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Biotransformation and oxidative stress responses in relation to tissue contaminant burden in *Clarias garipinus* exposed to leachate from a solid waste dumpsite in Calabar, Nigeria

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†In memoriam, May his soul rest in peace (RIP)

Abstract

In this study, we have investigated biotransformation and oxidative stress responses in relation to biota contaminant burden in *Clarias gariepinus* exposed to simulated leachate from a solid waste dumpsite in Calabar, Nigeria. Fish were exposed to simulated leachate, diluted to 0:0 (control), 1:10, 1:50, 1:100 and phenanthrene (a PAH: 50 μg/L used as a positive control) for 3, 7 and 14 days. Hepatic transcripts for *cat*, *sod*1, *gpx*1, *gr*, *gst*, *cyp*1a, *cyp*2d3, and *cyp*27 were analyzed by real-time PCR, while enzymatic assays for EROD, BROD, MROD, PROD, CAT, GPx, GR, GST, UDPGT and lipid peroxidase (LPO) were measured using standard methods. In addition, protein expression for CYP1A, CYP3A, metallotheionin (MT) and NF-Kappa-β were measured by immunoblotting. Fish samples were analyzed for selected group of contaminants after 14 days exposure showing significantly high uptake of heavy metals (Cd, Hg and Pb), polycyclic-aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorophenols, organochlorine (OC) and organophosphate pesticides in exposed fish, compared to control. We observed significant concentration-dependent and time-specific increases in biotransformation and oxidative stress responses at transcript and functional (enzyme and protein) levels, that paralleled tissue contaminants bioaccumulation pattern for PAHs, POPs and heavy metals, after exposure to the simulated leachates. Our results highlighted the potential environmental, wildlife and public health consequences from improper solid waste disposal. In addition, it also provides a scientific basis for local sensitization and inform legislative decisions and policy formulation towards sustainable environmental management in Nigeria and other developing countries.

Keywords: Solid waste, Dumpsites, POPs, Metals, Biotransformation, Oxidative stress, Fish, Environmental health

1. Introduction

The increases in solid waste production has been associated with rapid increase in human population with resulting negative consequences on ecosystem, wildlife and public health due to the presence of diverse categories of contaminants in sediment and leachates from dumpsites or landfills (Eggen et al., 2010; Arukwe et al., 2013; Olukunle and Okonkwo, 2015; Ogunlaja et al., 2019; Sibiya et al., 2019). In Nigeria, which is Africa's most populous and largest economy, with an estimated population of over 180 million, municipal solid waste production have been estimated at 0.58 kg of solid waste/person/day. Despite this large solid waste production there is no organized management plans or waste recycling methods available to handle these wastes (Ogwueleka, 2009). As a result, the usual, unhygienic and inefficient ways of waste management is the dumpsites, landfills and open burning (Bassey et al., 2015), and accounting for a significant proportion of total waste collection (Yukalang et al., 2017). Unfortunately, dumpsites or landfills operate below accepted international standards in developing countries (Oyeku and Eludoyin, 2010) by leaching toxic chemicals and gases into the environmental with detrimental effects on the ecosystem, wildlife and public health (Schrapp and Al-Mutairi, 2010; Laniyan et al., 2011).

A wide range of persistent organic pollutants (POPs), volatile organic contaminants (VOCs) and metals associated with dumpsites or landfills has been linked to severe human health problems. For example, reproductive and developmental effects, increased cases of cancer, disease outbreaks such as infectious viruses and bacterias were reported among human residents exposed to complex mixtures of hazardous chemicals from the Love canal landfill, USA (Janerich et al., 1981; Meyer, 1983; Vianna and Polan, 1984). Further, anaerobic decomposition of solid waste by bacterias and other microbes result to a significant production of methane, hydrogen sulphide and carbon dioxide, contributing to global warming and climate change that may affect the ecosystem and posing health risk to humans and wildlife (Stamps et al., 2016). Due to the ecological consequences arising from improper waste management, biomonitoring efforts have focused on identifying the presence of pathogenic bacterias and microbes associated with dumpsites and landfills (Allen et al., 1997; Jeremy, 2011; Kanchan et al., 2012; Flores-Tena et al., 2007; Allen et al., 2011; Ogunlaja et al., 2019). Similarly, other studies have focused on quantifying and characterizing environmental contaminants levels in soil and leachates from dumpsites or landfills. For example, elevated levels of individual or different groups of environmental contaminants have been reported from several

dumpsites/landfills (Urase and Miyashita, 2003; Minh et al., 2003; Eggen et al., 2010; Liu et al., 2010; Huset et al., 2011; Arukwe et al., 2013; Olukunle and Okonkwo, 2015; Wowkonowicz and Kijenska, 2017; Busch et al., 2010; Yan et al., 2015; Gallen et al., 2017; Fuertes et al., 2017; Sibiya et al., 2019).

