index (R_I) is calculated based on the equation:

$$R_I = \sum E_f^i \tag{7}$$

$$E_f^i = \sum T_r^i \left(C_s^i \middle/ C_n^i \right) \tag{8}$$

where R_I is calculated as the sum of individual risk factors for all trace metals in sediments, E_f^i is the monomial PERI for a single metal, C_s^i is the observed concentrations of metals in sediment samples, and C_n^i is the background values of trace metals, and T_r^i is the toxic response factor for a selected metal. The T_r^i for Cd, Cr, Ni, Pb and Zn are 30, 2, 5, 5 and 1 respectively [8, 35, 53].

Table 6 shows the potential ecological risk index of each trace metal at their respective studied sites as well as the integrated ecological risk index, R_I. The mean PERIs for individual metal stressors showed that the degree of trace metal pollution in the intertidal sediments was sequenced as Cd>Pb>Zn>Ni>Cr. The DC-V (E_f^i = 54.9) and DC-W (E_f^i = 59.40) sampling sites indicated moderate degree of potential ecological risk for Cd, while DC-X (E_f^i = 90.0), DC-Y (E_f^i =110.10) and DC-Z (E_f^i =114.3) exhibited considerable ecological risk. Nevertheless, the coastal ecosystem under investigation generally showed low potential ecological risk that was associated with Cr, Pb, Ni and Zn. Results of the multi-elemental potential ecological risk index (RI) computed for DC-Y and DC-Z sites indicated moderate potential ecological risk, while DC-V, DC-W and DC-X showed low ecological risk. However, the mean R_I for the entire intertidal estuarine ecosystem indicated the existence of low degree of potential ecological risk (R_I < 95).

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3.5 Ecotoxicological assessment of trace metals levels in sediments

To evaluate the sediment contamination and potential ecotoxicological effects associated with the observed concentration of contaminants, two sets of SQGs developed for marine and estuarine ecosystems [54,55] were applied in this study to assess the ecotoxicological potential of trace metal concentrations in sediments (a) the effect range-low (ERL)/effect range-median (ERM); and (b) the threshold effect level (TEL)/probable effect level (PEL) values (Table 7). Low range values (ERLs/TELs) are concentrations below which adverse effects upon sediment dwelling fauna would infrequently be expected. In contrast, the ERMs and PELs represent chemical concentrations above which adverse effects are likely to occur [55].

Two different ways of comparison that have been included in this study are: the number of single-species limits values exceeded and the mean quotient calculable from the two empirically derived sets of SQGs using PEL and ERM values. Table 7 gives the number of all samples in three ranges of chemical concentrations where adverse biological effects are expected rarely (<TEL/ERL), occasionally (≥TEL/ ERL and <PEL/ERM) and frequently (≥PEL/ERM). For Pb, Ni, Zn and Cr in all the sediment samples were in the minimal effect-range (<TEL/ERL); while for Cd at DC-X, DC-Y and DC-Z sampling locations were above the threshold effect level (> TEL). For all the metals studied, none of the sediments were in the probable effect-range and effect range median (≥PELs/ERMs).

In order to determine the possible biological effect of combined toxicant groups, one can calculate the mean quotient for a large range of contaminants. This mean ERM quotient (m-ERM-Q) has been calculated according to [56] as follows:

$$m-ERM-Q = \frac{\sum_{i=1}^{n}(C_i/ERM_i)}{n} \tag{9}$$

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where C_i is the sediment concentration of compound i, ERM_i is the ERM for compound i and n is the number of compound i. Similarly, the mean PEL quotient (m- PEL- Q) can be calculated according to the equation:

$$m - PEL - Q = \frac{\sum_{i=1}^{n}(C_i / PEL_i)}{n} \qquad (10)$$

where, PEL_i is the PEL for compound i.

Mean ERM quotients have been related to probability of toxicity [55, 57, 58] based on the analysis of matching chemical and toxicity data from 1068 samples from the USA estuaries. The mean ERM quotient of <0.1 has a 12% probability of being toxic; a mean ERM quotient of 0.11- 0.5 has a 30% probability of toxicity; a mean ERM quotient of 0.51- 1.5 has a 40% of being toxic and a mean ERM quotient of >1.50 has a 74% of toxicity. According to this classification, all the sediment samples studied can be classified as "medium–low priority" sites with 30% probability of toxicity (m-ERM-Q = 0.12 − 0.16). Similarly, the mean PEL quotient ranged from 0.19 for DC-V to 0.25 for DC-X and DC-Z. The mean PEL quotients have been classified into four grades as follows: low degree of contamination (≤0.1), medium-low degree of contamination (0.11–1.5), high-medium degree of contamination (1.51–2.3), and high degree of contamination (>2.3), respectively having a 8%, 21%, 49% and 73% probability of being toxic [57]. The values of mean PEL quotients obtained for these sediments are low (0.19-0.25), and as such will be expected to have medium-low degree of contamination with 21% probability of being toxic.

