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Resistance to Salts of Two Heavy Metals by *Batanga lebretonis* Obtained from Effluent Pathways in Lagos State, Nigeria

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Abstract: The toxicity of mercury and lead chloride against *Batanga lebretonis* was evaluated in laboratory bioassays. The aim of the study was to determine the toxicity tolerance of *Batanga lebretonis* so as to use determine the possibility of the fish being used as indicator of heavy metal pollution in acquatic environment. The fish were obtained from effluent pathways and brought to the laboratory in healthy conditions and kept in well designed culture tanks containing improvised feed of balanced nutrients. Concentrations of the metallic salts were prepared according to standard methods and introduced to the tanks containing the fish. Effect of acute lethal concentration of mercury chloride and lead chloride were also assessed in static bioassays. It was found that mercury chloride was 1.295 times more toxic to *Batanga lebretonis* than lead chloride. When the concentration of each chemical was subjected to statistical analysis using PROBIT and ANOVA test, it was observed that there were significant differences between the concentrations at ($P<0.05$). The toxicity tolerance of *Batanga lebretonis* to lead offers an indicator that can be used to assess lead salt pollution in the aquatic environment. These observations now form the basis of the present discussion.

Key words: Effluent · Lead · Mercury · Nutrients · Pollution

INTRODUCTION

The present magnitude of the world's population demands the increased application of technology to explore and exploit natural resources, produce more food, goods and services. This situation translates into increased industrialization, which inevitably results in the release of varied types and amounts of industrial wastes into the environment. These industrial wastes are complex mixtures of several classes of pollutants including synthetic chemicals of various descriptions, hydrocarbons, as well as the class referred to as "Heavy metals". Heavy metals are those with densities greater than $5gcm^{-3}$ and atomic number greater than that of cadmium. When such materials and or energy are disposed into the environment in such quantities that can cause deleterious effects to living resources and structure whose preservation is desirable, pollution is said to occur [1,2].

According to Gray and Vennjamer [3], heavy metals are an important source of pollution not just because they are toxic above a relatively low concentration but also because they are persistent, remaining in the environment long after the source of pollution has been removed. Many studies related to heavy metal pollution have been carried out in the industrialized countries of Europe, America and Asia. However, such studies in developing countries-including Nigeria are more recent with considerable information gaps in their occurrence pattern and biological effects on local species. In his book fish and river pollution, Taylor [4] asserted that pollution of rivers with effluent containing chemicals whether metallic or otherwise affects developmental stages of fish and reduces the number of catch by fishermen.

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As a result of industrial effluent discharge, Fadeke and Okoya [5] estimated that up to 600 tones of mercury are introduced into Minamata Bay in Japan annually. Gate (6) also reported that mercury is released into Swansea Bay along with industrial effluents. According to Flower [7], there is toxicity and other environmental problems associated with cadmium-containing effluent discharged into the environment. Similarly, Omanayi *et al*. [5] analyzed industrial effluence from Lagos, Ogun, Kwara and Sokoto State and found that they contained heavy metals ranging in concentration from Nil to 15.4, 4.2, 6.6 and 8.3 ppm respectively for Zn, Cu, Fe and Mn. The rate of entry of heavy metal pollutants into natural waters in Nigeria is therefore still largely unknown. Adebayo *et al*. [8] also described heavy metal concentrations' in Lagos lagoon sediments and biota without relating the measured levels to provenance and demonstrable biological effects while Chukwu [9] reported heavy metals levels in Shasha River. There are also a few reports on the biological effects of some heavy metals against local fauna without relating the derived level of toxicity to the local concentration and distribution of metal species in the aquatic environment. The biological effects of some selected heavy metals in terms of distribution and concentration can be meaningfully undertaken to facilitate proper control and management. Ilori [10] in his emphasized the role industries play in the pollution of the environment with special reference to bodies of water with chemicals containing heavy metals and their derivatives.

