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Species-specific toxicological responses in relation to body burden and bioaccumulation pattern of polycyclic aromatic hydrocarbons (PAHs) in a tropical estuarine food web

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ABSTRACT

The aim of this study was to investigate oxidative stress, biotransformation and bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) in tropical estuarine food webs including fish (*Chrysicthys nigrodigitatus*), fiddler crab (*Uca tangeri*), blue crab (*Calinectis amnicola*), prawn (*Macrobrachium vollenhovenii*), periwinkle (*Tympanotonus fuscatus*) and sediment samples at three sites, Adiabo (control site), Obutong and Nsidung representing different degrees of anthropogenic contamination along Cross River Estuary, Nigeria. Hepatic oxidative stress and biotransformation enzyme activities glutathione peroxidase (Gpx), glutathione reductase (Gr), glutathione S-transferase (Gst), uridine diphosphate glucuronosyltranferease (Udpgt), 7-ethoxy-, methoxy-, pentoxy-, and benzyloxyresorufin O-deethylase (EROD, MROD, PROD and BROD) and PAHs levels were determined. Data demonstrated species- and site-specific mediated toxicological effects in oxidative stress, biotransformation responses, and PAHs bioaccumulation in biota and sediments from contaminated sites (Obutong and Nsidung), compared to control (Adiabo). The EROD, MROD, BROD, PROD activities and GPx, Gr, Gst, Udpgt exhibited significant increase in biota collected from contaminated sites at Obutong and Nsidung compared with control Adiabo. These biomarker response observations paralleled PAHs accumulation at Obutong and Nsidung suggesting PAHs exposure induced oxidative and biotransformation biomarker responses. Principal component analysis (PCA) produced significant associations between variables indicating sites were major factors determining contaminants uptake and biomarker responses in biota (fish, crabs, prawn and periwinkle). Data demonstrated site and species-specific occurrence and concentrations of PAHs in sediment and tropical estuarine food webs with corresponding biotransformation and oxidative stress responses on resident biota. Concentrations of PAHs detected in these tropical food webs indicate serious human food safety and environmental health concerns.

Introduction

Multiple and complex environmental stress resulting from continuous and unregulated release of several classes of contaminants including polycyclic aromatic hydrocarbons (PAHs) and other persistence organic pollutants (POPs) into the aquatic ecosystem is of significant ecological and public health concerns in tropical ecosystems (Arukwe et al. 2015; Honda and Suzuki 2020; Hylland 2006; Ogunkoya, Sogbanmu, and Seiler 2024). PAHs are specific toxic compounds with two or more fused aromatic rings that are ubiquitous in several environmental matrices (Honda et al. 2018; Miki et al. 2014). Due to their potential rapid metabolism, these compounds are considered as priority environmental contaminants owing to their ability to bioaccumulate and biomagnify across several aquatic food fish species and food webs (Chormare and Kumar 2022; Saidon et al. 2024). Approximately four different sources of PAHs inputs into the aquatic ecosystem were identified including fuels (petrogenic), incomplete combustion process (pyrogenic), organic metabolism (biogenic) and transformation process in

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sediment (diagenetic), with petrogenic and pyrogenic sources noted as important and major contributors of PAHs contamination into the aquatic environment (Hylland 2006; Jafarabadi et al. 2020). Further, crude oil exploitation and exploration activities including spillage and bunkering (in developing countries) were identified amongst the major sources of PAHs contaminant in the aquatic and terrestrial ecosystems (Hayakawa 2018; Ladwani, Ladwani, and Ramteke 2013; Laffon, Pásaro, and Valdiglesias 2016; Takeshita et al. 2021; Uno et al. 2017).

While there are available data on global PAHs contamination in several aquatic food fish species across regions of the world (Teixeira et al. 2024), there are limited reports on PAHs mediated toxicological responses from low- and middle-income countries such as Nigeria due to limited research capacity and infrastructures. These problems are exacerbated by weak environmental regulations and enforcements in these crude oil-rich regions (Adeogun, Ibor, Regoli, et al. 2016b). Species-specific PAHs mediated toxicological responses provides a holistic biomonitoring approach and contributes significantly toward global ecotoxicological surveillance for aquatic ecosystems health from a developing country standpoint. Therefore, understanding individual species level differences and their mechanisms of contaminants biotransformation and metabolism may help in designing better ecotoxicological monitoring animal models and protocols that might improve our understanding of environmental and biota health.

These groups of contaminants were reported to produce a wide range of toxicological, physiological and metabolic effects in exposed organisms, including carcinogenic, immunotoxic, liver damage, DNA methylation (Cocci et al. 2018), teratogenic and mutagenic effects (Bandowe et al. 2014; Lohmann et al. 2013; Osman et al. 2010; Segner et al. 2021; Tairova et al. 2017). Other investigators demonstrated PAHs trophic dynamics and transfer across several food chains and webs (Goutte et al. 2020; Han et al. 2022; Liu et al. 2023; Navarro et al. 2013). Further, exposure of organisms to these xenobiotics results in the activation of physiological process that are targeted toward metabolizing and possibly elimination of these chemical contaminants from the body in a biological process referred to as biotransformation (Arukwe et al. 2015; Galloway et al. 2004; Ibor, Khan, and Arkuwe 2023). In the initial phase I of the biotransformation process, the parent compound is functionalized through the addition of a hydroxy (OH) group (Testa, Pedretti, and Vistoli 2012). In phase II, a covalent linkage is formed between the functional group and an endogenous molecule to produce water soluble conjugate that facilitates excretion of the xenobiotic compound (Lech and Vodicnik 1984). The induction of CYP enzymes measured as EROD, PROD, MROD and BROD in organisms play important role in the biotransformation process and are involved in detoxification and/or bioactivation mechanisms which is crucial in ecotoxicological monitoring and health consequences (Whyte et al. 2000). In addition, Huang et al. (2016) noted changes in enzymatic activities in biotransformation and oxidative stress responses in fish species as early warning sensitive biomarkers of environmental exposures to a wide range of contaminants including PAHs. A wide range of several environmental contaminants including PAHs are pro-oxidant stressors inducing the intracellular production of reactive oxygen species (ROS) resulting in oxidative stress (Ibor et al. Arukwe 2021). This is because, an array of enzymes involved in the oxidative stress defense network generally function as antioxidant and include glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and UDP-glucuronosyltransferases (UDPGT) (Giuliani et al. 2013; Regoli and Giuliani 2014; Regoli et al. 2014). Therefore, the induction of biotransformation and oxidative stress enzymes was adopted as sensitive and reliable biomarkers of exposure to environmental pollution in ecotoxicological monitoring (Goksøyr and Förlin 1992). Other investigators found that PAH exposure produced global DNA methylation in zebrafish (Fang et al. 2013), while specific PAH congener concentrations were positively associated with oxidative, biotransformation responses and global DNA methylation biomarker responses in loggerhead seas turtles from the Adriatic Sea, Italy (Cocci et al. 2018).

