Original Research Article

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

ABSTRACT

~~We investigated, for the first time,~~ This study lead on 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 ~~(10)~~ fish per test concentration were used for the study. 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 inhibited ~~($P < 0.05$)~~ the activities of GSH however, it did not have any significant ~~($P > 0.05$)~~ effects on the activities of catalase, superoxide dismutase and malondialdehyde. Ni caused a significant ~~($P < 0.05$)~~ elevation in the number of micronuclei in the test fish and induced heavy HSPs as well 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: *Heavy metal pollution; Nickel; Oxidative stress; Ecological relevance; Biomarker; Genotoxicity; Molecular biology.*

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1. Introduction

The discharge of partially treated and untreated industrial effluents ~~from industries~~ 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 are considered as elements that have relatively high densities, especially above 5g/cm [1]. ~~Heavy metals~~ They occur naturally as trace elements, ~~that are~~ present in the abiotic and biotic components of the ecosystem [2]. However, anthropogenic activities~~, however,~~ have increased the

concentrations of these metals in the environment which have resulted in heavy metal pollution [3]. According to [3], the sources of heavy metals in aquatic ecosystems are 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 health date back to 1956 when the cases of Minimata 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 impact of arsenic in aquatic orgainsms range from cytoxicity 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 located between longitude 3°03' and 3°07' and latitude 6°26' and 6°30' (Figure 1). It is the smallest of the lagoons that make up the Lagos lagoon system, which comprises Lagos lagoon, Lekki lagoon, Badagry creek and Ologe lagoon [22]. ~~The~~ Ologe lagoon is connected to the ocean through the Badagry lagoon and it receives effluents from

Agbara industrial estate through the Owo River. Give here its hydromorphetric characteristics (Area, watershed, length, width, average depth, etc.). Ologe lagoon is particularly important because it receives industrial and domestic wastes from Agbara industrial estate.

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2.2 Field Studies and Heavy Metal Analyses

Samples were collected from three stations in reference to the direction in which effluents are received from Agbara Industrial estate (Figure 1). Give more information about sampling sites (topographic data).~~Physicochemical~~ The physical and chemical parameters of the lagoon were determined *in-situ* with Horiba U50 Gmulti water quality meter**.** Water samples were collected in 1 L plastic container; sediments were collected with a Venn-Grab sampler and placed in foil wraps doing according (give here norm or references) respectively.~~;~~ The fish~~es~~ samples were collected with aid of local fishermen, and preserved with ice packs before digestion. The digestion of samples was done according to the procedure described by [23]. Heavy metal analysis was done with a Perkin Elmer atomic absorption spectrometer.

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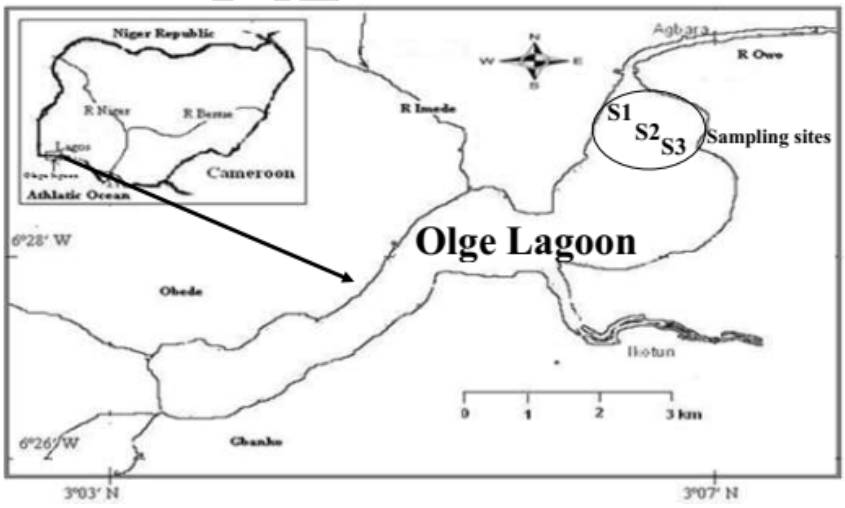

Figure 1: ~~Map Showing~~ Ologe Lagoon and sampling sites localization

2.3 Collection and Acclimatization of Experimental ~~Animals~~ fishes

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 Ecotoxicology Laboratory, Zoology Department, University of Lagos~~ to laboratory~~,~~ and kept in holding tanks (40 cm×30 cm×30 cm). During acclimatization, ~~the animals~~ these fishes 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 [24].