Industrial effluents containing methyl mercury when channelled into our stream, river....etc pollute the water and make it unfit for domestic use and consumption. A case of a small town in Mina Mata in Japan in the 1950s was recorded when people were poisoned by the consumption of fish containing high levels of methyl mercury from effluent discharged from a textile industry that use methyl chloride as catalyst. Several cases of wildlife poisoning from seeds treated with methyl mercury were document as well in Sweden during the period of 1948-1965. Thus, the methyl mercury is dominant toxic mercury species in the environment, consumption of fish and crustaceans being the man hazards to man higher animals.

When lead is released into the environment, it has a long residue time compared with most other pollutants [11]. Exposure of Lead to protozoa affects the growth rate of *Cristigera* [3]. Annelids appear resistant to lead toxicity [12]. Crustaceans also appear to be resistant to lead toxicity. Exposure to lead slightly affects their embryonic development e.g., the mud crab *Rihithroponopeus harrisii* [12].

Juvenile stages of molluscs are usually more sensitive to toxic substances than adults [13]. Hrs-Brenko [14] found that embryos development in lead solutions was affected by an increase in temperature and a reduction in salinity. Generally, the discharge of lead in an aquatic habitat affects the biota in the water body by reducing their reproduction and feeding rate. Aquatic biota like fish accumulates the lead in their system and can lead to death of organisms.

MATERIALS AND METHODS

Test Animals: Batanga lebretonis, Guppies were collected from open drainage located at the Montgomery Road, Yaba Lagos, Nigeria and taken to the laboratory in a plastic bucket half-filled with water from the source.

Acclimatization: The guppies were kept in holding tanks (25 litres) one quarter filled with dechlorinated tap water and allowed to acclimatize to laboratory conditions (28±2.0°C, 78.5% (Relative Humidity) (RH) for at least 14 days before using them in bioassay. The guppies were fed with fish feed once a day. They showed high degree of survival in the holding tanks, without aeration, although the water was changed twice a week to prevent accumulation of toxic waste metabolites.

Procurement, Processing and Preservation of Diet: All ingredients used for the test diets formulation were purchased from Tejuosho market, Yaba and individually processed as follows.

Fish Meal (FM): The fishmeal used was crayfish meal. About 250g of dried crayfish was bought from Tejuosho market, Yaba, finely ground into powder, sieved and stored in sealed plastic bags pending use.

Yellow Corn Meal (YCM): About 500g of dried corn dent yellow was bought from the market, ground into flour. The flour was stored in sealed plastic bags.

Liver Grounded Meat (LGM): About 250g of liver was bought from the market and ground into paste.

Soya Bean Meal (SBM): About 300g of soya bean seeds were soaked overnight in water. The soaked beans were

autoclaved at 115°C for 30mins. Thereafter the heat-treated beans were oven-dried at 50°C for 6 hours. The dried soya bean was milled into powder, sieved and stored up in plastic bags. The above ingredients were mixed together with water to form a paste, which was spread with a wooden spreader on a plane surface. The paste was baked to form flakes.

The nutrient composition of the feed formula was determined using the procedural methods of Felsine *et al*. [15] and the results obtained were as shown in the recipe below. The nutrient is highly rich in proteins, carbohydrates and fat which will as expected support the growth of the fish understudy.

Composition of Basic Diet Feed

Components	composition
Crude protein	32
Crude fat	3
Moisture	10
Sodium chloride	25
Carbohydrate	30

Test Compounds

Mercury Chloride: This is marketed as mercuric chloride by J.T. Baker-Nigerian Chemical Company. It is a white crystal or powdering substance with molecular weight of 271.52. It is odourless; its solubility in 100ml of water is 7.4g, with specific gravity of 5.4. It boils at 302°C and melt at 2.76°C at 1mmHg and vapour pressure at 20°C is 1.2 x 10^{-2} mmHg.

Lead Chloride: This is marketed as lead chloride by J.T. Baker-Nigerian Chemical Company. It is a hazardous chemical with the following physical and chemical properties.

Bioassay Containers: Plastic containers (Vol. = 1500ml, bottom diameter = 15cm) were employed in all bioassays.

Preparation of Toxicant Solutions: Predetermined volumes of test compound were pipette out into a measuring cylinder and made up to an appropriate volume by adding distilled water as the diluent. The amount of each chemical compounds present in the resultant stock solution was known and based on this, amount required to achieve a predetermined concentration in each bioassay treatment was calculated and pipette into the bioassay containers and made up to a fixed test media volume. Test media were made up to 500ml of water, which in preliminary trial was shown to support 20 guppies in a bioassay container for more than 7 days without dissolved oxygen problems.

