

Cytogenotoxicity Effects of Some Selected Heavy Metals on Oreochromis niloticus and Clarias gariepinus

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Abstract

The contamination of natural waters by heavy metals depreciates aquatic biota and poses considerable environmental risks and concerns. This study investigated the cytogenetic abnormalities associated to Oreochromis niloticus and Clarias gariepinus exposed to some selected heavy metals [Mercury (Hg), Zinc (Zn), Lead (Pb) and Nickel (Ni)] using micronucleus (MN) and nuclear abnormalities (NA) tests in peripheral erythrocytes. Acute toxicity bioassay was conducted in a semi static system to determine the 96-h LC50 value of the test chemicals following standard methods. The sublethal doses for all the heavy metals used were calculated as 75% of the obtained LC_{s0} [Sub Lethal dose 3 (SL3)]. Fishes were exposed to SL3 for 21 days after which they were harvested and the cytogenetic toxicity endpoint assessed. The NA shapes were scored into binucleated cells, micronucleated, notched, blebbed, vacuolated, dumb-bell shaped and deshaped nuclei. Result of acute toxicity showed that lethality increased with increase in heavy metal concentration. It was observed that, fish species showed significant sensitivity to the different heavy metals treatment. In general, the highest value of both MN and NA cells were significantly increased in the exposure and followed trend as $Hg > Pb^{>}Zn^{>}Ni$. On the other hand, C. gariepinus was more sensitive to the three heavy metals exposure than O. niloticus. The frequencies of each NA shape were found in all the fish species and in all the treatments as follows NT>LB>BN>BL. Results showed that the exposed fishes showed cytogenetic alterations in their genetic materials. Heavy metals in natural water body can be bio accumulated and biomagnified in fish and consumption of such fish contaminated with metals thus poses a risk to human health.

Keywords: Toxicity, Heavy Metals, Micronuclei, Nuclear Aberration, Fish.

Introduction

The contamination of aquatic ecosystems by heavy metals negatively affects aquatic biota and poses considerable environmental risks and concerns. The continuous input of pollutants into the water bodies has led to the advancement in techniques for evaluation and monitoring of the integrity of such ecosystems. Among these pollutants, heavy metals have been of great concern due to their toxicity, abundance, persistence, and subsequent accumulation in aquatic habitats (Ali *et al.*, 2019). Their toxicity can have several consequences in all ecological matrices (Engwa *et al.*, 2019). Heavy

metals are those metallic elements with relative atomic masses higher than iron, which have the tendency to accumulate almost in a nonbiodegradable form in water, sediments and organisms such as fish with some toxic effects (Izuchukwu *et al.*, 2017). Heavy metals tend to accumulate in the aquatic environment because they cannot be degraded. Ultimately, this leads to human exposure and results in serious environmental problems (Bawuro *et al.*, 2018; Ezemonye *et al.*, 2019).

Metals (such as, zinc (Zn), chromium (Cr), Copper (Cu), Nickel (Ni), Molybdenum (Mo) Cobalt (Co), and Iron (Fe)). The non-essential metals (e.g., Aluminum (Al), Lead (Pb) Mercury (Hg), cadmium (Cd), and Tin (Sn) have no proven useful biological function (also called xenobiotics or foreign elements), and their toxicity rises with increasing concentrations. For instance, all metals Mercury (Hg), Lead (Pb) and Nickel (Ni) when in excess are known to pose adverse effects on fish. Consumption of fish contaminated with metals thus poses a risk to human health.

Some of the heavy metals may be genotoxic to the sentinel species and/or to non-target species, causing deleterious effects in somatic or germ cells (Obiakor *et al.*, 2012). The possibility of using changes in DNA integrity to the genetic material as markers of exposure and effect of genotoxicants has been previously investigated (Lapuente *et al.*, 2015). Measurement of cytogenetic damage by MN presented an important assay in detection of pollution stress and load in aquatic ecosystems resulting in the decline of populations of particular species (Baršienė *et al.*, 2013).

