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Cytogenetic Studies on the Effect of Copper Sulfate and Lead Acetate Pollution on *Oreochromis niloticus* Fish

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Abstract: The present study is carried out in order to explore the capability of copper sulfate (CuSO₄) and lead acetate (CHCOO)₃ Pb in inducing chromosomal aberrations in aquatic organisms. To achieve such a purpose, Oreochromis niloticus was chosen as a test material for the study. The LC_{50} of the two chemicals was determined and the data indicated that LC_{50} of copper sulfate (CuSO₄) and lead acetate (CHCOO)₃ Pb were 40.6 and 422.5 mg L^{-1} , respectively. The effect of both chemicals on fish chromosomes and mitotic indices was investigated and the results revealed that gill cells of the treated fish by copper sulfate and lead acetate displayed lower mitotic activity than that of the control group, both pollutants were found to be positive inducer of macro-DNA damage which represented by different types of aberrations e.g., chromatid deletions, chromatid breaks, gaps, fragments, stickness, translocations, ring chromosomes and centromeric attenuation. We also observed that chromatid deletion, stickness and fragments were more frequent than other chromosomal aberrations.

Key words: O. niloticus, chromosomal aberrations, copper-lead

INTRODUCTION

Chemical pollution in water especially with heavy metals is among the most important health significance for human beings and animals consuming such water due to their toxicity and accumulative behavior playing a prominent role in aquatic ecosystems (De Gregori *et al.*, 1994).

The assessment of biological effects on aquatic vertebrate and invertebrate species is frequently employed to monitor water pollution because it provides meaningful information on bioavailability and effective concentration levels. Of special concern are genotoxic agents that induce DNA alterations at sub toxic exposure levels. Clastogenic (chromosome breaking) compounds are responsible for altered reproductive outcomes, genetic diseases and cancer (Bunton, 1999).

Oreochromis niloticus is considered as one of the most commercial and common freshwater fish species. Various species of tilapia have been favored by many earlier workers as test models for both cytogenetic and molecular studies (Martins *et al.*, 2000, 2002).

Lyne *et al.* (1992) stated that increased contamination of the environment by toxic chemicals has resulted in the need for sensitive assays to be used in risk assessment of polluted sites. Traditional tests are useful to detect and measure concentrations of chemicals in the environment and in tissues. However, physicochemical assays possess deficiencies that impair their use in evaluating complex

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environmental contamination. They have developed cytogenetic procedures, including chromosomal, micronucleus and flow cytometric assays, to assess the mutagenic damage of petrochemicals and low-level radioactivity on indigenous terrestrial and aquatic wildlife populations. These procedures are sensitive to the perturbation of DNA that results from exposure to mutagenic contaminants in both field and laboratory studies. The use of natural populations of animals in biomonitoring, combined with traditional chemical assays, will ultimately provide sufficient information to estimate the risk to human health and environmental quality from anthropogenic pollution.

In order to investigate the capability of copper sulfate and lead acetate to induce mutagenic effects in aquatic organisms. *Oreochromis nilotica* fish were chosen and employed. This study was carried out to the Determination of the acute lethal concentration $LC_{50}/72$ h of copper sulfate and lead acetate and to investigation of the effect of both pollutants on fish chromosomes, at different exposure intervals, at various concentrations levels.

MATERIALS AND METHODS

A total number of 300 apparently healthy fish namely *Oreochromis niloticus* were obtained from Al-Abbassa fish farm (Abo Hammad, Al-Sharkia province, Egypt). Fish were acclimatized to laboratory condition for at least two weeks before experiments during which they fed twice daily on the fine fish commercial diet.

Determination of the Acute Lethal Concentration Dose (LC $_{50}\!/72$ h) for Copper Sulfate and Lead Acetate

The $LC_{50}/72$ h Was calculated according to the standard procedure of Weil (1952) by the following equation.

$$Log LC_{so} = Log Da + d (f+1) For K = 3$$

Cytogenetic Analysis of Chromosomal Abnormalities in Gills of Oreochromis niloticus

The fish were treated with three doses of copper sulfate and lead acetate: 1/5th, 1/10th and 1/20th of the previously determined LC_{50} . The cytogenetic effect of copper sulfate and lead acetate were studied after 2, 4 and 6 weeks of each of the three doses treatment.

