DIFFERENTIAL TOXICITY AND INFLUENCE OF SALINITY ON ACUTE TOXICITY OF COPPER SULPHATE AND LEAD NITRATE AGAINST *Oreochromis niloticus*

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Abstract

This study investigated the salinity-tolerance of *Oreochromis niloticus* and the influence of salinity changes on the acute toxicities of copper sulphate ($CuSO_4$) and lead nitrate ($Pb[NO_3]_2$) against the fish species. On the basis of daily mortality assessment, *O. niloticus* was found to be unable to survive in media with salinity above 22‰ within a 24-hour period, but the species survived well at lower salinities. The 96-hour LC_{50} values of both $CuSO_4$ and $Pb(NO_3)_2$ against the species was lower indicating a higher toxicity at salinities lower (0 and 2‰) or higher (18‰) than 12‰ which had the highest 96-hour LC_{50} value and exerted lowest toxic action. $CuSO_4$ was however more toxic to the species than $Pb(NO_3)_2$ based on the recorded 96-hour LC_{50} values. The significance of this study in setting ecologically safe limits that would be relevant to the varying salinity conditions that characterize the lagoon ecosystems in the tropics for the discharge of heavy metals into lagoons was discussed. The advantages of breeding *O. niloticus* under typical brackish water conditions instead of freshwater were also emphasized in the text.

Keywords: *Oreochromis niloticus;* acute toxicity; salinity; copper sulphate, lead nitrate.

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Introduction

Lagoons are brackish water bodies that are characterized by varying salinities (1-35 ‰).They can be found all over the world including West Africa and are known to be productive, being rich in flora and fauna. The Lagos Lagoon is the largest among four others found along the West African Coast (Hill and Webb, 1958); it covers about 700 km on the western coast of Nigeria. Studies carried out in the middle of the last century have shown that the Lagos Lagoon had more varieties of pelagic and benthic organisms than are indicated by studies carried out in the last two decades (Oyewo, 1998). This major decline has been attributed to pollution which has led to a steady deterioration of the health of the lagoon (Amaeze *et al* 2012).

Increasing industrial activities have resulted in generation of large amount of effluents which contain a wide range of pollutants such as heavy metals, hydrocarbons and pesticides. The exact type and amount of pollutant depends on the type of industry, technology adopted, raw materials used and waste treatment facilities.

Heavy metals are metals with density greater than 5g/cm^3 and atomic number higher than that of calcium. They are toxic to living organisms and their non-degradable characteristic creates a significant environmental problem as the concentrations accumulate over time, whereas the toxicity persists in the ecosystems.

Various studies have been carried out in order to establish safe limits for the discharge of heavy metals into aquatic ecosystems including lagoons (Otitoloju,

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2003 and Ladigbolu, 2011). However, these studies were carried out on a narrow range of salinity (at freshwater or typical marine salinities) constituting a major limitation when using data from such studies to set safe limits for lagoon ecosystems that are characterized by varying salinities. In lagoon ecosystem, the major physico-chemical parameter that varies widely in response to the two hydrological seasons (Rainy and Dry) that characterize tropical regions is salinity, which varies 0.5 and 30 ‰. Animals that inhabit lagoon ecosystems are able to tolerate the wide variations in salinity using various forms of adaptation which may include behavioural, morphological and/or physiological (Willmer *et al*, 2009). Common examples of such animals include *Monodactylus argenteus, Tilapia guinensis, Oreochromis niloticus* and *Clibanarius africanus,* they are also known as euryhaline species. Salinity variation in lagoon ecosystems in response to the two seasons can influence heavy metal toxicity and therefore the damage potential of metals to aquatic organisms (Oyewo, 1998).

Oyewo (1998) and Riba *et al* (2006) are two of the few workers who have studied the effect of changing salinity on toxicity of some of the heavy metals in Lagos Lagoon. The authors reported that salinity influenced the toxicity of heavy metals, with higher toxicity recorded at lower salinities. For example, the toxicity of either copper (Cu) or mercury (Hg) against three test organisms (*Tilapia guinensis, Clibanarius africanus,* and *Nerita senegalesis*) was minimal at intermediate or typical brackish water salinity between 10-20% but metal toxicity increased significantly with increasing salinity, especially above 25% and decreasing salinity, particularly below 5%. Based on the 96-hour LC_{50} values computed at varying salinities, it was found that the toxicities of Cu or Hg increased by as much as 1.5-2.8, 1.4-3.0, 2.5-5.0 times for Cu; 1.8-13.0, 1.9-2.8, 1.6-3.2 times for Hg against *T. guinensis, C. africanus* and *N. senegalesis* respectively at salinities above 25% or below 5% when compared to the toxicity of equivalent metals at 15% (Otitoloju and Don Pedro, 2002). Hence there is need to carry out more research spanning wide salinities that are typical of lagoon ecosystems in order to set reliable safe limits for heavy metals in lagoon ecosystems.