The Calabar river is an ecological and economically important river in southern Nigeria (Moses 2000) and receives complex mixtures of untreated

industrial, agricultural and domestic effluents. Due to its strategic location as port of entry into the Atlantic Ocean, the river plays an important economic role in southern Nigeran as a major route for crude oil exploitation and exploration avenue including oil spillage and bunkering (Moses 2000). Further, the Calabar River serves as a major fishing ground and fisheries including breeding and nursery for several aquatic food fish (Holzloehner et al. 2007). Thus, the Calabar River is believed to be heavily polluted due to poor enforcements and regulations of environmental laws in Nigeria. Previously, the effects of environmental perturbations including climate change and ecological distribution of ichthyofauna assemblages of the river were reported (Ama-Abasi et al. 2022; Andem, Ekanem, and Oku 2016). Other investigators identified the occurrence and distribution of several emerging and reemerging groups of environmental contaminated in biota, water and sediment samples from the river (Adebusuyi, Sojinu, and Aleshinloye 2022; Ilechukwu et al. 2018; Oyo-Ita et al. 2014). Despite these reports on the occurrence and concentrations of these contaminants, information on biomarker responses on resident biota is limited or nonexistence. At present the relationship between specific PAH congeners with catalytic biotransformation and oxidative stress responses in tropical estuarine food webs from the Cross River Estuary, Nigeria is not known. Therefore, the aim of the study was to investigate the oxidative stress and biotransformation responses in relation to PAHs bioaccumulation in tropical estuarine food webs using the Cross River Estuary in Nigeria, as a model. This study constitutes an ongoing integrated effort aimed at biomarker development and validation in tropical aquatic food fish species with respect to sustainable environmental, food safety and human health management from a developing country perspective.

Materials and methods

Chemicals and reagents

All chemicals and reagents were of the highest commercially available grades (see SI file).

Study area

The Calabar River, is part of the Cross River estuary located in the tropical rain forest belt of southern Nigeria and lies between latitude 4°54′ and 5°50′N and longitude 8°and 8°20′E (Figure 1). This river is dredged by the Nigerian Port Authority (NPA) for sea-going vessels and the shoreline consist of soft-dark mud flats which are usually uncovered during the low tide. The Calabar River has a semi-diurnal tide with a tidal difference of 3 m, estimated length of 65 km and 1.5 km wide at its maximum. The Calabar River is a significant recipient of industrial including oil exploration and exploitation, agricultural, domestic effluents, and areal runoffs. Three sampling stations were selected across the length of the Calabar River, namely Adiabo (a putative control site with no visible industrial, agricultural and domestic inputs), Obutong (a heavily industrial site, receiving point and diffuse sources of effluents from petroleum industries/tank farms, cement factory and domestic waste effluents), and Nsidung (receiving effluents of agricultural and domestic origin). The water physicochemical conditions were temperature: 26.24 ± 0.61°C; pH: 6.52 ± 0.21 and dissolved oxygen (DO): 4.42 ± 0.13 mg/L).

Sample collection and preparation

A total of 253 fin and shellfish samples of *C. nigrodigitatus* ($n = 44$), fiddler crab (*U. tangeri*; $n = 21$), Blue crab (*C. amnicola;* $n = 24$), prawn (*M. vollenhovenii*; $n = 42$), periwinkle (*T. fuscatus*; $n = 122$) were collected between March and April, 2022 between 07:00–14:00 hr with a combination of gill and cast nets (mesh sizes 50-55 mm) at the different sampling locations with the aid of artisanal fishermen from the three (3) sampling stations; Adiabo ($n = 62$), Obutung ($n = 93$) and Nsidung ($n = 98$) along the Calabar River. Collected biota were identified according to Idodo-Umeh (2003) and anesthetized with MS-222 (tricaine methanesulfonate) on ice. Liver samples were extracted, and a portion was snap frozen in liquid nitrogen for enzyme assays. Fish muscle samples were also