Given that many solid waste dumpsites/landfills contain complex mixtures of environmental contaminants and pathogens (Arukwe et al., 2013; Ogunlaja et al., 2019), a comprehensive monitoring scheme of all potential chemicals and disease causing organisms is almost impossible, due to cost, lack of chemical standards, including limited available analytical methods. To address these limitations, there is need to develop and incorporate a robust, sensitive, reliable, comprehensive and systematic evaluation method which represents a cause-and-effect approach. This approach may highlight biota contaminants burden with adverse toxicological endpoints such as biotransformation and oxidative stress responses. These methods are very unique and appropriate in measuring exposure effects due to the presence of contminants (Galloway et al., 2004; Arukwe et al., 2015; Adeogun et al., 2016b, Ibor et al., 2017, 2019). A wide range of environmental contaminants have been reported to induce biotransformation responses via the aryl hydrocarbon receptor (AhR), interfere with oxygen consumption by producing reactive oxygen species leading to oxidative stress with severe consequences on proteins, lipids and macromolecules such as DNA (Yadetie et al., 2014; Arukwe et al., 2015; Dale et al., 2019).

The aim of this study was to investigate toxicological effects (biotransformation and oxidative stress responses) and tissue contaminant burden in the African sharptooth catfsi (*Clarias gariepinus)* exposed to simulated leachate from the Lemna solid waste dumpsite. The Lemna solid dumpsite is a major municipal waste dumpsite in Calabar, Southern, Nigeria. The African sharptooth catfish is an ecologically and economically, important food fish species, and relevant model species due to it´s sensitivity, easy to handle and available scientific data.

2. Materials and Methods

2.1. Chemical and reagents

Primers, technical grade phenanthrene (CAS Number 50-28-2, 98% purity), bovine serum albumin (BSA), NADPH, 1-chloro-2, 4-dinitrobenzene (CNDB), tertbutyl hydroperoxide, glutathione peroxidase and reductase, ethoxyresorufin, benzyloxyresorufin, methoxyresorufin and

pentoxyresorufin were purchased from Sigma-Aldrich Oslo, Norway. Polyclonal CYP1A and MT antibodies was purchased from Biosense Laboratory, (Bergen, Norway), CYP3A antiserum was a gift from Dr. Malin Celander, while NF-Kβ p65 antibody were purchased from Santa Cruz Biotechnology, USA. RNA isolation kits were purchased from Zymo Research Corporation, Irvine, CA USA, iScriptTM cDNA synthesis kit, iTaq DNA polymerase, dNTPmix, iTaqTM Sybr® Green supermix with ROX were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Other reagents and chemicals were of the highest commercially available grade.

2.2. Fish exposure and sampling

Five (5) weeks old juvenile African sharptooth catfish (*Clarias gariepinus*: 9 ± 0.4 g weight and 10.5 ± 0.5 cm length) were obtained from the hatchery at the University of Calabar fish farm and transferred to transparent plastic holding tank (50 liters) at the Department of Zoology and Environmental Biology Research Laboratory, University of Calabar. Fish were maintained at 27.5 ± 1.6 °C, dissolved oxygen (DO) of 6.8 ± 0.2 mg/L and pH of 6.9 ± 1.8 under a 12h:12 h light and dark photoperiod. Fish were fed two times daily with fish feed (40% crude protein) throughout the experimental period. The experiment was conducted in accordance with the guidelines for fish toxicity testing as stipulated by Organization of Economic Co-operation and Development (OECD, 2010) and approved by the appropriate authority at the University of Calabar.