3.6 Health risk assessment of trace metals

The health risk assessment associated with trace metal pollution was evaluated using the edible muscle tissues of *Leptodius exarata* by calculating the estimated dietary intake (EDI) and target hazard quotients (THQ).

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3.6.1 Estimated daily intake (EDI)

The daily intake (DI) of contaminants is a primary function of its concentration in food matrix, the daily consumption of food and the body weight of the food consumer [46, 59, 60]. In view of these factors, the estimated daily intake (EDI) of trace metals by adults and children that consumes crabs was evaluated using the following equation:

$$EDI = \frac{C_{metal} \ x \ DNI \ x \ C_f}{Bw} \qquad (11)$$

where C_{metal} is the concentration (mg kg^{-1}) of the trace metals in the muscle tissue of *L. exarata*, DNI is the daily nutritional intake in (g day^{-1}), and C_f is the factor for conversion of fresh crab tissues to dry constant weight. The average moisture content in *L. exarata* was 74.25% and the C_f (0.2250) was calculated using the equation as reported by Abubakar et al. [61]. The average body weight for adults and children (age range 6–18 years), in Nigeria were 70 kg and 48 kg, respectively. The daily nutritional intake of crabs was evaluated by adopting the ingestion rate for Nigeria based on 2011 estimate by FAO. The DNI for adults and children were 62.60 g capita^{-1} day^{-1} and 60.0 g day^{-1}, respectively [62].

3.6.2 Target hazard quotients (THQ)

Health risk assessment associated with non-carcinogenic contaminants is typically expressed in terms of the ratio of the determined dose of a contaminant to the reference dose (Rf_D) below which such contaminants might likely pose any appreciable health risk. This noncancer risk assessment method is the target hazard quotient (*THQ*) and was determined in this study as described by US EPA [63].

$$THQ = \frac{EF \ x \ ED \ x \ FIR \ x \ C_{metal}^i}{RfD \ x \ Bw \ x \ ATn} \ x \ 10^{-3} \qquad (12)$$

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$$THQ_{tot} = \sum_{i=1}^{i=5} THQ_i \tag{13}$$

where *EF* is exposure frequency (365 d/year); *ED* is the exposure duration (52.5 years) (World Bank 2013 estimate for average life expectancy in Nigeria) [64]; *FIR* is the seafood ingestion rate (62.6 and 60 g capita^{-1}day^{-1} for adults and children, respectively); C_{metal} is the concentration of trace metal in (mg kg^{-1}); BW is the average body weight (70 and 48 kg for adults and children, respectively), and ATn is the averaging exposure time for non-carcinogens (365 d year^{-1} × *ED*); *RfD* is the oral reference dose (mg kg^{-1} day^{-1}). The *RfDs* for Cd, Cr, Ni, Pb and Zn are 0.001, 1.5, 0.02, 0.035 and 0.3 mg kg^{-1} d^{-1}, respectively [65]. The total *THQ* (*THQ_{tot}*) was used to evaluate the overall exposure to the metal toxicants under consideration in order to assess their combined health effects. The total *THQ* was treated as the arithmetic sum of the individual metal *THQ* values [46,66].

Results indicated that the estimated dietary intakes (*EDIs*) for Cd, Cr, Ni, Pb and Zn through possible consumption of crabs harvested from the mangrove ecosystem were 0.10, 1.36, 2.70, 1.12 and 42.02 mg kg^{-1} day^{-1} for adults, and 0.36, 4.81, 9.54, 3.95 and 148.39 mg kg^{-1} day^{-1} for children aged between 6–18 years. The *EDIs* of trace metals investigated were relatively higher for children than those obtained for adults. Results showed that the average *EDIs* exceeded the *RfDs* for adults (except Cr) and children indicating that the consumption of crabs harvested from the studied ecosystem would likely result in noncancer health risks. The daily nutritional intake (*DNI*) of Cr did not exceed the *RfD* in adults. However, the excessive consumption of crabs may possibly result in eventual bioaccumulation and bioconcentration of Cr in adults leading to serious deleterious effects. Fig. 3 shows the *THQ_{tot}* for adults and children associated with consumption of crabs. The THQs of each trace metal obtained for adults were 0.39, 0.03, 0.52, 1.24 and 0.54 for Cd, Cr, Ni, Pb and Zn, respectively. Also, the THQs calculated for children were 1.38, 0.01, 1.85, 4.39 and 1.93 for

