Original Research Article

BIOCHEMICALAND GENOTOXIC EFFECTS OF ECOLOGICALLY RELEVANT CONCENTRATIONS OF NICKEL IN *CLARIAS GARIEPINUS*

ABSTRACT

This study was aimed at determining the biochemical and genotoxiceffects 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) and nuclear abnormalities were assessed in fish chronically exposed to ecologically relevant concentrations of Ni. Environmentally relevant concentrations of Ni did not have any significant effects on the levels of reduced glutathione, catalase, superoxide dismutase and malondialdehyde after 30 days of exposure. Similarly, Ni had no significant effects on all tested parameters of genotoxicity after day 30. Therefore, environmentally relevant concentrations of Ni may not have any deleterious effects in terms of oxidative stress and genotoxicity.

Keywords: *Biomarker; Ecological relevance; Genotoxicity; Nickel; Ologe Lagoon; Oxidative stress.*

1. Introduction

The discharge of partially treated 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]. They occur naturally as trace elements, and are present 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 health date 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"N and 6°29'50"N, and longitudes 3°5'60"E and3°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 samples were 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 *Clariasgariepinus* (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].

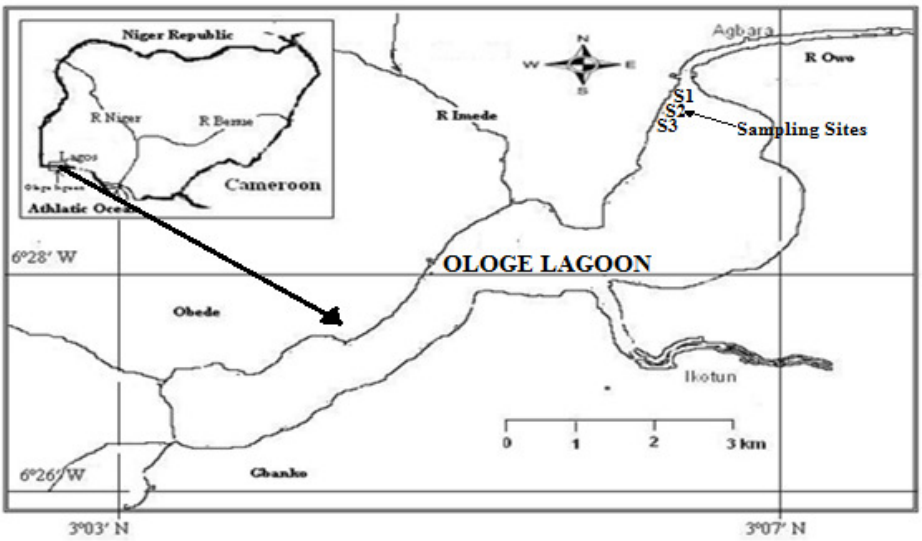


Figure 1: Ologe Lagoon and Sampling Sites Localization

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 by Nunes et 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 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. [34]. 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 [35]. The slides were analyzed at 100x 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 [36].

2.7 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 results of 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 [37]. TDS and turbidity values of 0.24±0.02 g/L and 66.63±17.04 NTU respectively were above NESREA safe limits [37] (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[38] 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's Surface Water

Parameter	Sampling Stations				NESREA Limit [37]
	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±0.00 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 NiT were significantly ($p < 0.05$) increased while NiW had no significant ($p > 0.05$) effect on GSH activities. The mean values ranged from 19.86±4.79 to 43.07±2.72µmol/ml and 18.10±3.11 to 27.54±5.14 µmol/ml on the 15th and 30th day respectively (Figure 2).However, NiT and NiW did not have any significant ($p > 0.05$) effects on the activities of GSH after 30 days of exposure. The results of GSH obtained afterNiTexposure agree with the transitory pattern observed by Kuroshima[39] and Lange et al. [40]. GSH is the primary line of defence against reactive oxygen species (ROS) [41]. The initial elevation of GSH may be attributed to an increased flux of Ni through the liver, with the removal of GSH by metals probably stimulating the synthesis of GSH [42].