Chromosomal Preparation

Chromosome preparations were made following the procedure described by Bertolla et al. (1978).

Mitotic Index

Mitotic indices of control and treated fish (*Oreochromis niloticus*) groups were calculated according to the equation:

$$MI = \frac{No. \text{ of metaphase cells}}{Total \text{ No. of cells}} \times 100$$

where, the number of examined metaphase cells represented the mitotic division due to injection of colchicine substance which blocked the mitotic division at metaphase stage.

Statistical Analysis

An ANOVA included the main effects of treatment, concentration and interval. Data were analyzed using SAS Statistical Analysis System Package (Littell et al., 1991). Significant

differences between each two means were evaluated utilizing Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

RESULTS AND DISCUSSION

According to the equation of Weil (1952) the $LC_{50}/72$ h of copper sulfate and lead acetate for *Oreochromis nilotica* were 40.6 and 422.5 mg L^{-1} , respectively.

Cytogeneic Investigation

Mitotic Index

The obtained results of mitotic activity of gill filaments of fish (*Oreochromis niloticus*) treated with copper sulfate and lead acetate for three different concentrations and exposure times where found to be lower than that of control group (Table 1). Such a result is expected, since gills represent the first target for the aquatic contaminants. This result was agreed with that of Raid and Hafez (1996) investigated the effect of pollution upon the genetic material of aquatic organisms. In order to achieve such a purpose, *Tilapia nilotica* and *Tilapia zillii* were chosen and caught from two regions varying in their environmental stress; the first is River Nile and the second is a closed drain which receives domestic sewage. Mitotic activity, chromosomal aberrations and *in vivo* induction of sister chromatid exchanges were examined in gills as well as in kidneys. The results obtained revealed that the environmental stress was proven to be capable of causing inhibition of cell proliferation; clastogenic effect and primary DNA damage in gills and kidneys of both species with differential effect.

Chromosomal Aberrations

Examination of chromosome abnormalities after treatment with copper sulfate and lead acetate showed that they capable of inducing macro-DNA damage which represented by different types of aberrations e.g., chromatid deletions, chromatid breaks, gaps, fragments, stickness, translocations, ring chromosomes and centromeric attenuation (Table 2, 3 and Fig. 2-7).

Similar findings are shown by the study of Yadav and Trivedi (2006) that evaluated the genotoxic potential of chromium [Cr(VI)] on aquatic bio-system, bottom feeding fishes, Channa punctata, as model fish, were exposed to [Cr(VI)]. The chromosomal aberration test (CAT) was used as biomarker of [Cr (VI)] induced toxicity. The fish were divided into three groups: Group 1 non-treated controls; group 2 positive controls, treated with an intra-muscular injection of mitomycin-C at 1 mg kg $^{-1}$ b.wt.; group 3 exposed to a sub lethal concentration (7.689 mg L $^{-1}$) of [Cr (VI)], dissolved in the water. For CAT estimation, short term static bioassays were conducted and samples were collected from the kidneys of fish after 24, 48, 72, 96 and 168 h of exposure. The remarkable chromosomal aberrations recorded in the present investigation included chromatid breaks, chromosome breaks, chromatid

 $\underline{\textbf{Table 1:}} \ \textbf{Mitotic index of metaphases from gill filaments of fish treated with copper sulfate and lead acetate}$

		Intervals					
Treatment	Concentration	a	b	С			
Control		27	27	27			
Copper	C_1	16	18	13			
	C_2	21	20	14			
	C_3	26	21	17			
Lead	\mathbf{L}_1	14	21	18			
	L_2	15	15	16			
	L_3	22	23	13			