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2.4 Bioassay Procedure

A total of ten (10) acclimatized fish each were 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 animals 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 by [25]. In this study, NiW means test concentration derived from Ni surface water concentration in Ologe Lagoon while NiT is the test concentration derived from Ni concentration in fish from Ologe Lagoon

2.5 Measurement of Anti-oxidative Stress Enzymes and Lipid Peroxidation

93 The reduced glutathione (GSH) of liver tissue as non-protein sulphydryls was determined
94 according to the procedure described by [26]. Superoxide Dismutase activity was determined
95 by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in
96 absorbance at 480nm as described by [27]. Catalase activity was determined according to the
97 methodology of [28]. Thiobarbituric acid reactions (TBARS) assay was used to determine the
98 levels of the lipid peroxidation product, Malondialdehyde (MDA) [29].

99 **2.6 Determination of Heat Shock Protein Induction**

100 The methodology adopted is fully described by [30]. Briefly, the liver and gill samples of
101 dissected fish were washed with phosphate buffered saline and dried on filter paper, and
102 soluble proteins were extracted by mixing selected organs with equal volume of SDS loading
103 buffer (2×). The mixture was boiled for 7 min and immediately cooled, then the protein
104 content was assayed using the Biuret method, and protein profile was analysed by SDS-
105 polyacrylamide (10 %) gel electrophoresis under denaturing conditions, using the
106 discontinuous buffer system of Laemmli. Equal amounts of protein were loaded per lane on
107 each gel. The Sm0441 Fermentas (Thermo scientific) was used as the protein ladder.

108 **2.7 Nuclear Abnormalities**

109 The staining procedure was performed on blood smears obtained from fish samples. The
110 smear of the peripheral blood collected using heparinized syringe from the caudal vein of fish
111 sample was made on clean glass slides. Glass slides prepared per group were processed in
112 accordance with [31]. The smeared slides were allowed to air-dry at room temperature, fixed
113 in methanol for 15 minutes and then stained with May-Grunwald stain and allowed to air dry
114 for 6 hours, then slightly rinsed out with distilled water before staining with 5% Giemsa stain;
115 then left to dry for 12 hours [32]. The slides were analyzed at 1000x for micronuclei and
116 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 [33]. At least 500 erythrocytes per fish were examined to determine the frequencies of micronucleated erythrocytes and nuclear abnormalities.

2.8 Data Analysis

The mean and standard error (Mean±S.E.) and comparison of means were analysed using Statistical Package for 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 ~~Physiochemical~~ Physical and chemical Parameters of Ologe Lagoon

~~The physicochemistry~~ the physical and chemical parameter evaluated in ~~the three stations~~ sampling sites is presented in Table 1. The mean values of temperature and pH were within NESREA recommended limits (Put here references). TDS and turbidity values of 0.24±0.02 g/L and 66.63±17.04 NTU were above NESREA safe limits (Put here references) (Table 1). The high amount of TDS might have been as a result of the discharge of effluents into the lagoon by nearby industries. [34] assessed the implications of industrial pollution on source of water supply and found 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. 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. 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).

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Table 1: Physicochemical Characteristics of Lagoon Surface Water

Parameter	Sampling Stations				NESREA Limit
	Station 1	Station 2	Station 3	Mean±SE	(references)
Temperature (°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

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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	Asernic (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 GSH activity in fish exposed to NiW was 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 15th and 30th day respectively (Figure 2). GSH is the primary line of defense against Reactive Oxygen Species (ROS) [35]. Oxidative stress occurs when the number of ROS increases significantly [36]. [37] attributed GSH reduction to an increased need for GSH as a reducing agent in cellular processes. [38] observed that GSH activities in the testes of mice were inhibited after exposure to Ni (II).

The results showed that there was no significant difference ($p > 0.05$) between the SOD (give it definition) 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 activity of CAT in fish from the treated groups was not significantly different ($p > 0.05$) from that 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). [39] associated the inactivity of CAT to the high activity of Glutathione peroxidase (GPX), which acts as a defense against the production of H_2O_2. [40] observed that the activities of catalase in the liver of *Rutilus rutilus* exposed to diazinon were not altered.

There was no significant difference ($p > 0.05$) between the level of lipid peroxidation product, malondialdehyde (MDA) in the control and treated ~~animals~~ fishes 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). [41] 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. [42] observed that MDA level remained unchanged despite a marked increase of ROS generation observed in *Oreochromis niloticus* exposed to 15 mg L^{-1} of PFOS. [42] went further to state that the reasons for the insignificant change in MDA level in the fish were unknown.

