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Genotoxicology: Single and Joint Action of Copper and Zinc to *Synodontis clarias* and *Tilapia nilotica*

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ABSTRACT: The genotoxicity of copper, zinc and their binary mixture was examined in *Synodontis clarias* and *Tilapia nilotica* using the sensitive micronucleus assay in fish genome. Increased formation of the micronuclei were observed in all the three concentrations studied ($0.25LC_{50}$, $0.125LC_{50}$ and $0.0625LC_{50}$). The frequency of micronuclei was observed to increase heavily ($p<0.05$) when the fish species were exposed to binary mixture of the heavy metals. Individual metal (Cu and Zn), acting alone, produced significant ($p<0.05$) levels of micronucleated erythrocytes in both species. After 96-hour recovery in clean water of the fish species exposed to the binary mixture of the heavy metals, the levels of micronuclei in *Tilapia nilotica* decreased ($p<0.05$) while the levels in *Synodontis clarias* displayed no meaningful decrease ($p>0.05$) at the highest and lowest concentrations studied. *Synodontis clarias* was numerically observed to exhibit higher incidence of micronuclei in the sampled blood than *Tilapia nilotica*.@ JASEM

Capacity of the water body to support aquatic life as well as its suitability for other uses depends on many factors among which are trace element concentrations. Some metals such as manganese, zinc, copper, and nickel, when present in trace concentrations are important for the physiological functions of living tissue and regulate many biochemical processes (Sanders, 1997). Generally, trace amount of metals are always present in freshwaters from the weathering of rocks and soils and other natural mechanisms. Some metals when discharged into natural waters at increased concentration in sewage, industrial effluent or from mining and refining operations can have severe toxicological effects on aquatic environment and humans (Merian, 1991; DWAF, 1996). In addition, heavy metal becomes toxic when their concentration exceeds the threshold level.

Most of the studies that have been carried out on the toxicity of heavy metals have been focused on single compound activity (Enserink *et al*., 1991; Don-Pedro, 1996). The main value of single action toxicity is to establish toxicity scales or ranking orders employing sensitive species in local ecosystems. In Nigeria, Ogunsua *et al.* (1991) reported environmental levels of heavy metals in sediment and animals collected from different parts of the Lagos Lagoon without relating the measured levels to any demonstrable biological effects. Chukwu (1991) also reported the contamination of River Sasa in Lagos by heavy metals originating from industrial effluents being discharged into the river but not that of the metallic constituents. [*]

However, aquatic ecosystems are usually exposed simultaneously to a mixture of toxic substances, where different interactions among metals are possible. Oyewo (1998) and Otitiloju (2001) documented that the effects of heavy metals when acting jointly could differ from its single toxicity to living organisms. Investigations into joint action toxicity of metal compounds have revealed that different types of interactions do occur when mixtures of metals jointly impact aquatic organisms. Genotoxicity studies conducted in a variety of test systems have failed to provide evidence for mutagenicity of zinc. However, there are indications of weak clastogenic effects following zinc exposure (ATSDR, 1990). Various investigations have been made in copper genotoxicity (Guecheva, 2001; Bagdonas and Vosyliene, 2006) on aquatic organisms but actual mechanisms of its genotoxicity are poorly discussed (Bagdonas and Vosyliene, 2006). The later authors documented the genotoxicity of copper and zinc, observing non dose-dependency of all the concentrations of the toxicants employed in the study.

Due to the increasing environmental exposure to heavy metals, the need for biomonitoring of copper and zinc using sensitive genetic marker becomes paramount to forestall possible outbreak of congenital and genetic anomalies and diseases, since the freshwater fish has recently occupied

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considerable position in local food menu. Therefore, the study was designed to evaluate the genotoxicity of copper, zinc and their binary mixture to the preponderant freshwater fish species, *Synodontis clarias* and *Tilapia nilotica* using the Micronucleus Assay in Fish Genome.