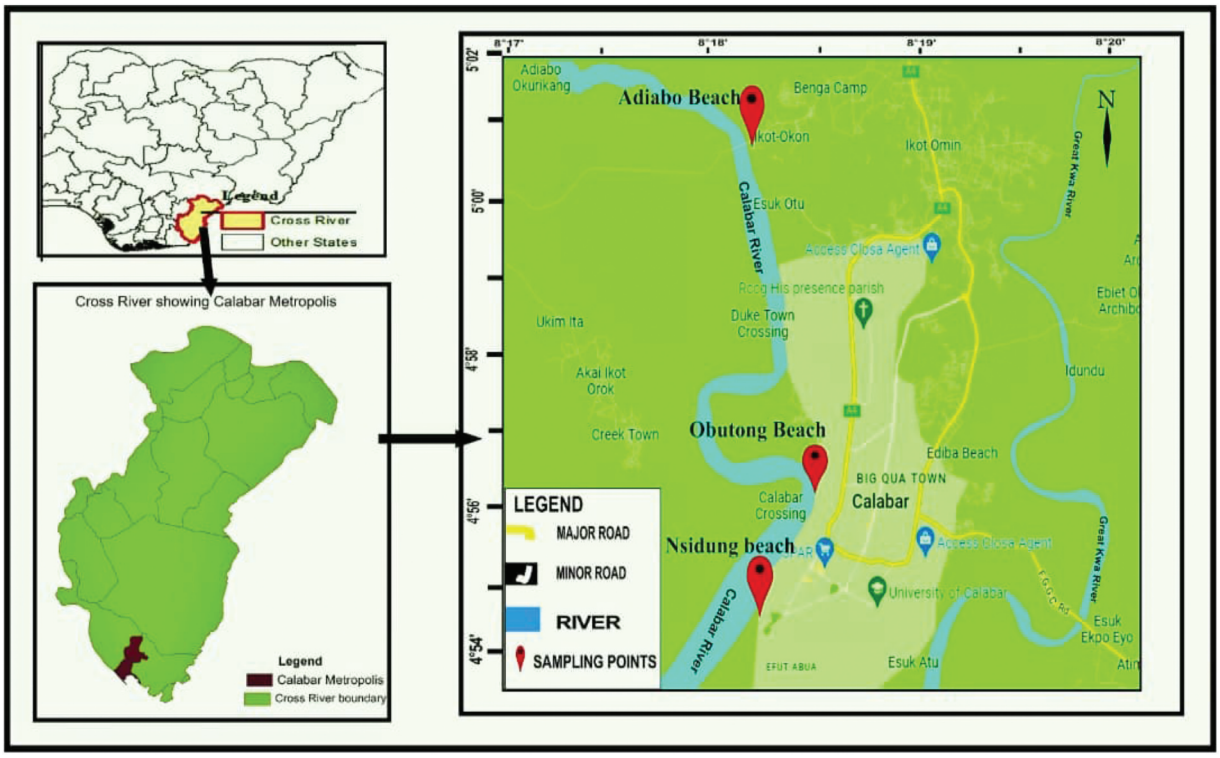

Figure 1. Map of Calabar River indicating the sampling sites and surrounding environments.

collected in aluminum foil from the three sampling sites and analyzed for PAHs. The van Veen grab was used to collect sediment samples in triplicates from the three (3) sites into aluminum foil and further prepared for analysis.

Fish morphometric measurements

Fish biometric data – total length (cm), body weight and gonad weight (g) were measured with digitally using Vernier Caliper (Tresna Instruments) and Ohaun (Metler Instruments), respectively. Condition factor (CF) was calculated, while organ somatic indices were calculated as the organ weight related to the total body weight and expressed in percentage (%).

Preparation of post-mitochondrial fraction (PMF) and enzyme activity assays

A 500 mg of individual liver samples were homogenized with 0.1 M sodium phosphate, 100 mM EDTA, 1 mM dithiothreitol and 10% glycerol buffer (pH 7.4) using a ratio 1:4 (liver:buffer). The homogenized samples were centrifuged at 12,000 ×g at 4°C and 20 min.

The activities of phase I (EROD, BROD, PROD and MROD) and phase II biotransformation enzymes (Gst and Ugt) (Bello et al. 2001) and oxidative stress (Gr and Gpx) (Badary et al. 2005; Wheeler et al. 1990) were measured using standard protocols. All enzyme activities were normalized against Bradford (1976) determined total protein concentration with bovine serum albumin (BSA) as a standard.

Chemical analysis

PAHs were measured in fish muscle, whole body of crabs, prawns, and periwinkles and sediments by conventional procedures based upon high performance liquid chromatography (HPLC) with diode array and fluorometric detection, as previously described (Regoli et al. 2014). Detailed protocol is presented in the supplementary information (SI) file.

Statistical analysis

All data were presented as mean ± standard error and was subjected to one-way ANOVA using Origin 8 software, (Originlab software, USA) and dataset normal distribution was analyzed with the Shapiro-Wilks test, while variance homogeneity was determined using the Levene's test. Relationship between biological variables (enzymatic responses) and group of chemical contaminants (PAHs), across sampling sites were assessed using principal component analysis (PCA). Extraction of principal component and biplot were achieved using Statistica^{TM} for iWindows version 8.0 (Statsoft. Inc. USA).

Results

Biotransformation and oxidative stress biomarker responses

Phase I (EROD, BROD, PROD and MROD) and II (GST and UDPGT) biotransformation and oxidative stress (GPx and GR) biomarker responses were determined in fish (*C. nigrodigitatus*), fiddler crab (*U. tangeri*), blue crab (*C. amnicola*), prawn (*M. vollenhovenii*), periwinkle (*T. fuscatus*) samples collected from across the three sampling sites along the Calabar River showing variable and dynamic differences in biomarkers responses across the sites (Figures 2 and 3). EROD activity was significantly higher in *U. tangeri, M. vollenhovenii, T. fuscatus, and C. nigrodigitatus* from downstream stations Obutong and Nsidung, compared with upstream control Adiabo (Figure 2(A)). Similarly, MROD activity was significantly elevated in *C. nigrodigitatus, U. tangeri* and *M. vollenhovenii* from Obutong and Nsidung, compared with upstream control site at Adiabo (Figure 2(B)). However, *C. amnicola* exhibited significantly higher EROD activity at the control Adiabo site, compared to Obutong and Nsidung (Figure 2(A)). For BROD activity, a significant increase was observed in *C. nigrodigitatus, U. tangeria*, from Obutong and Nsidung, *M. vollenhovenii* from