 C_1 : First concentration of copper sulfate (1/5 LC₅₀), C_2 : Second concentration of copper sulfate (1/10 LC₅₀), C_3 : Third concentration of copper sulfate (1/20 LC₅₀), a: Two weeks exposure time, b: Four weeks exposure time, c: Six weeks exposure time, L₁: First concentration of lead acetate (1/5 LC₅₀), L₂: Second concentration of lead acetate (1/10 LC₅₀), L₃: Third concentration of lead acetate (1/20 LC₅₀)

Table 2: Chromosomal aberrations in fish (O. nilotica) treated with different concentrations of copper sulfate for three different exposure times

No. of						Type of aberration							
		examined	Normal	Aberrant									
Conc.	Interval	cells	cells	cells	Cd	Cb	G	F	St	T	R	CA	
Cont.		200	176	24	11	1	2	5	2	0	0	3	
C_1	a	200	148	52	15	2	9	9	5	2	3	7	
	b	200	132	68	17	4	9	10	13	3	5	7	
	c	200	140	60	17	3	11	12	9	1	2	5	
C_2	a	200	153	47	13	1	4	10	7	2	5	5	
	b	200	146	54	12	2	4	14	13	2	3	6	
	c	200	146	54	13	4	7	14	4	1	3	3	
C_3	a	200	156	44	12	2	6	6	10	1	3	5	
	b	200	152	48	12	2	5	10	12	1	2	5	
	c	200	151	49	11	3	5	9	12	1	2	6	

 $\label{eq:continuous} Cd = Chromatid \ deletion, \ Cb = Chromatid \ break, \ G = Gap, \ \ F = Fragment, \ St = Stickness, \ T = Translocation, \ R = Ring \ chromosome, \ CA = Centromeric \ Attenuation$

Table 3: Chromosomal aberrations in fish (O. nilotica) treated with three different concentrations of lead acetate for different exposure times

No. of					Type of aberration							
		examined	Normal	Aberrant								
Conc.	Interval	cells	cells	cells	Cd	Cb	G	F	St	T	R	CA
Cont.		200	176	24	11	1	2	5	2	0	0	3
L_1	a	200	136	64	19	2	5	12	10	3	4	9
	b	200	122	78	24	4	5	13	13	5	5	11
	c	200	116	83	23	10	11	16	12	0	5	10
L_2	a	200	145	55	19	3	6	12	6	1	3	5
	b	200	134	66	16	2	4	15	21	1	3	5
	c	200	132	68	16	1	5	18	21	2	2	5
L_3	a	200	152	48	19	2	4	12	7	0	2	4
	b	200	144	56	16	2	5	12	11	2	3	5
	c	200	143	57	15	4	8	10	9	3	2	7

 $\label{eq:continuous} Cd = Chromatid \ deletion, \ Cb = Chromatid \ break, \ G = Gap, \ \ F = Fragment, \ St = Stickness, \ T = Translocation, \ R = Ring \ chromosome, \ CA = Centromeric \ Attenuation$

deletions, fragments, acentric fragments and ring and di-centric chromosomes, along with chromatid and chromosome gaps. A significant increase in chromosomal aberrations was observed after 72 h of [Cr (VI)] exposure.

Samanta *et al.* (2005) deals with the measurement of five heavy metals viz., Cd, Cu, Mn, Pb and Zn in water of the rivers Hooghly and Haldi at Haldia during June 1999 to October 2002. The average concentrations of the studied metals were Cd 2-14, Cu 5-19, Mn 8-88, Pb 17-41 and Zn 22-37 microg I(-1). Comparison of the data with the Criterion Continuous Concentration (CCC) of USA revealed that Cd, Cu and Pb were the pollutants present at alarming level to disturb the aquatic life process in the zone. The effect was found to reflect on the tissue level aberrations in the residential fishes. The other two metals viz., Mn and Zn were probably less harmful to the aquatic ecosystem.

Also, Petrova *et al.* (2007) studied the polymorphism of natural populations of Chironomus plumosus from lakes of Kaliningrad with the presence of heavy metals (Pb, Cd, Fe, Zn, Cu, Mn, Ni, Cr, Co) in the ground was investigated. An increase in the number of heterozygous inversions per individual in the reservoir with the highest concentration of heavy metals is revealed. The rare inversions pluA6 and pluF5 were found in the most polluted reservoirs.