MATERIALS AND METHODS

Synodontis clarias and *Tilapia nilotica* of similar live weight were obtained from the commercial hatchery and kept in aquaria of 60L capacity, where they remained until acclimation. Water of high quality was used as their medium. The water in the aquarium was changed daily and fish were fed trice a day with commercial feed food.

The concentrations of Cu and Zn were chosen based on studies of Svecevičius and Vosylienė (1996) and Svecevičius (1999) cited in Bagdonas and Vosyliene (2006), which indicated that the 96-hour LC_{50} of copper was 0.65mg/l and the 96 –hour LC_{50} of zinc was 3.79mg/l. Chemically pure salts of copper sulphate ($CUSO_4.5H_20$) and zinc sulphate ($ZnSo_4.7H_2O$) dissolved in distilled water were used as toxicants. Expressions of the three metal concentrations studied are shown in Table 1. Fish were divided into exposure groups for every concentration studied. Fish in twenties were exposed to copper and zinc, forty fish to the mixture of metals, and twenty fish were kept in clean water as control. After a 96 –hour exposure, twenty fish from the metal mixture exposed group were transferred to clean (metal free) water for recovery for the next 96 – hours. After a 96 –hour exposure, fish under study and control ones were caught and blood was taken from the incision of caudal vessels for the micronucleus test.

Micronucleus Test: Fish were caught and blood samples collected. The blood smears were obtained through the incision of caudal vessels. The slides were then, air-dried for 24h, fixed in methanol for 10min, followed by 10% Giemsa (v/v) staining. 1000 erythrocytes of each fish were examined from the blood. To determine micronuclei in erythrocytes, the slides were examined using oil-immersion (x 1000). For the scoring of micronuclei, the following criteria were adopted from Fenech *et al.,* (2003); the diameter of the micronucleus (MN) should be less than one-third of the main nucleus; MN should be separated from or marginally overlap with main nucleus as long as there is clears identification of the nuclear boundary; and MN should have similar staining as the main nucleus. Statistical analysis was performed using Analysis of Variance (ANOVA) to portray the significance of the micronuclei frequency formed at various concentrations and bar charts to show its distribution. The limit of significance was settled down with alpha error of 0.05.

RESULTS AND DISCUSSION

The frequencies of the micronuclei are shown in Table 2 and 3 and Figure1 and 2 while Figure 3 displays the photomicrograph of the micronuclei formed in the fish species employed in the study. The highest levels of micronuclei ($p<0.05$) were induced in fish species exposed to Cu and Zn metal mixtures, especially in groups exposed to 0.25 and 0.0625 96-hour LC_{50} concentrations. Significantly ($p<0.05$) increased frequencies of micronucleated erythrocytes were also found in fish exposed to the highest concentrations of Cu (0.16mg/l) and Zn (0.948mg/l) and lowest concentration of Zn (0.238mg/l). After a 96-hour recovery of fish species exposed to a mixture of metals, there was a significant ($p<0.05$) decrease in micronuclei levels of *Tilapia nilotica* while *Synodontis clarias* displayed no meaningful decrease (Table 2 and 3 and Figure 1 and 2).

Table 1 Concentration of Cu, Zn and their Binary Mixture Studied

Metals	Concentrations	
	Part of LC_{50}	Mg/l
Cu	0.25	0.16
Zn	0.25	0.948
Mixture of Cu and Zn	0.25+0.25	0.16+0.948
Cu	0.125	0.08
Zn	0.125	0.474
Mixture of Cu and Zn	0.125+0.125	0.08+0.474
Cu	0.0625	0.04
Zn	0.0625	0.238
Mixture of Cu and Zn	0.0625+0.0625	0.04+0.238

Source: Bagdonas and Vosyliene (2006)

Table 2 Variation in micronucleus frequency induced by Cu and Zn acting singly and in binary mixture against *Synodontis clarias*

Metals	*Synodontis clarias*		
	$0.25LC_{50}$	$0.125LC_{50}$	$0.0625LC_{50}$
Cu	2.50±1.8*	1.80± 0.2	2.00± 1.4
Zn	3.00± 0.2*	1.10± 0.7	2.80± 1.2*
Cu + Zn	3.70± 2.4*	2.40± 0.3	3.50± 2.1*
Cu + Zn 96-hr in clean water	3.50± 2.2$^{\$}$	2.30± 1.6$^{\$}$	3.30± 1.5$^{\$}$
Control	0.90± 0.3		