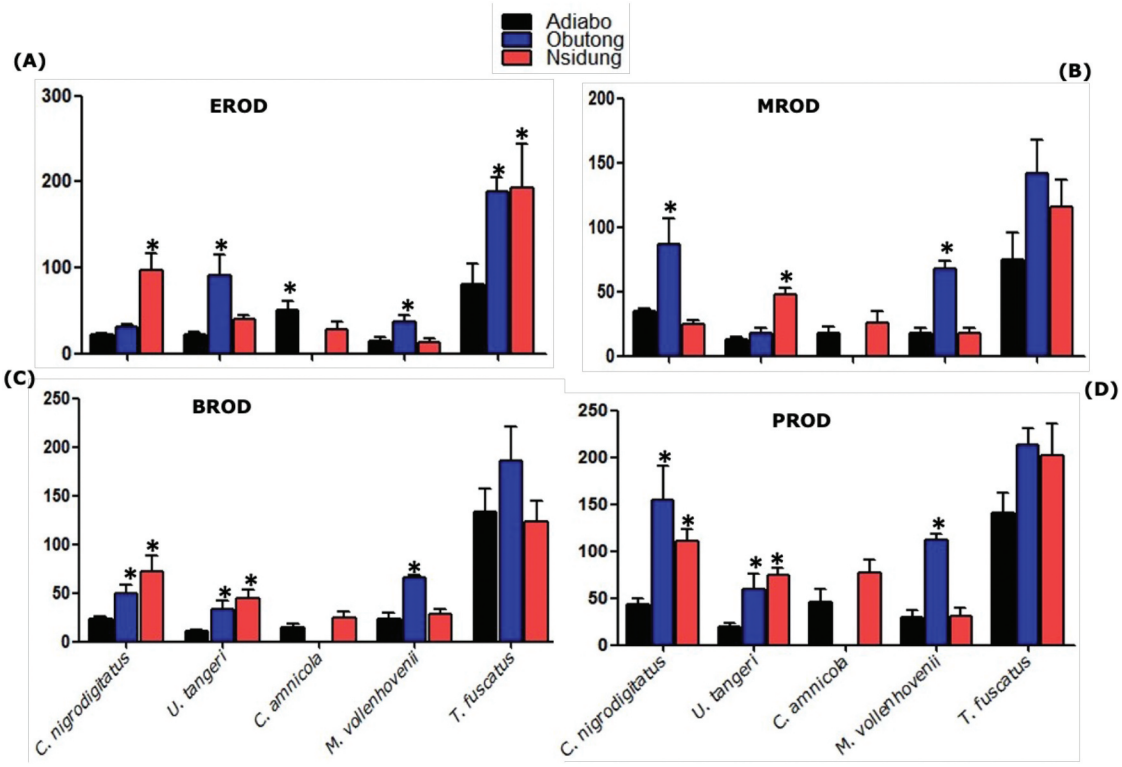

Figure 2. Changes in 7-ethoxyresorufin (EROD: A), 7-methoxyresorufin (MROD: B), 7-benzyloxyresorufin (BROD: C) and 7-pentoxyresorufin O-deethylase (PROD: D) in *chrysicthys nigrodigitatus*, *uca tangeri*, *calinectis amnicola, macrobrachium vollenhovenii*, and *tympanotonos fuscatus* collected at different sites of the Calabar River. Data are presented as mean ± standard error (SE) of $n = 6$. Asterisk (*) denote significant difference between control and downstream sampling site groups. *significant from control $p < 0.05$. Enzyme activities expressed as pmol/mg protein/min.

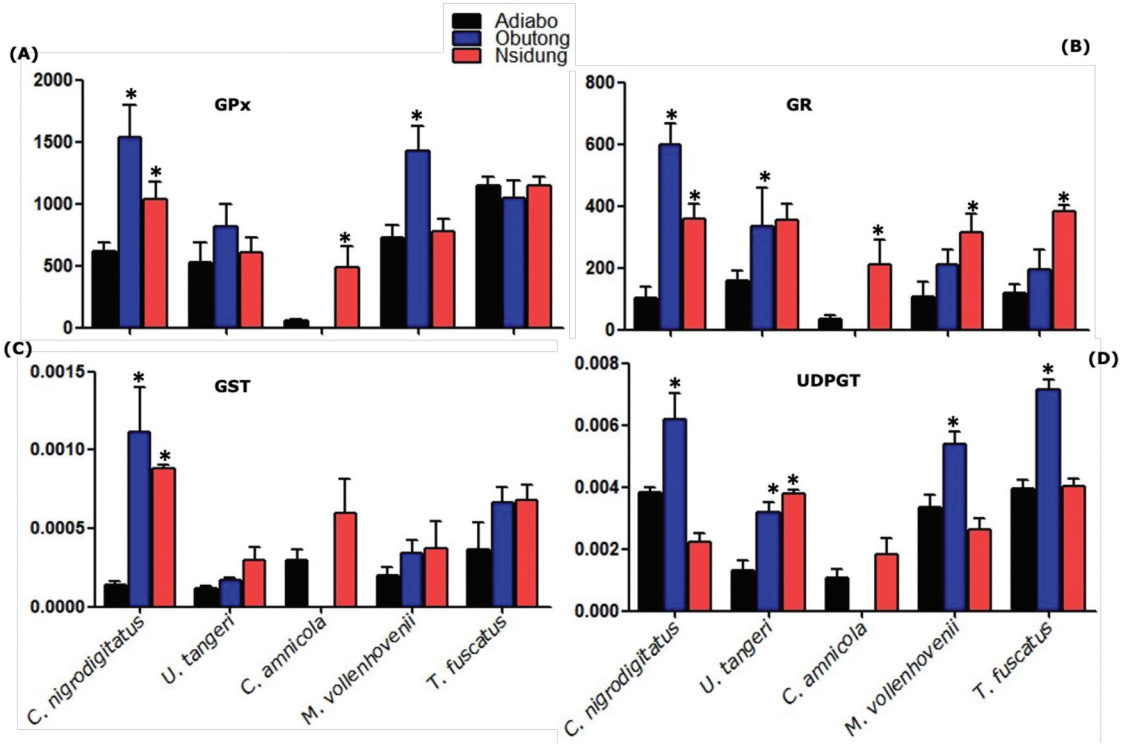

Figure 3. Changes in oxidative stress biomarker responses – glutathione peroxidase (Gpx: A), glutathione reductase (Gr: B), glutathione S-transferase (Gst; C), and UDP glucuronsoyl transferase (Udpgt: D) in *chrysicthys nigrodigitatus*, *uca tangeri*, *calinectis amnicola, macrobrachium vollenhovenii*, and *tympanotonos fuscatus* collected at different sites of the Calabar River. Data are presented as mean ± standard error (SE) of $n = 6$. Asterisk (*) denote significant difference between control and downstream sampling site groups. *significant from control $p < 0.05$. Enzyme activities expressed as pmol/mg protein/min.

Obutong compared with Adiabo (Figure 2(C)). Further, PROD activity was significantly elevated in *C. nigrodigitatus, U. tangeri and M. vollenhovenii* from downstream Obutong and Nsidung, compared with Adiabo (Figure 2(D)).

The oxidative stress biomarker responses (GPx and GR) demonstrated significant elevated levels on variable and significant rise across the different sites of the Calabar River, with GPx activity displaying a significant increase in *C. nigrodigitatus* at Obutong and Nsidung, *C. amnicola* at Nsidung, *M. vollenhovenii* at Obutong, compared with Adiabo (Figure 3 (A)). For GR, significantly higher activity was observed in all the species examined (*C. nigrodigitatus, U. tangeri, C. amnicola, M. vollenhovenii* and *T. fuscatus*) at Obutong and Nisdung, compared with Adiabo (Figure 3(B)). The phase II biotransformation responses were significantly increased in GST activity only in *C. nigrodigitatus*, mainly at Obutong, compared to Adiabo (Figure 3 (C)), while UDPGT activity was significantly elevated in all species, mainly at the Obutong compared to Adiabo except for *C. Amnicola* (Figure 3(D)).