Soil samples were collected at a depth of about 0-10 cm from fifteen (15) different points at the Lemna solid waste dumpsite (SWD) in Calabar, Nigeria, using stainless steel spoon and transferred into glass containers. Thereafter, the samples were transported to the laboratory and pooled to make a composite sample and air-dried, thereafter. Leachate simulation was carried out following the methods described by the American Society for Testing and Materials (ASTM-D 3987-35) and the United States Environmental Protection Agency (EPA 823/B-01-002) with slight modifications. Briefly, to 1kg of air dried soil, we added 2 L of distilled water and mixed thoroughly for complete dissolution of the sediment in water. The solution was then placed on a shaker for 24 h at room temperature, after which the mixture was allowed to settle, before filtration using a Watman filter paper. Fish were divided into 4 experimental groups (n=25 individuals) and exposed to the simulated leachate at 0:0 (control), 1:10, 1:50, 1:100 dilutions. In addition, phenanthrene (a PAH at 50 µg/L) was used as a positive control group. All experimental groups

were exposed for 3, 7 and 14 days. The test medium was renewed after each sampling day with new leachate added to restore the desired dilution within each tank.

During sample collection, fish weight and length were measured with a digital weighing balance and a digital caliper. Fish were anaesthetized on ice for blood collection by cardiac puncture using a 2 mL syringe and then transferred into heparinized vacutainer. Thereafter, fish were dissected and a portion of the liver was harvested and immediately preserved for enzyme, RNA isolation and protein analysis.

2.3. Post-mitochondrial supernatant (PMS) preparation.

Liver samples (0.2 g) from individual fish was sliced with a pair of scissors and homogenized in 1:4 ratio of liver weight and volume of 0.1 M sodium phosphate buffer (containing 100 mM EDTA, 1 mM dithiothreitol at pH 7.4), with 4-6 up and down strokes using a potter-elvehjem type teflon glass homogenizer. The homogenate was centrifuged for 20 min at 12000 x g at 4°C and the resulting supernatant was stored in aliquots at -80 °C, until analyzed.

2.4 Biochemical assays and lipid peroxidation (LPO)

Hepatic enzymatic activities for ethoxy-, buthoxy-, methoxy- and pentoxyresorufin *O*-deethylase (EROD, BROD, MROD and PROD, respecyively) were measured in PMS fraction as enzyme biomarkers for phase I biotransformation responses in *C. garirpinus* exposed to simulated leachates and a positive control (PAH) in a black flurometric 96-well plate according to standard protocols. The reaction was initiated by addition of NADPH and 1 µL of the ethoxyresorufin, buthoxyresorufin, methroxyresorufin and pentoxyresorufin (180 µM in DMSO) and the fluorescence was measured in a microplate reader for 20 min (ex: 535 nm; em: 590 mm) according to Burke and Mayer (1974).

The CAT, GPx, GR, GST, UDPGT and LPO activities were analyzed in PMS fractions. GST activity was analyzed using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate as described by (Habig et al., 1974). We measured GPx activity indirectly by a coupled reaction with glutathione reductase (GR). Oxidized glutathione (GSSG), produced upon reduction of hydroperoxide by GPx, is recycled to its reduced state by GR and NADPH. CAT activity was determined according to (Aebi, 1984) by following the consumption of 35 mM H_2O_2 at 340 nm. One unit of CAT activity

was defined as the amount of enzyme, required to produce 1 nmol of formaldehyde. UDPGT activity toward p-nitrophenol was measured as described by Andersson et al. (1985), while lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS) and expressed in terms of malondialdehyde (MDA) equivalent according to the method proposed by Ohkawa et al. (1979). All enzyme activities were assayed at room temperature.

Total amount of PMS protein was determined with the method of Bradford (Bradford, 1976.), using bovine serum albumin (BSA) as standard. All enzymes and protein measurements were simplified using a Synergy HT microplate reader from Bio-Tek Instruments Inc. (Winnoski, Vermont, USA) for absorbance readings.

2.5 RNA isolation and quantitative PCR

Total RNA was isolated from 50 mg of liver tissue, using Direct-zol RNA MiniPrep Kit following the manufacturer's protocol (Zymo Research Corporation, Irvine, CA USA). Complementary DNA (cDNA) was generated from 1 µg total RNA using a combination of oligo (dT) and random hexamer primers from iScript cDNA synthesis kit, as described by the manufacturer (Bio-Rad, Oslo Norway). Real-time PCR was performed with gene-specific primers (SI Table 1) for *cat*, *sod*1, *gpx*1, *gr*, *gst*, *cyp*1a, *cyp*2d3 and *cyp*27 using Mx3000P real-time PCR system (Stratagene, La Jolla, CA). A serial dilution of plasmid cloned with amplicon of interest was used to generate a standard curve and standard plots of cycle threshold (Ct-value) versus log copy number were used to quantify the expression of the target gene in unknown samples.

