PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (2) 533-541 February 2024

Analysis of Heavy Metals and Gene Expression of African Arowana Fish (*Heterotis niloticus*) obtained from Igbalegbe River, Ughelli, Delta State, Nigeria

***OKORO, B; TAWARI-FUFEYIN, P**

Department of Environmental Management and Toxicology, College of Science, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria

**Corresponding Author Email: baoken2003@yahoo.com*
Co-Author Email: prekeyi@gmail.com

ABSTRACT: Heavy metal pollution in aquatic ecosystems can be assessed with suitable biomarkers in fish. This study was carried out to determine the concentration of heavy metals and gene expression of African Arowana Fish **(***Heterotis niloticus*) obtained from Igbalegbe River**,** Ughelli, Delta State, Nigeria using Atomic Absorption Spectrophotometer (Varian 220 Fast sequential) for the metals and Polymerase chain reaction (PCR) for the gene expression study. Results of the study showed that fishes in the downstream sectionof the river and the effluent discharge point recorded higher metal pollution Index (MPI) compared to the upstream station. The concentration of heavy metals in the fish tissue were generally within the limits of Food and Agricultural Organisation (FAO)/World health Organisation (WHO) except for that of Cadmium and Lead in the discharge station. The selected genes investigated were biomarkers for general stress (HSP70), xenobiotic metabolism (CYPA1), antioxidative defence (SOD), Insulin growth factor-1 (IGF-1) and hypoxia inducible factor (HIF) respectively. The expression of the genes revealed significant variations ($P<0.05$) in the fishes obtained from the different stations of the river. Some of the genes were down-regulated and suppressed while others were upregulated to enable the fish cope with stress while adapting to environmental pollution. Changes in biomarkers can therefore be considered as early signals of stress in the selected fish species.

DOI: https://dx.doi.org/10.4314/jasem.v28i2.26

Cite this paper as: OKORO, B; TAWARI-FUFEYIN, P. (2024). Analysis of Heavy Metals and Gene Expression of African Arowana Fish (*Heterotis Niloticus*) obtained from Igbalegbe River, Ughelli, Delta State, Nigeria. *J. Appl. Sci. Environ. Manage.* 28 (2) 533-541

Dates: Received: 16 December 2023; Revised: 02 February 2024; Accepted: 24 February 2024 Published: 28 February 2024

Keywords: Heavy metals; gene expression; Heterotis niloticus; effluent; biomarkers

The presence of heavy metals in water bodies can result in the accumulation of heavy metals in the tissues and organs of fish. This can cause a wide range of biological effects with possibilities of inducing molecular and biochemical changes in organisms while exposing humans who consume such fishes to heavy metal poisoning (Amoatey, 2019). Some of these organisms may have their genes expression altered in order to adapt to environmental stress after long-term exposure to such contaminant (Hamilton *et al*., 2017). Hence, the condition of aquatic organisms in polluted environment can therefore be assessed and monitored through the use of suitable biomarkers in fish. *Heterotis niloticus* is a pelagic fish with hard flesh and scales. It is widely distributed in Nigeria inland waters, most especially in freshwaters. It is considered endangered and has been reported to be on the decline as it is the only species of the genus Heterotis (Mustapha, 2010). Analysis of biomarkers in fish obtained from the field can provide relevant information on their health status as well as the contamination level of the environment. Another aspect of fish biomarker is that of the molecular changes associated with their adaptive strategy to

chemical stress (Tenji *et al*., 2020). The genetic basis for such tolerance requires further investigations considering the fact that these adaptive changes can modify ecosystem impacts. Understanding how fish adapt and respond to pollution especially at wastewater discharge point becomes imperative, this will provide information on evolutionary dynamics associated with anthropogenic disturbance. Studies reported on gene expression of fish in relation to water pollution include works of Tenji *et al*. (2020), Veliz *et al*. (2020) and Thomas *et al.* (2014). It was however observed that there was limited knowledge on the effect of glass industry effluent on the molecular biology of fish. The objective of this study was to determine the concentration of heavy metals and gene expression of African Arowana Fish (*Heterotis niloticus*) obtained from Igbalegbe River, Ughelli, Delta State, Nigeria

MATERIALS AND METHODS

Description of study area: This study was carried out in Igbalegbe river, located beside a glass manufacturing company in Ekerhavwe, Ughelli, Delta State. The river lies between Latitude 5° 31' 57.06"N and 5° 33' 8.89"N and Longitude 5° 55' 6.43"E and 5°58' 46.28"E. It originates from Isiokolo in Ethiope East LGA, flows through Ekapamre, Ughevwughe, Iwhrekeka down to Okpare river. The study area is characterized by tropical equatorial climate with two distinct seasons; the wet and dry season. The area has mangrove vegetation with forest trees, Oil palm (*Elaeis guinensis*), India bamboo (*Bambusa sp*), Rubber tree (*Hevea brasiliensis*) with abundant shrubs and grasses. Farmlands within the study area had crops like cassava (*Manihot esculenta*), maize (*Zea mays*), cocoyam (*Colocasia and Xanthosoma sp.),* plantain (*Musa paradisiaca*), banana (*Musa sapientum*), pawpaw (*Cariaca papaya*) and a variety of leafy vegetables. Human and industrial activities within the study area include sand dredging for glass production and building construction**,** loading and offloading of goods in the trailer park beside the factory, a mechanic workshop, road and bridge for passage of vehicles, fishing, swimming, laundry and washing of trailers beside the river.

