Original Research Article

OXIDATIVE STRESS, MOLECULAR AND GENOTOXIC EFFECTS OF ECOLOGICALLY RELEVANT CONCENTRATIONS OF NICKEL IN *CLARIAS GARIEPINUS*

ABSTRACT

This study was aimed at determining the biochemical, genotoxic, and molecular effects of ecologically relevant concentrations of Nickel (Ni) in Ologe Lagoon; which constitutes its originality. An initial field study was conducted to determine the concentrations of some heavy metals (Arsenic, nickel and lead) in surface water, sediments, and fish from Ologe Lagoon. Ten fish per test concentration were used for the bioassay. Oxidative stress indicators (superoxide dismutase, catalase, reduced glutathione, and malondialdehyde), nuclear abnormalities and heat shock proteins were assessed in fish chronically exposed to ecologically relevant concentrations of Ni. Ni inhibitedthe activities of GSH, however, it did not have any significant effects on the activities of catalase, superoxide dismutase and malondialdehyde. Ni caused a significant elevation in the number of micronuclei and induced heavy HSPs, as well as other HSPs such as HSP 40, chaperonins and HSP 70, in gills and livers of the test species. Results from this study suggest that Ni can induce deleterious effects in aquatic organisms inhabiting Ologe Lagoon.

Keywords: *Biomarker; Ecological relevance; Genotoxicity; Heavy metal pollution; Molecular biology; Nickel; Oxidative stress.*

1. Introduction

The discharge of partiallytreated and untreated industrial effluents is considered as a major source of water pollution in Nigeria. Industrial growth is increasing the levels of toxicants that may have catastrophic impacts on Nigerian water bodies. These toxicants include organic compounds and heavy metals. Heavy metals refer to elements that have relatively high densities, especially above 5g/cm [1]. Theyoccur naturally as trace elements, and arepresent in abiotic and biotic components of the ecosystem [2]. However, anthropogenic activities have increased the

concentrations of these metals in the environment which have resulted in heavy metal pollution [3]. According to Don-Pedro et al. [3], the sources of heavy metals in aquatic ecosystems are the direct discharge of domestic and industrial effluents, and runoff from urban and agricultural lands. In aquatic ecosystems, heavy metals are highly persistent and can be amplified along the food chain [4]. Heavy metals concentrations in most Nigerian rivers were found to be above acceptable and permissible levels [5,6]. The impacts of heavy metals on human healthdate back to 1956 when the cases of Minamata disease in humans caused by methyl-mercury were reported in Japan [7]. Others include Itai-Itai disease caused by cadmium poisoning [8]. High levels of manganese in drinking water induced intellectual dysfunctions in children in Araihazar, Bangladesh [9]. The effects of arsenic in aquatic organisms range from cytotoxicity in fish cell lines [10,11] to oxidative stress [12-14]. Lead, yet another toxicologically important heavy metal has been a culprit in several biological effects that include haematological [15], neurological [16], and physiological effects [17]. Carcinogenicity [18], immune-suppression [19,20], and respiratory disorder [21] have been observed in aquatic organisms exposed to Nickel.

The objectives of this study were to determine the current levels of some heavy metals in surface water, sediments and fish from Ologe Lagoon, and to conduct an ecotoxicological assessment of the predominant heavy metal in the water body.

2. Materials and Methods

2.1 Study Site

Ologe Lagoon is a part of the Lagos Lagoon system that consists of Lagos Lagoon, Lekki Lagoon, and Badagry Creek [22].

Ologe Lagoon's edges are shallow while the centre is deep [23,24]. It has an average depth of 2.42 m with a wide navigable mouth that allows for recreation, fishing, and transportation [23,24].

2.2 Field Studies and Heavy Metal Analyses

Samples were collected from three sampling sites, lying between latitudes 6°29'41"Nand 6°29'50"N, and longitudes 3°5'60"E and 3°6'5"E,in reference to the direction where effluents are received from Agbara Industrial Estate (Figure 1).Temperature, pH, conductivity, turbidity, salinity, dissolved oxygen, and total dissolved solids (TDS) of the lagoon surface water were determined *in-situ* with Horiba U50 Gmulti water quality meter**.** Water samples were collected using a 1 L plastic container; sediments were collected with a Venn-Grab sampler and placed in foil wraps [25].The fish sampleswere collected with the aid of local fishermen, and preserved with ice packs before digestion. The digestion of samples was done according to the procedure described by Zheljazkov and Nielson [26]. Heavy metal analysis was done with a Perkin Elmer atomic absorption spectrometer.

2.3 Collection and Acclimatization of Experimental Fish

A total of 100 post-juvenile *Clarias gariepinus* (weight 18–20 g, and length 10 – 15 cm), were purchased from a fish farm in Ikorodu, Lagos State. They were transported in a 50 L capacity rectangular tank containing aerated water to the laboratory, and kept in holding tanks (40 cm×30 cm×30 cm). During acclimatization, these fish were fed with "catfish grower", twice daily (morning and evening). The acclimatization was for a week, and water was changed every 3 days to prevent accumulation of toxic waste metabolites. Laboratory conditions were kept at 27–28

°C, 65–75 % humidity, and 10-h/14-h light/dark cycle for 2 weeks before bioassay in accordance with APHA/AWWA/WPCF [27].