The results showed that the total aberrant metaphases in the control group were found to be 12%. It ranged from 26-34, 23.5-27 and 22-24.5% for 1st, 2nd and 3rd concentrations of copper sulfate, respectively. Das and John (1999) studied the genotoxic potential of methyl parathion and phosphamidon, two commercial formulations of organophosphorus pesticides through induction of Sister Chromatid Exchanges (SCE) and chromosome aberrations in fish gill tissues. Fishes exposed to

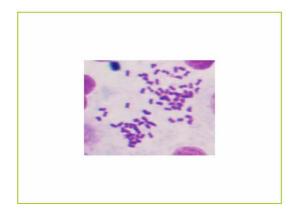


Fig. 1: A photomicrograph shows a normal mitotic metaphase of O. niloticus (Giemsa, x 4000)

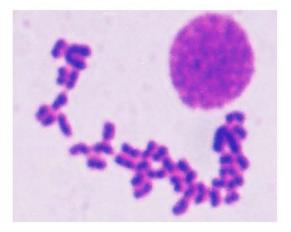


Fig. 2: A photomicrograph shows a mitotic metaphase stage of O. niloticus with chromatid Deletion after treatment with copper (1/10 LC_{50} for 2 weeks) Giemsa, x 400

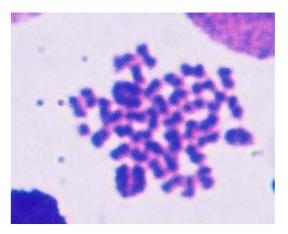


Fig. 3: A photomicrograph shows a mitotic metaphase stage of O. niloticus with gaps after treatment with copper (1/5 LC_{50} for 2 weeks). Giemsa, x 4000

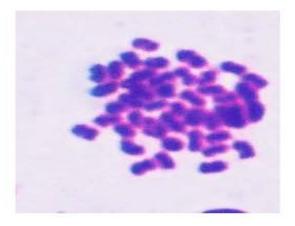


Fig. 4: A photomicrograph shows a mitotic metaphase stage of $\it O.mloticus$ with ring chromosome after treatment with copper (1/20 LC so for 6 weeks) Giemsa, x 4000

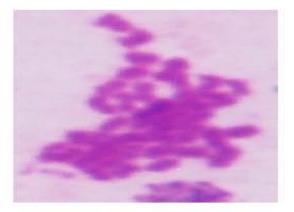


Fig. 5: A photomicrograph shows a mitotic metaphase stage of C ruloticus with stickness after treatment with lead (1/5 LC₅₀ for 4 weeks). Giemsa, x 4000

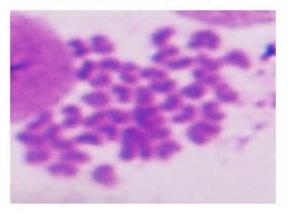


Fig. 6: A photomicrograph shows a mitotic metaphase stage of O. niloticus With chromatid break after Treatment with lead (1/10 LC $_{50}$ for 2 weeks)

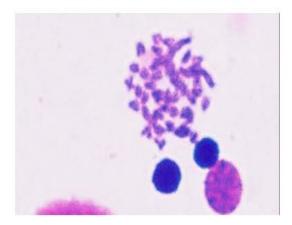


Fig. 7: A photomicrograph shows a mitotic metaphase stage of *O. niloticus* with chromosomal fusion after treatment with lead (1/5 LC₅₀ for 2 weeks)

the medium containing 0.05, 0.1 and 0.2 ppm of methyl parathion or 0.5, 1.0 and 2.0 ppm of phosphamidon for a duration of 96 h revealed significant increase in the number of SCE and chromosome aberrations against control values.