Nuclear abnormalities [NA] (such as notched nuclei, blebbed, lobbed, budding, fragmenting nuclei and bi nucleated cells) are considered as high-quality indicators of cytotoxicity (Kirschbaum *et al.*, 2009). These tests rely on the premise that any changes to genetic materials may have chronic, long-lasting and profound consequences (Osman, 2014). Recently, reports on the occurrences of malignancies and other biological conditions in aquatic organisms following exposure to suspected genotoxins (heavy metal) have increased. Such exposure of aquatic fauna not only poses a high risk for non-target organisms including man via food chain in the ecological context, but may also lead to heritable mutate ones (Osman, 2014). The biomonitoring of genotoxicity in aquatic organisms is important for several reasons. First, from the ecological perspective, the protection of genetic diversity in natural populations is important for population survival, and avoiding contaminant-induced mutations that skew genetic diversity (Sabzar *et al.*, 2016). Second, the detection of carcinogenic effects in aquatic organisms is needed to assess the health of aquatic organisms, as well as to prevent carcinogens from entering the food chain of humans (Lee and Steinert, 2003).

Fishes are good model animals for cytogenotoxic studies and provide early warnings for toxicants induced environmental alterations and degradations (Pawar, 2012) Fishes are considered to be most important biomonitors in aquatic systems for the estimation of metal pollution level (Authman, 2008). They provide several specific advantages in describing the natural characteristics of aquatic systems and in assessing changes to habitats (Lamas et al., 2007). Fish have the ability to uptake and concentrate metals directly from the surrounding water or indirectly from other organisms such as small fish, invertebrates, and aquatic vegetation (Polat et al., 2015). Fish, in comparison with invertebrates, are more sensitive to many toxicants and are a convenient test subject for indication of ecosystem health (Authman et al., 2015). They are also readily available and have high consumer acceptability. C. gariepinus and Oreochromis niloticus are used in this study because of their economic importance.

Hence, this study investigated the cytogenetic abnormalities associated to *Oreochromis niloticus* and *Clarias gariepinus* exposed to some selected heavy metals [Mercury (Hg), Zinc (Zn), Lead (Pb) and Nickel (Ni)] using micronucleus (MN) and nuclear abnormalities (NA) tests in peripheral erythrocytes.

Materials and Methods

Experimental fish

Two hundred and sixty (260) apparently healthy adult *O. niloticus* and *C. gariepinus* were procured from reputable farms in Onitsha. *O. niloticus* and *C. gariepinus* were one hundred and thirty (130) each used for the biotoxicity tests in this study. *Oreochromis niloticus* (mean weight: $458.5 \pm 3.14g$ and mean length: $34.3\pm 2.62cm$), and *Clarias*

gariepinus (mean weight: 658.4 ± 24.66 g and mean length: 36.2 ± 2.17 cm). The specimens were acclimatized to the laboratory conditions for two weeks under a 12-h photoperiod and fed commercial diets at 2% of body weight for the period.

Tested heavy metals

The chemicals (heavy metals) evaluated in this present study were Mercury (Hg), Zinc (Zn), Lead (Pb) and Nickel (Ni). Standard metal solutions used for biotoxicity assays were prepared using the standard methods of Reish and Oshida (1987) and Nsofor *et al.* (2014).

Determination of median lethal concentration (LC_{50})

The acute toxicity bioassay was conducted in a semi static system to determine the 96-h LC50 value of the test chemical following standard methods (USEPA, 2002). A preliminary toxicity range-finding test was carried out to determine approximate range by selecting the six concentrations of the test metal solution (Ni, Hg, Pb and Zn) for the definite test. The stock solution for the definite test was prepared in doubledistilled water. Test concentrations were prepared by diluting the test chamber tap water with the appropriate quantity of stock solution and reaching the final volume of 20 L. The fish did not feed for 24 h before and during the experiment, as recommended (Reish and Oshida, 1987). Proper oxygenation was provided to the test solution with showers fixed above the test chamber. Seven acclimatized specimens were randomly selected and exposed individually to seven test concentrations, along with a control in tap water. The experiment was repeated thrice under normal day/night illumination to obtain the 96-h LC₅₀ value of the test chemical for the test species. Fish mortality was recorded as 10%, 20%, 50%, 70%, 80%, and 100% at 96 h after exposure for respective concentrations of the test chemical. No mortality was observed in the control experiment. The 96-h LC₅₀ value of the test chemical was determined using probit analysis, as described by Hamilton et al., (1977). Water quality of the test solution was determined using standard procedures (OECD, 2000).