2.6 Western blot analysis

For immunoblotting, 50 µg hepatic protein were separated using 12% precast sodium dodecyl sulphate polyacrylamide gel (Bio-Rad Laboratories) electrophoresis (SDS-PAGE) according to Laemli (1970). The gel was blotted onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad) as described by Towbin et al (1979). The CYP1A, CYP3 and MT proteins were detected using specific antibodies as described previously (Arukwe et al., 2015). The polyclonal CYP1A (1:500), NF-Kβ p65 (1:1000), CYP3 and MT (1:2000) antibodies were diluted in 5% non-fat milk and used for detections. After primary antibody incubation at 4°C overnight, membranes were washed with TTBS and incubated with the secondary antibody, peroxidase conjugated goat anti-rabbit antibodies

(GAR-HRP: Bio-Rad) diluted 1:3000 in TTBS containing 5% non-fat milk. The membranes were thereafter developed using Immun-Star^{TM} Western^{CTM} Chemiluminescent Kit (Bio-Rad) before visualizing using the Eastman KODAK Company's Molecular Imaging Systems (Rochester, NY, USA)

2.7. Tissue contaminant analysis

Trace metals (As, Ba, Cu, Hg, Cd, Cr, Fe, Mn, Ni, Pb, V, Zn), aliphatic hydrocarbons (C10-C40) and polycyclic aromatic hydrocarbons (PAHs), organophosphate (OPs) and organochlorine pesticides (OCPs), chlorophenols (CPs) and polychlorinated biphenyl (PCBs) were analyzed in fish muscle by conventional procedures based on atomic absorption spectrophotometry, gas-chromatography with flame ionization detector, electron capture detector and mass detector, high performance liquid chromatography (HPLC) with diode array and fluorometric detection. Detailed analytical methods and procedures for quality assurance/quality control are given in Supplementary information (SI).

2.8. Statistical analysis

Data obtained were presented as mean ± SEM and checked for normal distribution using the Shapiro-Wilk test and homogeneity of variance with Levene's test. One-way analysis of variance (ANOVA) was performed followed by post-hoc analysis (Tukey's test) between exposure concentrations and control group, values were considered significantly different at $p<0.05$. Statistical analysis was performed using the Prism GraphPad 5 (GraphPad software, La Jolla, USA).

3. Results

3.1 Tissue contaminant levels

Fish muscle tissues were analyzed for trace metals, aliphatic hydrocarbons (C10-C40) and PAHs, OPs and OCPs, CPs and PCBs after 14 days exposure to simulated leachate (Table 1). For all measured traced metals, only Cd, Hg and Pb were significantly higher in exposed fish compared with control (Table 1). The sum of the total aliphatic (ΣTAHs) and aromatic hydrocarbons concentrations in fish muscle significantly increased in exposed fish compared with control, with

both low and high molecular weight (LMW and HMW-PAHs) concentrations significantly higher in fish exposed to the highest leachate dilution (1:10) showing respective 171.0 ± 18.0, 47.4 ± 18.0 µg/g, compared with control with respective 59.0 ± 4.8, 15.2 ± 3.4 µg/g values (Table 1). Generally, the LMW-PAHs were more abundant in the muscle and detected at significant higher concentrations compared with the HMW-PAHs (Table 1). For OPs and OCPs, only d-lindane (313.3 ± 72.0, 575.2 ± 71.0 ng/g, respectively) and dichlorvos (16.0 ± 2.4 ng/g) were detected in fish exposed to 1:50-100 leachate dilutions and below limit of detection (<LOD) in control and PAH exposed fish (Table 1). Among all measured chlorophenols, only 2,4 dichlorophenol (110.4 ±1 .0 ng/g) was detected above the LOD in fish exposed to the 1:100 leachate dilution (Table 1). PCB 13 and 28 were detected above LOD in the 1:50 and 100 exposure groups, respectively, with total PCBs concentrations of 45.2 and 22.0 ng/g recorded in the 1:50 and 100 exposed fish respectively (Table 1).