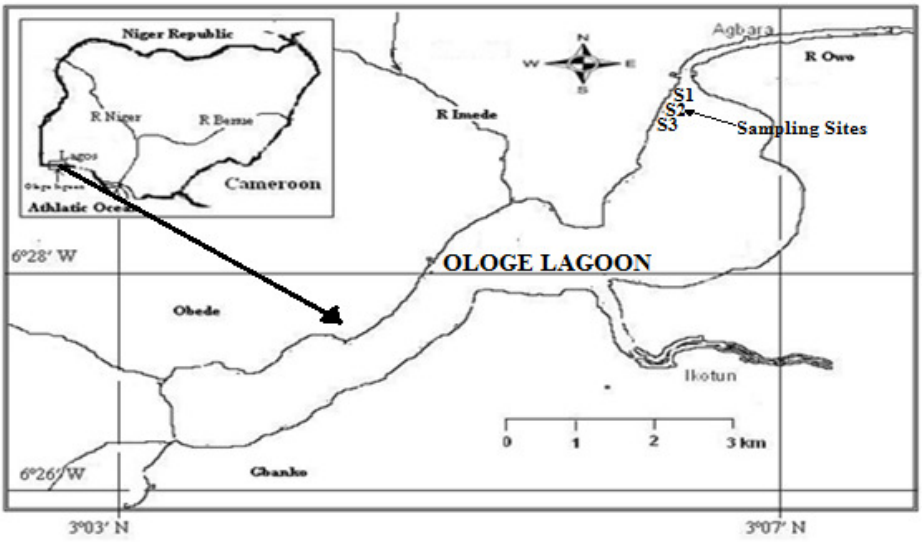


Figure 1: Ologe Lagoon and Sampling Sites Localization

Adapted from [28]

2.4 Bioassay Procedure

A total of ten (10) acclimatized fish eachwere randomly caught using a plastic sieve from the stock in the holding tank, and carefully transferred to the different concentrations of the chemical as well as in control in each bioassay tanks. The respective concentrations of the test chemical were duplicated making 5 fish per test concentration. The fish were not fed for 24 hours before exposure. The test containers were labelled with each concentration and filled with 6 L of water each. The test solution was prepared using the method described byNuneset al. [29]. In this study, NiW meanstest concentration derived from Ni surface water concentration in Ologe

Lagoon while NiT is thetest concentration derived from Ni concentration in fish from Ologe Lagoon.

2.5 Measurement of Anti-oxidative Stress Enzymes and Lipid Peroxidation

The reduced glutathione (GSH) of liver tissue as non-protein sulphydryls was determined according to the procedure described by Sedlak and Lindsay [30]. Superoxide dismutase (SOD) activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Sun and Zigma [31]. Catalase (CAT) activity was determined according to the methodology of Sinha [32]. Thiobarbituric acid reactions (TBARS) assay was used to determine the levels of the lipid peroxidation product, malondialdehyde (MDA) [33].

2.6 Determination of Heat Shock Protein Induction

The methodology used in this study is described in detail by Amaeze et al. [34]. Briefly, the liver and gill samples of dissected fish were washed with phosphate buffered saline and dried on filter paper, and soluble proteins were extracted by mixing selected organs with an equal volume of SDS loading buffer (2×). The mixture was boiled for 7 mins and immediately cooled, then the protein content was assayed using the Biuret method, and protein profile was analysed by SDS-polyacrylamide (10 %) gel electrophoresis under denaturing conditions, using the discontinuous buffer system of Laemmli. Equal amounts of protein were loaded per lane on each gel. The Sm0441 Fermentas (Thermo Scientific) was used as the protein ladder.

2.7 Nuclear Abnormalities

The staining procedure was performed on blood smears obtained from fish samples. The smear of the peripheral blood collected using a heparinized syringe from the caudal vein of the fish sample was made on clean glass slides. Glass slides prepared per group were processed in accordance with Singh et al. [35]. The smeared slides were allowed to air-dry at room temperature, fixed in methanol for 15 minutes and then stained with May-Grunwald stain and allowed to air dry for 6 hours, then rinsed out with distilled water before staining with 5% Giemsa stain; then left to dry for 12 hours [36]. The slides were analyzed at 1000x for micronuclei and nuclear abnormalities. Micronucleus was smaller than one-third of the main nucleus and did not touch the main nucleus. Cells having two nuclei with approximately equal sizes were scored as binucleated, while cells with round appearances and basophilic cytoplasm were scored as immature erythrocytes [37]. At least 500 erythrocytes per fish were examined to determine the frequencies of nuclear abnormalities.

2.8 Data Analysis

The mean and standard error (Mean±S.E.), and comparison of means were analysed using the Statistical Package for the Social Sciences (SPSS) Version 20. One-way ANOVA was used to test for significant difference between means, and differences in means were considered significant when $P<0.05$ and separated using Duncan's Multiple Range (DMR) test.

3. Results and Discussion

3.1 Physical and Chemical Parameters of Ologe Lagoon

The physical and chemical parameters evaluated in the three stations are presented in Table 1. The mean values of temperature and pH were within NESREA recommended limits[38]. TDS and turbidity values of 0.24±0.02 g/L and 66.63±17.04 NTU respectively were above NESREA

safe limits[38] (Table 1). The high amount of TDS might have been as a result of the discharge of effluents into the lagoon by nearby industries. Dan'azumi and Bichi [39] assessed the implications of industrial pollution on a source of water supply and documented that effluents discharged into water bodies by industries consequently increased the quantity of solid dissolved in the water.

The mean concentration of Arsenic was 0.0007±0.0003 mg/kg in sediments while Lead levels were 11.89±1.61mg/kg and 1.34±1.02 mg/L in sediments and surface water respectively (Table 2). The mean concentrations of Nickel in surface water and sediments were 6.56±2.35 mg/kg and 1.85±0.22 mg/L respectively (Table 2). Arsenic and Lead were not detected in the homogenate sample of fish, but Nickel was found to be 3.72 mg/kg in the homogenate sample (Table 2). The Bioaccumulation Factor (BAF) and Biota to Sediment Accumulation Factor (BSAF) for nickel were 2.01 and 0.57 respectively (Table 2).

