RESEARCH ARTICLE

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Oxidative stress responses to heavy metal burden in African catfish (*Clarias gariepinus*) from Warri River, Niger-Delta, Southern Nigeria

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Abstract

Despite the degradation and threat of ubiquitous contaminants, such as heavy metals, in Warri River, Southern Nigeria, little is known about the ecological effects of such pollution using pro-oxidant variables. This study investigated oxidative stress responses to heavy metals (lead (Pb), cadmium (Cd), manganese (Mn), nickel (Ni) and copper (Cu)) burden in the heart, kidney, liver and gills of African catfish inhabiting Warri River. Fish (N=30) were collected during August-September, 2018 from two contaminated sites along the course of Warri River and a fish farm which was considered a reference site. The concentrations of Pb, Cd and Ni in the two contaminated sites were above the WHO permissible limits and significantly higher (p<0.05) as compared to the fish collected from the reference site. The trend of accumulation of the metals followed the order: Reference site (site 1)-Mn>Ni>Cu>Pb>Cd; site 2-Pb>Cu>Cd>Mn>Ni and site 3-Ni>Mn>Cu>Cd and Pb respectively. The activities of catalase (CAT) and superoxide dismutase (SOD) were significantly higher (p<0.05) in the organs of fish caught at the two contaminated sites. Malondialdehyde (MDA) was significantly elevated (p<0.05), in the liver and heart of fish obtained from site 3, relative to sites 2 and 1. Fish obtained from the two contaminated sites had significantly decreased (p<0.05) levels of reduced glutathione (GSH) in their organs, however, the reduction was more in site 2. The results of this study confirm that environmental stressors, such as heavy metals, can alter antioxidant enzymes and glutathione systems, as well as induce lipid peroxidation, leading to oxidative stress in aquatic organisms.

Introduction

Warri River is an important river located in the Niger Delta region of Southern Nigeria. This river has been the focus of increasing attention over the years, due to the high degree levels of pollution caused by the pervasive presence and operation of oil and gas companies, indiscriminate discharge of industrial effluents and residential wastes (Obafemi *et al.,* 2012). Among the major pollutants, heavy metal contamination has been reported in the water, sediment and biota (Nduka *et al.,* 2010; Oluowo &

Omoregie, 2016; Wogu & Okaka, 2011), with concentrations that exceed the threshold of concern. The prevalence of heavy metals pollution in Warri River has been regarded as a significant contaminant as they are ubiquitous in the environment and can bio-accumulate in organisms, potentially reaching toxic levels (Balali-Mood *et al.*, 2021). Chronic exposure to high concentrations can affect the health and survival of non-target organisms such as fish (Hamilton *et al.*, 2015). Studies have shown that fish species ingesting environmental contaminants may catalyse reactions that generate reactive oxygen species (ROS) which may in turn lead to oxidative stress (Jiri *et al.*, 2018; El-Agri *et al.*, 2022). Oxidative stress is an imbalance between the production of ROS and the antioxidant defence system in an organism (Hoseinifar *et al.*, 2020). In an organism, reactive oxygen species can attack cellular macromolecules (proteins, lipids and DNA) (Valon *et al.*, 2013). Structural and enzymatic proteins are the primary targets for oxidative damage leading to impairment of many cellular functions. To counteract the impacts of ROS, biological systems employ antioxidant enzymes to detoxify and degrade the contaminant's harmful consequences (Tjalkens *et al.*, 1998). Fishes possess both enzymatic (superoxide dismutase (SOD)); catalase (CAT) and non-enzymatic (reduced glutathione (GSH)) antioxidants defence systems (Abhijith *et al.*, 2016), located in virtually all tissues. Similarly, lipid peroxidation products such as malondialdehyde (MDA), are utilized as biomarkers to reflect severe oxidative damage (Van der Oost *et al.*, 2003) in an organism. Essential for the maintenance of redox status in fishes, antioxidant systems have been demonstrated to be excellent biomarkers of oxidative stress (Yadav *et al.*, 2015). Heavy metals can induce oxidative stress (Mahboob, 2013) and assessing antioxidant defences system and oxidative damage in fishes can indicate heavy metal contamination in the aquatic environment, as well as the magnitude of the fish population's response to heavy metals (Akinsanya *et al.*, 2020). This study was aimed at assessing oxidative stress biomarkers in *Clarias gariepinus* to determine heavy metal contamination in Warri River.