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Cd, Cr, Ni, Pb and Zn, respectively. In the adults, the *THQ* value for Pb was greater than 1 through the consumption of crabs. This implies that there could be possible health risks associated with Pb.

For the children, there are greater health risks that would be linked to consumption of crabs since the *THQs* of Cd, Ni, Pb and Zn were greater than 1. Obviously, the children are more vulnerable to health risks associated with trace metals contamination than adults. In general, the THQ_{tot} for children ($THQ_{tot} = 9.58$) was higher compared to the THQ_{tot} for the adults ($THQ_{tot} = 2.70$).

3.7 Factor analysis

Factor analysis (FA) was applied to interpret relationships between variables and we have used the technique of correlation analysis and Principal Component Analysis (PCA) to extract significant principal components (PCs) and further reduce the contribution of variables with minor significance. The XLSTAT software (version 2015.1.03) was used for the PCA. Pearson correlation analysis indicated that a significant correlation (r = 0.799, *p<0.05*) was found between Pb and Cr indicating that these trace metals might have originated from same pollution source(s) [9, 10, 49, 67]. Correlation coefficients derived from interrelationships among investigated trace metals were either negative or positive but not significant. Results of n-Pearson PCA conducted, primarily elucidated the interrelationships among the five studied trace metals and also identify their possible sources (Table 8). The factors loading presented in Table 9 indicated that the concentrations of trace metals for the intertidal sediments of the studied ecosystem were grouped into a two principal component model. The loading plot and the biplot of the PCs are presented in Fig. 4. The Eigen values of the PCs are greater than 1 and in general accounted for 74.12% of the variability in concentrations of trace metals found in the intertidal sediments. The PC1 indicated that 44.31% of the total variance was positively related to Cd, Cr, Pb and Ni, with

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Cr and Pb showing relatively strong relationships. Zn was moderately negatively related to PC1. However, the loading of Ni and Cd with PC1 suggested possible contamination could be influenced by anthropogenic pollution sources into the sediment in addition to background contributions. PC2, which explained 29.81% of the total variance indicated positive interrelationships with Cd, Ni and Zn, while Pb and Cr were negatively related to PC2.

4. Conclusion

Trace metals (Cd, Cr, Pb, Ni, and Zn) were detected in *Leptodius exarata* epipellic sediment and surface water collected from Douglas Creek, Qua Iboe estuary. The concentrations of the metals exhibited significant variability between sampling sites and environmental matrices. The mean concentration of some trace metals exceeded the recommended guideline values in most matrices, implying that the aquatic ecosystem and its biota may be exposed to short- and long-term metals pollution. Cd levels in *L. exarata* obtained at DC-Y and DC-Z, and Pb in all locations exceeded the FAO/WHO permissible level of 0.5 mg/kg. Trace metal enrichment in the tissues of *L. exarata* ranked in the following order: Zn > Pb >Ni > Cd > Cr. However, the modified degree of contamination (mCd) and pollution load index (PLI) for all analysed metals indicated very low degree of contamination at all sites. On the contrary, the contamination factor (CF) values showed that sediments were moderately contaminated with Cd and Zn, and considerably contaminated with Cd at DC-Y and DC-Z. Most of the calculated *Igeo* values for Zn showed that the sediments were practically uncontaminated at all sites (*Igeo* < 0) except at DC-X site. The sediments from DC-V, DC-W and DC-X were uncontaminated to moderately contaminated (*Igeo* > 0) with Cd and Zn (only at DC-X) as a result of anthropogenic activities. However,

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epipellic sediments from DC-Y and DC-Z sites were moderately contaminated ($Igeo > 1$) with Cd.