Table 1: Physicochemical Characteristics of Ologe Lagoon Surface Water

Parameter	**Sampling Stations**				**NESREA Limit**[38]
	Station 1	**Station 2**	**Station 3**	**Mean±SE**	
Temperature (^{o}C)	32.27	32.41	39.49	34.72±2.38	< 40
pH	6.65	6.60	7.03	6.76±0.14	6 - 9
Conductivity (ms/cm)	0.344	0.427	0.393	0.39±0.03	NA
Turbidity (NTU)	94.5	69.7	35.7	66.63±17.04	10
Salinity (ppt)	0.20	0.20	0.20	0.20±0	NA
Dissolved oxygen (mg/L)	6.50	5.12	5.02	5.55±0.48	5.0
TDS (g/L)	0.23	0.28	0.20	0.24±0.02	0.2

NESREA -National Environmental Standards and Regulatory Enforcement Agency

NA -Not Available

Table 2: Levels of Arsenic (As), Lead (Pb), and Nickel (Ni) in Sediments, Surface water and Fish Tissues from Ologe Lagoon

Media	Sampling Stations	Arsenic (As)	Lead (Pb)	Nickel (Ni)
Sediment	**Station 1**	0.001 mg/kg	9.45 mg/kg	2.24 mg/kg
	Station 2	0.001 mg/kg	14.94 mg/kg	7.11 mg/kg
	Station 3	ND	11.29 mg/kg	10.34 mg/kg
	Mean±SE	0.0007±0.0003 mg/kg	11.89±1.61 mg/kg	6.56±2.35 mg/kg
Surface water	**Station 1**	ND	0.66mg/L	2.05 mg/L
	Station 2	ND	ND	1.41 mg/L
	Station 3	ND	3.36 mg/L	2.08 mg/L
	Mean±SE	ND	1.34±1.02 mg/L	1.85±0.22 mg/L
Fish Tissues (Homogenate of 5 fish samples)		ND	ND	3.72mg/kg
Bioaccumulation Factor (BAF)		0.00	0.00	2.01
Biota to sediment accumulation factor (BSAF)		0.00	0.00	0.57

ND: Not detected using AAS. ND means values < 0.001

3.2 Oxidative Stress

After 15d of exposure, the reduced glutathione (GSH) activities in fish exposed to NiW were inhibited ($p < 0.05$). The mean values ranged from 19.86±4.79 to 43.07±2.72 and 18.10±3.11 to 27.54±5.14 µmol/ml/min/mg pro on the 15^{th} and 30^{th} day respectively (Figure 2).This result is in agreement with the findings of Murawska-Ciałowicz et al. [40] who observed that GSH activities in the testes of mice were inhibited after exposure to Ni (II).GSH is the primary line of defence against reactive oxygen species (ROS) [41]. Oxidative stress occurs when the number of ROS

increases significantly [42]. Henning et al. [43] attributed GSH reduction to an increased need for GSH as a reducing agent in cellular processes.

The results showed that there was no significant difference ($p > 0.05$) between the superoxide dismutase (SOD) levels of control and the exposed groups after 15 and 30 days of exposure. The mean values ranged from 4.28±0.20 to 4.61±0.50 µmol/ml/min/mg pro and 4.35±0.11 to 5.01±0.62 µmol/ml/min/mg pro on the 15th and 30th day respectively (Figure 3). The activities of catalase (CAT) in fish from the treated groups were not significantly different ($p > 0.05$) from those of the control after 15 and 30 days of exposure. The mean values ranged from 17.85±2.39 to 25.13±3.53µmol/ml/min/mg pro and 17.37±4.59 to 27.00±4.76 µmol/ml/min/mg pro on the 15th and 30th day respectively (Figure 4). Keramati and Ramin [44] observed that the activities of catalase in the liver of *Rutilus rutilus* exposed to diazinon were not altered.Ahmad et al. [45] associated the inactivity of CAT to the high activity of Glutathione peroxidase (GPX), which acts as a defence against the production of H_2O_2.

There was no significant difference ($p > 0.05$) between the level of lipid peroxidation product, malondialdehyde (MDA) in the control and treated fish after 15 and 30 days of exposure. The mean values ranged from 1.80±0.19 to 2.05±0.15 µmol/ml/min/mg pro and 1.36±0.08 to 1.42±0.29 µmol/ml/min/mg pro on the 15th and 30th day respectively (Figure 5). Palermo et al. [46] observed an increase in the level of MDA in the liver of *Prochilodus lineatus* exposed to 2500 µg/L of Ni, however, the level of MDA in the gills of *P. lineatus* remained unchanged. Liu et al. [47] reported that MDA level remained unchanged despite a marked increase of ROS generation observed in *Oreochromis niloticus* exposed to 15 mg L^{-1} of PFOS. Liu et al. [47] went further to state that the reasons for the insignificant change in MDA level in the fish were unknown.