PAHs levels in sediment and biota

Total PAHs (Σ_{19}PAHs), high- and low molecular weight (HMW and LMW, respectively), showed sediment occurrence at all sites including the control site at Adiabo, with Obutung demonstrating significantly higher abundance levels than Nsidung, which was higher than Adiabo (Figure 4(A)). PAHs were detected in sediment and biota samples in *C. nigrodigitatus, U. tangeri, C. amnicola, M. vollenhovenii* and *T. fuscatus*. All measured PAHs were present in sediment samples from Obutong, except for acenaphthylene, 1-methylnaphthalene, acenaphthene, dibenzo(ah)anthracene and indeno (123 cd) pyrene which was not detected in sediment samples from Adiabo and Obutong (Figure 4(A)).

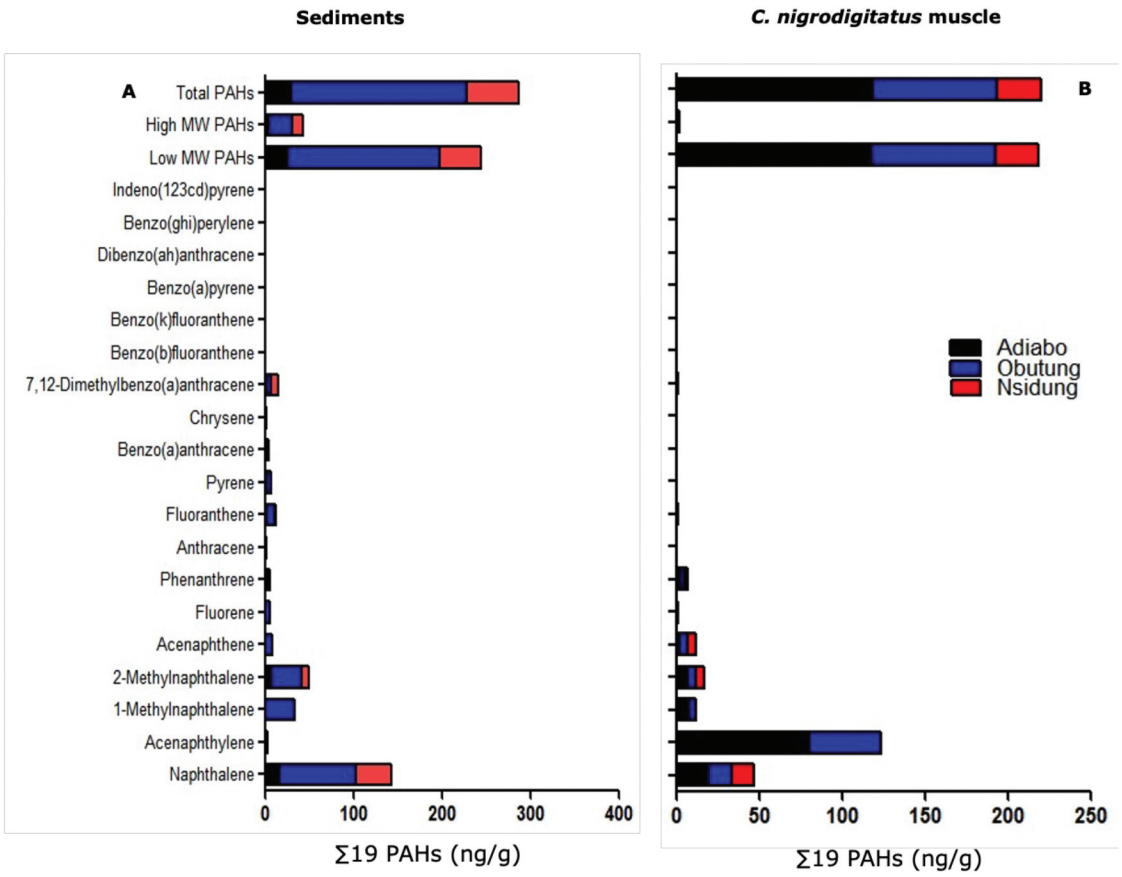

Figure 4. Mean concentrations of polycyclic aromatic hydrocarbons (PAHs) in (sediment: A), (*chrysicthys nigrodigitatus*: B) collected at different sites of the Calabar River. Data are presented as mean ± standard error (SE) of $n = 3$. PAH concentrations are expressed as ng/g.

All measured sediment PAHs concentrations including the low and high molecular weight PAHs were significantly higher at Obutong and Nsidung compared with Adiabo (Figure 4(A)). The sum of total PAHs (Σ_{19}PAHs) exhibited site-specific concentrations with higher levels recorded at Obutong (197.9 ± 5.9 ng/g) and Nsidung (61.5 ± 4.3 ng/g), compared with Adiabo (29.6 ± 2.3 ng/g) (Figure 4 (A)). In biota, variability was noted in PAHs dynamics across the species evaluated. Interestingly for *C. nigrodigitatus* (fish) muscle, Σ_{19}PAHs, HMW and LMW levels were significantly higher at Adiabo (control), compared with the polluted sites at Obutung and Nsidung (Figure 4(B)).

For the invertebrates (*U. tangeri, C. amnicola, M. vollenhovenii*), the Σ_{19}PAHs concentrations were significantly higher at Obutong and Nsidung compared to Adiabo, which also exhibited relatively high concentration levels (Figure 5(A-C)). Overall, invertebrates demonstrated variable concentrations of several PAH congeners at Adiabo, and some cases higher than the comminated sites at Obutong and Nsidung (Figure 5 (A-C)).

Principal component analysis (PCA)

The extracted principal component (PC), % variation, PCA biplot of the relationship between sites, biomarkers (biotransformation and oxidative stress responses) and PAHs burden in biota are ilustrated in (Figure 6(A-B)). Due to multiple biomarker responses, site and contaminant (PAHs) variables measured in the present study, the PCA biplot of variables was separated into two PCs showing different associations between contaminants, site, and measured biomarker responses. Two principal components were extracted accounting for 59- and 60% of the total variation in the entire dataset and