Materials and Methods
Study area
Warri River lies between latitudes 5^021^1 - 6^000^1N and longitudes 5^024^1 - 6^021^1E), in the Niger Delta region of Southern Nigeria. It takes its origin around Utagba -Uno traversing through the oil prospecting parts of Warri City, where it flows across many markets, residential areas and petroleum refineries parking lots and waste dumps before emptying into the Atlantic ocean. This river has been documented among the polluted coastal rivers in Southern Nigeria (Aghoghovwia *et al.*, 2016) due to frequent oil spills induced by willful acts of vandalization, ageing facilities, accidents and illegal bunkering. Three sampling sites (Figure 1) were selected; A reference fish farm (site 1), Mclver (site 2) and Gbomiomio (site 3) along the course of Warri River. Site 1: A fish farm ($5^034'30.54''$ N, $5^043'8.98''$ E) located in Jeddo, Okpe Local Government Area of Delta State. Fish farming in this location is practised in concrete ponds of 3m by 2.5m in length and breadth. Site 2. Mclver ($5^030'38.84''$ N, $5^0 45'1.30''$ E) is located near the Mclver market, a potentially contaminated area and site 3: Gbomiomio ($5^030'55.15''$ N, $5^047'4.13''$ E) located near the community, which harbours small artisan oil industries.

Fish collection
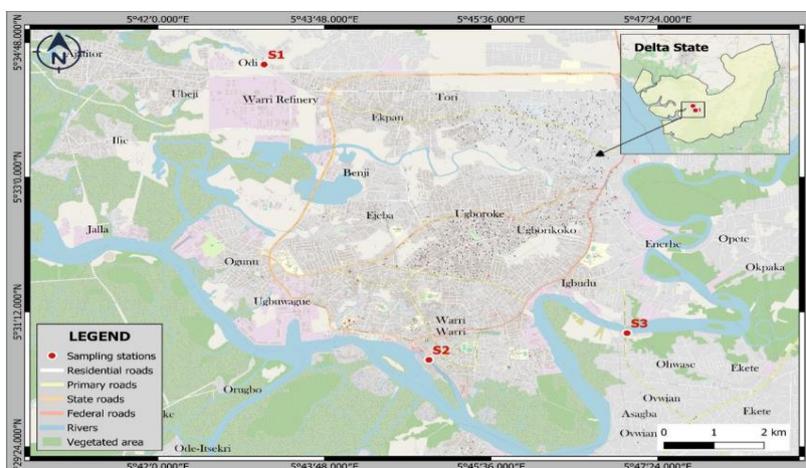African catfish (*Clarias gariepinus*) weighing between 250g and 400g with lengths of 25.8cm-30.5cm were collected from a fish farm in Jeddo, and two sites (Mclver & Gbomiomio) along the course of Warri River. Control catfish samples purchased from the reference farm was assumed to be devoid of any pollution that could affect the biochemical responses of the control. All catfish samples were transported to the laboratory in ice-cold containers (0-4°C) and identified according to the method of Idodo-Umeh (2003). The fish (N=30) were sacrificed by medullary transection (Lucky, 1977) and the liver, heart, gills,

Figure 1: Map of Warri River showing the study area. S1=a fish farm; S2= Mclver; S3= Gbomiomio

and kidney were quickly harvested.

Heavy metal analysis
All fish tissues (heart, kidney, liver and gills) were oven dried at 105 °C for 24 h. They were then transferred to a desiccator until it attained a constant weight. Fish sample of 1g was pounded with mortar and pestle before being transferred to a digestion flask containing 5 mL of nitric acid and heated until a clear solution was visible. The solution was then filtered with Whatman No. 1 filter paper and used for heavy metal analysis. To determine the concentration of heavy metals (Pb, Cd, Mn, Ni and Cu) in fish tissues, Atomic Absorption Spectrophotometer (AAS, 2000 series) was used. All chemical reagents were analytical reagent grade (Sigma).

Biochemical assay
 The liver, heart, gills and kidney tissues were washed in ice-cold 1.15% potassium chloride (KCl) solution, blotted and weighed. They were then homogenized in four volumes of buffer (50 mM Tris – HCl combined with 1.15% KCl and the pH adjusted to 7.5). The homogenate was centrifuged at 3,000 rpm for 20 minutes. To separate the nuclear debris, the homogenate was filtered with Whatman No. 1 and the supernatant was collected and preserved at -20°C until needed for further biochemical analysis (Arojojoye & Adeosun, 2016).