Estimation of sublethal concentrations and in vivo exposure

Using the 96-h LC₅₀ sublethal test concentrations of the chemical (75% of LC₅₀) [SL3] were calculated for the in vivo experiment, O. niloticus (Ni=0.008, Hg=0.016, Pb= 0.006, Zn=0.598) and C. gariepinus (Ni=0.032, Hg=0.32, Pb= 0.035, Zn=0.627). This study was conducted under semistatic test conditions following OECD Guideline No. 203 (16). The specimens were exposed to the test concentrations continuously for 21 days. Blood samples were collected at the same time intervals at a rate of five specimens per sampling per group by puncturing the caudal vein with a heparinized syringe and processed for Micronucleus and nuclei abnormality assay. The physiochemical properties of the test water, namely temperature, pH, conductivity, dissolved oxygen, chloride, and total hardness, were analysed by standard methods APHA(2005).

Micronucleus (MN) and Nuclear Abnormalities (NA) Assay

Blood samples were taken from the caudal vein to determine the normality of micronucleus. A single drop of blood was placed on the surface of a clean and grease-free microscope slide at a distance of 2 cm from one end. The blood smear was created by carefully and uniformly extending the drop of blood with the edge of another slide held at a 45^{o} angle to the first. Once prepared, the blood smear slide was dried by gently waving it in the air. These were carried out according to the methods described by Fenech *et al.*, (2003).

Statistical analysis

Data generated were analysed using the IBM SPSS v25 (IBM SPSS Inc., USA). Treatment groups were compared using Analysis of variance (ANOVA) and mean differences were separated using the Duncan Multiple Range Test (DMRT). Differences were considered significant at 5% level of significance.

Result

Physiochemical parameters of test water

During experimentation, test water temperature varied from 30.0 to 32.4 °C and pH ranged from 7.5 to 8.0. The dissolved oxygen varied from 7.0 to 8.5 mg L–1. Total hardness, Chloride content and total alkalinities were within good range.

Estimation of median lethal and sublethal concentrations

No mortality or visible behavioural changes were observed in control group. In the treated groups, fish mortality heightened with the increase in test concentrations of the chemical, and the specimens swarmed to the surface more often than the control group. Both cell and nuclei abnormalities significantly differed [Micronuclei (F=19.87, p < 0.01), binucleated (F = 24.07, p < 0.01), notched (F = 4.49, p < 0.05), blebbed (F=21.92, p < 0.01), vacuolated (F=16.31, p < 0.01), dumb-bell shaped (F = 16.09, p < 0.01), deshaped (F = 11.76, p < 0.01)] between heavy metal treated groups in *O. niloticus*.

The frequency of micronuclei cells was highest in mercury treated group (31.67 ± 11.24) although not significantly different from lead treated group (29.00 ± 6.93) and least in zinc treated group (Figure 2). Binucleated cells were observed highest in mercury treated group (17.00 ± 2.00) and least in zinc treated group (1.67 ± 1.15) . There was no significant difference between nickel and lead treated samples (Figure 3). Notched nucleus was highest in fishes

Micronucleus (MN) and Nuclear Abnormalities (NA)



Figure 1: Photomicrographs showing various micronucleus and nuclear abnormalities *a:normal, b:blebbed nucleic, deshaped nuclei, d: micronucleus, e:binucleated cell, f: notched nuclei, g:vacuolated nuclei*

Nuclei abnormalities	Control	Heavy metal				Б
		Nickel	Mercury	Lead	Zinc	г
Total Cells Counted	1000.00 ± 0.00	1000.00 ± 0.00	1000.00 ± 0.00	1000.00 ± 0.00	1000.00 ± 0.00	
Micronucleated	$0.00{\pm}0.00^{a}$	$4.33{\pm}2.08^{a}$	31.67±11.24 ^b	29.00 ± 6.93^{b}	$3.00{\pm}0.00^{a}$	19.87**
Binucleated	$0.00{\pm}0.00^{a}$	11.00 ± 4.36^{b}	$17.00 \pm 2.00^{\circ}$	9.67 ± 2.52^{b}	1.67 ± 1.15^{a}	24.07**
Notched	$0.00{\pm}0.00^{a}$	10.33 ± 5.51^{b}	10.334.93 ^b	12.67 ± 3.06^{b}	4.67 ± 5.03^{a}	4.49*
Blebbed	$0.00{\pm}0.00^{a}$	$6.00{\pm}2.65^{ab}$	10.33 ± 3.21^{b}	$33.33{\pm}10.02^{\circ}$	$2.67{\pm}2.08^{ab}$	21.92**
Vacuolated	$0.00{\pm}0.00^{a}$	4.33 ± 2.08^{ab}	13.00 ± 7.00^{b}	26.67±7.77°	0.67 ± 1.15^{a}	16.31**
Dumb-bell shaped	$0.00{\pm}0.00^{a}$	$2.33{\pm}1.53^{a}$	14.00±5.29 ^c	8.33 ± 1.15^{b}	$1.00{\pm}1.00^{a}$	16.09**
Deshaped	$0.00{\pm}0.00^{a}$	11.00 ± 7.81^{ab}	21.33±16.65 ^b	54.00±13.89°	13.00±2.65 ^{ab}	11.76**