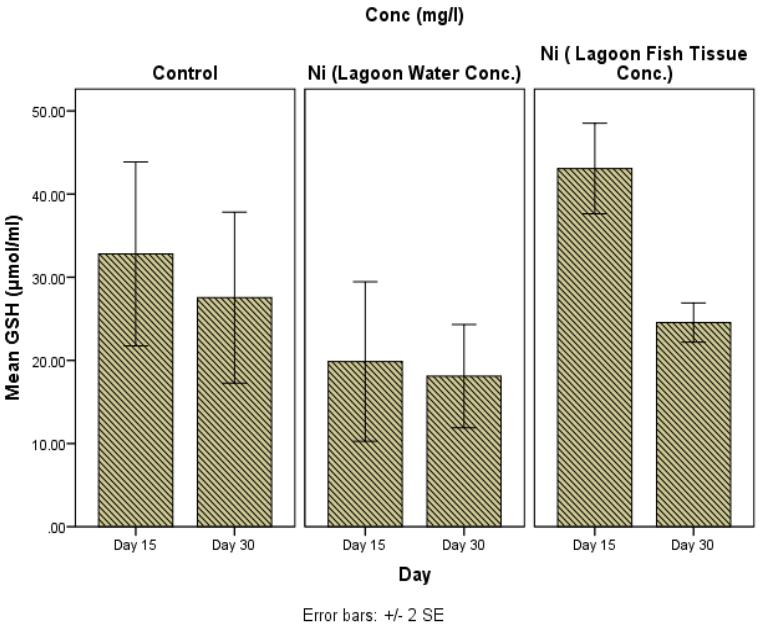


Figure 2: GSH levels in fish exposed to NiW and NiT for 15 and 30 days

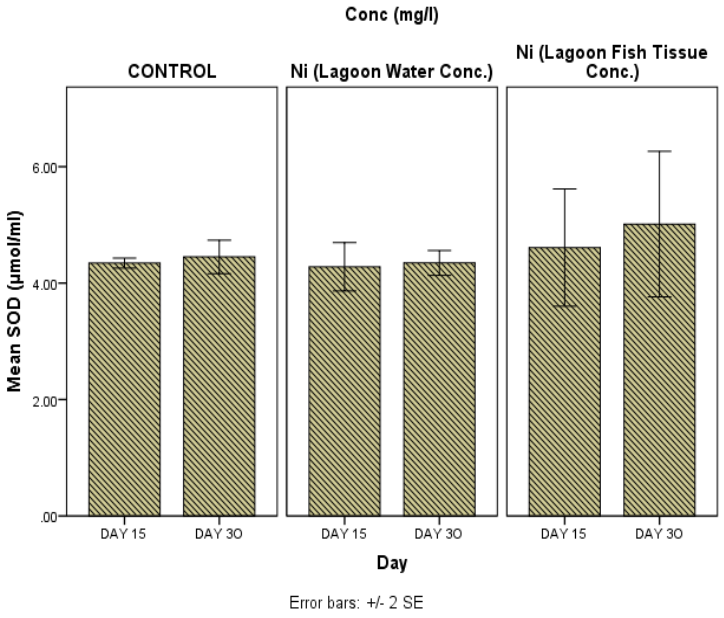


Figure 3: SOD levels in fish exposed to NiW and NiT for 15 and 30 days

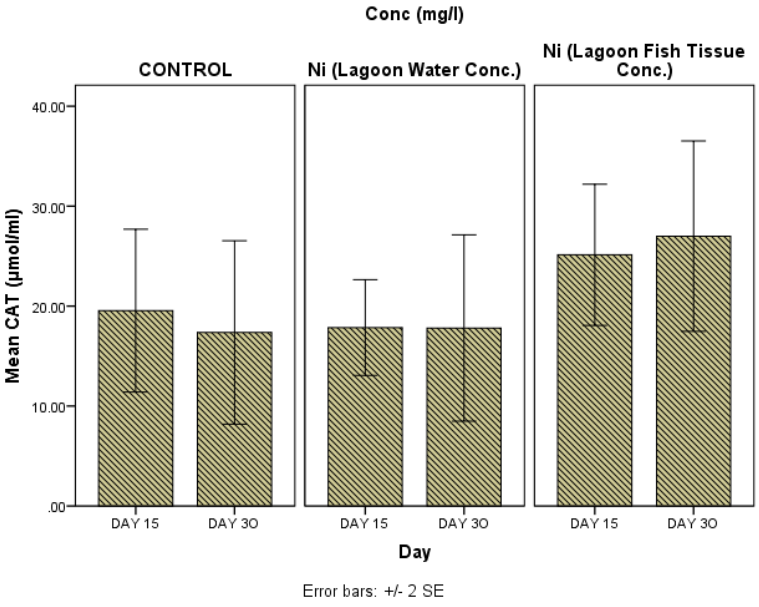


Figure 4: CAT levels in fish exposed to NiW and NiT for 15 and 30 days

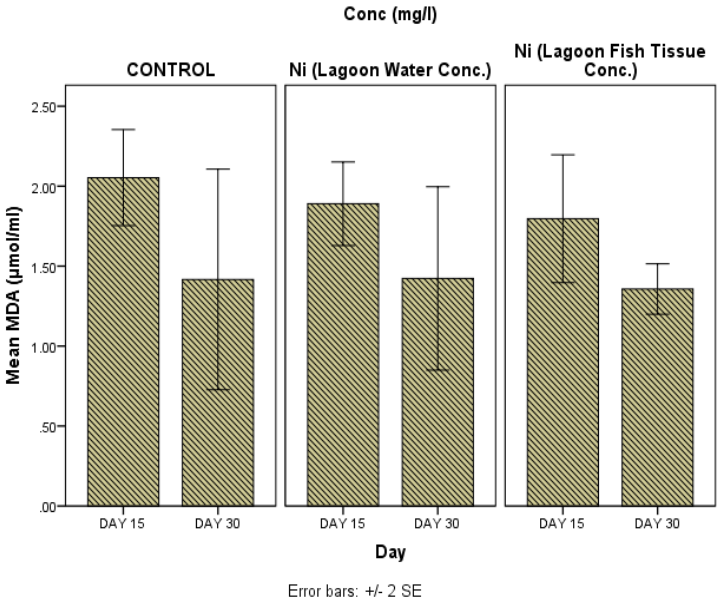


Figure 5: MDA levels in fish exposed to NiW and NiT for 15 and 30 days

3.3 Induction of Heat Shock Proteins

After 15 days of exposure, small heat shock proteins (sHSPs), HSP 40, chaperonins, HSP 70 and heavy HSPs were induced in the control organs (liver and gill samples) and organs (liver and gill samples) of fish exposed to NiW and NiT (Plate 1). However, at day 30 post-exposure, the expressions of HSPs were different in all the groups. Heavy HSPs, as well as other HSPs such as HSP 40, chaperonins and HSP 70, were observed in the gills and livers of the fish exposed to NiW and NiT (Plate 2). Bauman et al. [48] reported that heat-shock proteins contributed to the survival of cells following a variety of stresses. Bauman et al. [48] investigated the induction of heat-shock proteins in response to metals and found that arsenic, cadmium, and zinc increased the heat-shock proteins in the hepatocytes of rats. Nickel also increased the levels of heat-shock proteins in the hepatocytes but not as high as that of the aforementioned metals [48]. Heavy HSPs are located in the cytoplasm [49]. Heavy HSPs work with HSP70 to counter stress for cell survival [50]. Sharaf-ElDeen[51] demonstrated that HSP 70 increased in the hepatocytes of *Oreochromis niloticus* exposed to different concentrations of copper.