Markers of oxidative stress
Superoxide dismutase activity was measured by the inhibition of auto-oxidation of adrenaline at pH 10.2 (30ºC) as described by Mistra & Fridovich (1972). The reaction was first added with an aliquot of the sample (200 µL) to 2.7 mL carbonate buffer (0.05 M pH 10.2) containing 0.01% epinephrine. Absorbance change was 480 nm against a blank containing all the components except the enzyme source and the activity was expressed as units per gram of tissue. Catalase activity was assayed by the method of Claiborne (1985). The reaction was conducted at 25 º C with the following reaction mixture: 50 mM potassium phosphate buffer, pH 7.4, 19 mM hydrogen peroxide and 10% proxymonosulfate (PMS). The absorbance change was recorded at 240 nm. In terms of moles of H_2O_2 consumed per minute per gram of protein, CAT activity was calculated. Using the fluorimetric method of Del Rio *et al.* (2003), malondialdehyde (MDA) was estimated, using fluorescence spectroscopy (Bulk scientific model 210). At room temperature, 700 mL of 0.1 M HCL and 200 mL of the sample were incubated for 20 min. The mixture was added with 900 mL of 0.025 M thiobarbituric acid and incubated at 37°C

for 65 minutes, 400 µL of Tris– EDTA protein extraction buffer was then added. Malondialdehyde concentration was calculated and expressed as nmoles of MDA/ Mg protein. Assay of glutathione reductase was measured using the glutathione reductase kit (Sigma Aldrich, UK) following manufacturer's instruction. This method used the rate of oxidation of NADPH to NADP+(Cooper & Hanigan, 2018). Absorbance at 340 nm decreased as a result of NADPH's oxidation, which is directly correlated with the glutathione reductase activity in the sample. Reduced glutathione (GSH) levels were calculated and expressed as nmoles/mg of protein.

Statistical analysis
All data were presented as mean ± S.E (standard error). One-way analysis of variance (ANOVA) was used to test for significant differences between means and group means compared by the Duncan multiple range test. All statistical analyses were performed using Microsoft excel 2016 and SPSS 23. 0 version 2018.

Results
Table 1 shows the mean levels of heavy metals in the heart of *Clarias gariepinus* from reference site and polluted sites. Manganese mean concentration was high (8.81mg/kg) in fish from site 1, while Ni had the lowest mean (0.03mg/kg) in fish from site 2. The mean of Pb, Ni and Mn were significantly higher (p< 0.05) across the different sites.

Table 2 shows the mean levels of heavy metals in the kidney of *Clarias gariepinus* from reference site and polluted sites, which ranged between (0.03mg/kg - 4.93mg/kg). The highest mean (4.93mg/kg) was observed for Mn for fish from site 1 and the lowest mean value (0.03mg/kg) was in Ni of fish from site 2. Analysis of Variance (ANOVA) showed that there was a significant difference in the Mn and Ni mean values among the sites (P<0.05).

Mean levels of heavy metals in the liver of *Clarias gariepinus* from reference site and polluted sites are shown in Table 3. Manganese had the highest (8.45mg/kg) mean level in fish from site 1 and the lowest mean (0.02mg/kg) was in fish from site 2 for Mn. There was a significant difference in the mean values (P<0.05) of all metals in the study sites except for Cu.

The mean levels of heavy metals in the gills of *Clarias gariepinus* from the reference site and polluted sites were between 0.35mg/kg and 5.13mg/kg (Table 4). Manganese had the highest (5.13 mg/kg) mean level in fish from site 1 and the lowest mean (0.31mg/kg) was in fish from site 2 for Ni. Nickel and Mn showed significant differences (p<0.05) across the sites.