Table 1: Micronucleus analysis in O. niloticus

**p < 0.05; values represent mean \pm standard deviation (n = 3) followed by the same alphabet between treatments (columns) are not significant (p > 0.05)

 Table 2: Micronucleus analysis in C. gariepinus

Na dataka ka ana dista	Control	Heavy metal				Б
Nuclei abilormanties		Nickel	Mercury	Lead	Zinc	г
Total Cells Counted	1000.00 ± 0.00	1000.00 ± 0.00	1000.00 ± 0.00	1000.00 ± 0.00	1000.00 ± 0.00	
Micronucleated	$1.67{\pm}2.89^{a}$	4.33 ± 2.08^{a}	43.00±15.52 ^b	$40.00{\pm}6.08^{ m b}$	$3.00{\pm}0.00^{a}$	23.05**
Binucleated	$0.00{\pm}0.00^{\mathrm{a}}$	11.00 ± 4.36^{b}	31.67±7.23°	9.67 ± 2.52^{b}	1.67 ± 1.15^{a}	30.23**
Notched	0.00±0.00a	$12.00{\pm}1.00^{ab}$	19.00±12.12 ^{ab}	23.00 ± 17.58^{b}	11.00±4.36 ^{ab}	2.44ns
Blebbed	$0.67{\pm}0.58^{a}$	6.67±3.51 ^a	18.33 ± 7.51^{b}	38.67±10.07 ^c	9.67 ± 2.52^{ab}	18.56**
Vacuolated	$0.00{\pm}0.00^{\mathrm{a}}$	$5.33{\pm}0.58^{a}$	$13.00{\pm}7.00^{b}$	43.00±5.29 ^c	13.00 ± 2.65^{b}	49.37**
Dumb-bell shaped	$0.00{\pm}0.00^{\mathrm{a}}$	3.33±1.53 ^b	17.67 ± 0.58^{d}	13.00±2.65°	4.67±1.53 ^b	67.18**
Deshaped	$0.67{\pm}0.58^{a}$	8.67 ± 3.21^{a}	68.33±21.98°	$58.33 \pm 8.50^{\circ}$	32.00 ± 1.00^{b}	23.35**

** p < 0.05; ns: not significant



Figure 2: Frequency of micronucleated cells (MN) per 1000 cells in *O. niloticus* and *C. gariepinus*

(** p < 0.05; bars (Mean \pm SD) followed by the same alphabet are not significantly different using Duncan Multiple Range Test)

treated with lead (12.67 ± 3.06) and least in zinc treated samples (4.67 ± 5.03) . There was no significant difference in the frequency of notched nuclei observed in samples treated with nickel, mercury and lead (Figure 4). The frequency of blebbed number per 1000 cells counted was highest in lead treated samples (33.33 ± 10.07) and





C. gariepinus



least in zinc treated samples (2.67 ± 2.08) . Blebbed nuclei showed no significant difference between nickel and mercury treated samples (Figure 5). Lead treated samples gave significantly higher vacuolated nuclei (26.67 ± 7.77) than the other heavy metal treatments. However, vacuolated nuclei were observed least in zinc treated (0.67 ± 1.15) samples (Figure 6). The highest dumb-bell shaped nuclei number was observed in mercury treated (14.00 ± 5.29) samples while the least was observed in zinc treated (1.00 ± 1.00) samples (Figure 7). Deshaped cells were observed highest in lead treated samples (54.00 ± 13.89) and least in nickel treated samples (11.00 ± 7.81) . The frequency of deshaped cells observed in nickel treated samples did not significantly differ to the frequency of deshaped cells observed in both mercury and zinc treated samples (Figure 8).

