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Full Length Research Paper

Mortality assessment of *Oreochromis niloticus* fingerlings in varying salinity and influence of salinity changes on acute toxicity of lead

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The setting of safe limits for lead (Pb) into the lagoon and protection of fishes such as *Oreochromis niloticus* has been based on its toxicity ignoring the influence of salinity, an important parameter in the lagoon. The study therefore investigates the salinity tolerance and relative acute toxicity of lead nitrate [$Pb(NO_3)_2$] under fresh water and varying salinity against *O. niloticus* fingerlings. A total of 280 fingerlings were used as test animals and $Pb(NO_3)_2$ as test chemical. Concentrations were 1, 2, 4, 6, 8, 10, 30, 40, 50, 60, 80, 120, 160 and 180mg/l at salinity levels of 2, 12, 18, 22, 32, 35 parts per thousand (ppt) and fresh water. Dose response data were analyzed by Probit. Mortality assessment showed that the fingerlings could not survive at salinities of 22, 32 and 35 ppt within 24 h but survived well in 2 ppt. The 96 h LC_{50} was 130.094 mg/l at 12 ppt but below 12 ppt there was a steady increase in toxicity (3.255 and 6.243 mg/l) in fresh water and 2 ppt respectively. Similarly, the toxicity also increased with an increase in salinity from 12.1 to 18 ppt (113.191 mg/l). The significance of this study shows the need for inclusion of varying salinity in setting of safe limits to confer protection on the delicate biotic components of the rich lagoon biodiversity.

Key words: Lead nitrate, *Oreochromis niloticus*, salinity.

INTRODUCTION

There is paucity of data on the setting of safe limits for discharge of heavy metals such as lead into the lagoon ecosystems, aimed at protecting brackish water organisms at varying salinity conditions. Salinity is one of the major factors (Lawson and Anetekhai, 2011) affecting organisms including fishes such as Tilapia in aquatic medium (Lagoon) and also has the ability to influence the toxicity of pollutants such as heavy metals (Oyewo, 1998; Lawson, 2011). There have been a lot of studies on the influence on the changing salinity on various physicochemical parameters, pollutant toxicity (heavy metals and pesticides) and their effects on fishes (Oyewo, 1998; Chukwu and Okpe, 2006; Breves et al., 2010; Lawson, 2011; Samy et al., 2011). The contamination, persistence, bioaccumulation and biomagnification of heavy metals (manganese, nickel, lead and zinc) in natural brackish are of global concern (Bhupander et al., 2011; Iulia et al., 2012; Marleen et al.,

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2013). Researchers have established high levels of heavy metals in the Lagos lagoon (Oyewo, 1998; Aderinola et al., 2009). Lead (Pb) is one of the most common heavy metals detected in the Lagos lagoon (Oyewo, 1998) which is not useful in living systems and extremely toxic at low concentration thus an indicator of pollution. In Nigeria, the recent increase in the environmental Pb has been attributed to industrial discharges and mining activities. The toxicity of Pb to aquatic organisms and its accumulation in the aquatic biota are well documented. Leblond and Hontela (1999) reported that the 96 h median tolerance limit value for fathead minnows and brook trout were 40 to 70 mg/L in soft water and 440 to 480 mg/L in hard water. The 96-h LC_{50} for Lead acetate in bluegill was found to be approximately 400 mg/L (McKim, 1985).

The principal toxic effects of chronic Pb exposure to fish cause hematological (Małgorzata et al., 2010; Usama et al., 2013), neurological (Talia et al., 2009) and renal (Patel et al., 2006) disorders. In water bodies contaminated with Pb, it has been observed in fishes that it causes the disruption of Na^+, Cl^- and Ca^{2+} regulation during acute exposure, development of black tails and spinal curvature during chronic exposure as well as disruption in hemoglobin synthesis (Sippel et al., 1983; Rogers et al., 2003; Rogers et al., 2005). Concentration of Pb in pond water ranged from 0.03 - 0.16 g mL^{-1} (Himadri and Anilava, 2000). In polluted areas dissolved Pb has been observed in concentration of 50400 $nmolL^{-1}$ (Bowles et al., 2006) while Pb toxicity has been reported for some freshwater species at concentrations as low as 50 nmol L^{-1} (Grosell et al., 2006).