Table 1: The mean levels of heavy metals in the heart of *Clarias gariepinus* from reference site and polluted sites

Heavy metals	Reference site (Site 1)	Site 2	Site 3	P-value	Significant Level
	$\overline{X}\pm$S. E. (Min-Max)	$\overline{X}\pm$S. E (Min-Max)	$\overline{X}\pm$S. E (Min-Max)		
Pb	0.40 ± 0.21^b (0.00-1.11)	1.85 ± 0.85^a (0.00-4.56)	0.00 ± 0.00^c (0.00-0.00)	0.059 0.059	p<0.05*
Cd	0.00 ± 0.00 0.00 ± 0.00	0.15 ± 0.07 0.15 ± 0.07	0.09 ± 0.03 0.09 ± 0.03	0.155 0.155	p>0.05
Mn	8.81 ± 0.44^a (7.74-9.53)	0.05 ± 0.03^b (0.00-0.16)	0.32 ± 0.20^b (0.00-9.62)	0.000 0.000	p<0.001***
Ni	0.07 ± 0.07^b (0.00-0.31)	0.03 ± 0.03^b (0.00-0.15)	3.78 ± 1.41^a (0.00-8.88)	0.011 0.011	p<0.05*
Cu	0.22 ± 0.11 (0.00-6.25)	0.33 ± 0.06 (0.15-0.52)	0.22 ± 0.13 (0.00-0.79)	0.657 0.657	p>0.05

Data represents mean ± S.E (n=30 fish). Values within a row with different superscripts differ significantly according to Duncan's test (p<0.05). * p values <0.05 regarded as statistically significant, ***p<0.001 high significant difference

Table 2: The mean levels of heavy metals in the kidney of *Clarias gariepinus* from reference site and polluted sites

Heavy metals	Reference site (Site 1)	Site 2	Site 3	P-value	Significant Level
	$\overline{X}\pm$S. E (Min-Max)	$\overline{X}\pm$S. E (Min-Max)	$\overline{X}\pm$S. E (Min-Max)		
Pb	0.07 ± 0.07 (0.00-0.34)	0.25 ± 0.25 (0.00-1.51)	0.00 ± 0.00 (0.00-0.00)	0.508 0.508	p>0.05
Cd	0.00 ± 0.00 0.00 ± 0.00	0.06 ± 0.06 0.06 ± 0.06	0.08 ± 0.01 0.08 ± 0.01	0.371	p>0.05
Mn	4.94 ± 2.14^a (0.00-9.52)	0.05 ± 0.03^b (0.00-0.16)	0.32 ± 0.20^b (0.00-0.96)	0.013	p<0.05*
Ni	0.17 ± 0.11^b (0.00-0.54)	0.03 ± 0.03^b (0.00-0.19)	4.77 ± 2.01^a (0.00-11.84)	0.025	p<0.05*
Cu	0.09 ± 0.06 (0.00-0.31)	0.26 ± 0.07 (0.00-0.52)	0.35 ± 0.09 (0.26-0.78)	0.089	p>0.05

Data represents mean ± S.E (n=30 fish). Values within a row with different superscripts differ significantly according to Duncan's test (p<0.05). * p values <0.05 are regarded as statistically significant

Table 3: The mean levels of heavy metals in the liver of *Clarias gariepinus* from reference site and polluted sites

Heavy metals	Reference site (Site 1) $\overline{X}\pm$S. E (Min-Max)	Site 2 $\overline{X}\pm$S. E (Min-Max)	Site 3 $\overline{X}\pm$S. E (Min-Max)	P-value	Significant Level
Pb	0.07 ± 0.07^b (0.00-0.37)	1.65 ± 0.79^a (0.00-4.55)	0.00 ± 0.00^b (0.00-0.00)	0.050	p<0.05*
Cd	0.00 ± 0.00^b (0.00-0.00)	0.24 ± 0.08^a (0.00-0.36)	0.11 ± 0.05^b (0.00-0.28)	0.032	p<0.05*
Mn	8.45 ± 1.91^a (1.79-11.31)	0.03 ± 0.03^b (0.00-0.16)	0.16 ± 0.16^b (0.00-0.96)	0.000	p<0.001***
Ni	0.54 ± 0.42^b (0.00-2.17)	0.02 ± 0.02^b (0.00-0.09)	3.78 ± 1.47^a (0.00-9.87)	0.023	p<0.05*
Cu	0.23 ± 0.12 (0.00-0.63)	0.25 ± 0.045 (0.10-0.41)	0.18 ± 0.11 (0.00-0.53)	0.841	p>0.05

Data represents mean ± S.E (n=30 fish). Values within a row with different superscripts differ significantly according to Duncan's test (p<0.05). * p values <0.05 regarded as statistically significant, ***p<0.001 very high significant difference