Copper is commonly detected in the Lagos Lagoon (Oyewo, 1998) and although it is useful in living systems, it becomes toxic at high enough concentrations.

Lead is a non-essential heavy metals that has no biological function in living systems, it is toxic at low concentrations and is one of the heavy metals commonly detected in the Lagos Lagoon.

*Oreochromis niloticus*is one of the most common edible fish species that inhabit the Lagos Lagoon, it is of high commercial value as it is a major source of protein for human population. *O.niloticus*is also bred in commercial fish farms using freshwater which varies from the water in its natural habitat in terms of salinity. This study aims to determine and compare the influence of salinity changes on acute toxicity of copper and lead against *Oreochromis niloticus.*

Materials and method

Test animals

O. niloticus fingerlings were used as test animals in this study. Fingerlings (age; 3-4 weeks old, mean snout to tail length; 6.00 ± 2.00 cm, mean weight; 5.00 ± 1.00 g) were purchased from a local fish farm in Lagos State, Nigeria, and transported in 25 litre Jerry cans (opened at the top for aeration) with fish pond water to holding tanks (length: 50.00 cm, width: 30.00 cm, height: 35.00 cm and volume: 50 litres) in the laboratory.

The fingerlings were kept in the holding plastic tanks, half filled with dechlorinated tap water, to acclimatize to laboratory conditions (temperature: 28.00±2.00^{0}C, relative humidity: 70.00±2.00%) for a period of 7 days before they were used for the experiment. They were fed with fish food (Coppens), and the water was changed once every 48 hours, aerating it continuously with Cosmo air pump (Double type 1200).

Chemical preparation

The test compounds used were copper as $CuSO_4.5H_2O$ (molecular weight: 249.680 g) and lead as $Pb(NO_3)_2$ (molecular weight: 331.210 g). Both metals were of analytical grade and manufactured by BDH (British Drug House), purity; 98.5-101%. Stock solutions of both metals were prepared by taking computed amount (1 g) and made up to the desired volume (1liter) using distilled water to achieve a stock of 1 g L^{-1} for each metal. The stock solutions were serially diluted to obtain solutions with desired concentrations selected after range finding experiments.

Preparation of media of varying salinities

Media of varying salinity between 35.00 and 0.00 ‰ obtainable in brackish water ecosystems were used in this study. Test media were made up by serially diluting sea water with dechlorinated tap water in pre-determined proportions which depended on desired salinity to be obtained. The salinity of each prepared media was tested with a salinometer (AGRO® Master Refractometer) for confirmation of accurate salinity before being used in experiments.

Bioassays

Salinity tolerance of *O. niloticus*

Five active fingerlings were taken from plastic holding tanks with a sieve and randomly assigned to bioassay containers already holding test media at varying salinities as described below. Each treatment was replicated twice, giving a total of 10 fingerlings that were exposed per salinity level and fresh water. Mortality was assessed once every 24-hours for a period of 4 days. Fingerlings were exposed to salinities of 35.00, 32.00, 22.00, 18.00, 12.00, 2.00 and 0.00 ‰.

Relative acute toxicity of test heavy metals against *O. niloticus*

Based on salinity tolerance result obtained after carrying out experiment described above, fingerlings were then exposed to graded concentrations of the two heavy metals pre-determined after range finding tests, in media with salinity within their tolerance range as described below. Mortality was assessed once every 24-hours for a period of 4 days.

0%

$CuSO_4$ against fingerlings at; 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 mgL^{-1} and untreated control.

$Pb(NO_3)_2$ against fingerlings at; 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 $mgL^{-1}$and untreated control.

2%

$CuSO_4$ against fingerlings at; 8.0, 10.0, 12.0, 13.5, 15.0 $mgL^{-1}$and untreated control.

$Pb(NO_3)_2$ against fingerlings at; 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 $mgL^{-1}$and untreated control.

12%

$CuSO_4$ against fingerlings at; 16.0, 20.0, 22.0, 25.0, 28.0, 30.0, 40.0 $mgL^{-1}$and untreated control.

$Pb(NO_3)_2$ against fingerlings at; 30.0, 50.0, 80.0, 120.0, 160.0, 200.0 $mgL^{-1}$and untreated control

In 18ppt

$CuSO_4$ against fingerlings at; 12.0, 16.0, 20.0, 24.0, 28.0, 30.0mgL^{-1} and untreated control.

$Pb(NO_3)_2$ against fingerlings at; 40.0, 60.0, 80.0, 120.0, 180.0, 240.0mgL^{-1} and untreated control.

Statistics

The dose response data for freshwater and varying salinities were analyzed by probit analysis (Finney, 1971). Indices of measuring toxicity (LC_{95}, LC_{50}, LC_5) and their 95% confidence limits were employed.