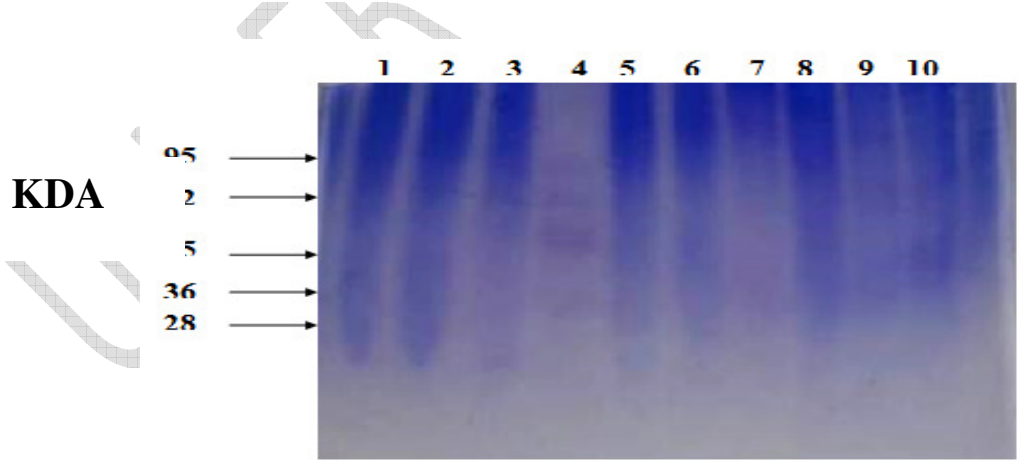


Plate 1: Heat shock proteins expression in the liver and gill of African catfish (*Clarias gariepinus*) exposed to NiW and NiT on 15d

Lane 1 (Control Liver); Lane 2 (NiW Liver 1A); Lane 3 (NiW Liver 1B); Lane 4 (NiT Liver 2A (Ladder); Lane 5 (NiT Liver 2B); Lane 6 (Control Gill) Lane 7 (NiW Gill 1A); Lane 8 (NiW Gill 1B); Lane 9 (NiT Gill 2A); Lane 10 (NiT Gill 2B)

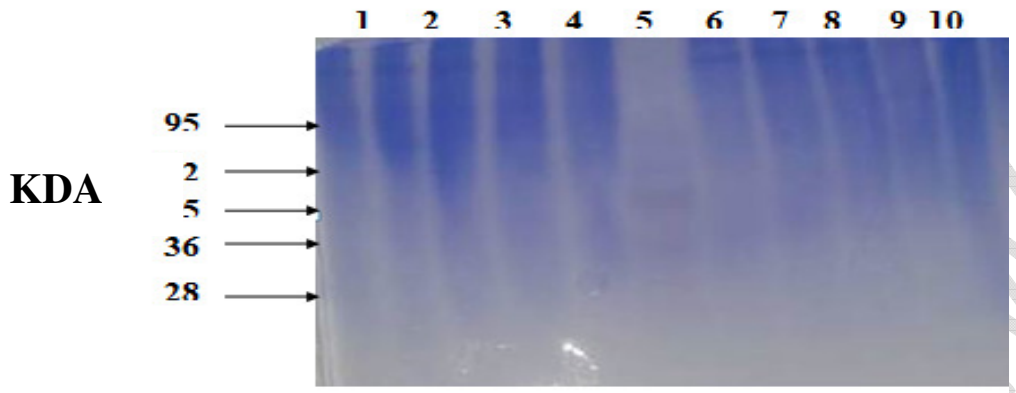
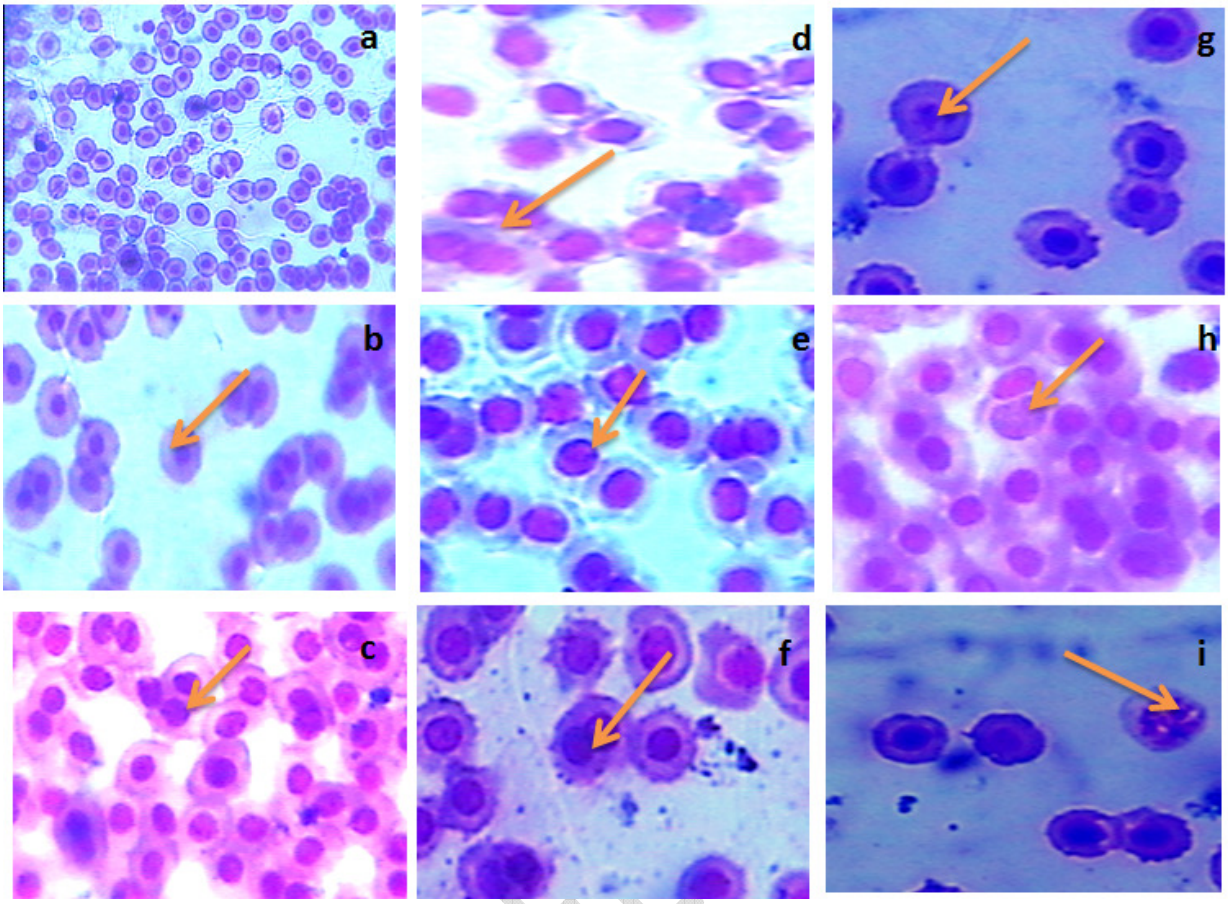