Table 4: The mean levels of heavy metals in the gills of *Clarias gariepinus* from reference site and polluted sites

Heavy metals	Reference site			P-value	Significant Level
	(Site 1) $\overline{X}$±S. E (Min-Max)	Site 2 $\overline{X}$±S. E (Min-Max)	Site 3 $\overline{X}$±S. E (Min-Max)		
Pb	0.00±0.00 (0.00-0.00)	0.89±0.42 (0.00-2.34)	0.00±0.00 (0.00-0.00)	0.099	P>0.05
Cd	(0.00-0.00) 0.00±0.00	(0.00-0.36) 0.12±0.08	(0.00-0.96) 0.21±0.19	0.608	P>0.05
Mn	5.05±2.49[a] (0.89-9.53)	0.00±0.00[b] (0.00-0.00)	0.58±0.24[b] (0.00-0.96)	0.008	p<0.01**
Ni	0.07±0.07[b] (0.00-0.22)	0.03±0.03[b] (0.00-0.19)	5.13±0.66[a] (2.96-6.91)	0.000	p<0.001***
Cu	0.37±0.19 (0.00-0.63)	0.36±0.09 (0.00-0.61)	0.16±0.06 (0.00-0.26)	0.298	p>0.05

Data represents mean ± S.E (n=30 fish). Values within a row with different superscripts differ significantly according to Duncan's test (p<0.05). * p values <0.05 regarded as statistically significant, ***p<0.001 very high significant difference
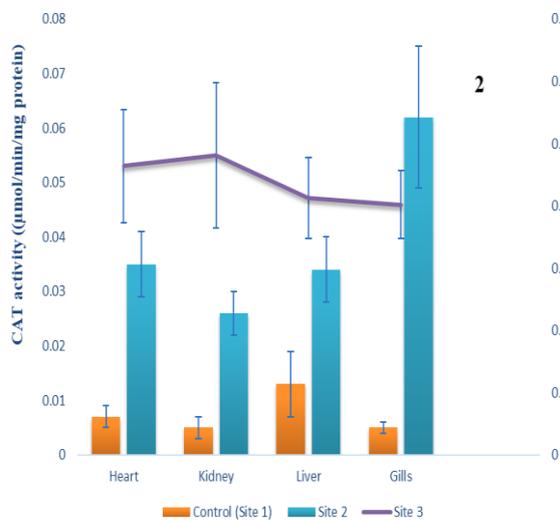

Figure 2: Catalase (CAT) activity in the heart, kidney, liver and gills of *Clarias gariepinus* from a reference site (site 1) and two polluted sites (site 2 and site 3)
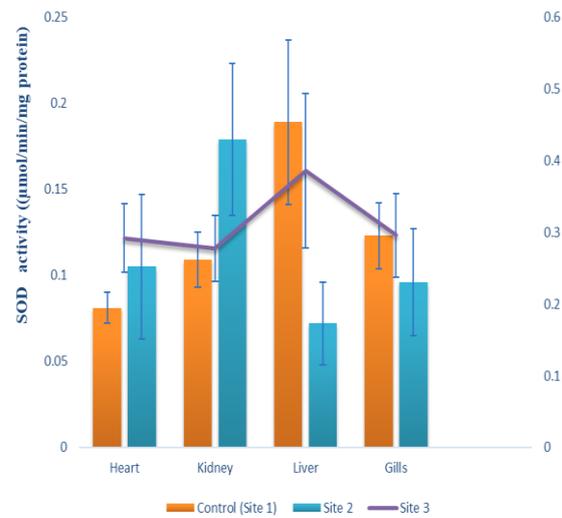

Figure 3: Superoxide dismutase (SOD) activity in the heart, kidney, liver and gills of *Clarias gariepinus* from a reference site (site 1) and two polluted sites (site 2 and site 3)
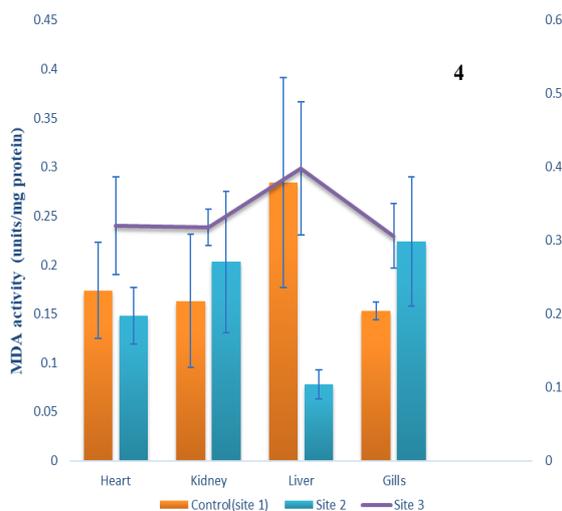