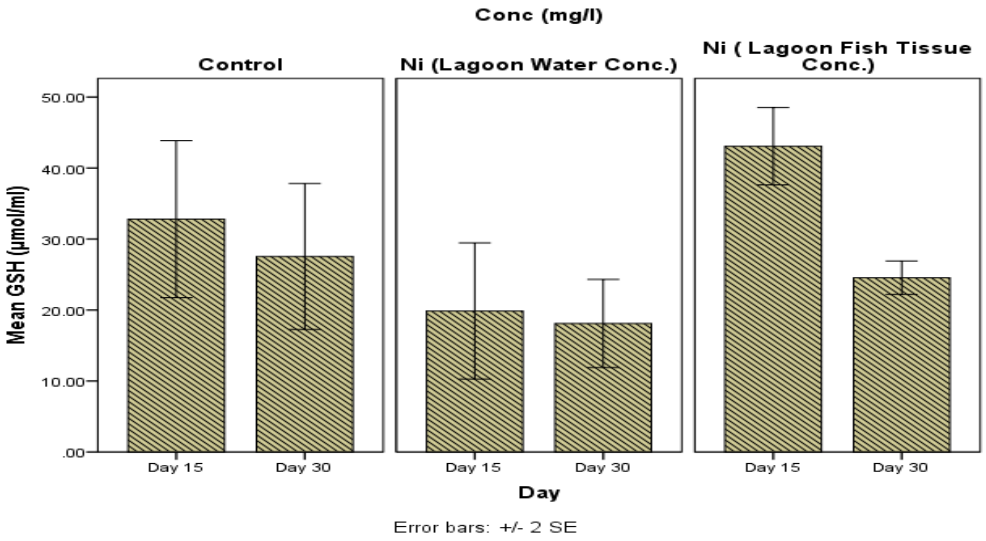

Figure 2: GSH levels in fish exposed to NiW and NiT for 15 and 30 days

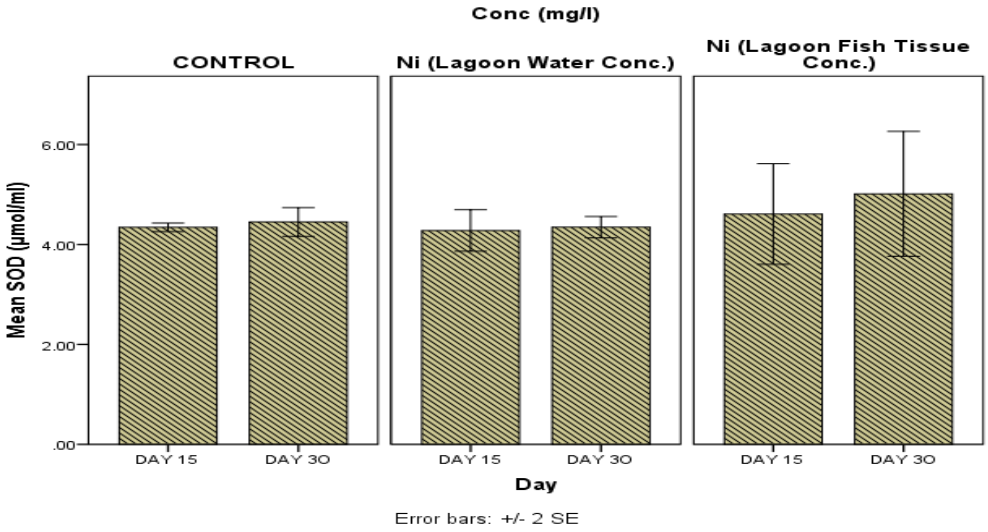

Figure 3: SOD levels in fish exposed to NiW and NiT for 15 and 30 days

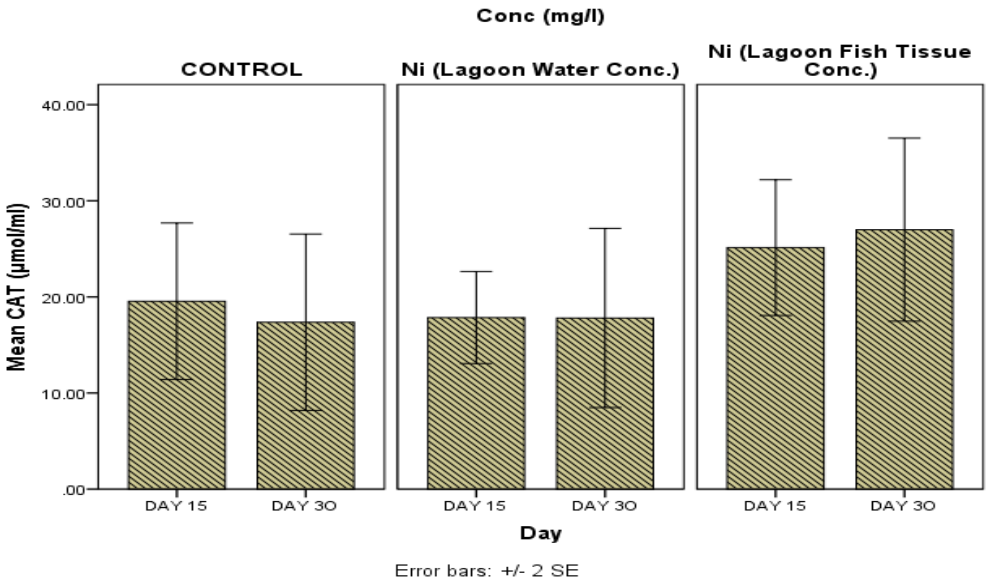

Figure 4: CAT levels in fish exposed to NiW and NiT for 15 and 30 days

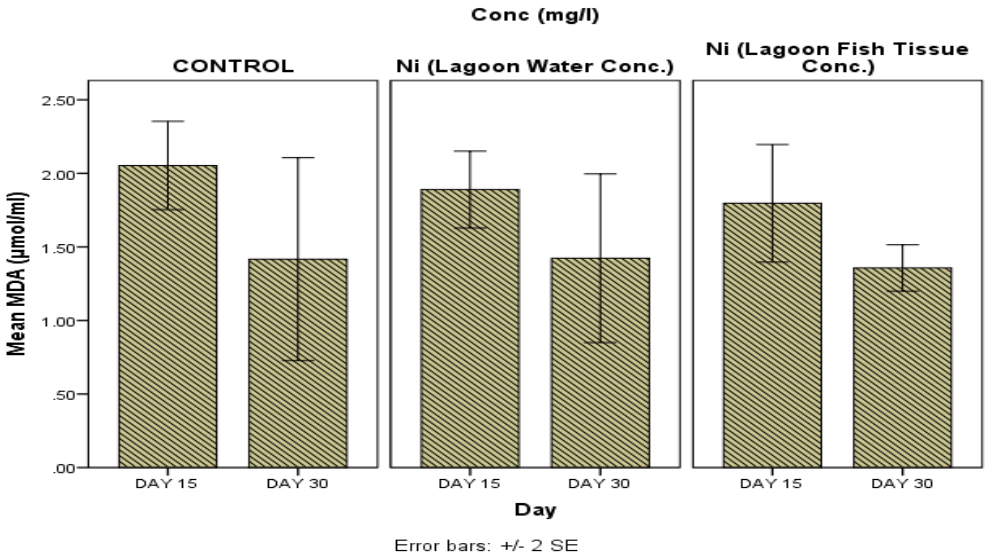

Figure 5: MDA levels in fish exposed to NiW and NiT for 15 and 30 days

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 other HSPs such as HSP 40, chaperonins and HSP 70 were observed in the gills and livers of the fishes exposed to NiW and NiT (Plate 2). [43] reported that heat-shock proteins contributed