Selection of Experimental Animals: Active guppies of similar sizes (Length 2.4cm and weight 0.15g) of unknown age were selected into a holding tank from where they were randomly assigned to bioassay containers with a hand net.

Quantal Response (Mortality): The guppies were taken to be dead if they failed to show anybody or fin movement even when probed with a glass rod.

Relative Acute Toxicity of Mercury and Lead Chloride against *Batanga lebretonis:* A total of 200 guppies in three replicates were randomly placed in each bioassay container and exposed to varied concentration of mercury chloride and lead chloride. There were three replicates per treatment excluding control, meaning 60 guppies were exposed per concentration.

Concentration of Mercury chloride tested against guppies was 0.012, 0.015, 0.018, 0.021, 0.024, 0.027,0.03, 0.033, 0.036, 0.039mg/litre.

Concentrations of Lead chloride tested against guppies were 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5, 35, 37.5mg/l. Mortality assessments were carried out once every 24hrs, 48hrs, 72hrs, 96hrs period.

Statistics: Toxicological data (dose response) were subjected to probit analysis [16] including deviations of equations for the probit lines was done by a computer run program designed and implemented by Gede Pattourial Imperial College, London run by an IBM computer, adopted by Don-Pedro [17]. The probit analysis depends on maximum likelihood intervals and was extrapolated as follows.

LC_{50}: Concentration that will kill 50% of the population
LC_{95}: Concentration that will kill 95% of the population
LC_{5}: Concentration that will kill 5% of the population

RESULTS

Differential Toxicity of Mercury Chloride and Lead Chloride Against

Batanga Lebretonis: Toxicity based on 96hr LC_{50} values showed that mercury chloride was 1.195 times more toxic than lead chloride against *Batanga lebretonis* in toxicity value not significant overlaps in 95%, in both chemicals,

Table 1: Probit Log Table (Mercury Chloride)

Concentration µg/l	Log-dose	Total organisms exposed	Response mortality	% Mortality	Probit
0.012	-1.9208	60	3	5	3.3551
0.015	-1.8239	60	11	18	4.0846
0.018	-1.6575	60	14	23	4.2612
0.021	-1.602	60	15	25	4.3255
0.024	-1.5228	60	25	41	4.7725
0.027	-1.4559	60	37	61	5.2793
0.03	-1.5229	60	44	73	5.8276
0.033	-1.4815	60	51	85	6.4246
0.036	-1.4437	60	56	93	6.7113
0.039	-1.4089	60	58	96	6.9764

Table 2: Probit Log Table (Lead Chloride)

Concentration µg/l	Log-dose	Total organisms exposed	Response Mortality	% Mortality	Probit
12.5	1.0969	60	3	5	3.3551
17.5	1.243	60	13	22	4.2278
22.5	1.3521	60	19	32	4.5323
27.5	1.4393	60	21	35	4.6147
32.5	1.5118	60	28	46	4.8996
37.5	1.574	60	29	48	4.9498

Table 3: Differential toxicity of mercuric chloride against *Batanga lebretonis*

Chemical	Time/h	LC50 (95%CL)	LC95 (95%C;)	LC_5 (95%CL)	Probit Equation	Slope ±S.E	d.f	Tf
HgCl2	24	0.042(0.058-0.035)	0.141(0.366-0.089)	0.012(0.015-0.008)	y = 9.31±3.14x	3.14±0.56	4	1.00
	48	0.039(0.053-0.033)	0.145(0.384-0.090)	0.011(0.013-0.007)	y= 9.07+2.90x	2.90±0.51	4	1.08
	72	0.035(0.096-0.027)	0.117(4.7-0.059)	0.011(0.015-0.002)	y = 9.59+3.16x	3.16±0.51	4	1.20
	96	0.033(0.039-0.029)	0.098(0.177-0.071)	0.011(0.013-0.007)	y =10.13+ 3.47x	3.47±0.51	4	1.27