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/mland 4.35±0.11 to 5.01±0.62 µmol/ml 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/mland 17.37±4.59 to 27.00±4.76 µmol/ml on the 15th and 30th day respectively (Figure 4).Keramati and Ramin [43] observed that the activities of catalase in the liver of *Rutilusrutilus*exposed to diazinon were not altered. Ahmad et al. [44] 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/mland 1.36±0.08 to 1.42±0.29 µmol/ml on the 15th and 30th day respectively (Figure 5). Palermo et al. [45] observed an increase in the level of MDA in the liver of *Prochiloduslineatus*exposed to 2500 µg/L of Ni, however, the level of MDA in the gills of *P. lineatus*remained unchanged. Liu et al. [46] reported that MDA level remained unchanged despite a marked increase of ROS generation observed in *Oreochromisniloticus*exposed to 15 mg L^{-1} of PFOS. Liu et al. [46] went further to state that the reasons for the insignificant change in MDA level in the fish were unknown. After 30 days of exposure, environmentally relevant concentrations of Ni had no significant ($p > 0.05$) effects on GSH, SOD, CAT, and MDA levels in the test fish. Therefore, environmentally relevant concentrations of Ni did not induce oxidative stress in *C. gariepinus.*

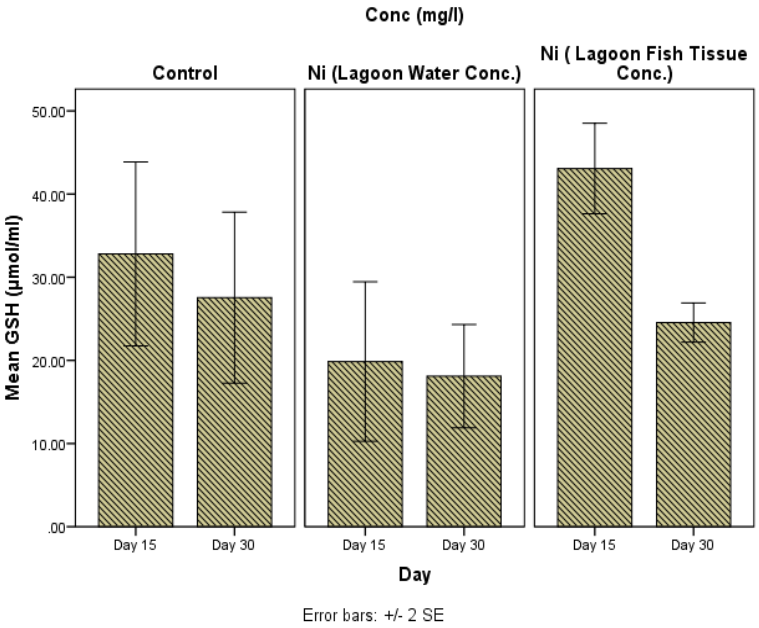


Figure 2: GSH levels in fish exposed to NiW and NiT for 15 and 30 days

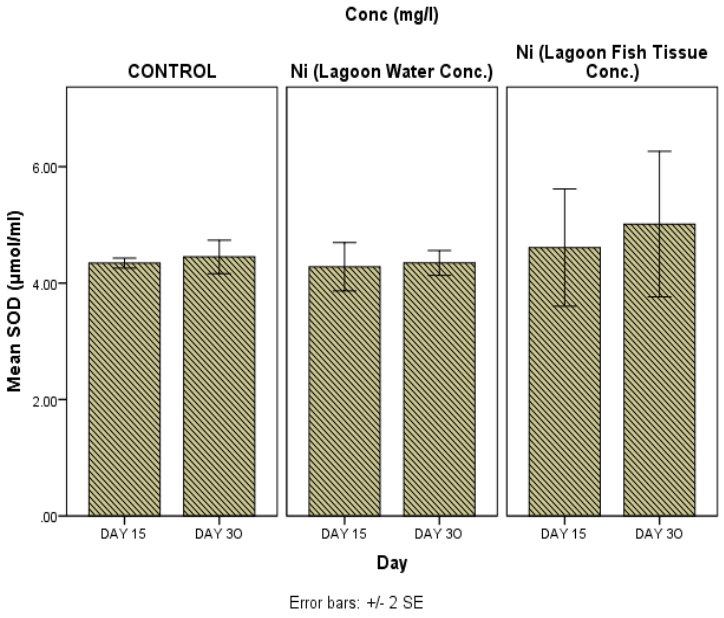


Figure 3: SOD levels in fish exposed to NiW and NiT for 15 and 30 days

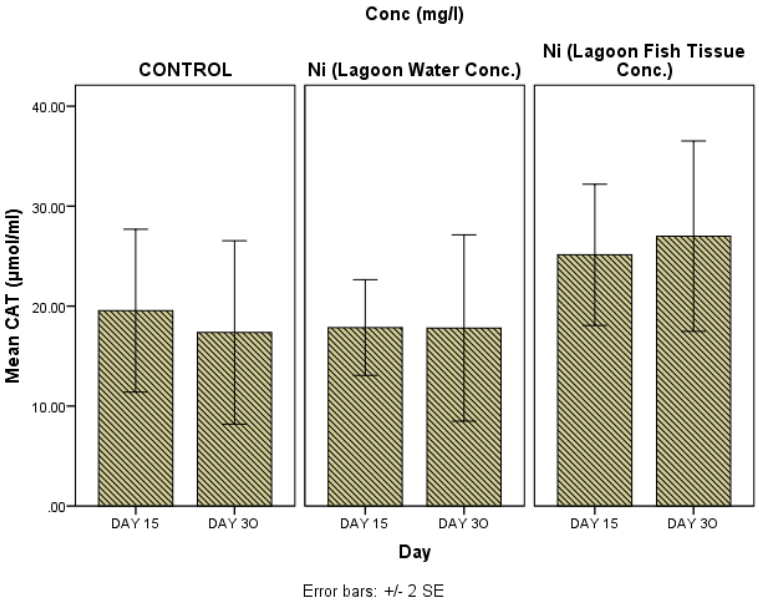


Figure 4: CAT levels in fish exposed to NiW and NiT for 15 and 30 days