The mean-ERM quotient and mean-PEL quotient of all the sediment samples were low, and as such will be expected to have medium-low degree of contamination with 30% and 21% probability of being toxic, respectively. The mean R_I for the entire intertidal estuarine system indicated the existence of low degree of potential ecological risk ($R_I < 150$). PCA has proved to be an effective tool for investigating the source(s) metal pollution. The results of our analysis revealed underlying relationships among the trace metal data, which had physical meanings. More specifically, PCA applied on trace metal pollution data resulted in components attributed to possible contamination, which could be influenced by anthropogenic pollution sources into the sediment in addition to background contributions. Health risks (*EDI* and *THQ*) associated with trace metals contamination in *L. exarata* were significant in children than adults.

Conflicts of interest
The authors declare no conflict of interest

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Table 1: Reference (SRM 8704) concentration values, analytical results and percentage recovery

Metals	RM 8704 reference values (mg/kg)	AAS results (mg/kg) (n=3)	Accuracy (%Recovery)
Cadmium	2.94±0.29	3.03±0.04	102.96
Chromium	121.90±3.80	119.47±1.64	98.01
Nickel	42.90±3.70	40.86±0.18	95.23
Lead	150.00±17.00	156.04±6.95	104.23
Zinc	408.00±15.00	398.60±10.54	97.67

Table 2. Trace metal levels (means, ±SD, n = 3) in sediment, crab (mg/kg dw) and surface water (mg/L) from Douglas Creek (DC)

Sample Type	Sample site	Zn	Pb	Cd	Ni	Cr
Sediment	DC-V	120.8±7.9	5.75±0.84	0.550±0.033	9.15±0.82	15.95±1.47
	DC-W	112.6±9.4	6.01±0.52	0.595±0.054	13.15±1.19	10.63±0.81
	DC-X	161.4±12.8	2.00±0.22	0.900±0.086	13.96±1.42	9.57±0.92
	DC-Y	91.5±6.4	3.45±0.28	1.100±0.098	10.45±0.96	13.28±0.88
	DC-Z	121.6±10.6	8.90±0.93	1.142±0.090	12.67±1.06	13.78±1.34
Crab	DC-V	184.45±9.56	7.35±0.78	0.250±0.025	4.45±0.38	5.13±0.46
	DC-W	175.20±8.93	6.60±0.44	0.162±0.032	11.65±1.46	3.81±0.39
	DC-X	157.50±13.83	3.05±0.54	0.291±0.018	11.20±0.79	7.11±0.74
	DC-Y	269.75±12.35	5.40±0.43	0.550±0.059	14.80±1.53	4.95±0.40
	DC-Z	125.55±8.54	1.90±0.22	0.931±0.075	17.15±1.47	8.62±0.88
Surface water (x 10^{-3})	DC-A	$4.0\pm3.5x0^{-1}$	$4.0\pm2.4x10^{-1}$	$1.0\pm7.3x10^{-2}$	$1.0\pm6.7x10^{-2}$	16.0±1.3
	DC-B	$1.0\pm5.2x10^{-2}$	$2.0\pm1.6x10^{-1}$	$1.0\pm4.6x10^{-2}$	$1.0\pm4.0x10^{-2}$	18.0±1.6
	DC-C	$3.0\pm2.8x10^{-1}$	$2.0\pm1.6x10^{-1}$	$2.0\pm8.2x10^{-2}$	$1.0\pm8.9x10^{-2}$	$17.0\pm8.5x10^{-1}$
	DC-D	$1.0\pm9.2x10^{-2}$	$2.0\pm2.1x10^{-1}$	$3.0\pm1.4x10^{-1}$	$4.0\pm3.4x10^{-1}$	31.0±2.4
	DC-E	$2.0\pm1.7x10^{-1}$	$2.0\pm1.9x10^{-1}$	$2.0\pm1.2x10^{-1}$	$5.0\pm4.2x10^{-1}$	18.0±2.8
Water quality criteria	WHO limit[a]	3000	10	3.0	20	50
	US EPA[b]	n.i	0	n.i	100	100
	USA Protection	180	10	n,i	50	50

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$(\mu g / L)$	of aquatic life					

[a]World Health Organization[68]; [b]USEPA [69], [c]Frits [70].

Table 3: Trace metal accumulation levels in tissue of crab (*loptodius exarata*) using biota to sediment accumulation and bioconcentration factors.