Plate 2: Heat shock proteins expression in the liver and gill of African catfish (*Clarias gariepinus*) exposed toNiW and NiT on 30d

Lane 1 (Control Liver); Lane 2 (NiW Liver 1A); Lane 3 (NiW Liver 1B); Lane 4 (NiT Liver 2A); Lane 5 (NiT Liver 2B (Ladder); Lane 6 (Control Gill); Lane 7 (NiW Gill 1A); Lane 8 (NiW Gill 1B); Lane 9 (NiT Gill 2A); Lane 10 (NiT Gill 2B)

3.4 Nuclear Abnormalities

Plates 3(a-i) illustrate the nuclear abnormalities in the blood of fish exposed to environmentally relevant concentrations of Nickel.The results showed that after 15 days of exposure, the frequency of micronuclei in the fish exposed to NiT was significantly ($P<0.05$) higher than that of the control while other nuclear abnormalities were not significantly ($P>0.05$) induced (Table 3). Çavaş and Ergene-Gözükara [52] stated that nuclear abnormalities were indicators of genotoxic damage. Previous studies have shown that heavy metals induce genotoxic effects in fishes by increasing the number of micronuclei in their blood [52,53]. Formation of micronuclei in animals could be an effectof chromosomal breakage or dysfunction of the spindle mechanism by genotoxic agents such as heavy metals [54].



Plates 3a-i: Photomicrograph of blood samples of *C. gariepinus* showing normal cells (control group) and nuclear abnormalities (exposed groups). **a-** Normal cells **b-** micronucleated cell (arrow) **c-** binucleatedcell (arrow) **d-** nuclear bud (arrow) **e-** notched nucleus (arrow)**f-** 8-Shaped nucleus(arrow) **g-** blebbed nucleus (arrow) **h-** polychromatic erythrocyte (PCE) (arrow) **i-**lobed nucleus (arrow)

Table 3: Frequencies of Nuclear Abnormalities in the Blood of the Fish (*Clarias gariepinus*)

Nuclear Abnormalities	Duration(days)	Control	NiW	NiT
Micronuclei (%)	**15**	0.00±0	0.03±0.03	1.2±0.51*
	30	0.13±0.09	0.57±0.33	0.13±0.13
Binuclei (%)	**15**	0.10±0.56	0.00±0	0.47±0.27
	30	0.07±0.03	1.43±1.23	0.50±0.32
Buds (%)	**15**	0.10±0.10	0.00±0	0.00±0
	30	0.00±0	0.03±0.03	0.67±0.03
Notched nuclei (%)	**15**	0.00±0	0.03±0.03	0.20±0.15
	30	0.00±0	0.07±0.03	0.13±0.09
8-Shaped Nuclei (%)	**15**	0.80±0.70	0.30±0.10	0.40±0.21
	30	0.00±0	0.07±0.03	0.03±0.03
Blebbed Nuclei (%)	**15**	0.03±0.03	0.07±0.07	0.07±0.03
	30	0.00±0	0.07±0.07	0.00±0
PCE (%)	**15**	0.00±0	0.53±0.03	0.23 ± 0.15^{A}
	30	0.63±0.19	0.60±0.10	0.40 ± 0.15^{B}
Lobed Nuclei (%)	**15**	0.07±0.07	0.00±0	0.43±0.75
	30	0.00±0	0.03±0.03	0.00±0

*means significantly different at $p < 0.05$ in rows while different letters (superscript) in the upper case means significantly different at $p < 0.05$ between durations of exposure.