to the survival of cells following a variety of stresses. [43] investigated the induction of heat-shock proteins in response to metals and found out 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 [43]. Heavy HSPs are located in the cytoplasm [44]. Heavy HSPs work with HSP70 to counter

stress for cell survival [45,46]. [47] reported that HSP 70 increased in the hepatocytes of *Oreochromis niloticus* exposed to different concentrations of copper.

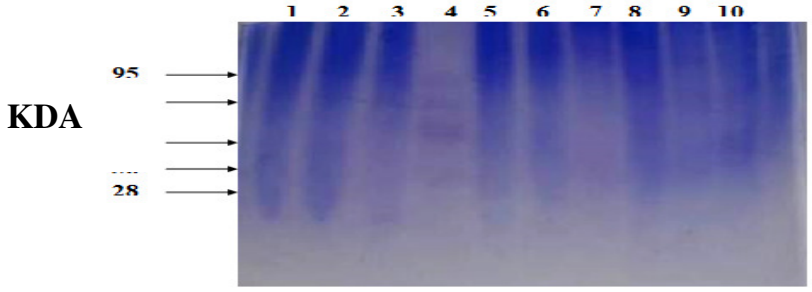

Plate 1: Heat shock proteins expression in the liver and gill of African catfish (*Clarias gariepinus*) exposed to NiW and NiT on 15d

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)