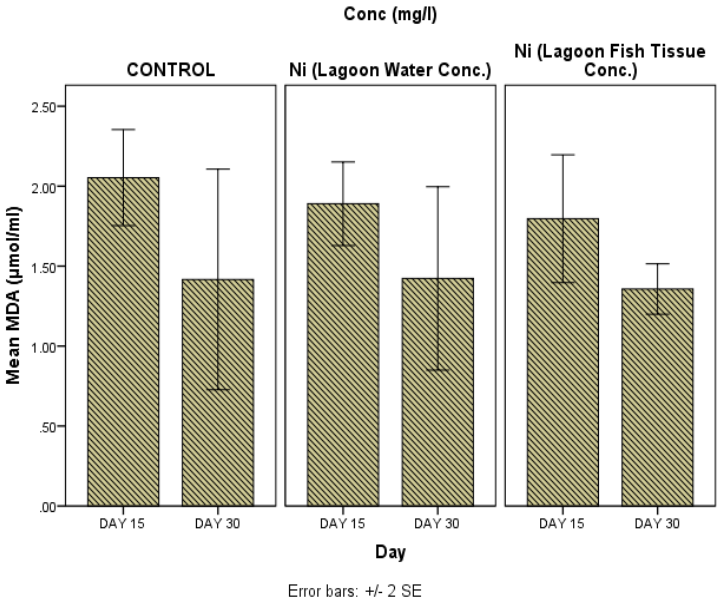
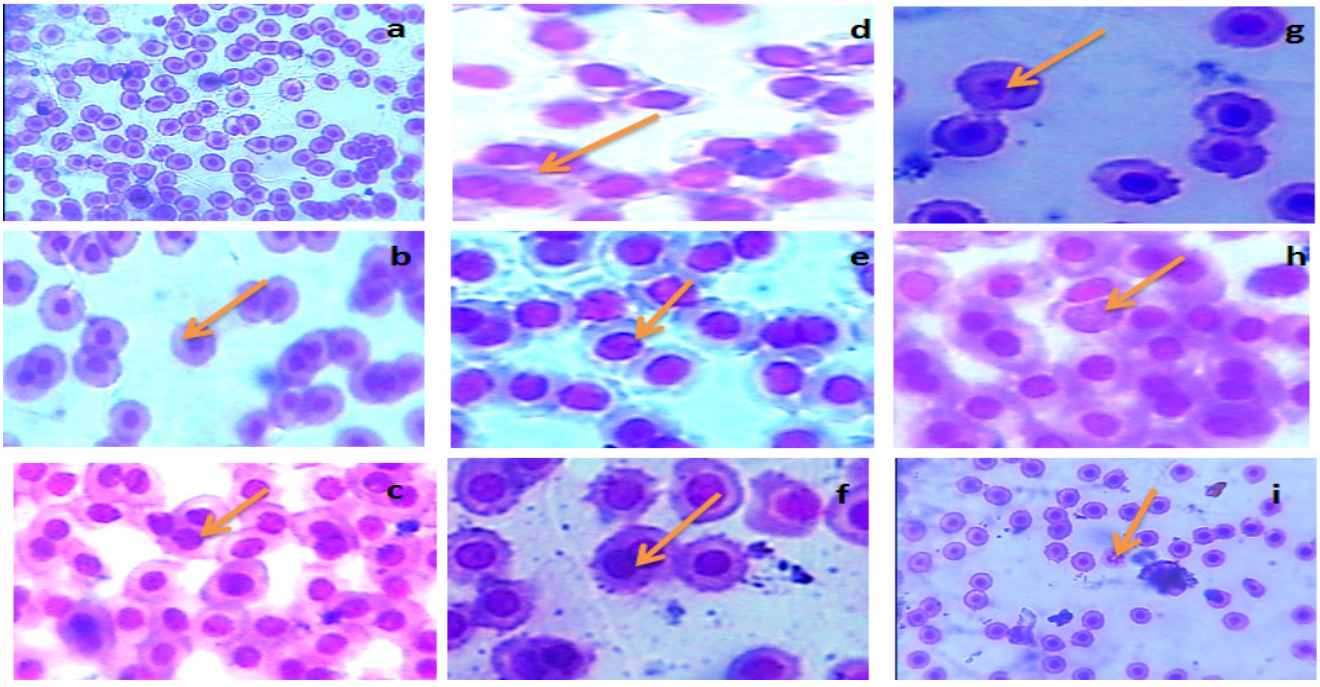
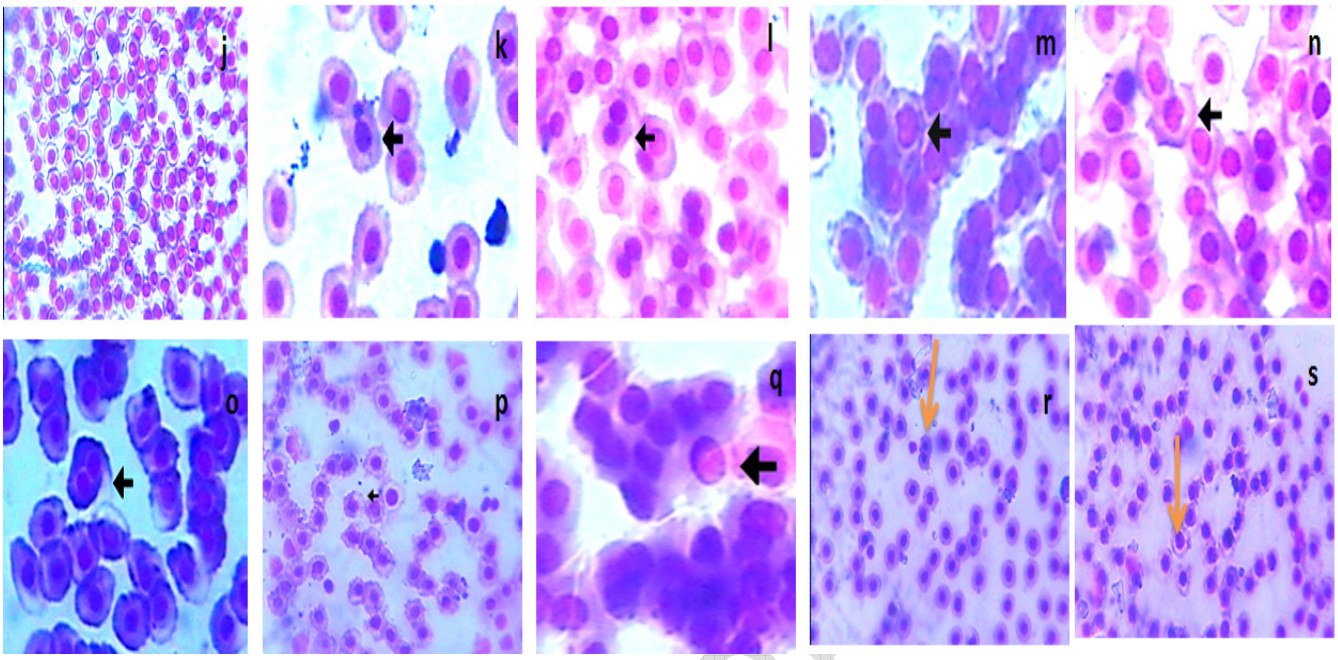


Figure 5: MDA levels in fish exposed to NiW and NiT for 15 and 30 days

3.3 Nuclear Abnormalities

Plates 1(a-s) 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 [47] 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 [47,48]. Formation of micronuclei in animals could be an effect of chromosomal breakage or dysfunction of the spindle mechanism by genotoxic agents such as heavy metals [49].However, at day 30 post-exposure there was no significant difference in the frequency of micronuclei in the control group and exposed group (Table 3). It is possible for organisms to undergo DNA repair after exposure to genotoxic agents [50].