Figure 4: Malondialdehyde (MDA) activity in the heart, kidney, liver and gills of *Clarias gariepinus* from a reference site (site 1) and two polluted sites (site 2 and site3)
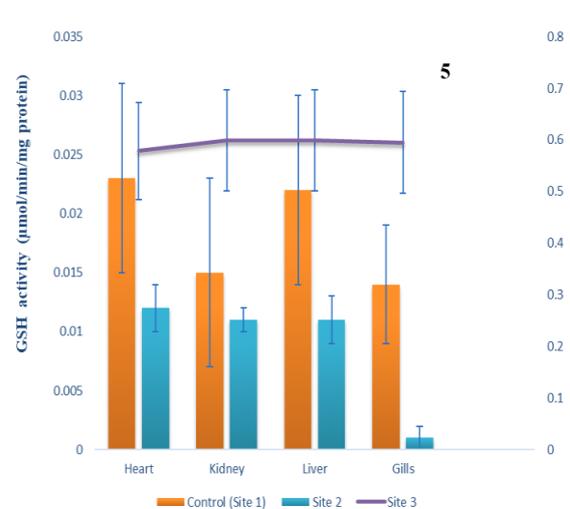

Figure 5: Glutathione (GSH) activity in the heart, kidney, liver and gills of *Clarias gariepinus* from a reference site (site 1) and two polluted sites (site 2 and site 3)

The order of concentration of metals in the organs of fish followed the sequence, Site 1: Pb-heart>liver>kidney>gills; Mn-gills> heart> kidney>liver; Ni-gills> kidney> liver>heart; Cu-heart>kidney>liver>gills. For fish at site 2 the sequence was: Pb-heart> kidney> liver> gills; Cd-gills> liver> heart>kidney; Mn-gills> heart> kidney> liver; Ni- gills> Kidney> heart> liver; Cu-kidney>heart>liver>gills; while for fish at Site 3 the sequence was: Cd-gills> liver> heart> kidney; Mn-gills> heart> kidney> liver; Cu-kidney>heart>liver>gills. The fish accumulated heavy metals in varying amounts in their organs. The trend of accumulation of the metals is as follows: Fish from Site 1-Mn>Ni>Cu>Pb>Cd; and for fish from site 2-Pb>Cu>Cd>Mn>Ni, while for fish from site 3 the trend of accumulation is-Ni>Mn>Cu>Cd and Pb.

As shown in figure 2, the CAT activity in the organs (heart, kidney, liver and gills) of *Clarias gariepinus* from reference site and polluted sites ranged between 0.01 and 0.48. The highest concentration of CAT (0.48) was in the kidney of fish from site 3 and the lowest mean (0.01) were in the heart, kidney and liver of fish from site 1. Catalase activity was significantly increased (p<0.001) in all the organs of fish caught from site 3 in comparison to sites 2 and 1. Figure 3 shows the SOD activity in the organs of *Clarias gariepinus*. Highest SOD activity (0.39) was in the liver of fish from site 3, while the lowest (0.07) mean was in the liver of fish from site 2. The activities of SOD in the fish organs were significantly elevated (p<0.05) in the fish from site 3 when compared to fish from sites 2 and 1.

The malondialdehyde activities in the organs of *Clarias gariepinus* from reference site and polluted sites were between 0.08 and 0.40 (figure 4). Malondialdehyde activities were highest in the liver of fish from site 3 (0.40), while the liver of fish from site 2 had the lowest MDA mean (0.08). Malondialdehyde activities in the heart and liver of fish from site 3 were significantly elevated (p<0.05) relative to sites 1 and 2.

The activities of GSH in the organs of *Clarias gariepinus* from reference site and polluted sites as shown in figure 5 were found high (0.60) in all the organs of fish from site 3. The lowest GSH mean (0.01) was observed in all the organs of fish from site 2. Fish obtained from the two polluted sites had significantly reduced levels (p< 0.001) of GSH in their organs and the reduction was more in site 2 than in sites 1 and 3.