Similarly, both cell and nuclei abnormalities significantly differed [Micronuclei (F = 23.05, p < 0.01), binucleated (F = 30.23, p < 0.01), blebbed (F = 18.56, p < 0.01), vacuolated (F = 49.37, p < 0.01),



Figure 4: Frequency of notched nucleus per 1000 cells in *O. niloticus* and *C. gariepinus*

(** p < 0.05; ns: not significant; bars (Mean \pm SD) followed by the same alphabet are not significantly different using Duncan Multiple Range Test)



Figure 5: Frequency of blebbed nucleus per 1000 cells in *O. niloticus* and *C. gariepinus*

(** p < 0.05; bars (Mean \pm SD) followed by the same alphabet are not significantly different using Duncan Multiple Range Test) dumb-bell shaped (F=67.18, p < 0.01), deshaped (F=23.35, p < 0.01)] between heavy metal treated groups in *C. gariepinus* except notched (F=2.44, p > 0.05).

The frequency of micronuclei cells was highest in mercury treated group (43.00 ± 15.52) although not significantly different from lead treated group (40.00 ± 6.08) and least in zinc treated group (Figure 2). Binucleated cells were observed highest in mercury treated group (31.67 ± 7.23) and least in zinc treated group (1.67 ± 1.15). In Figure 4, binucleated cells were higher in the nickel [11.00 ± 4.36 (*O. niloticus*) and 11.00 ± 4.36 (*C. gariepinus*)] treated fishes than in zinc (1.67 ± 1.15 ; 1.67 ± 1.15) treated fishes used for this study. The frequency of BN was the same mean in both fishes exposed to zinc treatments.





(** p < 0.05; bars (Mean \pm SD) followed by the same alphabetarenotsignificantly different using Duncan Multiple Range Test)



Figure 7: Frequency of dump-bell shaped nucleus per 1000 cells in *O. niloticus* and *C. gariepinus*

(** p < 0.05; bars (Mean ± SD) followed by the same alphabet are not significantly different using Duncan Multiple Range Test)



Figure 8: Frequency of deshaped cell per 1000 cells in O. niloticus and C. gariepinus (**p < 0.05; bars (Mean \pm SD) followed by the same alphabet are not significantly different using Duncan Multiple Range Test)

There was no significant difference between nickel and lead treated samples (Figure 3). There was no significant difference (p > 0.05) among treatment means for notched nuclei. Although, mercury treated samples showed the highest frequency of notched nuclei (Figure 4). The frequency of blebbed number per 1000 cells counted was highest in lead treated samples (38.67 ± 10.07) and least in nickel treated samples (6.67±3.51). Blebbed nuclei showed no significant difference between mercury and zinc treated samples (Figure 5). Lead treated samples gave significantly higher vacuolated nuclei (43.00 ± 5.29) than the other heavy metal treatments. However, vacuolated nuclei were observed least in nickel treated (5.33 ± 0.58) samples (Figure 6). The highest dumb-bell shaped nuclei number was observed in mercury treated (17.67±0.58) samples while the least was observed in nickel treated (3.33 ± 1.53) samples. There was no significant difference in the frequency of dumb-bell shape nuclei in nickel and zinc treated samples (Figure 7). Deshaped cells were observed highest in mercury treated samples (68.33±21.98) and least in nickel treated (8.67±3.21) samples (Figure 8). Also, dumb-bell shape in treated C. gariepinus was higher than in lead treated O. niloticus.

Similarly to MN, the frequencies of NA were also of significant greater number in fish treated with Hg followed by Pb and Zn. Otherwise, *C. gariepinus* was still the most sensitive to the four heavy metals exposure. The frequencies of NA in erythrocytes were analysed separately. It was observed that the frequencies of each nuclear abnormality shapes in all treatments were found as follow: NT>LB>BN>BL. Results of MN and NA revealed the order of heavy metals in ascending order using the frequency of abnormalities in both fishes: Hg>Pb>Zn>Ni (Table 1 and 2).

Similarly to MN, the frequencies of NA were also of significant greater number in fish treated with Hg followed by Pb and Zn. Otherwise, *C. gariepinus* was still the most sensitive to the four heavy metals exposure. The frequencies of NA in erythrocytes were analysed separately. It was observed that the frequencies of each nuclear abnormality shapes in all treatments were found as follow: NT>LB>BN >BL. Results of MN and NA revealed the order of heavy metals in ascending order using the frequency of abnormalities in both fishes: Hg>Pb>Zn>Ni (Table 1 and 2).