Total aberrant metaphases ranged from 32-41.5, 27.5-34 and 24-28.5% for 1st, 2nd and 3rd concentrations of lead acetate, respectively. This result was agreed with that of Mathew and Jahageerdar (1999). They found that when Channa punctatus were exposed to lead nitrate at the concentration of 0.012 mg L⁻¹. The total metaphase spreads with chromosomal aberrations was significantly higher than compared to control groups at all the three exposure times (96, 120 and 144 h). It was found that as the exposure time increased the percentage of metaphase spreads with chromosomal abnormalities also increased.

Our data recorded that the structural chromosomal aberrations were more observed due to pollution than numerical chromosomal aberrations, although Barsiene et al. (2002) studied cell aneuploidy and polyploidy in somatic cells of bivalves Anodonta cygnea and Unio turnidus and gastropods Viviparus viviparous inhibiting different sites of the Neris River. The amount of cytogenetic injuries in indigenous molluscs from contaminated sites was 1.8-4 times higher than in organisms from the upstream zones. Hypodiploidy of cells was the main type of aneugenic effects in the tissues of the molluscs studied.

Regarding the most prominent types of chromosomal aberrations after exposure to three different concentrations of copper sulfate for three different exposure times were chromatid deletion which ranged from $22.2(C_2b)$ to $28.8(C_1a)$ } and fragments which ranged from $\{13.6(C_3a)$ to $25.9(C_2b)\}$, while translocations were found to be the lowest frequency type of chromosomal aberrations and ranged from $\{1.7(C_1c)$ to $4.4(C_1b)\}$. Concerning the most prevalent types of chromosomal aberrations after exposure to different concentrations of lead acetate for three different exposure times were found to be chromatid deletion which ranged from $\{23.5 (L_2c) \text{ to } 59.5 (L_3a)\}$, stickness was ranged from $\{10.9(L_2a) \text{ to } 31.8 (L_2b)\}$ and fragments were ranged from $\{16.6 (L_1b) \text{ to } 26.4 (L_2c)\}$. While translocation was found to be the lowest chromosomal aberration and ranged from $\{1.5 (L_2b) \text{ to } 6.4 (L_1b)\}$. Chandra and Khuda-Bukhsh (2004) studied the genotoxic effects of cadmium chloride (CdCl₂) and azadirachtin (Aza) singly and conjointly in a fish, *Oreochromis mossambicus*, with endpoints such as chromosome aberrations, abnormal red cell nuclei, abnormal sperm morphology and protein content (both qualitative and quantitative) of selected tissues, namely, muscle, heart, eye, brain,

gill, liver, spleen and kidney. As compared with distilled water-treated controls, both $CdCl_2$ and Aza induced genotoxicity in O. mossambicus, the former in greater quantity than that produced by Aza. However, Cd-induced toxicity in O. mossambicus appeared to be ameliorated to some extent.

Statistical Analysis

Concerning Statistical analysis, Table 4 shows Means±SE of aberrations for control and treated groups of fish with three different concentrations of copper sulfate and lead acetate for three different exposure times. There was significant difference in the rate of aberrations for the treated groups compared with control one. Table 5 shows Means±SE of different types of aberrations for control and treated groups of fish with copper sulfate and lead acetate for different concentrations and exposure times. There was significant difference in stickness for the treated groups compared with control group. The most significant values obtained after treatment with lead acetate ($1/10 \text{ LC}_{50}$ for 4 and 6 weeks) were 1.0 ± 0.58 and 1.67 ± 0.33 , respectively followed by the values obtained after treatment with

Table 4: Mean±SE of aberrations for control and treated groups of fish with different concentrations of copper sulfate and