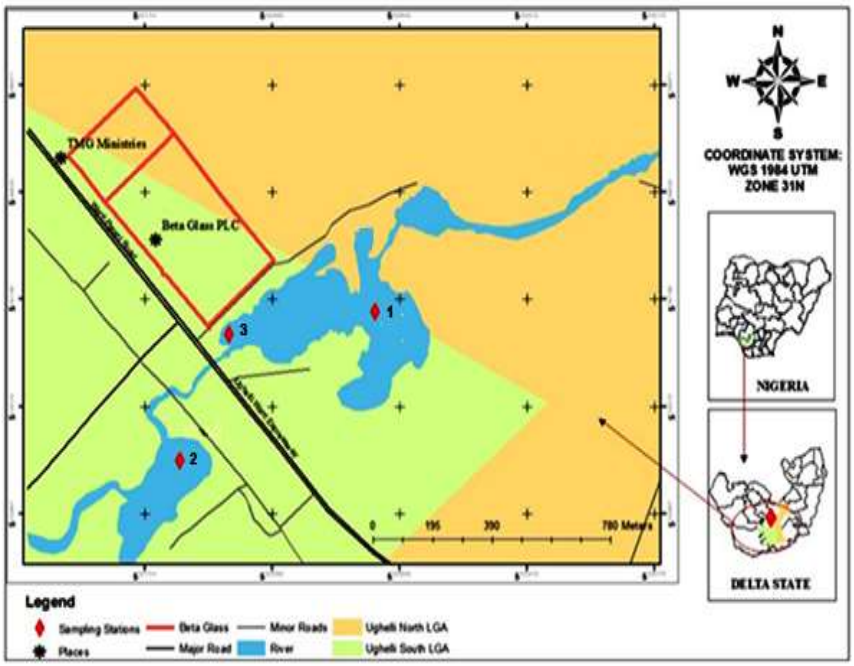

Fig 1: Map of the study area showing sampling stations: (Source: Self, 2022)

*Sampling stations:*Three sampling stations were selected along the stretch of the river coving the upstream, downstream and the effluent discharge area of a glass manufacturing company.

Station 1 is located in the upstream section of the river with respect to the discharge station. This station is located between latitude 5° 32' 27.75" N and longitude 5° 55' 39.85" E. There is human settlement at the bank of this station with activities like swimming, wood cutting and fishing. There are farmlands with crops such as Oil palm trees (*Elaeis guinensis*), Cassava (*Manihot esculenta*), Maize (*Zea mays*), Plantain (*Musa paradisiaca*), Mango (*Mangifera indica*) etc. The water surface at this station appears turbid and brownish as a result of the dredging operations.

Station 2 is in the downstream section of the river located between latitude 5° 32' 13.64" N and longitude 5° 55' 20.19" E. This station has less human activities. There were trees at the background with vegetations consisting of Indian bamboo (*Bambusa sp*), Rubber (*Hevea brasiliensis*), Oil palm trees (*Elaeis guinensis*), Plantain (*Musa paradisiaca*), Pawpaw (*Cariaca papaya*), Ferns (*Dryopteris sp*), shrubs and grasses.

Station 3 (Effluent discharge station). It is located between the upstream and the downstream station. It is between latitude 5° 32' 24.80" N and longitude 5° 55' 23.72" E. Wastewater from the factory is discharged into this station. The water at this station occasionally becomes oily during the study period. The area is surrounded by vegetation comprising Indian bamboo (*Bambusa sp*), grasses and shrubs. *Heterotis niloticus* were found to be thriving in this station.

Sample Collection: Heterotis niloticus species were obtained from the various stations with the help of local fishermen in the area using fish nets and traps on monthly basis (January-June 2022). Fish samples were sorted and identified using appropriate identification keys (Holden and Reed, 1991),weighed with electronic balance and measured using a measuring board before transferring them to the laboratory for further treatment. Fish samples were dissected before collecting the muscle tissue for heavy metal analysis. Fractions of the liver and gills were collected and preserved in TRIzol for genetic expression studies.

Plate 1: Samples of *Heterotis niloticus* obtained from discharge station

*Analysis of Heavy metals in Fish tissue:*Samples of the fish muscle tissue were oven dried at 105±20^{0}C before grinding into fine powder using a porcelain mortar. 2g of each homogenized tissue were weighed and digested with Conc. HNO_3, HF and $HClO_4$ in the ratio of 3:1:1 in a fume chamber at 120^{o}C for 20 minutes (Ayeloja *et al*., 2014). The digested samples were allowed to cool at room temperature, filtered through Whatman filter paper before adding deionised water to mark in a volumetric flask. The solution was aspirated into an Atomic Absorption Spectrophotometer (Varian 220 Fast Sequential) for analyses of the following heavy metals; Zinc, Iron, Copper, Manganese, Chromium, Cadmium and Lead (Mustapha *et al*., 2021).