A typical species that inhabit the brackish water is *Oreochromis niloticus.* It is among some of the edible commercial fish species that inhabit the Lagos lagoon and is also widely bred in commercial fish farms. *O. niloticus* can be cultured in fresh water coupled with slight challenges due to fluctuations in salinity and this makes it not so easy to be cultured in the laboratory. Since *O. niloticus is* sensitive to heavy metals (Taweel et al., 2013) it is therefore of interest to study the influence salinity changes will have on the effect of heavy metals such as lead which could be a biological constraint and crucial in setting of safe limits for this fish.

MATERIALS AND METHODS

Test animals

O. niloticus (Nile tilapia, Gnathastomato, Cichlidae) fingerlings of similar sizes (total length; 11 ± 6 cm, mean weight; 20 ± 4 g and age (4 - 6 weeks old) were purchased from Agboola farms and transported to the laboratory in 25 L container into holding tanks (50 X 30 X 35 cm), which contained fresh water from fish pond (opened at the top for aeration) in the laboratory. The fingerlings were kept in the holding plastic tanks, half filled (15 L) with dechlorinated tap water, to acclimatize to laboratory conditions (28 ± 2°C, R.H 70 ± 2%) for a period of seven days before they were used in the bioassays. The fingerlings were fed with fish food (Coppens) at 3% of their body weight and water was changed once every 48 h, aerated continuously with circular plastic bowls (volume = 0 5 L, bottom diameter = 18 cm) were used as bioassay container.

Test chemicals

The heavy metal, lead as [$Pb(NO_3)_2$] investigated in this work was obtained as metallic salts of Fisons laboratory reagents, Analar grades of molecular weight 331.21 g) with 98% purity manufactured by British Drug House (BDH). The choice of Pb as the heavy metal for this study was based on the available and common metal from the results of a chemical survey of industrial effluents that empty into the Lagos lagoon (Oyewo, 1998).

Preparation of treatments at varying salinities

Seawater was obtained in 25 L container, Lagos State and taken to the laboratory, where the salinity (35 ppt) was measured with a salinometer. On the basis of salinity level, computed volumes of the sea water were measured out and mixed with dechlorinated tap water to obtain sea water solutions at pre-determined salinity levels. The salinity of the prepared media was verified with a salinometer for more accurate determination of the salinity was recorded and used in appropriate experiments.

Preparation of treatments for acute toxicity test

A pre-determined amount of Pb compound was weighed (using an oertling 30TD top loading balance) and diluted with given volume of dechlorinated tapwater to obtain a stock solution of known strength. The resultant stock solution was serially diluted to obtain solutions of required concentrations. Actual concentration of Pb ions in each solution of known strength was computed based on molecular weight of test compound. Test media were always made up to 2 L because preliminary studies showed that 10 fingerlings survived well in 2 L of media for a period of 7 days without aeration. In range finding experiments, the fishes were exposed to a wide range of concentrations (600, 300, 100, 50, 25, 5 and 1 mg/l) of the test compound to obtain an effective range of activity, initially relying on trial and error techniques.

Assessment quantal response (mortality)

Fingerlings were taken to be dead if no body movements including the operculum were observed, even when prodded with a blunt glass rod and submerged in water.

Salinity tolerance of *O. niloticus*

Five active fingerlings of similar age and size (as described above) were taken from plastic holding tanks with a sieve (200 µm) and randomly assigned to bioassay containers already holding treatments at varying salinities as described below. Each treatment was replicated twice, giving a total of 10 fingerlings that were exposed per salinity level (35, 32, 22, 18, 12, 2 ppt) and fresh water. Mortality was assessed as described above, once every 24 h for a period of 7 days.

Acute toxicity of ($Pb(NO_3)_2$) against *O. niloticus* in varying salinity treatments

A similar set up as described above in the case of salinity tolerance

Table 1. Acute toxicity concentration at fresh water, 2, 12 and 18 ppt.

Fresh water (mg/l)	2 ppt	12 ppt	18 ppt
1.0	1.0	30.0	40.0
2.0	2.0	50.0	60.0
4.0	4.0	80.0	80.0
6.0	6.0	120.0	120.0
8.0	8.0	160.0	180.0
10.0	10.0	200.0	240.0

Table 2. Salinity tolerance of *O. niloticus* under varying salinity (mortality assessment).