Variable	Conc.	Interval	No.	Normal	Aberrant
Control			3	58.67±7.54°	8.00±1.53 ^d
Copper	C_1	a	3	49.33±3.84a	17.33±2.33°
		b	3	44.00±5.57°	22.67 ± 2.03 abc
		c	3	46.67±5.78°	20.00±3.61 abc
	C_2	a	3	51.00±6.93°	15.67 ± 3.38 ^{ed}
	_	b	3	48.67±2.73°	18.00±2.00 ^{bc}
		С	3	48.67±5.21°	18.00±3.79bc
	C_3	a	3	52.00±11.50°	14.67±1.76 ^{cd}
		b	3	50.67±0.67a	16.00 ± 1.00^{cd}
		С	3	50.33±2.85°	16.33±2.96 ^{cd}
Lead	$L_{\scriptscriptstyle 1}$	a	3	45.33±8.37 ^a	21.33 ± 2.03^{abc}
	-	b	3	40.67±7.75°	26.00±8.02ab
		С	3	39.00±5.57 ^a	27.67±2.73°
	L_2	a	3	48.33±6.89 ^a	18.33±2.91bc
	_	b	3	44.67±11.39°	22.00±1.53 ^{abc}
		С	3	44.00±13.58°	22.67±1.45 ^{abc}
	L_3	a	3	50.67±2.03°	16.00 ± 1.15^{cd}
	-	b	3	48.00±5.86°	18.67±2.40bc
		С	3	47.67±4.67a	19.00±1.53bc

Means having the same letter(s) in the same column within each class does not differ significantly (p>0.05)

Table 5: Mean±SE of the different types of aberrations in control and treated groups of fish

Variable	Conc.	Interval	No.	Cd	Cb	G	F
Control			3	3.67 ± 0.33^{d}	0.33 ± 0.33^{b}	0.67±0.33ª	1.67±0.88°
Copper	C_1	a	3	$5.00\pm0.58^{\text{cd}}$	0.67 ± 0.33^{b}	3.00 ± 0.58^a	3.00 ± 0.58^a
		b	3	$5.67 \pm 0.33^{\text{abcd}}$	1.33 ± 0.33^{b}	3.00±0.58°	3.33 ± 0.88^a
		c	3	$5.67\pm0.89^{\rm abcd}$	1.00±0.586	3.67 ± 0.88^a	4.00±0.58°
	C_2	a	3	$4.33\pm0.89^{\rm cd}$	0.33 ± 0.33^{b}	1.33 ± 0.67^{a}	3.33 ± 0.33^a
		b	3	4.00 ± 0.58^{cd}	0.67 ± 0.33^{b}	1.33 ± 0.67^a	4.67±0.88°
		c	3	4.33±0.67 ^{cd}	1.33 ± 0.33^{b}	2.33 ± 0.33^a	4.67±0.88°
	C_3	a	3	$4.00\pm1.15^{\rm cd}$	0.67 ± 0.33^{b}	2.00 ± 0.58^a	2.00 ± 0.58^a
		b	3	4.00 ± 0.58^{cd}	0.67 ± 0.33^{b}	1.67 ± 0.33^a	3.33 ± 0.33^a
		c	3	3.67 ± 0.89^{d}	1.00±0.586	1.67 ± 0.33^a	3.00±0.58°
Lead	L_1	a	3	6.33±0.33abc	0.67 ± 0.33^{b}	1.67 ± 0.33^a	4.00 ± 0.00^a
		b	3	8.00±1.73°	$1.33\pm0.89^{\circ}$	1.67±1.20°	4.33 ± 1.86^a
		c	3	7.67 ± 0.89^{ab}	3.33 ± 0.33^a	3.67 ± 1.76^a	5.33 ± 1.86^a
	L_2	a	3	6.33 ± 0.89^{abc}	1.00 ± 0.58^{b}	2.00 ± 0.58^{a}	4.00 ± 1.00^a
		b	3	5.33 ± 0.89^{bcd}	0.67 ± 0.33^{b}	1.33 ± 0.33^a	5.00±0.58°
		c	3	5.33±0.89 ^{bcd}	0.33 ± 0.33^{b}	1.67 ± 0.33^a	6.00±0.58°
	L_3	a	3	6.33 ± 0.89^{abc}	0.67 ± 0.33^{b}	1.33 ± 0.33^a	4.00 ± 0.58^a
		b	3	5.33±1.20bcd	0.67 ± 0.33^{b}	1.67±0.33ª	4.00±0.58°
		c	3	5.00±0.58°d	1.33±0.33 ^b	2.67±0.33ª	3.33±0.33ª