* = Significantly different (p<0.05); $ = Not significantly lower (p>0.05), after 96-hr in clean water

Many earlier authors in different parts of the world including Nigeria (Vosyliene and Svecevičius, 1995; Vosyliene *et al*, 2003; Kazlauskienė *et al.,* 2003; Kazlauskienė and Burba, 1997; Otitoloju, 2001; Oyewo, 1998) have similarly observed and recorded differential toxicity of heavy metal compounds against different test organisms. The observed differential toxicity of heavy metals can be attributed to several factors such as the type of heavy metal tested, solubility of the compound, predominant ions in test solution, physico –chemical characteristics of the test solution and the mechanism(s) of action of the different metals. All of these factors determine the availability, including the penetrability of the metals into the test animals and hence, their toxicity. Other factors which may affect metal toxicity or susceptibility of test animals include the formation of complexes with protein (e.g. metallothionien), metabolism and excretability; all of which are expected to be considerably different between the test organisms and heavy metal types tested in this work.

Table 3 Variation in micronucleus frequency induced by Cu and Zn acting singly and in binary mixture against *Tilapia nilotica*

Metals	*Tilapia nilotica*		
	$0.25LC_{50}$	$0.125LC_{50}$	$0.0625LC_{50}$
Cu	$0.25LC_{50}$	1.60± 1.8	1.90± 1.2
Zn	2.20±1.4*	0.80± 1.5	2.40± 2.0*
Cu + Zn	2.70± 1.6*	1.90± 1.3	3.10± 1.4*
Cu + Zn 96-hr in clean water	0.90± 0.8#	0.80± 0.7#	1.00± 1.7#
Control	0.60± 0.2		

** = Significantly different (p<0.05); # = Significantly lower (p<0.05), after 96-hr in clean water*

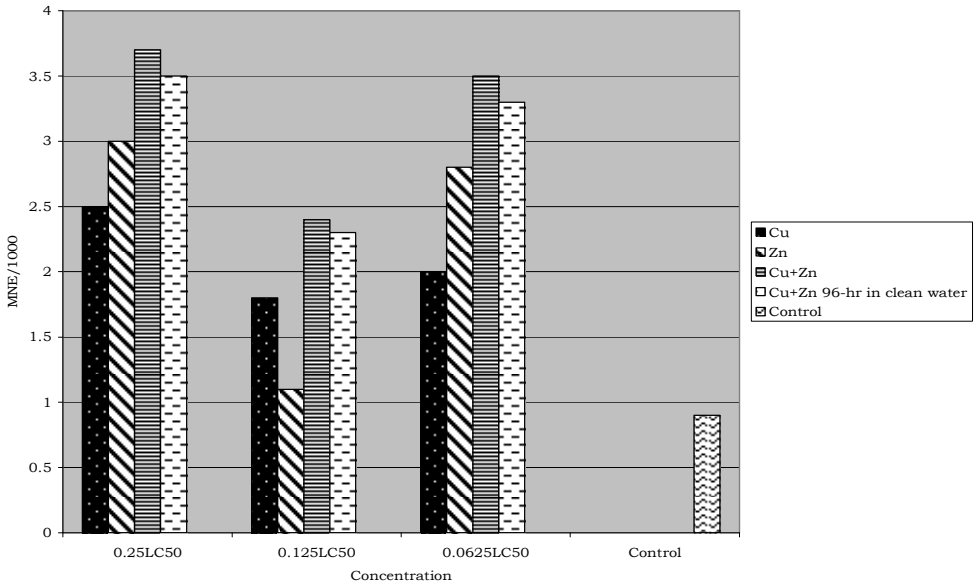

Fig 1. Frequency of micronucleated erythrocytes in blood of *Synodontis clarias* exposed to three concentrations of Cu, Zn and their binary mixture.