4. Conclusion

The current levels of the physical and chemical parameters of Ologe Lagoon showed that temperature and pH were within NESREA's safe limits whereas turbidity and NTU were above NESREA's safe limits. The field assessment of heavy metals in the lagoon indicated that Nickel was the predominant metal in surface water and fish from the lagoon. Subsequently, data obtained from the toxicological assessment suggest that Ni may cause deleterious effects in aquatic organisms that inhabit Ologe Lagoon. This shows that Ni is of environmental importance, and the biological endpoints employed in this study can be used as biomarkers to monitor the impact of Ni pollution on the lagoon and other water bodies.

REFERENCES

1. Holleman AF, Wiberg E. Textbook of Inorganic Chemistry. Berlin: Walter de Gruyter; 1995.
2. Wintz H, Fox T, Vulpe C. Functional genomics and gene regulation in biometals research. Biochem Soc Transactions. 2002;30:766-768.
3. Don-Pedro KN, Otitoloju, AA, Don-Pedro PO. Man and the Environmental Crisis. 2nd ed. Lagos: Cheers Book Series; 2013.
4. Armitage PD, Bomes MJ, Vincent HM. Long-term changes in macroinvertebrate communities of a heavy metal polluted stream: the River Nentcumbria, UK after 28 years. River Research and Applications, 2007;23:997-1015.
5. Essoka PA, Umaru JM. Industrial effluent and water pollution in Kakuri area, Kaduna South, Nigeria. Journal of Industrial Pollution and Control. 2006;22(1).
6. Eniola EB, Chukwu LO, Olaide BS. Hydro-Chemistry, Macro-invertebrate fauna and fish production of acdja fishing sites in a tropical lagoonal ecosystem. Journal of American Science. 2007;6(1).
7. Jarup L. Hazard of heavy metal contamination. Br Med Bull. 2003;68: 167-82.
8. Noda M, Kitagawa M. A quantitative study of iliac bone histopathology on 62 cases with itai-itai disease. Calcif Tissue Int. 1990;47:66-74.

9. Khan K, Wasserman GA, Liu X, Ahmed E, Parvez F, Slavkovich V, Factor-Litvak P. Manganese exposure from drinking water and children's academic achievement. Neurotoxicology, 2012;33(1):91-97.
10. Wang YC, Chaung RT, Tung LC. Comparison of the cytotoxicity induced by different exposure to sodium arsenite in two fish cell lines. Aquat. Toxicol. 2004;69:67–69.
11. Seok SH., Baek MW, Lee H., Kim DJ, Na YR, Noh KJ, Park SH, Lee HK., Lee BH, Ryu DY, Park JH, Arsenite-induced apoptosis is prevented by antioxidants in zebrafish liver cell line. Toxicol In Vitro. 2007;21:870–877.
12. Bhattacharya A, Bhattacharya S. Induction of oxidative stress by arsenic in *Clarias batrachus*: involvement of peroxisomes. Ecotoxicol Environ Saf. 2007;66:178–187.
13. Ventura-Lima J, Sandrini JZ, Ferreira-Cravo M, Piedras FR, Moraes TB, Fattorini D, Notti A, Regoli F, Geracitano LA., Marins LF, Monserrat JM. Toxicological responses in *Laeonereis acuta* (Annelida, Polychaeta) after arsenic exposure. Environ Int. 2007;33: 559–564.
14. Bagnyukova TV, Luzhna LI, Pogribny IP, Lushchak VI. Oxidative stress and antioxidant defenses in goldfish liver in response to short-term exposure to arsenite. Environ Mol Mut. 2007;48:658–665.
15. Ogbuagu DH, Adebayo ET, Ayoade AA, Ugwu OB, Mba DO. Lead accumulation in and its haematological effects on African catfish Clarias gariepinus. African Journal of Aquatic Science, 2015;40(2):201-204.
16. Green AJ, Planchart A. The neurological toxicity of heavy metals: A fish perspective. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 2018;208:12-19.
17. Salmerón-Flores P, Melendez-Camargo ME, Martinez-Tabche L. Hepatotoxic and nephrotoxic effect of lead on tilapia(*Sarotherodon aureus*). An Esc Nac Cienc Biol Mex. 1990;33:147-156.
18. Cameron KS, Buchner V, Tchounwou PB. Exploring the molecular mechanisms of nickel-induced genotoxicity and carcinogenicity: a literature review. Reviews on Environmental Health, 2011;26(2):81-92.
19. Harkin A, Hynes MJ, Masterson E, Kelly JP, O'Donnell JM, Connor TJ. A toxicokinetic study of nickel-induced immunosuppression in rats. Immunopharmacol Immunotoxicol. 2003;25(4):655–670.
20. Sun HX, Dang Z, Xia Q, Tang WC, Zhang GR. The effect of dietary nickel on the immune responses of *Spodoptera litura* Fabricius larvae. J Insect Physiol. 2011;57:954–961.
21. Abou-Hadeed AH, Ibrahim KM, El-Sharkawy NI, Sakr FS, El-Hamed, SAA. Experimental studies on nickel toxicity in Nile tilapia health. In 8th international symposium on tilapia in aquaculture;2008.
22. Webb JB. The ecology of Lagos lagoon. Philosophical Transaction of the Royal Society. 1958;683:307-419.