Plates 1a-s: Photomicrograph of blood samples of *C. gariepinus* showing normal cells (control group) and nuclear abnormalities (exposed groups) (100x). **a&j-** Normal cells**b,k&s-** micronucleated cell (arrow)**c,l&r-** binucleated cell (arrow)**d&m-** nuclear bud (arrow) **e&n-** notched nucleus (arrow) **f&o-** 8-Shaped nucleus (arrow) **g&p-** blebbed nucleus (arrow) **h&q-** polychromatic erythrocyte (PCE) (arrow) **i-** lobed nucleus (arrow).

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

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.

Results from the biochemical analysis indicated that environmentally relevant concentrations of Ni in Ologe Lagoon may not be a causal factor of oxidative stress or peroxidative damage.

Data from the genotoxicity assaysuggest that the test fish may have the ability to recover from the genotoxic effect of the current concentrations of Ni in the lagoon. However, there is a need for the continuous monitoring of the impact of ecologically relevant concentrations of Ni especially with other biomarkers not utilized in the present study.

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. BiochemSoc 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. ToxicolIn Vitro. 2007;21:870–877.
12. Bhattacharya A, Bhattacharya S. Induction of oxidative stress by arsenic in *Clariasbatrachus*: 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 *Laeonereisacuta*(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 MolMut. 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 Clariasgariepinus. 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 (*Sarotherodonaureus*). An Esc NacCiencBiol 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. ImmunopharmacolImmunotoxicol. 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 *Spodopteralitura*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 *Macrobrachiumvollenhovenii* 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 (*Chrysichthysnigrodigitatus*) (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 *Leuciscuscephalus* 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 *Scophthalmusmaximus*: 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. 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.
35. 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.
36. 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.
37. National Environmental Standards Regulation and Enforcement Agency (NESREA). Guidelines to Standards for Environmental Pollution Control in Nigeria, Lagos: Federal Government Press, Nigeria; 2011.
38. 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.
39. Kuroshima R. Hepatic metallothionein and glutathione levels in Red Sea bream. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology. 1995; 110(1): 95-100.
40. Lange A, Ausseil O, Segner H. Alterations of tissue glutathione levels and metallothionein mRNA in rainbow trout during single and combined exposure to cadmium and zinc. Comparative Biochemistry and Physiology Part C: toxicology and Pharmacology. 2002; 131(3): 231-243.
41. Halliwell B, Gutteridge JM. Free radicals in Biology and Medicine. Oxford: Oxford University Press; 2005.
42. Thomas P, Wofford HW. Effects of metals and organic compounds on hepatic glutathione, cysteine, and acid-soluble thiol levels in mullet (*Mugilcephalus*L.). Toxicol. Appl. Pharmacol. 1984; 76: 172–182.
43. Keramati V, Ramin M. Effect of diazinon on catalase antioxidant enzyme activity in liver tissue of *Rutilusrutilus*. Journal of Fisheries and Aquatic Science. 2010;5(5):368-376.
44. 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.
45. Palermo FF, Risso WE, Simonato JD, Martinez CB. Bioaccumulation of nickel and its biochemical and genotoxic effects on juveniles of the neotropical fish *Prochiloduslineatus*. Ecotoxicology and Environmental Safety. 2015;116:19-28.

46. 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 (*Oreochromisniloticus*). Aquatic Toxicology. 2007;82(2):135-143.
47. Çavaş T, Ergene-Gözükara S. Induction of micronuclei and nuclear abnormalities in Oreochromisniloticus following exposure to petroleum refinery and chromium processing plant effluents. Aquatic Toxicology. 2005;74(3):264-271.
48. 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.
49. 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.
50. Qiao M, Wang C, Huang S, Wang D, Wang Z. Composition, sources, and potential toxicological significance of PAHs in the surface sediments of the Meiliang Bay, Taihu Lake, China. Environment International. 2006;32(1): 28-33.