In our study, the micronucleus test showed the potential genotoxicity of Cu, Zn and their binary mixture to *Synodontis clarias* and *Tilapia nilotica*. Earlier observations on the genotoxicity of Cu have been reported by Bagdonas and Vosyliene (2006) and Guecheva *et al.* (2001). Guecheva *et al.* (2001) elucidated the genotoxicity of copper sulphates to planaria by means of comet assay, discovering elevated levels of DNA strand breakage and inhibition of DNA repair of planaria preexposed to methylmethan sulphonate. This inhibition of DNA repair enzymes according to the authors could be

caused by a non-specific binding of Cu^{2+} cations to essential sites in the enzyme molecule. This reflects the formation of micronuclei due to this DNA damage and inhibition of repair by Cu compound. One of the possible paths of copper genotoxicity is induction of oxidative stress and production of DNA damaging reactive oxygen species (Gabbianelli *et al.,* 2003). Significant ($p<0.05$) levels of micronuclei frequencies were observed in the fish species exposed to both highest and lowest concentrations of Zn (Table 2 and 3 and Figure 1 and 2), contrasting Bagdonas and Vosyliene (2006), who only recorded significant levels of micronucleated erythrocytes (MNE) in freshwater fish, rainbow trout with similar lower concentration to our study. Going by the same authors, they documented that comparative analysis of micronucleated erythrocyte levels of all concentrations studied did not display any significant difference ($p<0.05$), which in effect confirms our observation that there was no dose-dependent changes in micronuclei frequencies induced by highest and lowest concentrations of Zn ($p<0.05$). Consequently, the micronucleus values obtained (Table 2, 3 and Figure 1 and 2) were slightly higher than the ranges reported by Bagdonas and Vosyliene (2006) for Rainbow trout using similar micronucleus test. These observations could be due to the age and species of fish used, which may have resulted in biological-response variability and subsequent micronuclei frequency differentiations. After a 96-hour recovery of the two species of fish exposed to a mixture of metals, *Tilapia nilotica* showed a significant decrease ($p<0.05$) while *Synodontis clarias* displayed no meaningful decrease ($p>0.05$) in its micronuclei frequency. This might be due to an efficient metal detoxification and excretion from *Tilapia nilotica*, while *Synodontis clarias* lacks those mechanisms of metal removal. However, these observations could also be explained by the incessant capacity of *Synodontis clarias* recording higher values of micronuclei profile than *Tilapia nilotica* in our work, showing *Synodontis clarias* to be hypothetically, a good bioaccumlator of toxicants and that its genome well tolerates such cytogenetic damage (micronucleus formation) without apoptosis. From the micronucleus assay data, we could make assumptions that Cu and Zn act as aneugens, they induce aneuploidy (change in chromosome number-Hartwell *et al*., 2000) resulting in micronucleus formation and/or can result in chromosomal rearrangement (Hartwell *et al*., 2000).