Cl = Confidence Limits, S.E = Standard Error, d.f = degree of freedom,

Tf = Toxicity factor,

*Tfl = 24hrLC50 Value

48h, 72, 96h values

Table 4: Differential toxicity of Lead chloride against *Batanga lebretonis*

Chemical	Time/h	LC_{50} (95%CL)	LC_{95} (95%C;)	LC_5 (95%CL)	Probit equation	Slope±S.E	d.f	Tfl
$PbCl_2$	24	38.31(47.92-33.53)	124.95(267.19-84.24)	11.74(14.55-7.89)	y=-0.07+3.20x	3.20±0.53	4	1.00
	48	37.74(48.47-32.66)	144.34(362.01-91.55)	9.87(12.81-5.90)	y=-0.55+2.82x	2.82±0.49	4	1.02
	72	36.40(45.64-31.79)	135.04(316.13-87.83)	9.81(12.68-5.98)	y=-0.49+2.89x	2.82±0.49	4	1.05
	96	35.87(44.43-31.48)	129.26(288.77-85.54)	9.96(12.78-6.20)	y= 0.41+2.95x	2.95±0.49	4	1.06

Cl = Confidence limits, S.E = Standard Error, d.f = degree of freedom, T.f = Toxicity factor

*Tfl = 24hrLC50 Value

48h, 72, 96h values

toxicity increased (decreasing LC_{50} values) between 24 and 96h exposure periods, although the concentration of the chemicals differ. The toxicity of both chemicals is depicted by the 96h LC_{50} values (Tables 1 and 2).

The 96h-log dose probit tables depicting the differential toxicity profiles of mercury and lead chloride against *Batanga lebretonis* are shown differently in Tables (3 and 4) respectively. Those tables show the various concentrations of both chemicals required to kill 50, 95 and 5% respectively of the *Batanga lebretonis*. Computed toxicity factors (based on LC_{50} ratio) show the 24h LC_{50} of both chemicals was approximately lower than the value of 96h showing the usual increase in toxicity with exposure time (Table 5). The toxicity factor for Mercuric and Lead chlorides were (0.033g/l) and (36.40g/l) respectively. These were the concentrations that killed 50% of the experimental gobies at 96h. The observed result shows that time of exposure plays a significant role in the toxicity of chemicals. From the result in Table 3 it is clear that mercuric chloride is several factors more toxic than Lead chloride (1.195 greater in toxicity).

Table 5: Computed Toxicity factors of mercury chloride and lead chloride (based on LC_{50} ratio)

Chemical	Time/h	LC_{50}	Tf
Mercury chloride	24	0.042	1.00
	48	0.039	1.08
	72	0.035	1.20
	96	0.033	27
Lead chloride	24	38.31	1.00
	48	37.74	1.02
	72	36.40	1.05
	96	35.87	1.06

Table 6: Differential Toxicity of mercury chloride and lead chloride against *Batanga lebretonis*

Mercury Chloride					
		Mortality/Time Interval			
Treatment	Number Exposed	24h	48h	72h	96h
1	60	1	2	2	3
2	60	9	12	13	11
3	60	9	12	13	14
4	60	10	13	15	15
5	60	20	21	23	25
6	60	26	30	34	37
Control	60	0	0	0	0
Lead Chloride					
Treatment					
1	60	1	3	3	3
2	60	12	13	13	13
3	60	15	17	19	19
4	60	18	21	21	21
5	60	26	27	28	28
6	60	27	27	28	29
Control	60	0	0	0	0

It was also observed that of the 60 exposed *Batanga lebretonis* to Mercuric chloride for 24h one died, between 24-72h only two died, while those exposed for 96h three died. However, for the sixth treatment, after 24, 48, 72 and 96h of exposure 26, 30, 34 and 37 died respectively. For lead chloride, at first treatment after exposure for 24, 48, 72 and 96h, 1, 3, 3 and 3 *Batanga lebretonis* died respectively. However, at the sixth treatment and exposure for 24, 48, 72 and 96h, 27, 27, 28 and 29 experimental fish died respectively (Table 6). When the toxicity of Mercuric and Lead chlorides was compared it was noted that salt of lead was less toxic against the *Batanga lebretonis* in spite of the high concentration of lead salt used in the study.