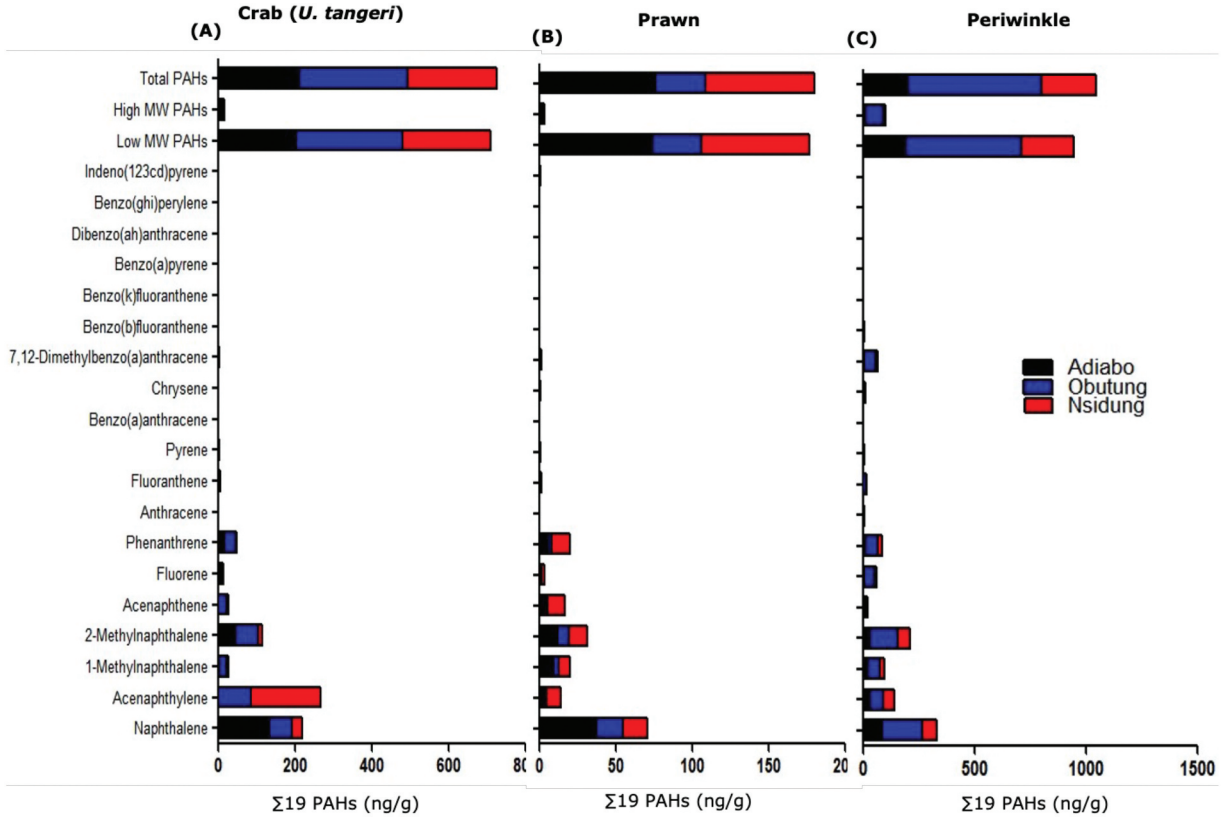

Figure 5. Mean concentrations of polycyclic aromatic hydrocarbons (PAHs) in (*Uca tangeri*: A), (*Macrobrachium vollenhovenii*: B), (*Tympanotonos fuscatus*: C) collected at different sites of the Calabar River. Data are presented as mean ± standard error (SE) of n=3. PAH concentrations are expressed as ng/g.

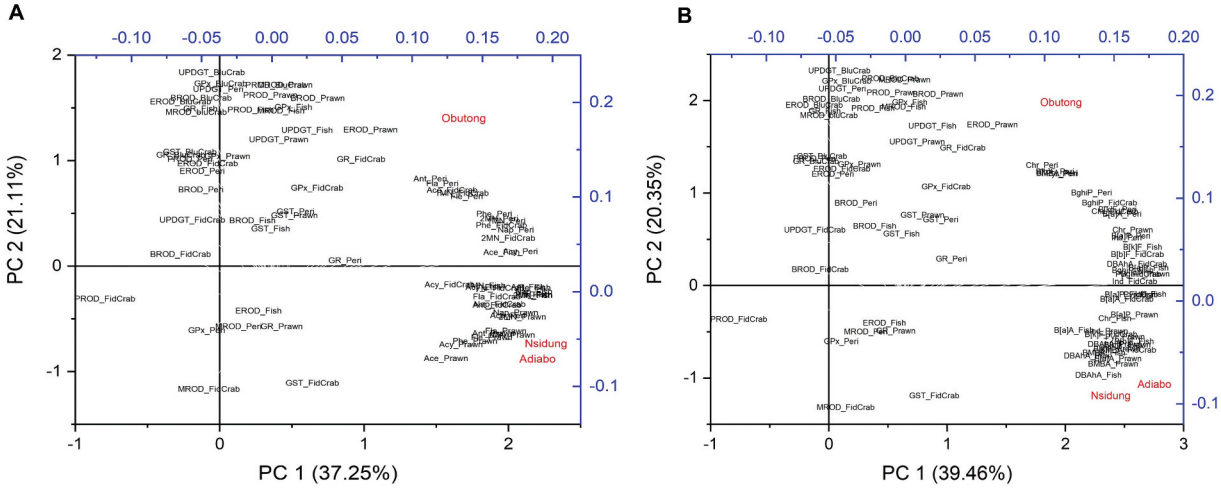

Figure 6. (A and B) Biplot of biotransformation and oxidative stress responses, and polycyclic aromatic hydrocarbons (PAHs) concentrations of environmental contaminants measured in sediments, and in *chrysicthys nigrodigitatus*, *Uca tangeri*, *Calinectis amnicola, Macrobrachium vollenhovenii*, and *Tympanotonos fuscatus* from Calabar River, Nigeria.

displaying different groupings for sites, PAHs and biomarker responses (Figure 6(A-B), respectively). For both PCAs, PC1 accounted for 37.2- and 40%, respectively and showing an arrangement of variables on ordination space with marked indications that sites were major factors determining bioaccumulation of contaminants and biomarker responses in fish, crabs, prawn and periwinkle. For example, all biota samples from Obutong demonstrated

positive correlation with contaminant levels (PAHs) and biological variables (biotransformation and oxidative stress responses; (Figure 6 (A-B)).

Discussion

Environmental pollution due to constant and unregulated release, uptake and bioaccumulation of contaminants in aquatic biota and sediments remains one of the biggest global banes of modern civilization (Bhat et al. 2023; Qayoom et al. 2024). The environment plays significant roles in sustaining ecosystem life, and all biota with dependence on environmental resources for growth and development, reproduction and overt survival (Munang, Thiaw, and Rivington 2011). At present, multiple environmental stressors such climate change, pollution (water and air) have increased with consequent effect on livelihood, global economies, policies, and resulting in increases in vector and disease burden with severe public health consequences (Yadav and Upadhyay 2023).