Discussion
In this study, evidence of heavy metal contamination in Warri River is demonstrated by their concentrations in the fish organs. The heavy metals concentration in the organs (heart, kidney, liver and gills) of *Clarias gariepinus* obtained from Warri River were higher in site 3 and above the WHO 1985 permissible limits. However, two of the metals (Pb and Cu) were within the permissible limits of 2.30 mg/kg and 3.00 mg/kg respectively in all the sites. The gills of fish caught at site 3 accumulated significantly higher concentrations of Ni and Mn than at site 2. This may be attributed to the gills in fishes functioning as respiratory organs that absorb metal ions. In contrast to other fish organs, the gills are in direct contact with the contaminated water and having the thinnest epithelia, allow metals to penetrate easily as suggested by Bebianno *et al.* (2004). Concentration of Cd in the heart, liver and gills of fish from site 2 was observed to be above the WHO (1985) permissible limits of 0.50 mg/kg. It is likely that the heart and liver accumulated heavy metals via metal-binding proteins as reported by Kargin & Cogun (1999). Other studies have reported high concentrations of Cd in the liver and heart organs in fish samples (Farombi *et al.,* 2007; Rajeshkumar & Xiaoyu 2018). Interestingly the reference fish farm (site 1) had a higher concentration of Mn in all the organs and was above the WHO (1985) permissible limit of 0.50 mg/kg. Although the metabolism of Mn is not well understood, previous studies have demonstrated that a deficiency of Mn can lead to skeletal deformities in fish (Lali & Kaushik, 2021). Thus, Prabhu *et al.* (2019) reported that Mn is a biological significant micronutrient required in fish feed for proper growth and development. The high level of Mn in fish from the reference fish farm may be as a result of the fish meal they were fed with. In general, the concentration of the studied metals (Pb, Cd, Mn, Ni and Cu) in *Clarias gariepinus* organs were higher in the two contaminated sites than in site 1 (reference fish farm). This is logical since Warri River, for many years, has been a sink for various industrial and residential wastes.

Elevated levels of CAT increased significantly in all the organs of *Clarias gariepinus* caught in site 3 relative to site 2 and site 1. This was probably due to the high heavy metal levels in the organs, which triggered antioxidant defences to mitigate biological stress. Similar findings have been reported by other authors (Osioma *et al.,* 2013; Aly *et al.,* 2020). In this study, SOD activity was elevated significantly in all the organs of fish obtained from the two polluted sites; the exception being in the liver and gills of fish caught

in site 2. Decreased SOD activity may be caused by the inhibition of the enzyme by high fluxes of ROS (Thitiya *et al.,* 2021). The results of this study are consistent with those of Lopes *et al.* (2002) and Achuba & Osakwe (2003). Malondialdehyde (MDA) level was increased significantly in all the organs of the two polluted sites, suggesting that the levels of heavy metals in the fish successfully induced oxidative stress in the organs. The results appear to suggest that antioxidant defences were successfully established in the heart and liver of fish caught in site 2 and reference fish farm (site 1) as demonstrated by the reduced MDA levels. Fish obtained from the two polluted sites had significantly reduced levels of GSH in their organs and the reduction was more in site 1 than in sites 2 and 3. The elevated level of GSH in fish obtained from reference fish farm suggests that the molecule plays a protective role against oxidative stress induced by heavy metals (Adeogun *et al.,* 2012). It could be also due to the high level of Mn in the organs of fish obtained from reference fish farm, as high levels of Mn were reported to interfere with biochemical and physiological mechanisms in fish (Wang *et al.,* 2022). Alterations in the level of GSH in the organs of fish have been suggested to be the consequence of organ-specific responses (Sayeed *et al.,* 2003; Ali *et al.,* 2004). These results agree with the reports of Wilhelm-Filho *et al.* (2001) and Javed *et al.* (2017).

In conclusion, the heavy metals (Pb, Cd, Mn, Ni and Cu) accumulated in the organs of *Clarias gariepinus* collected from Warri River exerted environmental pressure on *Clarias gariepinus* resulting in oxidative stress in the fish. This study has provided supporting evidence for the use of oxidative biomarkers in bio-monitoring studies, particularly in threatened aquatic ecosystems.

Conflict of Interest
The authors declare that there is no conflict of interest.

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