Plate 2: Variation in colour of *Heterotis niloticus* obtained from the upstream station

Analysis of gene expression in Fish: Gene expression study was carried out using polymerase chain reaction (PCR). The selected genes were amplified using the different primer set shown in table 1. The following genes: HSP70 (Heat Shock Protein for General Stress), SOD (Superoxide Dismutase for Oxidative Stress), CYPA1 (Cytochrome for xenobiotics metabolism), and IGF-1 (Insulin growth factor for growth) were analyzed in the liver of the fish while HIF (Hypoxia Induced Factor for Hypoxic condition) was assessed in the gills of the fish. Analysis was done in triplicate.

In the RNA extraction and cDNA synthesis, the excised liver and gill tissue required for gene expression profiling were homogenized in TRIzol reagent, followed by the addition of Chloroform to partition it into three phases. This was later centrifuged for 10 minutes. The upper layer was carefully removed into a new tube and a precipitating medium (isoamyl alcohol) was added to the solution containing RNA pellets and vortex for 30 minutes. The supernatant was later decanted to obtain the RNA pellet. Further precipitation and cleaning of RNA pellet were done by adding 70% ethanol and the sample was further treated with DNAse to remove any DNA contamination in order to obtain a DNase free RNA. After this treatment, the RNA were later converted to cDNA using ProtoScriptFirst Strand cDNA Synthesis Kit.

*Agarose gel electrophoresis:*In the agarose gel electrophoresis stage, amplicons from the PCR were submitted for a densitometric run and evaluation in agarose 2% gel electrophoresis using TBE buffer solution (BioConcept Switzerland). Snapshots taken under blue-light documentation were used to reveal the relative density of DNA bands while the intensities of the bands were quantified densitometrically using Image J software (http://imagej.en.softonic.com/). The gene expression levels of the selected genes were presented relative to B-actin.

Table 1: Primer sequences and polymerase chain reaction conditions

	Description	**Primer sequence (5 –3)**	**Annealing Temperature**	**PCR product size (bp)**
HSP70	Heat Shock Protein 70 for General Stress	F:CAAACGCAACACCACCATTC R:CATGGCTCTCTCACCTTCATAC	55	106
CYPA1	Cytochrome for xenobiotics metabolism	F: GCAGGGACTATCGCATCTTT R: CAAAGCCAAAGCCCAAACTC	50	102
HIF	Hypoxia Induced Factor for Hypoxic condition	F:ACTTCTTCCCTACCCAACATTAC R: CCTCCAGGCCAATGGTATTT	56	127
IGF-1	Insulin-like growth factor for growth	F: GTGGAGAGAGAAGGGTTCATTT R: AAGCAGCACTCATCCACTATC	52	94
SOD	Superoxide Dismutase for Oxidative Stress)	F: AGCCTGCCCTCAAGTTTAAT R: CCTCCATTAGCTCTCCTTGTG	55	105
Actin	β-Actin Internal Control	F: CTACAATGAGCTGCGTGTGG R: AAGGAAGGCTGGAAGAGTGC	57	143

Data Analysis: Statistical analysis was carried out using SPSS version 25.0 and Microsoft Excel. Analysis of variance (ANOVA) at $p < 0.05$ was used to determine significant differences in the parameters across the various stations. Where significant differences were obtained, Duncan's multiple-range test was applied to identify the source of variation. Gene expression data were analysed with Graphpad prism version 9.5. Results obtained were expressed as mean ± standard deviation (SD).

*Metal Pollution Index (MPI):*The Metal pollution index (MPI) was used to compare the overall metal content in different stations. MPI was estimated using the formula described by El-Agri *et al.* (2022) and Ahmed *et al.* (2019) where MPI= $(Zn \times Fe \times Cu \times Mn \times Cr \times Cd \times Pb)^{1/n}$.

MPI was grouped into six categories as described by Caerio *et al*.(2005) where : Class 1 (very pure) < 0.3; class II (Pure)=0.3-1.0; class III (Slightly affected)=1.0–2.0; class IV(Moderately affected)= 2.0–4.0; class V (Strongly affected)=4.0–6.0 and class VI (Seriously affected)>6.0