3.2. Changes in transcript levels

We analyzed hepatic mRNA expression at 3, 7 and 14 days after exposure for biotransformation and oxidative stress in *C. gariepinus* showing a concentration-specific effecte of the simulated leachate (Fig. 1-4). For example, an apparent concentration- and time-specific increase in *cyp1a* and *cyp2d3* mRNA levels were observed in exposed fish, compared with negative control group across all sampling days (Fig. 1A-F). The *cyp*27 mRNA levels were significantly elevated at day 3 in exposed fish (Fig. 2A) and thereafter, decreased, but still significantly above negative control level at day 7 and 14 (Fig. 2B-C). For *gpx* mRNA, a concentration-dependent significant increase was observed at day 3 and 7, which decreased at day 14, but still above the negative control levels (Fig. 2D-F). Similarly, concentration- and time-dependent significant increases were observed in *gr* and *sod*1 mRNA levels in exposed fish, compared with negative control group (Fig. 3A-F). On the hand, *gst* mRNA showed a consistent time and simulated leachate concentration dependent significant increase in exposed fish, compared with negative control group, across all exposure days (Fig. 4A-C). For *cat* mRNA concentration and time-specific significant increases were observed, compared with negative control group ((Fig. 4D-F).

3.3. Changes in functional enzyme and protein responses

Hepatic enzyme responses for phase I and II biotransformation (EROD, BROD, MROD, PROD, GST and UDPGT), oxidative stress (CAT, GPx, GR) and lipid peroxidation (LPO) in *C. gariepinus* exposed to simulated leachate from the Lemna dumpsite and analyzed at 3, 7 and 14 days in this study showing that leachate exposed fish and phenanthrene significantly modulated these enzyme levels, compared with the negative control fish, across all sampling days (Fig. 5-9). The EROD, BROD, MROD and PROD activities showed an apparent concentration- and time-specific significant increase in simulated leachate and phenanthrene exposed fish, compared with the negative control group (Fig. 5- and 6A-F, respectively).

Similarly, CAT and GPx activities showed significant increases in simulated leachate exposed fish, compared with the negative control group, at all sampling days (Fig. 7A-F). Further, UDPGT and GST activities were significantly elevated at day 3 and thereafter, decreased, but still above negative control group at day 7 and 14, in exposed fish, compared with the negative control group (Fig. 8A-F). We also observed significant increases in GR and LPO activities at day 3 and 7, which decreased at day 14, but still above negative control group in simulated leachate and PAH exposed fish (Fig. 9A-F).

Immunochemical analysis of CYP1A and CYP3A proteins after 14 days exposure showed detectable CYP1A and CYP3A protein reactions in *C. gariepinus* exposure groups and positive (PAH) control with higher protein band intensities verified by densitometric analysis, compared with the negative control group (Fig. 10A and B, respectively). Similarly, for the functional MT and NF-Kβ proteins, we observed higher protein band intensities in positive control and simulated leachate exposure groups, compared with the negative control group (Fig. 10C and D, respectively).

4. Discussion

Municipal solid waste dumpsites and landfills remain a global problem with negative environmental, wildlife and human health impacts. This is because they are significant sources of POPs, VOCs and trace metals to the environment (Eggen et al., 2010; Arukwe et al., 2013). The problems arising from improper waste management are worst in many low income countries due to legislative negligence by government, inadequate waste recycling and re-use techniques including lack of systematic approach for waste management. In an effort to develop important and crucial research and scientific material that will serve as a technical and scientific basis for local

sensitization and inform legislative decisions and policies for proper waste disposal and management, we have evaluated the contaminant burden and biological responses in fish exposed to simulated leachates from a solid waste dumpsite (Lemna) in Calabar, Southern Nigeria. We showed that *C. gariepinus* exposed to simulated leachate from the dumpsite accumulated significant amount of PAHs, OCPs, CPs, PCBs and trace metals (Cd, Hg and Pb) with resulting induction of biotransformation and oxidative stress biomarkers.

4.1 Tissue contaminant burden

In the present study, we measured muscle concentrations of targeted contaminants (PAHs, OCPs, CPs, PCBs) and trace metals in *C. gariepinus* exposed to simulated leachates from the Lemna dumpsite after 14 days exposure and detected significant amount of these contaminants, indicating their uptake and bioaccumulation in the experimental fish. The accumulated high concentrations of these contaminants, most likely reflect, the wide industrial application and use of these contaminants in the production of several consumer products and subsequent leaching from the dumpsite. The waste materials deposited at the dumpsite ranged from paints, tyres, batteries, plastics, electrical gadgets, papers, oil and petroleum products, metals, dyes, woods, pharmaceutical products, leather, textile, electroplaters, agrochemicals (such as pesticides), other house-hold products and cooking utensils. All these inductrial and domestic products are indiscriminately deposited at the Lemna dumpsite and have been reported in dumpsites and landfills in Nigerian and elsewhere (Hamer, 2003; Eggen et al., 2010; Arukwe et al., 2013). Similarly, given that these contaminants are non-biodegradable, continued and long-term deposition of these waste products at the dumpsite may result in accumulation of these contaminants into soil and further leach out to surrounding environments.