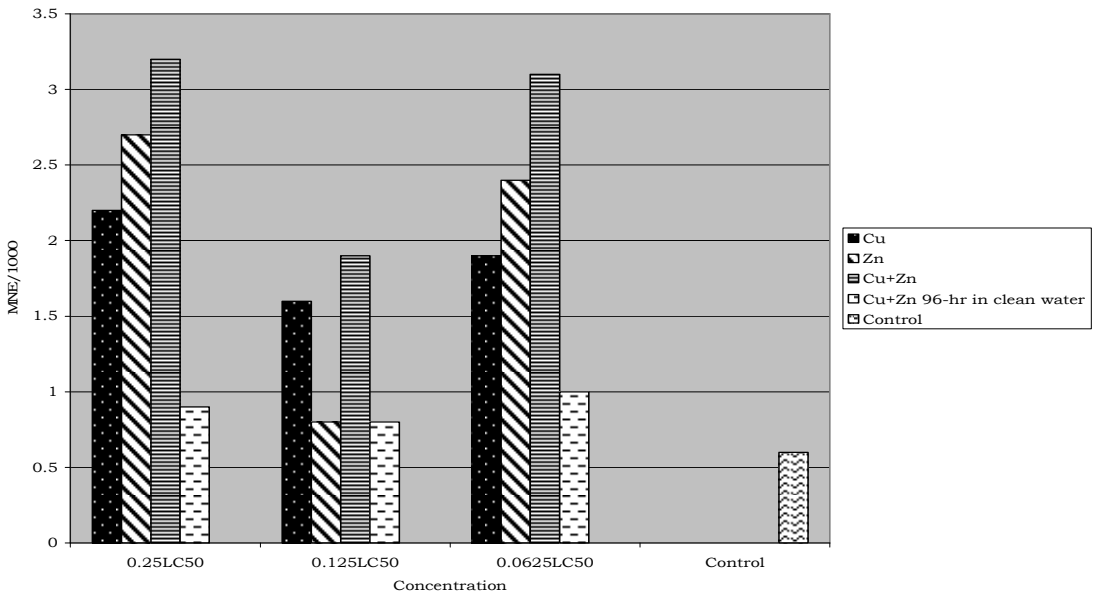

Fig 2. Frequency of micronucleated erythrocytes in blood of *Tilapia nilotica* exposed to three concentrations of Cu, Zn and their binary mixture.

Several studies have assessed the genotoxicity of copper sulphate following oral or parenteral exposure in various multicellular cells. Positive results have been found in studies testing for DNA damage *in vitro* in multicellular organisms. Errors in DNA synthesis by viral DNA polymerase (Sirover and Loeb, 1976), a reduction in DNA synthesis (Garrett and Lewtas, 1983; Sirover and Loeb, 1976), and an

increase in the occurrence of DNA strand breaks (Sideris *et al.,* 1988; Sina *et al.,* 1983) have been observed. The increased in sister chromatid exchange in Chinese hamster cells (Sideris *et al.,* 1988) is consistent with the clastogenic effects observed in *in vivo* assays. There are also indications of weak clastogenic effects following zinc exposure. A dominant lethal study in mice failed to show a mutagenic potential for zinc. However chromosomal aberrations have been observed in bone marrow cells following *in vivo* exposure to zinc (Vilkina *et al.,* 1978). This effect was observed in rats exposed to 14.8mg zinc/kg/day as zinc chlorate in drinking water (kowalska-Wochna *et al.,* 1988), mice given intraperitoneal infections of 3.6mg zinc/kg/day as zinc chloride (Gupta *et al.,* 1991), and mice exposed to zinc oxide by inhalation (Voroshilin *et al.,* 1978), chromosomal aberrations caused by zinc were also observed in the bone marrow cell of mice maintained on a low calcium diet (Deknudt and Gerber, 1979). Calcium may be displaced by zinc in calcium-depleted conditions, leading to chromosome breaks and/or interfering in the repair process (Deknudt and Gerber, 1979). *In vivo* exposure of zinc also resulted in single strand breaks, as measure by the comet assay in mice (Banu *et al.,* 2001). An increased incidence of sister chromatid exchange was observed in bone marrow cells of rats exposed to 17.5mg zinc/kg/day as zinc chlorate in drinking water (Kowalska-Wochna *et al.,* 1988).

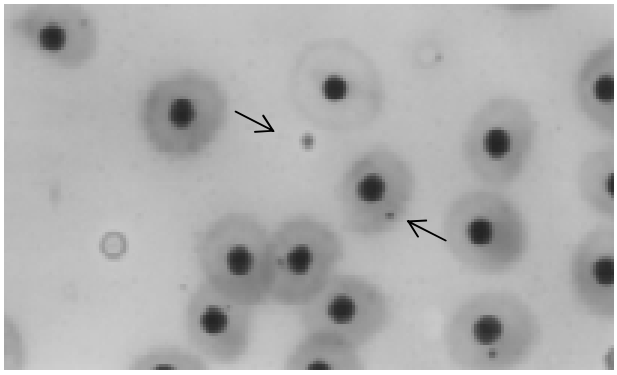

Fig 3. Photomicrograph showing micronucleated erythrocytes (arrows) in fish species after treatment with binary mixture of Cu and Zn.