RESULT AND DISCUSSION

*Heavy metals in the fish tissue:*Table 2 shows the mean concentration of heavy metals in the tissue and the standard deviation. The P-value, the limits of FAO/WHO and the metal pollution index are also indicated. The result of this study showed that *Heterotis niloticus* accumulated different heavy metals found in the different stations at varying concentrations. The mean concentration of Zinc ranged between 0.238mg/kg and 0.839mg/kg with the highest mean value recorded in the downstream section of the river. The mean value of zinc showed no significant difference (P>0.05) across the studied stations and were within the permissible limit of FAO/WHO. Fish obtained from the downstream section of the river had relatively higher concentration of zinc than that in the upstream section and the discharge station. Nwosu *et al*. (2014) reported the presence of zinc in *Heterotis niloticus* up to a concentration of 4.90mg/kg in whole fish obtained from Oguta lake in Imo state, Nigeria while Ibemenuga *et al*. (2019) reported bioaccumulation of zinc up to concentration of 5.419mg/kg in the muscle tissue of *tilapia zilli* obtained from river Niger in Anambra State. Iron ranged from 5.711mg/kg to 9.468mg/kg. The mean value of iron in the tissue showed significant difference (P<0.05) across the studied stations and were below FAO/WHO permissible limit in fish. The highest concentration of iron was recorded in fishes obtained from the downstream station. Bioaccumulation of iron up to concentration of 5.90mg/kg has been reported in *Heterotis niloticus* (*Osteoglossidae*) collected from Oguta lake in Imo state, Nigeria (Nwosu, 2014). Omar *et al*. (2014) asserted that high iron in water may affect the liver of fish and has been shown to cause respiratory disruption of the gills due to physical clogging. This can also cause suffocation and death of the fish. Copper ranged between 0.280mg/kg and 0.500mg/kg across stations. The mean value of copper in the tissue showed no significant difference (P>0.05) across the studied stations and were below the FAO/WHO permissible limit for Copper in fish. Copper is an essential micronutrient required for cellular metabolism. It is a major component of enzymes involved in metabolic processes. The mean concentration of Copper in the fish tissue was lower

than the limits set by FAO/WHO for fish food. Ndimele *et al.* (2017) recorded higher range (10.90–41.10) of copper in the tissue of *Oreochromis niloticus* obtained from a section of Lagos lagoon exposed to effluent from Agbara Industrial Estate. Ayeloja *et al*. (2014) reported a range of 0.042-0.236mg/l for copper in the tissues of selected fish species obtained from Eleyele reservoir. Exposure to high concentration of copper in fish can cause a number of toxic effects ranging from histological alterations, external lesion and discoloration in the gills, liver and kidney tissues.

Table 2: Heavy metal concentration in the fish tissue

	Station 1 Upstream $\bar{x}$**±SD**	**Station 2 Downstream** $\bar{x}$**±SD**	**Station 3 Effluent DP** $\bar{x}$**±SD**	**P-Value**	**FAO / HO 1989**
Zinc	0.238±0.961[a]	0.839±1.317[a]	0.320±0.395[a]	P>0.05	40
Iron	6.128±3.816[a]	9.468±2.118[b]	5.711±3.721[a]	P<0.05	100
Copper	0.280±0.558[a]	0.500±0.887[a]	0.392±0.441[a]	P>0.05	30
Manganese	3.741±2.458[a]	7.512±1.875[b]	3.049±2.095[a]	P<0.05	N/A
Chromium	0.265±0.518[a]	0.619±1.552[a]	0.512±0.692[a]	P>0.05	0.5
Cadmium	0.101±0.424[a]	0.462±0.661[a]	1.176±1.320[b]	P<0.05	0.5
Lead	0.199±0.407[a]	0.207±0.431[a]	0.512±0.700[b]	P<0.05	0.5
MPI	**0.502**	**1.084**	**0.945**		

MPI= Metal pollution Index

Manganese ranged between 3.049mg/kg and 7.512mg/kg. The mean value of Manganese in the tissue showed significant difference (P<0.05) across the studied stations. Manganese is a mineral that naturally occurs in the environment. It is one of the most abundant essential element that is needed by living organisms. It is a component of many enzymes and plays important role in physiological process in the body. Manganese content obtained in this study was within the range of values recorded by Mustapha *et al.* (2021) who reported 0.625mg/l-7.48 mg/kg in a study of different fish species obtained from Epe lagoon receiving effluents from industries in the Eastern part of Lagos State, Nigeria. Ayeloja *et al*. (2014) reported a range of 0.008-0.162mg/l for Manganese in the tissues of selected fish species obtained from Eleyele reservoir. Studies have shown that long term exposure to Manganese in low dose can cause parkinsonism and problems associated with memory and motor skills, while elevated level of it can result in a number of toxic effect in different organisms. Chromium ranged between 0.265mg/kg and 0.619mg/kg. The mean value of chromium in the tissue showed no significant difference (P<0.05) across the studied stations. Chromium is an essential nutrient that is needed by the body for carbohydrate metabolism. It usually enters into the environment through the discharge of effluent from industries and can rise to a level that is harmful to fish. Ambedkar *et al*. (2011) attributed the presence of chromium in fish obtained from in Kollidam river to the effluent and wastewater discharge into the water body. Mulk *et al*, (2017) also reported that the bioaccumulation of chromium in fish was caused by effluent discharge from marble industry into Barandu river in Pakistan. Toxic effects of chromium in fish include, histological, morphological and haematological alterations in the gill, kidney and liver. It can result in the production of reactive oxygen species (ROS) as well as the impairment of immune function.