Anthropogenic activities are believed to be important drivers of pollution and the continuous input of these chemicals contamination into the ecosystem represent a serious societal concern (Ogidi and Akpan 2022). For developing countries, most of these contaminated aquatic ecosystems constitute the major drinking water source and are often used for other domestic activities including cooking. Further, aquatic food species form a major composition of cheap protein source and tissue contaminant burden highlight potential public health and food safety concerns. In this study, toxicological responses were investigated in relation to PAHs bioaccumulation levels in a tropical estuarine food web from the Cross River Estuary, Nigeria. Data demonstrated that occurrence of significantly higher concentrations of PAHs in sediment and estuarine food webs (fish, crabs, prawns and periwinkle) across all study sites (Adiabo (putative control), Obutong and Adiabo) with resulting increases of phase I- and II- biotransformation and oxidative stress biomarker responses. Using PCA, results demonstrated a cause-and-effect relationship between PAHs levels and biomarker responses in these important estuarine food webs suggesting a strong relationship between sediment and tissue PAHs burden and toxicological responses.

An array of chemical contaminants was found to produce effects on biotransformation and oxidative responses and are implicated in the detoxification and subsequent elimination and/or activation of xenobiotic compounds (Arukwe et al. 2015; Nelson 2009; Yadetie et al. 2014). CYP enzymes metabolize a wide range of compounds, and these reactions are further conjugated to endogenous molecules through UGT and GST, functioning as major pathways for inactivation, activation, detoxification and elimination of xenobiotics (Leaver, Clarke, and George 1992). Our investigations examined biotransformation and oxidative stress responses in estuarine food webs (fish, crabs, prawns and periwinkle) from the Calabar River, and noting significant elevation in of EROD, MROD, BROD and PROD), II (UDPGT and GST) and oxidative stress (GPx and GR) enzymatic responses of this organism at Obutong and Nsidung compared with control (Adiabo). In addition, our study demonstrates species and site-specific responses in these biomarker activities that parallel biota and sediment PAHs concentrations. In agreement with our findings, biotransformation and oxidative stress induction was related to PAHs exposure as evidenced by other investigators (Fang et al. 2020; Gaber et al. 2021; Şeker 2012; Sun et al. 2020).

The biotransformation mechanisms of PAHs were linked to aryl hydrocarbon receptor (AhR) which regulates several cellular activities including xenobiotic metalizing enzymes. The binding of PAHs to the AhR initiates the induction of P450 enzymes resulting in biotransformation processes and thus considered as the first step in PAHs biotransformation (Pampanin et al. 2016; Zhou et al. 2010). Some levels of species and site-specific variability and dynamics were noted in the catalytic activities for EROD, BROD, MROD and PROD; however, this was not surprising considering unique and different degrees of anthropogenic contamination of these sampling sites. Further, the observed variability and dynamics in CYP enzymes inductions found in this study may suggest the presence and occurrence of other CYP inducing compounds in the studied environment. Our findings support established knowledge

indicating the occurrence of other CYP inducing contaminants, such as PCBs and OCPs in sediment and biota samples from Calabar (Ilechukwu et al. 2018; Oyo-Ita et al. 2014). Consistent with our data, several previous investigators demonstrated induction of catalytic and transcriptional biotransformation responses in aquatic organisms from contaminated water bodies (Dévier et al. 2013; Head et al. 2015; Regoli et al. 2011; Van der Oost, Beyer, and Vermeulen 2003; Woo 2022). For example, a significant rise in Cyp1a protein and mRNA levels were reported in PAHs contaminated Western Sea of Korea (Jung et al. 2011). In *Oreochromis niloticus* collected from contaminated Guanda River Rio de Janeiro, Brazil, a linear relationship was established between Cyp1a protein levels and catalytic (EROD and MROD) activities (Parente et al. 2004). Other field studies reported induction of transcriptional and catalytic biotransformation response in tilapia species in relation to contaminants burden from Nigerian freshwater ecosystems, namely – Ogun River (Adeogun, Ibor, Regoli, et al. 2016b), Awba dam and Eleyele lake (Adeogun, Ibor, Onoja, et al. 2016a; Ibor et al. 2019).

The phase II biotransformation enzymes (GST and UDPGT) initiate the covalent linkage between the functional group and a soluble conjugate for detoxification and possible elimination (Lech and Vodicnik 1984). In this study, site and specific significant inductions in GST and UDPGT were detected in biota from Obutong and Nsidung compared with Adiabo (the control site). This observation may reflect the contaminant levels of PAHs and other phase II inducing contaminant present at these sites and indicate enhanced metabolism, detoxification and possible elimination of these contaminants by these species. Regoli and Giuliani (2014) established that the antioxidant defense system is a protective system that forms a network against oxidative stress and damage. The responses and inductions were associated with production of free radicals following exposure of organisms to several groups of environmental contaminants where these radicals play important roles in cellular protection against contaminant-mediated oxidative stress. The biotransformation of xenobiotics involves an array of genes which are regulated by AhR and activated by various exogenous and endogenous molecules (Gu et al. 2000). CYP enzymes regulate the oxidative metabolism of several substrates, specifically, Cyp1, 2 and 3 enzyme super-families metabolize several planar compounds to produce metabolites that are substrates for phase II enzymes (Ugt and Gst), that subsequently inactivate and eliminate lipophilic xenobiotics (Leaver, Clarke, and George 1992). The cyp1 isoforms, Ugt and Gst expressions are controlled by ligand-activated AhR (Bradshaw et al. 2002; Nelson et al. 1996). Owing to the integral roles of Cyp enzymes in xenobiotic detoxification and activation, a change in their expression and activity affect the possible risks and benefits of xenobiotics metabolism and relevant in assessing environmental health through monitoring (Williams, Lech, and Buhler 1998). Our findings demonstrated a significant rise in antioxidant responses (GPx and GR) that parallel elevated levels of PAHs in biota from Obutong and Nsidung, compared with Adiabo suggesting a possible adaptive and protective responses against PAHs mediated ROS oxidative stress (Guan et al. 2020; Ranjit et al. 2016). In agreement with our observations, several investigators reported contaminant-induced ROS production with resulting oxidative effects in exposed organisms (Arojojoye, Oyagbemi, and Gbemisola 2019; Dale et al. 2019).