Bioaccumulation Factors	Sample code	Zn	Pb	Cd	Ni	Cr
BSAF	DC-V	1.53	1.28	0.45	0.49	0.32
	DC-W	1.56	1.10	0.27	0.88	0.36
	DC-X	0.98	1.52	0.32	0.80	0.74
	DC-Y	2.95	1.56	0.50	1.42	0.37
	DC-Z	1.03	0.21	0.82	1.35	0.62
BCF	DC-V	46112.5	1837.5	250	4450	320.62
	DC-W	175,200	3300	0.162	11650	211.67
	DC-X	52,500	1525	145.5	11200	418.24
	DC-Y	269,750	2700	183.33	3700	159.68
	DC-Z	62,775	950	465.5	3430	478.89

Table 4. Trace metal concentrations (mg/kg) of sediments, SQG by US EPA, and Pollution Load index (PLI) of metals in sediment types from Douglas Creek

Trace metals	Sediment codes					Natural background concentration	SQG		
	DC-V	DC-W	DC-X	DC-Y	DC-Z		Non-polluted	Moderately polluted	Heavily polluted
Zn	120.8	112.6	161.4	91.5	121.6	95.16 ± 0.20[a]	<90	90–200	> 200
	(1.27)	(1.18)	(1.7)	(0.96)	(1.28)				
Pb	5.75	6.01	2.00	3.45	8.90	20.08 ± 0.18	<40	40–60	> 60
	(0.29)	(0.3)	(0.1)	(0.17)	(0.44)				
Cd	0.55	0.595	0.900	1.10	1.142	0.30 ± 0.00	–	–	> 6
	(1.83)	(1.98)	(3)	(3.67)	(3.81)				
Ni	9.15	13.15	13.96	10.45	12.67	68.48 ± 1.79	<20	20–50	> 50
	(0.13)	(0.19)	(0.21)	(0.15)	(0.19)				
Cr	15.95	10.63	9.57	13.28	13.78	88.72 ± 15.67	<25	25–75	> 75
	(0.18)	(0.11)	(0.15)	(0.15)	(0.13)				
mCd	0.74	0.75	1.03	1.02	1.17				
PLI[b]	0.44	0.43	0.44	0.42	0.56				

Values in parenthesis are the contamination factors; [a]Average natural background concentration ($\pm SD$, n=3), [b]Gong et al.[71].

Table 5. I_{geo} classes[a] of trace metals in sediments from Douglas Creek of Qua Iboe estuary

Trace metals	$Igeo$[b]				
	DC-V	DC-W	DC-X	DC-Y	DC-Z
Zn	0	0	1	0	0
Pb	0	0	0	0	0
Cd	1	1	1	2	2
Ni	0	0	0	0	0
Cr	0	0	0	0	0

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[a] >5, extremely contaminated; 4–5, strongly to extremely strongly contaminated; 3–4, strongly contaminated; 2–3, moderately to strongly contaminated; 1–2, moderately contaminated; 0–1, uncontaminated to moderately contaminated; <0, uncontaminated, [b]Buccolieri et al. [72],

Table 6: The potential ecological risk assessment[a] for investigated trace metals in epipellic sediments from Douglas creek, Qua Iboe Estuary

Sampling sites	Calculated E^i_f of individual trace metals[a]					R_I^b
	Zn	Pb	Cd	Ni	Cr	
DC-V	1.27	1.45	54.90	0.65	0.36	58.63
DC-W	1.18	1.50	59.40	0.95	0.22	63.25
DC-X	1.70	0.50	90.00	1.05	0.30	93.55
DC-Y	0.96	0.85	110.10	0.75	0.20	112.96
DC-Z	1.28	2.20	114.30	0.95	0.01	118.74
Min.	0.96	0.50	54.90	0.65	0.01	58.63
Max.	1.28	2.20	114.30	1.05	0.36	118.74
Mean	1.27	1.30	85.74	0.87	0.24	89.43

[a]$E^i_f < 40$, Low risk; $40 \leq E^i_f < 80$, Moderate risk; $80 \leq E^i_f < 160$, Considerable risk; $160 \leq E^i_f < 320$, High risk; $E^i_f \geq 320$, Very high risk; [b]$R_I < 150$, Low risk; $150 \leq R_I < 300$, Moderate risk; $300 \leq R_I < 600$, High risk; $R_I \geq 600$, Very high risk.