Cadmium ranged between 0.101mg/kg and 1.175mg/kg. There was significant difference (P<0.05) across the studied stations. Cadmium concentration was higher in the discharge station compared to other stations. The mean values were below the limits of FAO/ WHO limits except in the discharge station. Cadmium is a non essential trace element that occurs naturally in the environment and has the tendency to bioaccumulate in the tissues of living organisms. It is not easily degraded and can alter aquatic trophic level for many years when it is released into the aquatic environment. Findings of this study is comparable to the values obtained by Bawuro *et al*. (2018) who recorded cadmium concentration range of 0.35-0.39mg/kg in *Heterotis niloticus* obtained from Geriyo Lake which received influx of polluted water in Adamawa State. Mustapha *et al.* (2021) reported cadmium range of 2.001mg/kg- 2.750mg/kg in fish species obtained from Lagos Lagoon receiving effluents from various industries in the area. High dose of cadmium have been reported to cause lesions, discoloration and necrosis in the livers of fish species.

Lead is a persistent and hazardous metal that occurs naturally, however its concentration can be increased by industrial and anthropogenic activities including metal mining, battery manufacturing, lead base paint, gasoline and wastewater. Lead ranged between 0.199mg/kg and 0.512mg/kg.There was significant difference (P<0.05) in lead content across the studied stations, however, the discharge station recorded relatively higher lead content compared to the other stations. The mean values were below the limits of

FAO/ WHO limits except in the discharge station which was slightly higher. The findings of this investigation indicated higher lead concentration in the discharge station. A number of studies have been carried out to determine the concentration of lead in fishes exposed to effluent discharge. Ndimele *et al.* (2017) reported lead concentration range of 0.12–1.81mg/kg in *Oreochromis niloticus* exposed to effluent from Agbara Industrial Estate. Bawuro *et al*. (2018) reported lead range of 3.51-7.12 mg/kg *in Heterotis niloticus* obtained from Geriyo Lake which received influx of polluted water in Adamawa State. Ayeloja *et al*. (2014) reported lead concentration range of 0.393-0.663mg/l in Tilapia, catfish and the Africa pike obtained from Eleyele reservoir in Ibadan, Oyo State. Exposure to lead can depletes antioxidants and enzymes in the cells causing oxidative stress and damage to lipids, protein, blood cells and DNA in fish (Erca *et al*., 2001). The Metal Pollution Index (MPI) for *Heterotis noloticus* in the different stations showed that the downstream station recorded MPI value that was above 1 (MPI>1). This falls into the "slightly affected category" as described by Caerio *et al*. (2005). The upstream and discharge station were below 1.00. This can be attributed to the heavy metal load in the downstream section of the river. The observation in the discharge station could be attributed to occasional migration of the fish species from the station. However, the MPI value was higher than the upstream section of the river. The presence of heavy metals in the tissue of fish is potentially dangerous to the fish and also poses threat to man when it becomes biomagnified. This is usually caused by its persistence, toxicity and bioaccumulation properties. The effect depends on the level of intake, storage and elimination from the body of the fish. Factor that can affect the amount of absorption and the concentration of heavy metal include the physical and chemical condition of the environment (water temperature, pH and hardness etc) as well as the physiology, exposure, sex, weight, age and feeding habit of the fish (Hashim *et al*., 2014).

Gene Expression: Table 3 shows the gene expression result of fishes obtained from the different stations.

Table 3: Results of gene expression

Gene	Organ	Station 1 Upstream $\bar{x}$±SD	Station 2 Downstream $\bar{x}$±SD	Station 3 Effluent DP $\bar{x}$±SD	P-Value
HSP70	**Liver**	8.55 ± 0.50^{a}	10.10 ± 0.14^{b}	11.30 ± 0.28^{c}	$p < 0.05$
CYPA1	**Liver**	10.30 ± 0.28^{a}	14.25 ± 0.35^{c}	15.69 ± 0.21^{d}	$p < 0.05$
HIF	**Gills**	11.65 ± 0.21^{a}	13.85 ± 0.49^{b}	13.40 ± 0.57^{b}	$p < 0.05$
IGF-1	**Liver**	13.40 ± 0.14^{b}	12.25 ± 0.21^{ab}	11.65 ± 0.21^{a}	$p < 0.05$
SOD	**Liver**	14.10 ± 0.14^{b}	13.45 ± 0.07^{ab}	12.70 ± 0.14^{a}	$p < 0.05$

Means with the different superscript are significantly different from one another (P<0.05)

Figure 2-6 show the snapshot representation of HSP70, CYPA1, HIF IGF-1 and SOD respectively. The results showed that fish samples obtained from the discharge station had the highest gene expression level of HSP70 followed by the downstream section. The level of expression were significantly different from one another (P<0.05) across the different stations. Heat Shock protein 70 is a gene that is associated with stress due to exposure to contaminants in the environment. It can serve as a source of energy in fish by aiding degradation of protein when the body is under stress, hence it is usually up-regulated by heat shock, thermal stress, heavy metal and oxidative stress (Umam *et al*., 2016). In this study, the level of expression of HSP70 was significantly higher in the discharge station compared to other stations under investigation. This station was exposed to effluent discharge and may likely have higher amount of heavy metal contamination. High HSP70 expression highlights the relevance and role of this gene as an adaptive mechanism to stress experienced by the fish in contaminated environment. This is in agreement with the finding of tenji *et al.* (2020) who reported an up- regulation of this gene in *Abramis brama* fish exposed to chemical stress.