Results

Salinity tolerance of *O. niloticus*

Survival of *O.niloticus* was dependent on salinity based on mortality assessments. *O. niloticus* could not survive for any appreciable period in media with salinity higher than 22% (Table 1). In media with salinity of 18%, 60% mortality was recorded at 48-hours and 70% at 72-hours, but no further mortality was recorded after 72-hours ill the end of the exposure period (4-days). At 2 and 12%, mortality occurred at low levels and remained at 30% after 72-hours (Table 1). No mortality was recorded in fingerlings exposed to media with 0% (freshwater).

Salinity (‰)	Mortality (%)			
	24-hours	48-hours	72-hours	96- hours
0	0	0	0	0
2	0	10	10	10
12	20	20	30	30
18	50	60	70	70
22	90	100	100	100
32	100	100	100	100
35	100	100	100	100

Influence of salinity on the acute toxicity of $CuSO_4$ and $Pb(NO_3)_2$ against *O. niloticus*

The toxicity of both heavy metals ($CuSO_4$ and $Pb[NO_3]_2$) against the fish species increased as salinity increased or decreased beyond 12%, where it was minimal. Based on the 96-hour LC_{50} values, the toxicity of $CuSO_4$ against *O. niloticus* was 27.785 mgL^{-1} at 12%, as compared to 2.492 mgL^{-1}, 10.008 mgL^{-1} and 16.786 mgL^{-1} at fresh water, 2% and 18% respectively while that of $Pb(NO_3)_2$was 130.094 mgL^{-1} at 12% as compared to 3.255 mgL^{-1}, 6.243 mgL^{-1} and 113.191 mgL^{-1} at fresh water, 2% and 18% respectively (Table 2). It was observed that both heavy metals had a similar trend of acute toxicity against the fish species (freshwater >2% >18% >12%) with both of them being least toxic at 12‰.$CuSO_4$was however more toxic to the fish as compared to $Pb(NO_3)_2$ based on lower 96-hour LC_{50} values obtained for $CuSO_4$ indicating a higher toxicity (Table 2).

Table 2. 96-hour LC_{50} Vvlues of $CuSO_4$ and $Pb(NO_3)_2$ against *O.niloticus* at different salinities.

Heavy Metals	0%	2%	12%	18%
$CuSO_4$ (mgL^{-1})	2.492	10.008	**27.785**	16.780
$Pb(NO_3)_2$(mgL^{-1})	3.255	6.243	**130.094**	113.191

Discussion

O.niloticus is a brackish water fish species that retains its innate brackish water characteristics; this was evident from results obtained in this study that showed that the fish species could not survive in water with salinities higher than 22% which is tending towards marine salinity concentrations. This observation is in agreement with findings of Thomas and Masser (1999) that studied the life and biology of *O. niloticus* and reported that the fish can survive in waters with salinity between 0-15%, but the optimum salinity range for the fish is 10-15%. A major physiological characteristic that contributes to the success of this species is their form of osmoregulation. Euryhaline species, like *O. niloticus*, carry out hyper-hypo-osmoregulation, switching from hyper-osmoregulation to hypo osmoregulation and *vice-versa* during varying salinity conditions in their habitat. However, variations tending towards the extremities (22-35%) may result in stress or death, as was demonstrated with this fish at extremely high salinities in this work.

The acute toxicities of the heavy metals investigated against the species were influenced by salinity changes as shown by results obtained in this study. The toxicities of both metals increased with corresponding increase or decrease in salinity beyond 12% were it was minimagfrl. This finding corroborates the findings of Oyewo (1998) who also showed that other 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% and above 25%), but were several folds more tolerant at salinities of up to 15% which falls within typical brackish water salinity (10-20%). The two heavy metals investigated in this study were most toxic to the fish at freshwater. This is an interesting observation because it would be assumed that since the fish can be bred in freshwater, they should be able to tolerate the chemicals more than in other extreme salinities. However, it only buttresses the fact that *O. niloticus* retains its innate brackish water characteristics and would survive best in such ecosystems even though it can be bred in fresh water as done in fish farms.

Copper sulphate, even though being an essential heavy metal, was more toxic to the fish species than lead nitrate, a non-essential heavy metal as shown by the 96-hour LC_{50} values. This negates the idea that essential heavy metals due to their biological function in organisms may exert lower toxicities against organism than essential metals and has also been demonstrated by other researchers (Otitoloju and Don Pedro, 2002).

Conclusion

This study has shown that the varying salinities that characterize brackish water ecosystems should be strongly emphasized and put into consideration when setting ecologically sound safe limits for disposal of pollutants into such ecosystems. This study has also shown that it may be erroneous to assume that essential heavy metals may be less toxic to organisms than non essential heavy metals. Hence, the need to set safe limits for heavy metal disposal irrespective of their biological function in organisms since it has been established that heavy metals are all toxic at low concentrations. Also, for aquaculture purposes, it may be better to breed *O. niloticus,* if feasible in pond-water with salinities between 10-20% that are obtainable in brackish water ecosystems for most part of the year. This will ensure an increase in tolerance of the fish to pollution, especially that imposed by heavy metals.

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