Discussion

Observed behavioural changes during the toxicity tests, such as erratic swimming, gasping for air, sudden quick movement and somersaulting are similar to changes linked to the toxicity of xenobiotics on fish as have been reported in previous studies by Sarikaya and Yilmaz (2003). Perhaps, such behavioural changes could be attributed to both the direct toxicity of the heavy metals. The high operculum and tail fin movement indicated sign of oxidative stress; this conformed to the findings of Babatunde and Idris (2017).

Selection of peripheral blood erythrocytes of fish, in this study, as a target cell to investigate genotoxic damage was based on the important role of blood in movement of toxic substances absorbed. The formation of nuclear abnormalities along with micronucleus in peripheral erythrocytes of tested fishes in this study reinforces and validates the evidence that heavy metals induce genotoxic action and are therefore possible indicators of genotoxicity. The results showed that the tested metals (Ni, Hg, Pb and Zn) have cytogenotoxic

and also suggested that they have clastogenic (chromosomal breaking) and/or aneugenic (mitotic spindle dysfunction) ability capable of increasing DNA damage and genome instability in O. niloticus and C. gariepinus. Results obtained also showed concentration dependent increase in micronuclei and nuclear aberrations and speciesspecific genotoxic behaviour of tested metals. This implies that metal induced genotoxicity is concentration dependent and this conforms to the report of Kousar and Javed (2015). Also observed is the variability in the sensitivity of C. gariepinus and O. niloticus towards tested metals toxicity which is in accordance with Azmat et al., (2012). This may be related to DNA repair or other mechanism associated with resistance or tolerance to chemical contaminants (Braham et al., 2017).

Time-related MN elevation incidences were detected in all experimental groups compared to the control group of treated fishes. The elevation of micronuclei increased progressively with increasing the duration of exposure.

In the present study, it was observed that both mercury and lead showed significant difference in the number of micronuclei and binucleated, notched, blebbed, vacuolated dump-belled shaped and deshaped cell, aberrations in all sublethal doses when compare to the control group.

Mercury is a toxic heavy metal which is widely dispersed in nature. Most human exposure to mercury is caused by outgassing of mercury from dental amalgam, ingestion of contaminated fish, or occupational exposure, according to the World Health Organization (WHO, 1991). According to Robin (2012), mercury in all forms poisons cellular function by altering the tertiary and quaternary structure of proteins and by binding with sulfhydryl and selenohydryl groups. Consequently, mercury can potentially impair function of any organ, or any subcellular structure. Elemental and methyl mercury are toxic to the central and peripheral nervous systems (WHO, 2017). The continued release of mercury into the environment from human activity, the presence of mercury in the food chain, and the demonstrated adverse effects on humans are of such concern since it poses a health risk. Lead is the most important toxic heavy element in the environment. Globally it is an abundantly distributed, important yet dangerous environmental chemical (Wani et al., 2015). Lead is a highly poisonous metal affecting almost every

organ in the body especially the nervous system, which is the mostly affected target in lead toxicity, both in children and adults. Long-time exposure to lead has been reported to cause anaemia, along with an increase in blood pressure, and mainly in old and middle aged people (WHO, 2019). In this present study, mercury and lead caused high genetic material alterations than other heavy metals. Severe damage to the brain and kidneys, both in adults and children, were found to be linked to exposure to heavy lead levels resulting in death (Wani et al., 2015). Blood disorders and damage to the nervous system have a high occurrence in lead toxicity. Heavy metals can bind to phosphate and base residues of DNA, to alter its primary and secondary structures and can also interfere with protein structure and function to cause DNA damage. Therefore, free radicals generation and oxidative damage by metals may be responsible for the observed cytogenotoxic damage therein.

From the present study, micronucleus and nuclear aberration assay can be described as a vital tool for assessment of genotoxic potential of various chemical agents by using fish as a model. It is considered to be one of the most efficient approaches for the assessment of exposure to contaminants.

Conclusion

This study shows that heavy metals have deleterious effects on the genetic material of *O. niloticus* and *C. gariepinus*. Also, both fishes are vital in early detection and monitoring of genotoxins in aquatic environment because of their economic importance. Both fishes can maintain aquatic ecosystem integrity and prevent health hazard related to heavy metal contamination on fish and man.