Table 7. TEL, PEL, ERL and ERM guideline values for trace elements[a,b] and mean quotients using the PEL and ERM values

Trace metals	Sediments					TEL	PEL	ERL	ERM
	DC-V	DC-W	DC-X	DC-Y	DC-Z				
Zn	120.8	112.6	161.4	91.5	121.6	124.0	271.0	150.0	410.0
Pb	5.75	6.01	2.00	3.45	8.90	30.2	112.2	46.9	218.0
Cd	0.55	0.595	0.90	1.10	1.142	0.6	3.53	1.2	9.6
Ni	9.15	13.15	13.96	10.45	12.67	15.9	42.8	20.9	51.6
Cr	12.95	10.63	9.57	13.28	13.78	52.3	160.4	81.0	370.0
m-PEL-Q	0.19	0.20	0.25	0.20	0.25				
m-ERM-Q	0.12	0.13	0.16	0.12	0.15				

[a]Long et al. [56], [b]Concentrations are in mg/kg dry weight

Table 8: Pearson correlation coefficients ($p<0.05$) between different trace metals in epipellic sediment samples.

	Zn	Pb	Cd	Ni	Cr
Zn	**1**				
Pb	-0.308	**1**			
Cd	-0.107	0.038	**1**		
Ni	0.385	0.419	0.493	**1**	
Cr	-0.309	0.799	0.332	0.199	**1**

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Table 9: Loadings of variables on significant principal components for trace metals in sediment

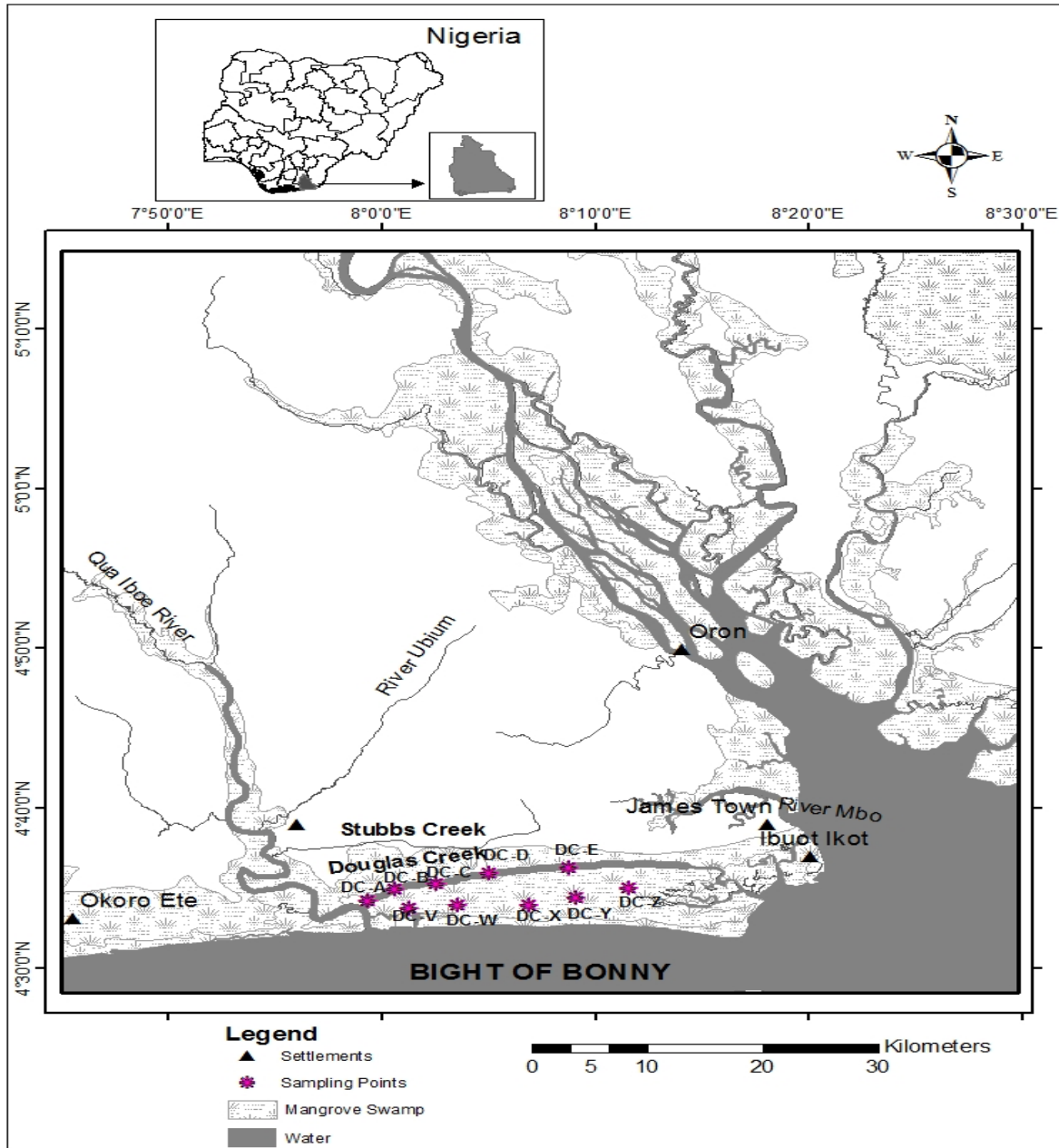|	PC1	PC2
Zn	-0.314	0.793
Pb	0.868	-0.207
Cd	0.522	0.417
Ni	0.557	0.766
Cr	0.884	-0.243
Eigenvalue	2.215	1.490
Variability (%)	44.310	29.805
Cumulative %	44.310	74.115