Salinity (ppt)	Time (h)							% Mortality
	24	48	72	96	120	144	168	
35	10	10	10	10	10	10	10	100
32	10	10	10	10	10	10	10	100
22	9	10	10	10	10	10	10	100
18	5	6	7	7	7	7	7	70
12	2	2	3	3	3	3	3	30
2	0	1	1	1	1	1	1	10
Fresh water	0	0	0	0	0	0	0	0

of *O. niloticus* was carried out in this case but mortality was assessed once every 24 h for a period of four days.

Acute Toxicity concentrations

Acute toxicity concentrations under which relative acute toxicity of test heavy metal against *O. niloticus* was investigated were as shown in Table 1.

Statistics

The dose response (mortality) data for freshwater and varying salinities were analyzed with a computer program using Probit analysis after Finney (1971) as adopted by Don Pedro (1989) and used as indices for measuring toxicity of the test organisms to the toxicant. The Indices of measuring toxicity derived from these analyses were Lethal Concentration that caused 95, 50 and 5% response [mortality] of exposed organisms (LC_{95}, LC_{50}, LC_5, respectively) and their 95% confidence limits.

RESULTS

Salinity tolerance of *O. niloticus*

The results of salinity tolerance of *O. niloticus* based on mortality assessment, showed that the fishes could not survive for any appreciable period in treatments with salinity at 22 ppt and above (Table 2). In treatments with salinity of 18 ppt, between 50 - 60 % mortality was recorded in 48 h but from 72 - 168 h, 70 % mortality was observed. At 2 and 12 ppt, mortality occurred at low levels (10 - 20 %) after 24 - 48 h respectively but stabilized at 30% after 72 h at 12 ppt (Table 1). No mortality was recorded in fingerlings exposed to media with fresh water.

Acute toxicity of $Pb(NO_3)_2$ against *O. niloticus* in varying salinity treatments

The acute toxicity of $Pb(NO_3)_2$, based on 96 h LC_{50} values against *O. niloticus* fingerlings at salinity of 12 ppt, was 130.094 mg/l whereas at 2 ppt and freshwater, it was 6.243 and 3.255 mg/l respectively which was significantly (no overlap in 95 % CL of 96 h LC_{50}) lower than the toxicity values at 12 and 18 ppt (113.191 mg/l) treatments evaluated (Figure 1).

Additionally, the relative toxicity of $Pb(NO_3)_2$ based on 24, 48 and 72 h LC_{50} values followed a similar trend of relative toxicity against the fingerlings as 96 h LC_{50} values in all test media (freshwater, 2,12 and 18 ppt) and there was no overlap in 95% confidence limit for 96 h LC_{50} values for all media. Figures 2, displays the Probit line graphs of the toxicity data for the test freshwater fish species under varying salinity conditions indicating increasing toxicity with exposure time.

DISCUSSION

The established salinity tolerance and salinity influence of Pb against *O. niloticus* at varying salinity range reported

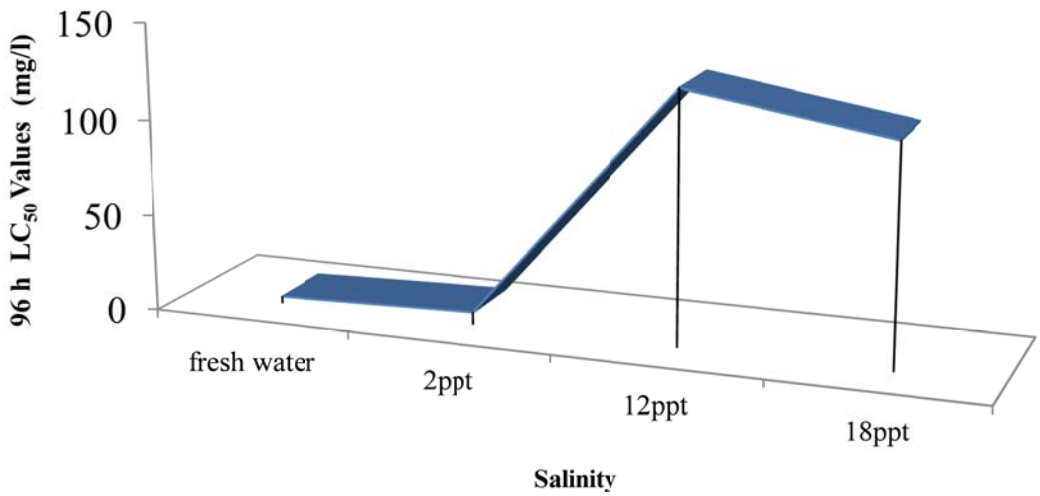

Figure 1. Relationship Between 96 h Lc_{50} values of $Pb(NO_3)_2$ against *O. niloticus* (n = 10) at different salinities.