Particularly for developing regions such as Nigeria, where climate change is a current environmental and societal issue with intense sun radiation, elevated temperature may potentially accelerate microbial decomposition of some of these waste materials, producing further toxic metabolites and complexes with severe toxicological effects (Arukwe et al., 2013). Further, we believe that the presence of these contaminants, specifically PAHs, may have resulted from the consistent and increased burning of these waste products at the dumpsite (which is currently the only waste management option observed at the dumpsite). The burning of several organic materials

have previously been identified as a significant source of PAHs (Abdel-Shafy and Mansour et al., 2016). Furthermore, the higher abundance of LMW-PAHS, may be related to their higher solubility, compared to HMW-PAHs that are more hydrophobic (Sinaei and Mashinchian, 2014). Consistent with our findings, significant levels of contaminants and trace metals have been reported in leachate, sediments and biota from dumpsites and landfills globally (Eggen et al., 2010; Busch et al., 2010; Arukwe et al., 2013; Olukunle and Okonkwo, 2015; Yan et al., 2015; Gallen et al., 2017; Fuertes et al., 2017; Ogunlaja et al., 2019; Sibiya et al., 2019).

4.2 Effects on biotransformation and oxidative stress responses

The biotransformation of environmental contaminant significantly affects their remedy and health effects, from a toxicological standpoint. The CYP enzymes are inducible by certain environmental contaminants, resulting in their use as effective biomarkers of exposure to these contaminants. In this study, we analyzed biomarker endpoints for phase I and II, and oxidative stress responses at both transcriptional and functional levels (enzymes and proteins). Overall, we demonstrated that simulated leachate from the Lemna dumpsite produced significant concentration and time-specific inductions at mRNA, enzyme and protein levels, indicating biotransformation and oxidative stress effects in the leachate exposed fish. Further, these responses paralleled the tissue contaminant burden measured in the exposed fish. These findings suggest that the leachate contains environmental contaminants that are producing biotransformation and oxidative stress effects through the activation of xenobiotic responsive elements and reactive oxygen species (ROS) signaling pathways (Gu et al., 2000; Ogunlaja et al., 2019).

The CYP systems play important roles in the phase I biotransformation of several environmental contaminants, by active participation in catalyzing multiple metabolic reactions through the introduction of a functional group on the parent compound (Nelson, 2009). Similarly, the phase II enzymes (GST and UDPGT) facilitate the introduction of a covalent linkage between the functional group and an endogenous hydro-soluble conjugate to facilitate excretion (Lech and Vodicnik, 1984). Therefore, from a toxicological standpoint, the observed significant increases in the hepatic phase I and II biotransformation responses at mRNA, enzyme and protein levels indicate exposure of fish to inducing contaminants. Interestingly, these responses parallel the observed tissue contaminant (PAHs, PCBs, OCPs and CPs) levels, thus, validating the application and use of these

responses in biomonitoring of contaminants exposure. In accordance to our findings, the induction of phase I and II biotransformation responses in organisms following exposure to a vast number of organic pollutants, including PAHs, PCBs, PBDEs, OCPs have been previously reported (Vega-López et al., 2007; He et al., 2008; Piazza et al., 2016; Lima et al., 2018).