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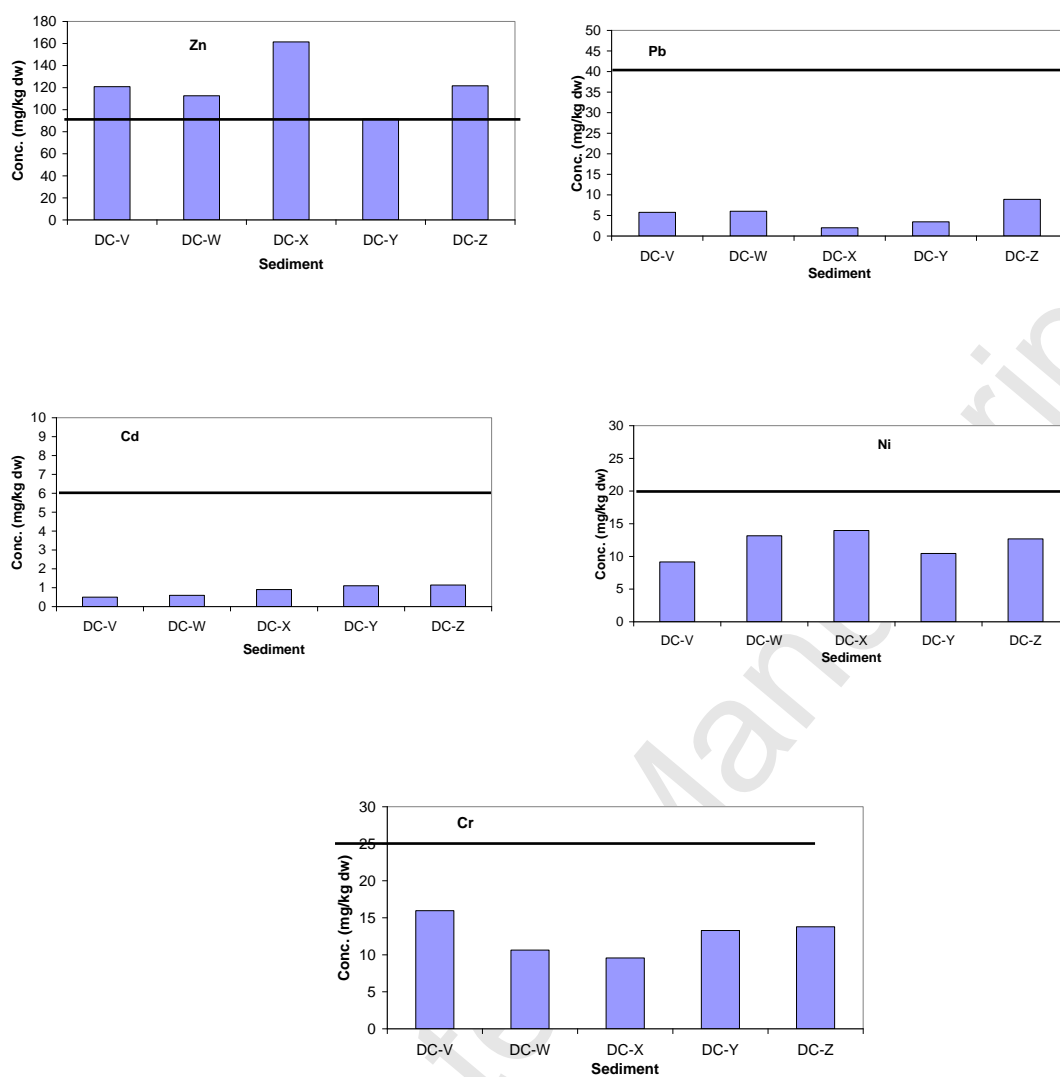Fig. 1: Qua Iboe Estuary mangrove ecosystem showing the sampling location along Douglas Creek. Insert: Map of Nigeria showing the location of the study area.