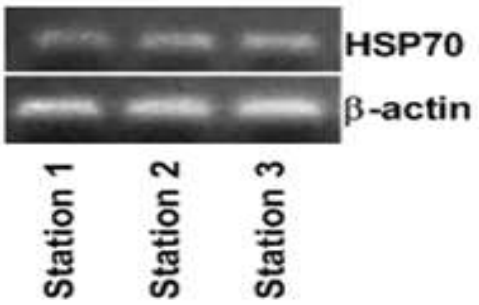

Fig 2: Expression of HSP70 gene

CYP1A belongs to a broad family of the CYPs. It is one of the most studied biomarker of environmental exposure to xenobiotics. It helps in the biotransformation of environmental contaminants and other endogenous substances like lipids, steriod and vitamins. In this study, CYPA1 was examined in the liver of the fish because it is highly expressed in this organ. The results showed significant variations

($p<0.05$) in the expression of this gene in fishes obtained from the different stations. The downstream and discharge stations recorded an up-regulation of this gene compared to the upstream station. However, the discharge station recorded the highest expression. It can be said that CYPA1 played an important role in adaptation and metabolism of xenobiotic chemicals in the fish species. Williams *et al.* (2022) reported the use of CYPA1 expression in fish as an indicator of pollution level in fish.

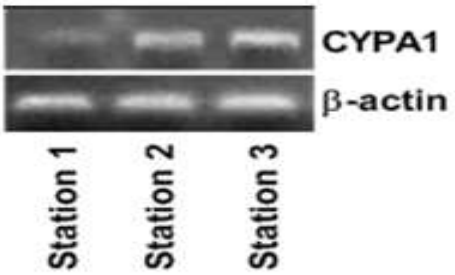

Fig 3: Expression of CYPA1 gene

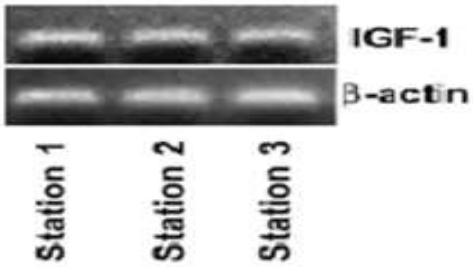

Fig 4. Expression of HIF gene

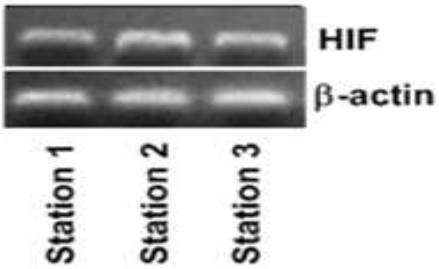

Fig 5: Expression of IGF-1 gene

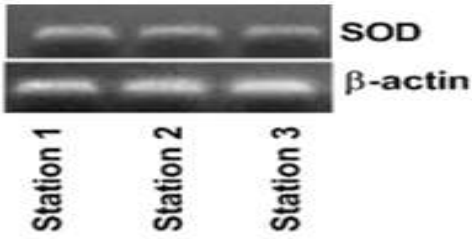

Fig 6: Expression of SOD gene

Findings of this study corroborates the works of Abd El Megid*et al.* (2020) who showed an up-regulation of CYP1A expression level due to chemical exposure, this change may alter the detoxification and metabolism activities of these compounds. However, this is not in agreement with observations made by Tenji *et al.* (2020) who recorded a down- regulation of this gene in the exposed fish population.

The results for HIF showed no significant difference in fishes obtained from the discharge station and that of the downstream station ($P>0.05$). However, both stations were significantly higher ($P<0.05$) than the upstream station. Fishes obtained from the downstream station recorded the highest mean gene expression level. The role of oxygen in regulating gene expression has received increasing attention, especially after the discovery of the hypoxia-inducible factor (HIF). Hypoxia can be considered as one of the stressors of aquatic organisms, it refers to a reduction of dissolved oxygen level in the aquatic environment usually below 2.0 mg/l. This can be caused by human activities such water pollution (Rogers *et al*., 2016). HIF gene expression was investigated in the gill of the fish, considering the fact that the gill is the organ of gaseous exchange and is capable of remodeling in response to changing level in dissolved oxygen (DO) by increasing the surface area.

The results of IGF-1 (Figure 5) showed that the gene expression of fish obtained from the discharge and the upstream stations were significantly different ($P<0.05$) from one another. The upstream station had an up-regulation of the gene with a significantly ($P<0.05$) higher level followed by downstream station. However, the downstream station was not significantly different from both the discharge and the upstream station. IGF-1 is a polypeptide growth factor that is structural similar to pro-insulin. It is mainly involved in growth regulation and development of many organs in the body, including the differentiation of the bone and skeletal muscles**.** Regulation of this gene transcription is therefore important for growth and energy utilization. The findings of this study indicating a down-regulation of this gene in the discharge station compared to the downstream station can be attributed to excessive and long-term exposure to contaminants in this section of the river. The suppression of this gene in the discharge station corroborates the findings of Abd El Megid *et al.* (2020) who made similar observations in fish exposed to pesticides. The up-regulation of this gene indicates the adaptive tolerance of the fish to the conditions of the environment.