Antioxidants enzymes such as CAT, SOD, GPx, GR and GST are cellular protective enzymes that are coded through the antioxidant responsive element (ARE), forming a network of protective machinery against oxidative stress (Giuliani et al., 2013; Regoli and Giuliani, 2014). These biomarkers are induced in response to ROS generation following contaminant exposure in organisms where they play significant roles in cellular defense and adaptation to contaminant-induced oxidative stress (Livingstone, 2001; Li et al., 2007; Adeogun et al., 2012). In this study, we observed significant increase in antioxidant responses (CAT, SOD, GPx and GR) that parallel elevated levels of metals (Cd, Hg and Pb) and POPs in exposed fish. These findings suggest a possible adaptive and protective response against contaminants mediated ROS oxidative stress (Limón-Pacheco and Gonsebatt, 2009; Regoli and Giuliani, 2014). Similarly, significant increase in antioxidant enzymes with resulting significant elevation in LPO and NF-Kβ may suggest a direct relationship between increased antioxidant levels and oxidative stress with negative effects on biomolecules such as lipids, proteins and DNA (Wang et al., 2006; Lin et al., 2007; Yadetie et al., 2014). Furthermore, this relationship may also highlight the important role of NF-Kβ activation in modulating oxidative stress (Hayden et al., 2004; Lingappan, 2018). Consistent with our findings, contaminant-mediated ROS-generation with downstream oxidative effects in organisms have been reported (Giuliani et al., 2013; Piazza et al., 2016; Lima et al., 2018; Arojoye et al., 2019; Dale et al., 2019). Due to elevated tissue concentrations of trace metals (Cd, Hg and Pb), we measured MT protein levels. MT is a metal-binding protein that is essential for metal detoxification (Sevcikova et al., 2011; Bervoets et al., 2013) We observed higher MT protein expression in exposed fish compared with control, suggesting homeostatic response to detoxify the elevated tissue concentrations of Cd, Hg and Pb (Huang et al., 2007; Bervoets et al., 2013).

For the sake of data validity, we have used phenanthrene, one of the USEPA priority PAHs that is bioavailable in the environment (Andersson and Achten, 2015; Keith, 2015) and described as the simplest PAH containing 3-fused aromatic rings (IARC, 2010), as a positive control. We showed that phenanthrene significantly induced biotransformation and oxidative stress responses in

C. gariepinus and this is consistent with previous reports (Piazza et al., 2016; Lima et al., 2018). However, contrary to our findings, it was recently reported that elevated bile phenanthrene bioaccumulation in Atlantic cod (*Gadus morhua*) caged at a capped waste disposal site in Kollevåg, Western Norway did not induce CYP1A (Dale et al., 2019). Thus, it should be noted that the reported discrepancies in the CYP1A induction ability of phenanthrene maybe related to differences in exposure regimes and species specific sensitivity to environmental stressors, which is also dependent on their genetic composition (Eide et al., 2018). For example, Dale et al. (2019) employed a field based-caged study, compared with the present study, which is a laboratory controlled experiment - as such responses are expected to differ to some degree. Furthermore, the species specific sensitivity to environmental stressors between *G. morhua* (temperate) and *C. gariepinus* (tropical) species may also account for the observed differences. Similarly, the loss or absence of the PXR (a crucial receptor that regulate transcriptional responses to environmental stressors) in *G. morhua* may suggest alternate compensatory mechanism for regulating chemical stressors (Eide et al., 2018), and this may also account for the observed species-specific differences

In conclusion, we present a robust, empirical and comprehensive evidence indicating significant tissue accumulation of environmental contaminants and heavy metals, with resulting toxicological responses at mRNA, enzyme and protein levels for biotransformation and oxidative stress, in *Clarias gariepinus* exposed to simulated leachates from a tropical solid waste dumpsite. Given the lack of solid waste recycling techniques and poor management methods in Nigeria and other low income countries, our results highlight the potential significant environmental, wildlife and public health problems arising from improper waste management, while also providing a scientific basis for local sensitization and inform legislative decisions and policy formulations towards sustainable environmental management in Nigeria and other developing countries.

Acknowledgments

This study was supported in part by the British Ecological Society, Ecologist in Africa Research Grant 2017 (BES Grant Reference: EA17/1366) and African Research Excellence Funds (MRF Grant Reference: MRF-157-0023-F-IBOR). We thank Randi Røsbak for technical assistance during laboratory analysis at the Department of Biology, NTNU.

Figure legends

Figure 1. Changes in hepatic ethoxyresorufin (EROD: A-C) and buthoxyresorufin (BROD: D-F), at day 3, 7 and 14 respectively after exposure of juvenile *Clarias gariepinus* to simulated leachates from Lemna solid waste dumpsite and a positive control (phenanthrene: PAH). Data are presented as mean ± standard error of the mean (SEM; n=5), different letters denote significant difference between exposure groups. Level of significant was set at $p<0.05$. Enzymatic activities are expressed as pmol/mg protein/min.