References

- Ali, H., Khan, E. and Ilahi, I. (2019). "Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation". *J. of Chemistry*, 1(2): 23-29.
- APHA (American Public Health Association) (2005). "American Water Works Association: Standard methods for the examination of water and wastewater". 22nd ed., Washington DC, USA, New York. Pp. 37.

- Authman, M.M.N., M.S. Zaki, E.A. Khallaf, and H.H.Abbas, (2015). "Use of fish as bio-indicator of the effects of heavy metals pollution". *Aquaculture Research & Development*, 6 (4): 1-13.
- Authman, M.N. (2008). "Oreochromis niloticus as a biomonitor of heavy metal pollution with emphasis on potential risk and relation to some biological aspects. *Global Vet.*, 2(3): 104-109. 28.
- Azmat, H., Javed, M. and Jabeen, G. (2012). "Acute toxicity of aluminum to the fish (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*)". *Pakistan Veterinary Journal*, 32: 85-87.
- Babatunde, M. M. and Idris, A.Y. (2017). "Acute toxicity test of Lead on *Clarias garienpinus* juveniles". *International Journal of Fisheries and aquatic studies*, 5 (2): 350-356.
- Baršienė, J., Rybakovas A., Lang T., Andreikėnaitė L. and Michailovas, A. (2013).
 "Environmental genotoxicity and cytotoxicity levels in fish from the North Sea offshore region and Atlantic coastal waters". *Mar. Poll. Bull.*,
- 68(1–2):106–116. Bawuro, A.A., Voegborlo, R. B. and Adimado, A. A. (2018). "Bioaccumulation of heavy metals in some tissues of fish in Lake Geriyo, Adamawa State, Nigeria," *Journal of Environmental and Public Health*, Article ID 1854892, pp. 1-7.
- Braham, R. S., Blazer, V., Shaw, K, and Mazik, P. M. and Blazer, V. S. (2017). "Micronuclei and other erythrocyte nuclear abnormalities in fishes from the Great Lakes Basin, USA". Environ Mol Mutagen. 58(8): 570–581.
- Engwa, G., Ferdinand, P.U., Nwalo, F. N. and Unachukwu, M.N. (2019). "Mechanism and Health Effects of Heavy Metal Toxicity in Human".
- Ezemonye, L., Adebayo, P.O., Enuneku, A. A., Tongo I. and Ogbomida, E. (2019). Potential health risk consequences of heavy metal concentrations in surface water, shrimp (*Macrobrachium macrobrachion*) and fish (*Brycinus longipinnis*) from Benin River, Nigeria". *Elsevier* Toxicology Reports 6(1): 1-9.
- Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland, N., Bonassi, S. and Zeiger, E. (2003). "HUMN project: detailed description of the scoring criteria for the Cytokinesis block micronucleus assay isolated human lymphocyte cultures". *Mutation Research*, 534: 65-75.
- Hamilton, M.A., Russo, R.C. and Thurston, R.V. (1977). Trimmed Spearman-Karber method for estimating median lethal concentration in Toxicity bioassays. *Environ. Sci. Technology* 7: 714-719.