23. Anetekhai MA, Akin-Oriola GA, Aderinola O, Akintola SL. Trace metal concentration in *Macrobrachium vollenhovenii* from Ologe Lagoon, Lagos, Nigeria. Afrotrop Zool. 2007;25-29.
24. Ndimele PE, Jenyo-Oni A, Jibuike CC. The levels of lead (Pb) in water, sediment and a commercially important fish species (*Chrysichthys nigrodigitatus*)(Lacepede 1803) from Ologe Lagoon, Lagos, Nigeria. Journal of Environmental Extension. 2009;8(1).
25. Demirak A, Yilmaz F, Tuna AL, Ozdemir N. Heavy metals in water, sediment and tissues of *Leuciscus cephalus* from a stream in southwestern Turkey. Chemosphere. 2006;63(9):1451-1458.
26. Zheljazkov VD, Nielson NE. Effect of heavy metals on peppermint and cornmint. Plant Soil. 1996;178:59–66.
27. APHA/AWWA/WPCF. Standard methods for the examination of water and wastewater. 16th ed. Washington, DC:American Public Health Association; 1995.
28. Okogwu OI. Physicochemical conditions and metal levels in Ologe Lagoon, Southwest, Nigeria. Afr J Environ Pollut Health. 2006;6(1):28-31.
29. Nunes B, Brandão F, Sérgio T, Rodrigues S, Gonçalves F, Correia AT. Effects of environmentally relevant concentrations of metallic compounds on the flatfish *Scophthalmus maximus*: biomarkers of neurotoxicity, oxidative stress and metabolism. Environmental Science and Pollution Research. 2014; 21 (12):7501-7511.
30. Sedlak J, Lindsay RH. Estimation of total protein bound and non-protein sulfhydryl groups in tissues with Ellman's reagent. Analytical Biochemistry. 1968;25:1192–1205.
31. Sun, M, Zigma, S. An improved spectophotometric assay of dismutase based on epinephrine antioxidation. Analytical Biochemistry. 1978;90:81–89.
32. Sinha AK. Colorimetric Assay of Catalase. Analytical Biochemistry. 1972;47:389-394
33. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods in Enzymology. 1978;52: 302–6310.
34. Amaeze NH, Adeyemi RO, Adebesin AO. Oxidative stress, heats shock protein and histopathological effects in the gills of African catfish, *Clarias gariepinus* induced by bridge runoffs. Environmental Monitoring and Assessment. 2015;187(4): 172.
35. Singh KP, Mohan D, Singh VK, Malik A. Studies on distribution and fractionation of heavy metals in Gomti river sediments--a tributary of the Ganges, India. Journal of Hydrology. 2005;31(1-4):14-27.
36. Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures, Mutat Res. 2003;534:65-75.
37. Kandroo M, Tripathi NK, Sharma I. Detection of micronuclei in gill cells and haemocytes of fresh water snails exposed to mercuric chloride. International Journal of Recent Scientific Research. 2015;6(8):5725-5730.

38. National Environmental Standards Regulation and Enforcement Agency (NESREA). Guidelines to Standards for Environmental Pollution Control in Nigeria, Lagos: Federal Government Press, Nigeria; 2011.
39. Dan'azumi S, Bichi MH. Industrial pollution and implication on source of water supply in Kano, Nigeria. International Journal of Engineering and Technology. 2010;10(1): 101-109.
40. Murawska-Ciałowicz E, Bal W, Januszewska L, Zawadzki M, Rychel J, Zuwała-Jagiełło J. Oxidative stress level in the testes of mice and rats during nickel intoxication. The Scientific World Journal. 2012;2012:1-5.
41. Halliwell B, Gutteridge JM. Free radicals in Biology and Medicine. Oxford: Oxford University Press; 2005.
42. Manoj K, Padhy PK. Oxidative stress and heavy metals: an appraisal with reference to environmental biology. International Research Journal of Biological Sciences. 2013;2:91-101.
43. Henning SM, Zhang JZ, McKee RW, Swendseid ME, Jacob RA. Glutathione blood levels and other oxidant defense indices in men fed diets low in vitamin C. The Journal of Nutrition. 1991;121 (12):1969-1975.
44. Keramati V, Ramin M. Effect of diazinon on catalase antioxidant enzyme activity in liver tissue of *Rutilus rutilus*. Journal of Fisheries and Aquatic Science. 2010;5(5):368-376.
45. Ahmad I, Oliveira M, Pacheco M, Santos MA. *Anguilla anguilla* L. oxidative stress biomarkers responses to copper exposure with or without β-naphthoflavone pre-exposure. Chemosphere. 2005;61(2):267-275.
46. Palermo FF, Risso WE, Simonato JD, Martinez CB. Bioaccumulation of nickel and its biochemical and genotoxic effects on juveniles of the neotropical fish *Prochilodus lineatus*. Ecotoxicology and Environmental Safety. 2015;116:19-28.
47. Liu C, Yu K, Shi X, Wang J, Lam PK, Wu RS, Zhou B. Induction of oxidative stress and apoptosis by PFOS and PFOA in primary cultured hepatocytes of freshwater tilapia (*Oreochromis niloticus*). Aquatic Toxicology. 2007;82(2):135-143.
48. Bauman JW, Liu J, Klaassen CD. Production of metallothionein and heat-shock proteins in response to metals. Toxicological Sciences. 1993;21(1):15-22.
49. Jee H. Size dependent classification of heat shock proteins: a mini-review. Journal ofExercise Rehabilitation. 2016;12(4):255-259.
50. Hartl FU. Molecular chaperones in cellular protein folding. Nature. 1996;381(6583):571-580.
51. Sharaf-ElDeen KM. Accumulation of a 70kda stressprotein in the Nile tilapia, *Oreochromis niloticus*, and its useas a biomarker of Cu exposure. Egyptian Journal of Aquatic Biology and Fisheries. 2006;10(2):19–31.

52. Çavaş T, Ergene-Gözükara S. Induction of micronuclei and nuclear abnormalities in Oreochromis niloticus following exposure to petroleum refinery and chromium processing plant effluents. Aquatic Toxicology. 2005;74(3):264-271.
53. Bolognesi C, Perrone E, Roggieri P, Pampanin DM, Sciutto A. Assessment of micronuclei induction in peripheral erythrocytes of fish exposed to xenobiotics under controlled conditions. Aquatic Toxicology. 2006;78: 93-98.
54. Lindberg H, Wang X, Jarventaus H, Ghita C, Falck M, Norppa M, Fenech M. Origin of nuclear buds and micronuclei in normal and folate-deprived human lymphocytes. Mutation Research.2007;617: 33-45.