Figure 2. Changes in hepatic methoxyresorufin (MROD: A-C) and pentoxyresorufin (PROD: D-F), at day 3, 7 and 14 respectively after exposure of juvenile *Clarias gariepinus* to simulated leachates from Lemna solid waste dumpsite and a positive control (phenanthrene: PAH). Data are presented as mean ± standard error of the mean (SEM; n=5), different letters denote significant difference between exposure groups. Level of significant was set at $p<0.05$. Enzymatic activities are expressed as pmol/mg protein/min.

Figure 3. Modulations in hepatic catalase (CAT: A-C) and glutathione peroxidase (GPx: D-F), at day 3, 7 and 14 respectively after exposure of juvenile *Clarias gariepinus* to simulated leachates from Lemna solid waste dumpsite and a positive control (phenanthrene: PAH). Data are presented as mean ± standard error of the mean (SEM; n=5), different letters denote significant difference between exposure groups. Level of significant was set at $p<0.05$. Enzymatic activities are expressed as nmol/min/mg protein.

Figure 4. Modulations in hepatic uridine 5'-diphospho-glucuronosyltransferase (UDPGT: A-C) and glutathione S-transferase (GST: D-F), at day 3, 7 and 14 respectively, after exposure of juvenile *Clarias gariepinus* to simulated leachate from Lemna solid waste dumpsite and a positive control (phenanthrene: PAH). Data are presented as mean ± standard error of the mean (SEM; n=5), different letters denote significant difference between exposure groups. Level of significant was set at $p<0.05$. Enzymatic activities are expressed as nmol/min/mg protein.

Figure 5. Changes in glutathione reductase (GR: A-C) and Lipid peroxidation (LPO: D-F), at day 3, 7 and 14 respectively after exposure of juvenile *Clarias gariepinus* to simulated leachates from Lemna solid waste dumpsite and a positive control (phenanthrene: PAH). Data are presented as mean ± standard error of the mean (SEM; n=5), different letters denote significant difference between exposure groups. Level of significant was set at $p<0.05$. GR activity is expressed as nmol/min/mg protein, while LPO is expressed as nmol MDA/mg protein.

Figure 6. Transcript levels for *cyp*1a (A-C) and *cyp*2d3 (D-F), at day 3, 7 and 14 respectively after exposure of juvenile *Clarias gariepinus* to simulated leachates from Lemna solid waste dumpsite and a positive control (phenanthrene: PAH). Data are presented as mean ± standard error of the mean (SEM; n=5), different letters denote significant difference between exposure groups. Level of significant was set at $p<0.05$. Messenger RNA (mRNA) expression was given as % of control.

Figure 7. Transcript levels for *cyp*27 (A-C) and *gpx*-1 (D-F), at day 3, 7 and 14 respectively after exposure of juvenile *Clarias gariepinus* to simulated leachates from Lemna solid waste dumpsite and a positive control (phenanthrene: PAH). Data are presented as mean ± standard error of the mean (SEM; n=5), different letters denote significant difference between exposure groups. Level of significant was set at $p<0.05$. Messenger RNA (mRNA) expression was given as % of control.

Figure 8. Transcript levels for *gr* (A-C) and *sod*-1 (D-F), at day 3, 7 and 14 respectively after exposure of juvenile *Clarias gariepinus* to simulated leachates from Lemna solid waste dumpsite and a positive control (phenanthrene: PAH). Data are presented as mean ± standard error of the mean (SEM; n=5), different letters denote significant difference between exposure groups. Level of significant was set at $p<0.05$. Messenger RNA (mRNA) expression was given as % of control.

Figure 9. Transcript levels for *gst* (A-C) and *cat* (D-F) at day 3, 7 and 14 respectively after exposure of juvenile *Clarias gariepinus* to simulated leachates from Lemna solid waste dumpsite and a positive control (phenanthrene: PAH). Data are presented as mean ± standard error of the mean (SEM; n=5), different letters denote significant difference between exposure groups. Level of significant was set at $p<0.05$. Messenger RNA (mRNA) expression was given as % of control.

Figure 10. Immunochemical detection using Western blotting of CYP1A (A), CYP3 (B), MT (C) and NF-Kβ in the liver of juvenile *Clarias gariepinus* exposed to different dilution (1:10. 1:50 and 1:100) of simulated leachate from Lemna solid waste dumpsite and a positive control (phenanthrene: PAH). 50 µg of total protein concentration was loaded per well. All arbitrary unit values represent mean ± standard error of the mean (SEM; n=5), different letters denote significant difference between exposure groups. Level of significant was set at $p<0.05$.