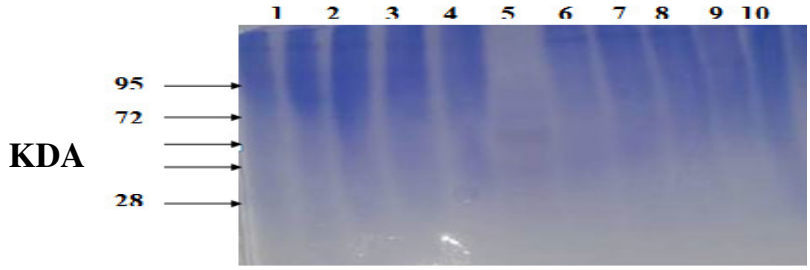

Plate 2: Heat shock proteins expression in the liver and gill of African catfish (*Clarias gariepinus*) exposed to NiW and NiT on 30d

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

The results showed that after the 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. The mean values ranged from 0.00±0 to 1.2±0.5 % and 0.13±0.09 to 0.57±0.33 % on the 15th and 30th day respectively (Table 3). [48] stated that nuclear abnormalities are indicators of genotoxic

218 damage. [49] observed the induction of the micronucleus and nuclear abnormalities in the
219 peripheral erythrocytes of *C. gariepinus* treated with process water. [48] reported the
220 formation of micronuclei and binuclei in fish cells exposed to cadmium, copper and
221 chromium. [50] found that micronuclei frequencies were elevated in fish exposed to both lead
222 acetate and mercury chloride.

223 The results of binuclei showed no statistical ($p > 0.05$) difference between the frequency of
224 binuclei in fish from the treated groups (NiW and NiT) and that of the control after 15 and 30
225 days of exposure. The mean values ranged from 0.00±0 to 0.47±0.27 % and 0.07±0.03 to
226 1.43±1.23 % on the 15th and 30th day respectively (Table 3). The percentage of buds in the
227 exposed fish samples did not significantly ($p > 0.05$) differ from that of the control after 15
228 days and 30 days of exposure. The mean values ranged from 0.00±0 to 0.10±0.10% and
229 0.00±0.0 to 0.67±0.03% on the 15th and 30th day respectively (Table 3). The results of
230 notched nuclei showed no statistical ($p > 0.05$) difference between the percentages of notched
231 nuclei in fish from the treated groups and that of the control after 15 and 30 days of exposure.
232 The mean values ranged from 0.00±0 to 0.20±0.15% and 0.00±0.0 to 0.13±0.09% on the 15th
233 and 30th day respectively (Table 3). The results of notched nuclei showed no statistical ($p >$
234 0.05) difference between the percentages of 8-shaped nuclei in fish from the treated groups
235 and that of the control after 15 and 30 days of exposure. The mean values ranged from
236 0.30±0.10 to 0.80±0.70% and 0.00±0.0 to 0.07±0.03% on the 15th and 30th day respectively
237 (Table 3). The results of blebbed nuclei showed no statistical ($p > 0.05$) difference between
238 the percentages of blebbed nuclei in fish from the treated groups and that of the control after
239 15 and 30 days of exposure. The mean values ranged from 0.03±0.03 to 0.07±0.03% and 0.00
240 ± 0.0 to 0.07±0.07% on the 15th and 30th day respectively (Table 3). The results of PCE
241 showed no statistical ($p > 0.05$) difference between the percentages of PCE in fish from the
242 treated groups and that of the control after 15 and 30 days of exposure. The percentage of

PCE exposed to NiT significantly ($p < 0.05$) increased from 0.23±0.15 on the 15th day to 0.40±0.15 on the 30th day (Table 3). The mean values ranged from 0.00±0 to 0.53±0.03% and 0.40±0.15 to 0.63±0.19% on the 15th and 30th day respectively (Table 3). The results of notched nuclei showed no statistical ($p > 0.05$) difference between the percentages of 8 shaped nuclei in fish from the treated groups and that of the control after 15 and 30 days of exposure. The mean values ranged from 0.00±0 to 0.43±0.75% and 0.00±0 to 0.03±0.03% on the 15th and 30th day respectively (Table 3). Some fish species have lower sensitivity to contaminants [51]. Such fishes include *Genyonemus lineatus* [52], *Phoxinus phoxinus* [53], and *Anguilla anguilla* [54]. [51] noted that some species had a very effective micronuclei removal system that prevented the increase in the level of micronuclei in peripheral blood.

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

Concentration (mg/L)	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 upper case means significantly different at $p < 0.05$ between durations of exposure.

4. Conclusion

The current levels of the ~~physicochemical~~ 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 in the present study suggest that Ni may cause deleterious effects in aquatic organisms that live in 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.

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