The establishment of the sensitivity scale of test fish species could serve as useful environmental management tool, for instance, in the derivation of safe limits of pollutants in water bodies meant for the protection of aquatic organisms; the genotoxicity index e.g. LC_{50} values of the test compounds against the most sensitive test organism are utilized in the determination of a minimum adverse concentration or a no effect level, from which safe limits are extrapolated (Mason, 1991). This safe limits or criteria are then employed in fixing realistic industrial effluent and water body limitation standards and guidelines. The study showed genotoxic effect of single and binary mixture of Cu and Zn, even at short exposure and its non dose-dependency. There was a significant departure when the toxicity levels of the mixtures were compared to the toxicity levels of the individual metals when acting alone against the test species. Further work with toxicity testing at higher concentration on fish will be very useful in assessing possible ecological risk of heavy metals.

REFERENCES

ATSDR (Agency for Toxic Substances and Disease Registry) (1990): *Toxicological Profile for Zinc*, ATSDR/TP.

Bagdonas E. and Vosyliene, M.Z (2006). A Study of Toxicity and Genotoxicity of Copper, Zinc and their Mixture to Rainbow Trout (*Oncorhynchus mykiss*). *Biologija*, 1: 8-13.

Banu, B.S., Devi, K.D and Mahboob, M, et al. (2001). *In Vivo* Genotoxic Effect of Zinc Sulfate in Mouse Peripheral Blood Leukocytes Using Comet Assay. *Drug Chem Toxicol*, 24(1): 63-73.

Chukwu, L.D. (1991). *Studies on Heavy Metal Contamination of Water, Sediment and Decapod Crustaceans from River Sasa.* Ph.D Thesis University of Lagos, 164p.

Deknudt, G and Gerber, G.B (1979). Chromosomal Aberrations in Bone-Marrow Cells of Mice given a Normal or a Calcium-deficient Diet Supplemented with various Heavy Metals. *Mutat Res,* 68: 163-168.

Don-Pedro, K.N (1996). Investigation of Single and Joint Fumigant Insecticidal Action of Citrus Peel Oil Components. *Pestic. Sci*, 45: 79-84.

DWAF (1996). South Africa Water Quality Guidelines, vol. 7, *Aquatic Ecosystem* 1st edition, Department of Water Affairs and Forestry.

Enserink, E.L., Maas-Dicpeven, J.L. and Van-Leeuwen, C.J. (1991). Combined Effects of Metlas: An Ecotoxicological Evaluation. *Wat. Res*., 25 (5): 579-687.

Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland, N., Bonassi, S. and Zeiger, E. (2003). Human Micronnucleus Project. HUMN Project: Detailed Description of the Scoring Criteria for the Cytokinesis-block Micronucleus Assay Using Isolated Human Lymphocyte Cultures. *Mutat. Res. 534* (1-2): 65-75.

Garrett, N.E and Lewtas, J (1983). Cellular Toxicity in Chinese Hamster Ovary Cells Culture I. *Environ Res*, 32: 455-465.

Guecheva, T., Henriques, J.A.P and Erdtmann, B (2001). Genotoxic Effects of Copper Sulphate in Freshwater Planarian *In vivo*, Studied with Single- Cell Gel Test (Comet Assay). *Mutation Research*, 497: 19-27.

Hartwell, L.H; Hood, L; Goldberg, M.L; Reynolds, A.E; Silver, L.M and Veres, R.C. (2000). Genetics: from Genes to Genomes. McGraw Hill Higher Education. ISBN 0-07-540923-2. Pp. 70-98, 144-169, 179-182, 341-351.

Kazlauskienė, N and Burba, A (1997) *Ekologija*, 2: 7–11.

Kazlauskienė, N., Marčiulionienė, D and Montvydienė, D (2003). *Environ Chem Physics* 25(3): 116–23.