The results of superoxide dismutase (SOD) showed that fish samples from the upstream section of the river recorded the highest gene expression level. The gene expression of fish obtained from the discharge station

and that of the downstream station were however not significantly different (P>0.05) from one another but both stations were significantly different (P<0.05) from the upstream station. The expression of SOD gene in the fish provides information on their response to oxidative stress. In this study, SOD level was repressed in the discharge station. This could be caused by long term exposure and the inhibitory effect of heavy metals on the expression of SOD. This is in opposition to the findings of Tenji *et al.* (2020) who reported an up-regulation of SOD gene in response to chemical pollution using *Abramis brama* fish species as case study. Gene expression provides useful information on the biological condition of fish that is exposed to contaminants. Heavy metals can alter the gene expression by interfering with the signal transduction pathway (Hamilton *et al*., 2017). This can cause oxidative stress as well as mutagenic and cacinogenic effect on living organisms, hence the need to regulate the uptake, storage and secretion of these metal by homeostasis.

*Conclusion:*This study has shown that *Heterotis niloticus* obtained from Igbalegbe river accumulated heavy metals in their muscle tissues with significant variations in the expression of the investigated genes. Effort should therefore be made by relevant authorities to improve on the quality of the environment in order to safeguard the health of aquatic organisms and human consumers

Acknowlegdement: We sincerely appreciate Dr. Olusola Elekofehinti of Federal University of Technology, Akure for the technical support and contributions during the studies. Special thanks to Dr. Ese Arigbe for financially supporting this research.

REFERENCES

Abd El Megid, A; Abd Al Fatah, ME; El Asely, A; El Senosi, Y; Moustafa, MM; Dawood, MA (2020). Impact of pyrethroids and organochlorine pesticides residue on IGF-1 and CYP1A genes expression and muscle protein patterns of cultured *Mugil capito*. *Ecotoxicol.Environ.Safety*, *188*, 109876.DOI: https://doi.org/10.1016/j.ecoenv.2019.109876

Ahmed, AS; Sultana, S; Habib, A; Ullah, H; Musa, N; Hossain, MB; Sarker, MSI (2019). Bioaccumulation of heavy metals in some commercially important fishes from a tropical river estuary suggests higher potential health risk in children than adults. *Plos One*, *14*(10), e0219336.DOI: https://doi.org/10.1371/journal.pone.0219336

Ambedkar, G;Muniyan, M (2011). Bioaccumulation of some heavy metals in the selected five freshwater fish from Kollidam River, Tamilnadu, India. *Adv. Appl. Sci. Res.*, *2*(5), 221-225.

Amoatey, P; Baawain, MS (2019). Effects of pollution on freshwater aquatic organisms. *Water Environ. Res.*, *91*(10), 1272-1287.DOI:https://doi.org/10.1002/wer.1221

Authman, MM; Zaki, MS; Khallaf, EA; Abbas, HH (2015). Use of fish as bio-indicator of the effects of heavy metals pollution. *J. Aquac. Research Dev.* *6*(4), 1-13.DOI: http://dx.doi.org/10.4172/2155-9546.1000328

Ayeloja, AA; George, FOA; Shorinmade, AY; Jimoh, WA; Afolabi,QO; Olawepo, KD(2014). Heavy metal concentration in selected fish species from Eleyele reservoir Ibadan Oyo State South-western Nigeria. *Afri. J. Environ. Sci. Tech*, *8*(7), 422-427. DOI:https://doi.org/10.5897/ajest2014.1700

Bawuro, AA; Voegborlo, RB; Adimado, AA (2018). Bioaccumulation of heavy metals in some tissues of fish in Lake Geriyo, Adamawa State, Nigeria. *J. Environ. and Public Health*, *2018*.

Caerio, S; Costa, MH; Ramos, TB; Fernandes, F; Silveira, N; Coimbra, A; Painho, M(2005). Assessing heavy metal contamination in Sado Estuary sediment: an index analysis approach. *EcolInd*,5:155–169

El-Agri, AM; Emam, MA; Gaber,HS; Hassan,EA; Hamdy, SM (2022). Integrated use of biomarkers to assess the impact of heavy metal pollution on Solea aegyptiaca fish in Lake Qarun. *Environ. Sci. Eur.* *34*(1),74. DOI:https://doi.org/10.1186/s12302-022-00656-6

Ercal, N; Gurer-Orhan, H; Aykin-Burns, N (2001). Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Current Topics Med.Chem.*, 1(6), 529-539.

FAO/WHO (1989). Evaluation of certain food additives and the contaminants mercury, lead and cadmium. WHO Technical Report, Series No. 505.