Using PCA, data demonstrated % variations that delineated sites, biomarkers (biotransformation and oxidative stress responses) and contaminant (PAHs) burden relationship in biota (fish, crabs, prawns and periwinkle). Two principal components were extracted with variables on ordination space showing solid indications that sites were important factors for uptake of contaminants and biomarker responses in fish, crabs, prawn and periwinkle. For example, all biota samples from Obutong demonstrated positive correlation with contaminant levels (PAHs) and biological variables (biotransformation and oxidative stress responses) and this observation may suggest a causal relationship between site, PAHs concentrations and biomarker responses in biota.

A holistic view of the presented data demonstrates a site and species-specific bioaccumulation of PAHs at Obutong and Nsidung, displaying the highest concentrations for certain PAHs compared

with Adiabo. Across all species investigated, significantly higher PAHs concentration was detected in periwinkles, crabs and sediment samples from Obutong compared with Nsidung and Adiabo, while PAHs bioaccumulation levels in fish showed the opposite trend. For example, fish from the putative control site (Adiabo) exhibited comparatively high level of fish (*C. nigrodigitatus*) muscle PAHs accumulation showing that acenaphthylene, sum and LMW PAHs at comparatively higher at Adiabo, compared with Obutung and Nsidung. Nevertheless, the higher body burden of these PAHs probably reflects the omnivorous feeding habit of this species and associated biomagnification of PAHs from lower trophic levels to *C. nigrodigitatus* (Han et al. 2022; Rahmanpour, Farzaneh Ghorghani, and Lotfi Ashtiyani 2014). The biota concentrations of PAHs recorded in the present study are significantly high and comparable with concentrations previously reported (Adeogun, Ibor, Regoli, et al. 2016b; Cocci et al. 2018; Ibor et al. 2019).

The analysis of phase I and II responses at functional enzyme levels in this study demonstrated significant site- and species related differences (higher in *C. nigrodigitatus* and *T. fuscatus*). Particularly for *T. fuscatus*, with the highest response levels, provides an interesting toxicological and physiological aspect of an invertebrate. While invertebrates are generally not prone to xenobiotic biotransformation activity levels with low inherent capability, even after exposure to contaminants (James 2024), the effects on *T. fuscatus* suggest that this species may represent a valuable species for ecotoxicological monitoring programs. In accordance with the CYPs, phase II enzymes are generally used as biomarker responses for xenobiotic compounds and exhibit isoform differences with substrates that overlap between endogenous and xenobiotic molecules (Clarke, George, and Burchell 1992). Herein, Ugt and Gst generally followed comparable response pattern with Cyp-mediated responses (EROD, BROD, MROD and PROD) and oxidative stress responses (Gpx and Gr) as previously reported in wild fish (Gadagbui and Goksøyr 1996; Vogelbein et al. 1990). The functional enzyme responses are in accordance with the increase in PAHs burden in the present study, suggesting the collective involvement of all the studied enzymes in xenobiotic metabolism and excretion (Regoli et al. 2014).

The observed, high occurrence and concentrations of PAHs in benthic organisms (periwinkles, crabs, prawn) and sediment suggest bioaccumulation, bioavailability and uptake of these contaminants by sediment-bound organisms and indicate the role of micro-habitant in contaminant dynamics and bioaccumulation (Gerig et al. 2019; Han et al. 2022). This is supported by the established knowledge suggesting that PAHs transfer within the aquatic ecosystem may occur by bioaccumulation, distribution, transformation and matrices exchange, and their high hydrophobicity affinity preferentially favors sediment accumulation and thus increasing their half-life (Han et al. 2022; Hu et al. 2014). The redistribution and resuspension of sediment resulting from physical disturbances or bioturbation by benthic-burrowing animals may promote their redistribution and release of PAHs from sediment and this may account for the high accumulation of PAHs in sediment-bound benthic organism (periwinkles and crabs) detected in this study (Han et al. 2022; Hu et al. 2014). Further, the observed site-specific prevalence of PAHs in Calabar River sediment and food webs may indicate direct influence of anthropogenic activities at these sites, particularly at the putative control site at Adiabo. For example, the high petroleum activities along the entire length of Calabar River (Obutong and Nsidung), including point and diffuse sources of effluents discharge from petrochemical industries and tank farms together with the high oil exploration and exploitation activities including oil bunkering and vandalization of crude oil vessels around the Calabar River as well as major shipping route for crude oil transportation into the Nigeria Ports might account for the observed significant high PAHs concentrations found at all the study sites, including Adiabo. Overall, this calls into question once again, if there are true reference (control) study sites in the environment. In addition, the PAHs concentrations recorded in this study are high and may be comparable to levels previously reported in aquatic food fish species from Nigeria (Adeogun, Ibor, Regoli, et al. 2016b; Adesina et al. 2024; Akinnusotu, Ukpebor, and Okieimen 2020; Apata et al. 2022; Ekere et al. 2019; Ibor et al. 2019;

Ogunbisi et al. 2023; Ololade et al. 2024; Tongo and Etor 2018).

Given the economic and ecological importance of the Calabar River and occurrence of significantly high concentrations of PAHs noted across the aquatic food webs, our study emphasizing an alarming and significant public health, food safety and environmental health concerns. Such is the case because, the Calabar River is utilized as a local source of domestic water supply (drinking, cooking and other domestic activities). In addition, these aquatic food fish species are a major source of cheap and available protein which are often consumed by the rural populations. Hence, the tissue residues of PAHs may contribute significantly to their diet and daily intake by humans and represent serious public health and food safety risk (Singh and Agarwal 2018). Due to the potential ecological effects and toxicity of PAHs including their carcinogenic, teratogenic, and mutagenic effects (Baird, Hooven, and Mahadevan 2005; da Silva Junior et al. 2021; Łuczyński et al. 2005; Pashin and Bakhitova 1979). Data presented herein represented an ongoing effort toward generating scientific information that may aid local sensitization and policy formulation, implementation and regulations for sustainable food safety and ecological management from a developing country standpoint.

Conclusions

Data demonstrated site and species-specific occurrence and concentrations of PAHs in sediment and tropical estuarine food webs with corresponding biotransformation and oxidative stress effects on resident biota. Our results indicate that contaminated sites were major factors determining the uptake of PAHs and biomarker responses in fish, crabs, prawn and periwinkle and this observation suggests a causal relationship between site, PAHs concentrations and biomarker responses in biota samples from Calabar River, Nigeria.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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