DISCUSSION

Several past studies have reported the toxicity of salts of heavy metals against terrestrial and aquatic animals. In the present study, it was observed that mercury chloride was more toxic than lead chloride against *Batanga lebretonis.* The differences in toxicity were significant since the F-calculated was greater than the F-tabulated for all periods (11.006>2.85, 21.495>2.85, 20.440>2.85, 40.417>2.85) for mercuric chloride and (163.06>2.85, 74.212>2.85, 40.091>2.85, 41.409>2.85) for lead. The toxicity of mercury and lead chloride has been reported by other workers. Branica and Konrad [18] determined the effect of lead against some marine

organisms (crustaceans) and found that lead chloride affects the mortality of the crustaceans. Brown [19] determined the effect of mercury chloride on mortality and growth of fish and observed that growth decreased with increase in mortality rate while some though survived were deformed.

The differences in the toxicity of the two chemicals are attributable to differences in their active constituents in content and proportion [20]. Their closeness in toxicity level may be indicative of similarities in chemical constituents; this is because both chemicals are heavy metals. The issue of the discharge of raw effluent containing heavy metals and their effect on the environment has been discussed extensively [21].

The result on Table 4 shows the mortality exposure period to mercury chloride and lead chloride. The difference in variation in this result is due to the different concentrations used. The stock preparation of mercury chloride was diluted before using in the bioassay while that of lead chloride was not. Although the mercury chloride heavily diluted it was still more effective as a toxicant than lead chloride, which is responsible for the differential toxicity observed against the test organism (*Batanga lebretonis*)

The concentrations of mercury chloride and lead chloride over the period of exposure were subjected to statistical analysis using PROBIT and ANOVA test. After analysis, it was observed that there were significant differences between the concentration of chemical used and in the toxicity factor of each chemical. Mercuric chloride had higher toxicity value at low concentration compared with Lead chloride. In a related study Onadeko and Kusemiju [22] discussed the effect of salinity on growth and survival of sleeper goby, *Batanga lebretonis* and reported that high salinity is detrimental to the growth and survival of the organism. In a similar study, Welch [23] studied the effects of cupric ions on temperature reference responses of Mozambique *Tilapia* and reported that the toxicity of the ions to tilapia increases with temperature. In another study, Renold [24] reported the acute toxicity of Kraft paper mill effluents on Bluegill. Exley [25] discussed the acute toxicity of aluminum to aquatic animals.

The various results obtained in this study demonstrate the effects of heavy metals, which are discharge at various level of concentration on aquatic and human lives. This is because the metals become biomagnified in the tissues of aquatic lives on which humans eventually depends. The results obtained further support that *Batanga lebretonis* can be safely used as heavy metal pollution indicator in aquatic environment judging from the remarkable high toxicity tolerance shown by the fish. It also demonstrates that remarkable tolerance the fish shown is a sine qua non of long time exposure to the salts of these metals. It is advised that policy makers in each country should put in place stringent policies on discharge of heavy metals by industries and ensure that offenders who violate the regulations are given appropriate punishment. This finding is an indication that the Lagos environment in Nigeria is highly under threat of heavy metal pollution. Our findings also point to the fact that this fish could be used as an index to determine heavy metal stress of water bodies judging from the resistance it offered to the two metallic salts tested.

CONCLUSION

In the course of this study it was shown that heavy metals are very toxic to aquatic organisms to the extent of causing death. Despite the high resistant potential of *Batanga lebretonis* to withstand pollution, it was still susceptibility to the heavy metals. This is an indication that some other fishes and shellfishes found in our aquatic environment, which are of economical value, are also at risk of this menace of heavy metals. Some edible Nigerian fishes and shellfishes found in coastal water have developed some resistance against pollution; however, the study showed that heavy metals had effect on their survival. In addition, heavy metals bioaccumulation in fishes and seafood, which in turn becomes biomagnified in man after consumption?

It is therefore important that the health status of most of these seafood and fishes are determined before consumption since they are likely to contain high levels of heavy metals. Efforts should also be geared towards controlling discharge of heavy metal containing effluent into natural bodies of water untreated. This finding is of public health significance.

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