Hamilton, PB; Rolshausen, G; Uren Webster, TM; Tyler, CR (2017). Adaptive capabilities and fitness consequences associated with pollution exposure in fish. *Philos. Transactions of the Royal Soc. B:*

Bio. Sci., *372*(1712), 20160042. DOI:https://doi.org/10.1098/rstb.2016.0042

Hashim, R; Song, TH; Muslim, NZM; Yen, TP(2014). Determination of heavy metal levels in fishes from the lower reach of the Kelantan River, Kelantan, Malaysia. *Trop.Life Sci.Res.*, *25*(2), 21.

Holden, M ;Reed, W(1991). *West African Freshwater Fish*. Longman Publishers Ltd., Singapore. p. 68

Ibemenuga, KN; Ezike, FA; Nwosu, MC; Anyaegbunam, LC; Okoye, EI; Eyo, JE (2019). Bioaccumulation of some heavy metals in some organs of three selected fish of commercial importance from Niger river, Onitsha shelf, Anambra state, Nigeria. *J. Fisheries Sci.*. *13*, 1-12.

Mulk, S; Korai, AL; Azizullah, A; Shahi, L; Khattak, MNK (2017). Marble industry effluents cause an increased bioaccumulation of heavy metals in Mahaseer (Tor putitora) in Barandu River, district Buner, Pakistan. *Environ. Sci. Pollut. Res.*, *24*(29), 23039-23056.

Mustapha, AM;Ugya, AY; Mustapha, Z(2021). Assessment of heavy metal levels in fish tissues, water and sediment from Epe lagoon, Lagos, Nigeria. *Sci. World J.*, *16*(4), 464-469.

Mustapha, MK (2010). Heterotis niloticus (Cuvier, 1829) a threatened fish species in Oyun reservoir, Offa, Nigeria; the need for its conservation. *Asian J. Exp. Bio. Sci.*, *1*(1-7), 511-514.

Ndimele, PE; Pedro, MO; Agboola, JI; Chukwuka, KS; Ekwu, AO (2017). Heavy metal accumulation in organs of Oreochromis niloticus (Linnaeus, 1758) from industrial effluent-polluted aquatic ecosystem in Lagos, Nigeria. *Environ.Monit. Assess.*,*189*, 1-15. DOI: https://doi.org/10.1007/s10661-017-5944-0

Nwosu, M; Ibemenuga, K; Arazu, V; Nwosu, C; Ekesiobi, A; Igbodika, M; Okeke, J (2014). Bioaccumulation of some heavy metals in two fish species, *heterotis niloticus* (*Osteoglossidae*) and tilapia zilli (*Cichlidae*) collected from oguta lake, Imo state, Nigeria.*Reiko Int.J. Sci. Technol.Res.*, 5 (2), 68-77

Omar, WA; Saleh, YS; Marie, MAS (2014). Integrating multiple fish biomarkers and risk assessment as indicators of metal pollution along the Red Sea coast of Hodeida, Yemen Republic. *Ecotoxicol. Environ. Safety*, *110*, 221-231.DOI:https://doi.org/10.1016/j.ecoenv.2014.09.004

Rogers, NJ; Urbina, MA; Reardon, EE; McKenzie, DJ; Wilson, RW (2016). A new analysis of hypoxia tolerance in fishes using a database of critical oxygen level (Pcrit) . *Conserv.Physiol.*, *4*(1).

Tenji, D; Micic, B; Sipos, S; Miljanovic, B; Teodorovic, I; Kaisarevic, S(2020). Fish biomarkers from a different perspective: evidence of adaptive strategy of *Abramis brama* (L.) to chemical stress. *Environ. Sci. Eur.*, *32*, 1-15. DOI:https://doi.org/10.1186/s12302-020-00316-7

Thomas, EG; Šrut, M; Štambuk, A; Klobučar, GI; Seitz, A; Griebeler, EM(2014). Effects of freshwater pollution on the genetics of zebra mussels (*Dreissena polymorpha*) at the molecular and population level. *BioMed. Res. Int.*, *2014*. DOI:https://doi.org/10.1155/2014/795481

Umam, K; Tang, CH; Lee, TH (2016). The roles of heat shock protein 70 (HSP70) and heat shock protein 90 (HSP90) in osmoregulatory and metabolic tissues of milkfish (*Chanos chanos*) under osmotic stress. *J. Aquatropica Asia*, *2*(2).

Veliz, D; Rojas-Hernández, N; Copaja, SV; Vega-Retter, C (2020). Temporal changes in gene expression and genotype frequency of the ornithine decarboxylase gene in native silverside Basilichthys microlepidotus: Impact of wastewater reduction due to implementation of public policies. *Evol. Appl.*, *13*(6), 1183-1194. DOI:https://doi.org/10.1111/eva.13000

Williams, R.; Taggart, T; Ganger, K; Koetsier, T; Johnson, S; Dinchman, A(2022). CYP1A expression in freshwater fish of western New York as an indicator of pollution levels. *Ecol. Evol.*, *12*(1),e8526. DOI:https://doi.org/10.22541/au.163250483.31206826/v1