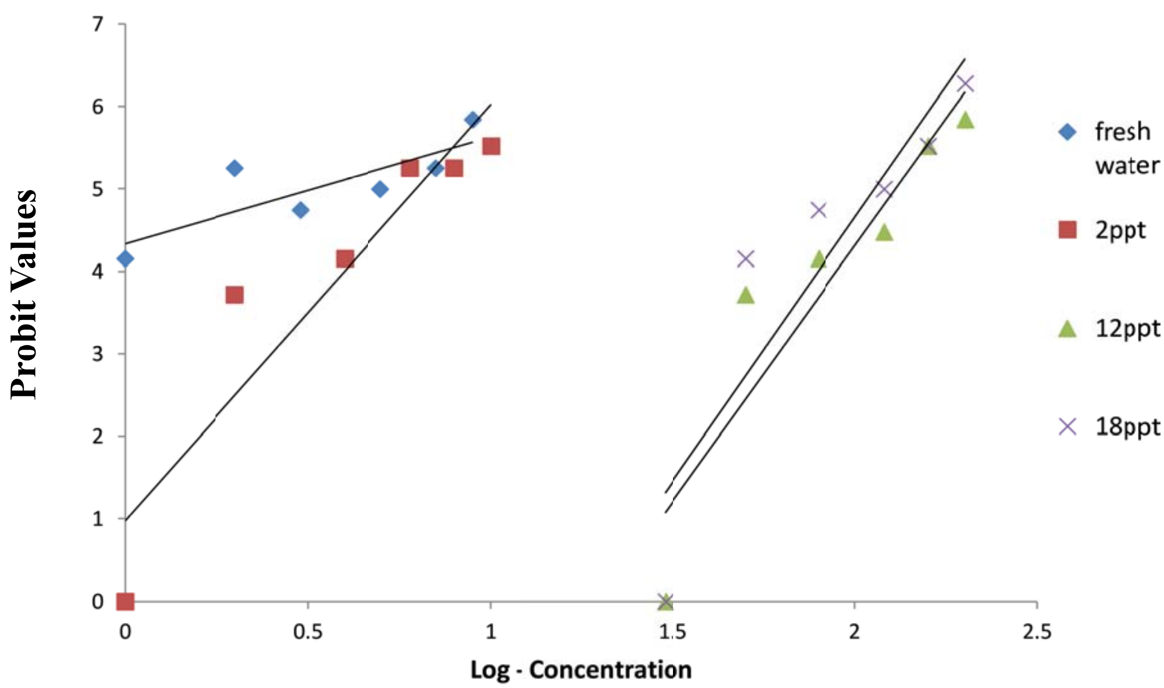

Figure 2. Probit transformed response of *O. niloticus* Log-concentration of $Pb(NO3)_2$ at fresh water, 2, 12 and 18 ppt conditions.

in this study showed that based on mortality assessment, *O. niloticus* fingerlings could not survive for any appreciable period in media with salinity equal to and higher than 22 ppt. This is not surprising because *O. niloticus* is a brackish water species and optimum survival is usually at salinity range between 10 - 15 ppt though can survive in waters with salinity between 0-15 ppt (Kurata, 1959, Thomas and Masser, 1999; Lawson and Anetekhai, 2011). Although the study conducted by Nugon (2003) showed that *O. niloticus* exhibited good survival (81%) in salinity regimes up to 20 ppt, which is in close agreement with the findings of this study and that of Osuala and Bawa-allah (2013).