Kowalska-Wochna, E., Moniuszko-Jakoniuk, J and Kulikowska, E, *et al.* (1988). The Effect of Orally Applied Aqueous Solutions of Lead and Zinc on Chromosome Aberrations and Induction of Sister Chromatid Exchanges in the Rat (*Rattus sp*.) *Genetica Polonica*, 29(2): 181-189.

Mason, C.F. (1991). Biology of Freshwater Pollution. 2nd Edition; Longman New York. 351 Pp.

Merian E. (1991). Metals and their Compounds in the Environment, Occurrence Analysis and Biological Relevance. UCH, Weintrein-New York.

Ogunsua, A.O., Adebona, M.B., Ako-Nai, K.O., Oluwole, A.F., Ruck, W. and Oyewo, E.O. (1991). Metals in the Lagos Lagoon. In: *Proceedings of International Conference on Heavy Metals in the Environment*, Toronto, Canada, September, 1983 (R.J. Allan & J.O. Nriagu Eds): 165-168.

Otitoloju, A.A. (2001*). Joint Action Toxicity of Heavy Metals and their Bioaccumulation by Benthic Animals in the Lagos Lagoon*. Ph.D Thesis, University of Lagos. 231p.

Oyewo, E.O (1998). *Industrial Sources and Distribution of Heavy Metals in Lagos Lagoon and their Biological Effects on Estuarine Animals*. Ph.D Thesis, University of Lagos, Nigeria, 230-274pp.

Sanders M.J. (1997). *A Field Evaluation of Freshwater River Crab, Potamonautes warreni, as a Bioaccumulative Indicator of Metal Pollution*.Thesis, Rand Afrikaans University, South Africa.

Sideris, E.G., Charalambous, S.C, Tsolomyty, A (1988). Mutagenesis, Carcinogenesis and the Metal Elements - DNA Interaction. *Prog Clin Biol Res*, 259: 13-25.

Sina, J.F., Bean, C.L and Dysart, G.R (1983). Evaluation of the Alkaline Elution/Rat Hepatocyte Assay as a Predictor of Carcinogenic/Mutagenic Potential. *Mutat Res*, 113: 357-391.

Sirover, M.A and Loeb, L.A (1976). Infidelity of DNA Synthesis *In Vitro*: Screening for Potential Metal Mutagens or Carcinogens. *Science,* 94: 1434-1436.

Svecevičius, G and Vosylienė, M.Z (1996). *Ekologija* (2): Pp. 17– 21 In: Bagdonas E. and Vosyliene, M.Z (2006). A Study of Toxicity and Genotoxicity of Copper, Zinc and their Mixture to Rainbow Trout (*Oncorhynchus mykiss*). *Biologija*, 1: Pp. 8-13.

Svecevičius, G (1999). *Acta Zool Lituanica Hydrobiol*, 9(2): Pp. 114–8 In: Bagdonas E. and Vosyliene, M.Z (2006). A Study of Toxicity and Genotoxicity of Copper, Zinc and their Mixture to Rainbow Trout (*Oncorhynchus mykiss*). *Biologija*, 1: 8-13.

Vilkina, G.A., Pomerantseva, M.D and Romaiia, L.K (1978). Lack of Mutagenic Activity of Cadmium and Zinc Salts in Somatic and Germ Mouse Cells. *Genetika (Moscow),* 14: 2212-2214.

Voroshilin, S.I., Plotko, E.G and Fink TV, et al. (1978). Cytogenetic Effects of Inorganic and Acetate Compounds of Tungsten, Zinc, Cadmium, and Cobalt in Animal and Human Somatic Cells. *Tsitol Genet*, 12(3): 241-243.

Vosylienė, M.Z and Svecevičius, G (1995). Fish Physiology, Toxicology, and Water Quality. *Proceedings of the Fourth International Symposium, Bozeman*, Montana, USA, September 19–21, 1995. EPA/600/R-97/098, 1997: 141–51.

Vosylienė, M.Z, Kazlauskiene, N and Sveceviius, G (2003). *Environ Sc and Pollut Res*, 10 (2): 103–107.