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Fig. 2 Trace metal concentrations in sediments from Douglas Creek of Qua Iboe estuary. *Straight horizontal line represents a moderately polluted level (except Cd, representing heavily polluted) as per SQG by USEPA*
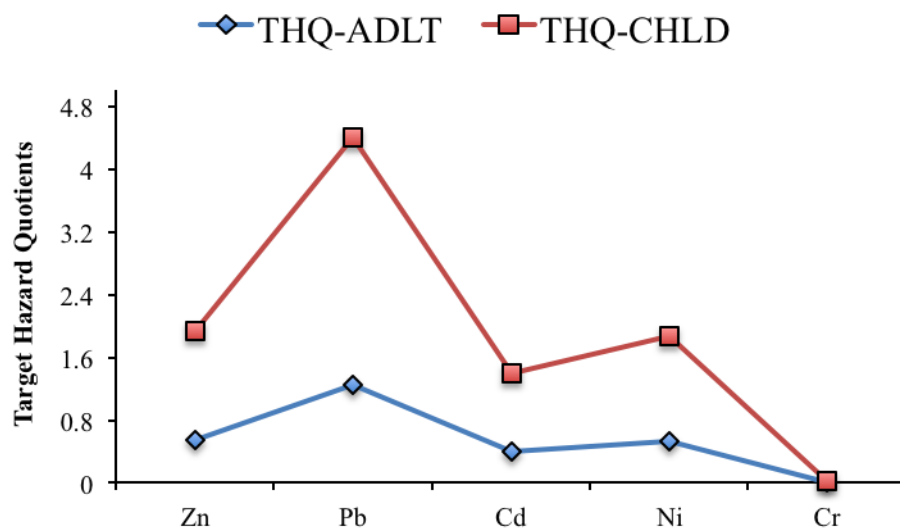
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Fig. 3. Total target hazard quotients due to consumption of *Leptodius exarata*
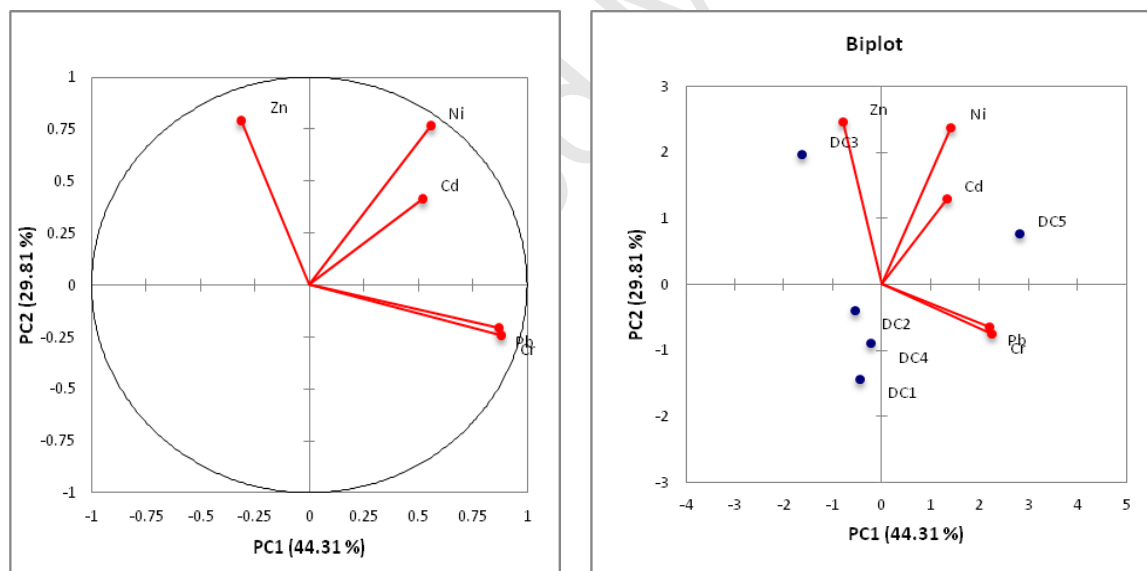

Fig. 4: Loading plot and biplot of the principal components obtained for trace metals at all investigated sites

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