- Izuchukwu, U. I., Okeke, D.O and Okpashi, V.E. (2017). "Determination of heavy metals in fish tissues, water and sediment from the Onitsha Segment of the River Niger, Anambra State Nigeria". J. Environmental Analytical Toxicology, 7(5): 507. DOI: 10.4172/2161.
- Kirschbaum, A. A., Seriani, R., Ranzani-Paiva, M. T., Abessa, D. S. and Pereira, C. S. (2009). "Cytogenotoxicity biomarkers in fat snook *Centropomus parallelus* from Cananéia and São Vicente estuaries, SP. Brazil". *Gen. Mol. Biol.*, 32(1):151–154.
- Kousar, S. and Javed, M. (2015). "Studies on induction of nuclear abnormalities in peripheral blood erythrocytes of fish exposed to coppe". *Turkish Journal of Fisheries and Aquatic Sciences*, 15: 879-886.
- Kris-Etherton, P., Harris, W. and Appel, L. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*, 106 pp. 2747-2757.
- Kumar, R., Pandey, S. and Sharma S. (2005). Study of sister chromatid exchanges for assessment of genotoxicity. In: Kapour D, Nagpour NS (eds), Training on genotoxic assays in fishes. National Bureau of Genetic Resources, Dilkusha, Telibagh, India pp: 68.
- Lamas, S., Fernández, J.A., Aboal, J. R. and Carballeira, A. (2007). "Testing the use of juvenile Salmo trutta L. as biomonitors of heavy metal pollution in freshwater". *Chemosphere* 67 (2) 221-8.
- Lapuente, J., Lourenço, J., Mendo, S.A., Borràs, M. G., Costa, P. M. and Pacheco, M. (2015). "The Comet Assay and its applications in the field of ecotoxicology: A mature tool that continues to expand its perspectives". *Frontiers in Genetics*, 6(1):180.
- Lee, R.F., Steinert, S. (2003). "Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals". *Mutation Research*, 544: 43–64.
- Nsofor, C. I. and Ikpeze, O. O. (2014). "Potential health hazards of consuming *Clarias gariepinus* fish contaminated with heavy metals of River Niger". *Glob J. Biol. Agric Health Sci* 3 (2): 69-74.
- Obiakor, M. O., Okonkwo, J. C., Nnabude, P. C. and Ezeonyejiaku, C. D. (2012). "Eco-Genotoxicology: Micronucleus assay in fish erythrocytes as in Situ Aquatic Pollution Biomarker: A Review". Journal of Animal Science Advances 2: 123-133.
- OECD (2000). "Guidelines for the testing of Chemicals.

Section 2: Effect on biotic systems test No.215: Fish, Juvenile Growth Test", Paris, France.

- Osman, A. G. (2014). "Genotoxicity tests and their contributions in aquatic environmental research". *Journal of Environmental Protection* 5:1391-1399.
- Pawar D.H. (2012). River water pollution, an environmental crisis: a case study of Panchaganga river of Kolhapur city. *Int. J. Ecol. Develop Sum.* 9 (1):131–133.
- Polat, F., Akın, Ş., Yıldırım, A. and Dal, T. (2015). "The effects of point pollutants originated heavy metals (lead, copper, iron, and cadmium) on fish living in Yeşilırmak River, Turkey". *Toxicol. Ind. Health*, 1-12.
- Reish, D. L, and Oshida, O. S. (1987). "Manual of methods in aquatic environment research. Part 10. Short-term static bioassays". *FAO Fisheries* Rome.62pages.
- Robin A. B. (2012). "Mercury toxicity and treatment: A review of the literature," *Journal of Environmental* and Public Health, Vol. 2012, Article ID 460508, 10 pages, 2012.

https://doi.org/10.1155/2012/460508.

- Sabzar, D. A., Yousuf, A. R., Balkhi, M. H. (2016). "An introduction about genotoxicology methods as tools for monitoring aquatic ecosystem: present status and future perspectives". *Fish Aquac J.*, 7(1): 158. doi:10.4172/2150-3508.1000158.
- Sarikaya, R. And Yılmaz, M. (2003). "Investigation of acute toxicity and the effect of 2,4-D (2,4-

dichlorophenoxyacetic acid) herbicide on the behaviour of the common carp (*Cyprinus carpio L.*, 1758; Pisces, *Cyprinidae*)". *Chemosphere*, 52:195-201.

- United States Environmental Protection Agency (USEPA) (2002). "Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms". 5th ed. EPA-821-R-02-012. U.S. Environmental Protection Agency, Office of Water, Washington, DC.
- Wani, A., Ara, A. and Usmani, J. (2015). Lead toxicity: a review *Interdiscip Toxicol.* 8(2): 55–64. doi: 10.1515/intox-2015-0009.
- World Health Organization (1991). "Inorganic mercury: environmental health criteria 118," in International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland. View at Google Scholar.
- World Health Organization (2019). Lead poisoning and health Geneva: World Health Organization; 2019 Fact sheets.
- World Health Organization (WHO) (2017). Guidelines for drinking-water quality. Fourth edn. World Health Organization, Geneva.
- Yadav, K. K. and Trivedi, S. P. (2009). "Sublethal exposure of heavy metals induces micronuclei in fish, *Channa punctatea*". *Chemosphere*, 77: 1495-1500.
- Zaki, M. N., El-Batraway, N. and Shalaby, S. (2017). "Heavy Metal on Fish". *Researcher*, 9(11):60-64.



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