However in fresh water and at 2 ppt the toxicity of Pb was higher than that observed at 12-18 ppt as depicted by the 96 h LC_{50} values suggesting that the toxicity of Pb against *O. niloticus* fingerlings may have synergized osmotic stress at lower salinities. Moreover, salinity played a significant role in influencing the acute toxicity of Pb. This probably resulted in the fish being less resistant and unable to regulate the body fluid to restore levels of osmotic pressure to near normal. Additionally it could be due to other toxicity modifying factors such as exposure duration and concentrations of Pb. Chemicals may have multiple effects on populations of organisms such as in mortality, reproductive failure, and productivity (Chukwu and Okpe, 2006). Sensitivity of populations depends upon such factors as age groups and temporal patterns of exposure. Passage of toxins or toxicants into an organism is also highly dependent on the specific physical-chemical characteristics of a given toxicant (Maheswaran et al., 2008; Ololade and Oginni, 2010). $Pb(NO_3)_2$, being the test chemical could be regarded as one of the major reasons for induced mortality due to its lipophilic and surfactant-containing nature (Ezemonye et al., 2007). Most of the test organisms survived initial attack at the early stage of test initiation and at the lower test concentrations of the toxicants. This could be attributed to protective adaptations as well as individual physiological nature of *O. niloticus* (Olalade and Oginni, 2010). However, as exposure progressed to 96 h, inevitable mortality could be due individual physiology and cumulative impact of the chemical toxicity. The results observed in this study are in agreement with other related studies (Omoregie et al., 1990; George and Clark, 2000; Johnson et al., 2005; Scarlett et al., 2005; Ezemonye et al., 2007). It is interesting to note that $Pb(NO_3)_2$ was most toxic to the fish at freshwater conditions, contrary to expectations that since the fish has the ability to survive at extremely low salinity such as in freshwater conditions, they should show more tolerance to the metal in freshwater. This is in agreement with findings of Oyewo (1998) who showed that similar brackish water adapted bony fishes such as *Tilapia guinensis* and *Nerite senegalensis* were known to be most susceptible to heavy metal pollutants including $CuSO_4$ at salinities tending towards the extremities (below 5 ppt and above 25 ppt), but were several folds more tolerant at salinity of up to 15ppt which falls within typical brackish water salinity (10-20 ppt).

The results reported for mean percentage mortality and 96 h LC_{50} values for the fresh water test could probably have been due to the physiology of *O. niloticus* under freshwater conditions, which have a body fluid concentration (about one-third) their surrounding environment, thus constantly taking in water by diffusion through their gills and skin for osmotic balance (Delbeek, 1987). Thus in a situation where there is damage to the skin and other tissues as is the case in exposure to high concentrations of surfactant-containing chemical ($Pb(NO_3)_2$), there is an influx of not only water but also the test chemical leading to a high lethal toxicity of the chemical and death rate of *O. niloticus* under freshwater conditions (Bury et al., 1999; Abel, 2006).

The authors' findings suggested that *O. niloticus* remains a brackish water fish as it retains its innate characteristics which allows it to do well in brackish water as its original natural habitat. Though, it can also strive relatively well in freshwater media because they are euryhaline (being able to adapt to a wide range of salinities due to their ability to osmoregulate especially in habitats where salinity changes regularly).

O. niloticus carry out hyper-hypo osmoregulation switching from hyper osmoregulation to hypo osmoregulation and vice versa during variations in salt content of water in their habitat. However, variations tending towards the extremities may result in stress or death, as was demonstrated with *O. niloticus* in extremely high salinities in this study.

Therefore, the practical significance of this study could be utilized in setting ecologically sound, safe limits for heavy metal (such as Pb) containing discharges into the lagoon ecosystems, aimed at protecting brackish water organisms. It is therefore suggested that data used for setting of safe limits should be based on those obtained from studies carried out over a wide range of salinity conditions.

Additionally this study provides data and information to already existing reviews on the influence of heavy metal pollutant in waters with varying salinity range against *O. niloticus*

Conclusion

This study has shown that salinity affects the acute toxicity of lead on fingerlings of *O. niloticus*. In the freshwater, 2, 12 and 18 ppt experimental media, death caused to *O. niloticus* may be detrimental since this could lead to elimination of potentially reproductive organisms in its natural brackish water habitat. The inclusion of salinity factor in the setting of safe limits would ensure amongst others that the delicate biotic components of the rich Lagoon biodiversity are prudently protected. The findings will assist our relevant agencies on the need to stipulate and implement stiffer regulations to control inflow of nutrient and chemical contaminants into brackish habitats coupled with enforcement of penalties imposed for illegal and unsustainable development that impacts these habitats.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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