Table 5: Continued

Table 5. C	onunueu						
Variable	Conc.	Interval	No.	St	T	R	CA
Control			3	0.67±0.67e	0.00 ± 0.00^a	0.00 ± 0.00^a	1.00±0.58°
Copper	C_1	a	3	$1.67\pm0.88^{\rm cde}$	0.67 ± 0.33^a	1.00 ± 0.58^a	2.33 ± 0.33^a
		b	3	4.33±0.886	1.00 ± 0.00^a	1.67 ± 0.33^a	2.33 ± 0.33^a
		c	3	$3.00\pm1.53b^{cde}$	0.33 ± 0.33^a	0.67 ± 0.33^a	1.67 ± 0.88^a
	C_2	a	3	$3.00\pm0.58^{\text{bcde}}$	0.67 ± 0.33^a	1.67±0.88°	1.33 ± 0.67^{a}
		b	3	4.33±1.20 ^b	0.67 ± 0.67^a	1.33 ± 0.33^a	2.00 ± 0.58^a
		c	3	1.33 ± 1.33^{de}	0.33 ± 0.33^a	1.00±0.58°	1.00 ± 1.00^a
	C_3	a	3	3.33 ± 0.33^{bcd}	0.33 ± 0.33^a	1.00±0.58°	1.67 ± 0.88^a
		b	3	4.00 ± 0.58^{bc}	0.33 ± 0.33^a	0.66 ± 0.66^a	1.67 ± 0.33^a
		c	3	4.00 ± 0.58^{bc}	0.33 ± 0.33^a	0.66 ± 0.33^a	1.67 ± 0.88^a
Lead	L_1	a	3	3.33 ± 0.88^{bcd}	1.00 ± 0.58^a	1.33 ± 0.33^a	3.00 ± 0.58^a
		b	3	4.33±0.886	1.67 ± 0.33^a	1.67 ± 0.67^a	3.67 ± 0.88^a
		c	3	4.00 ± 1.73^{bc}	0.00 ± 0.00^a	1.67±0.88°	3.33 ± 0.88^a
	L_2	a	3	2.00 ± 0.58^{bcde}	0.33 ± 0.33^a	1.00±0.58°	1.67 ± 0.88^a
		b	3	7.00±0.58 ^a	0.33 ± 0.33^a	1.00±0.58°	1.67 ± 0.88^a
		c	3	7.67±0.33°	0.67 ± 0.67^a	0.67 ± 0.33^a	1.67 ± 0.88^a
	L_3	a	3	$2.33\pm0.88b^{cde}$	0.00 ± 0.00^a	0.67 ± 0.33^a	1.33 ± 0.88^a
		b	3	$3.67\pm0.88b^{cd}$	0.67 ± 0.33^a	1.00 ± 0.58^a	1.67 ± 0.33^a
		c	3	3.00±0.58bcde	1.00±0.58 ^a	0.67 ± 0.33^a	2.33 ± 0.33^a

Means having the same letter(s) in the same column within each class does not differ significantly (p>0.05)

copper sulfate $1/10~LC_{50}$ for 4 weeks (4.33 ± 1.20) and lead acetate $1/5~LC_{50}$ for 4 weeks (4.33 ± 0.88) , respectively. The most significant value in case of chromatid deletion was obtained after treatment with lead acetate $1/5~LC_{50}$ for 4 weeks (8.0 ± 1.7) while the least significant value was obtained after treatment with copper sulfate $1/20~LC_{50}$ for 6 weeks (3.67 ± 0.89) . In case of chromatid break, the only significant value was obtained after treatment with lead acetate $1/5~LC_{50}$ for 6 weeks (3.33 ± 0.3) .

This research concluded that the water pollution especially with heavy metals have clastogenetic effect on fishes exposed to it which may lead to health risks among humans through chronic consumption of such fishes. This work also recommends the use of *Oreochromis niloticus* in order to assess the extent of pollution in aquatic environment.

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