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TOXICITY OF COPPER SULPHATE AND BEHAVIORAL LOCOMOTOR RESPONSE OF TILAPIA (*Oreochromis Niloticus)* AND CATFISH (*Clarias Gariepinus)* SPECIES

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ORIGINAL ARTICLE

ABSTRACT: *Acute toxicity of copper sulphate ($CuSO_4.5H2O$) to tilapia (Oreochromis niloticus) and catfish (Clarias gariepinus) species was investigated using toxicity index of 96 hours LC_{50} and the quantal response determined by the statistical probit analysis method. In response to the lethality of the copper toxicant, behavioral anomalies (locomotor response) of the exposed fish species were studied as indication of toxic effects of the heavy metal. Fish species shows different mortality responses to the varying concentrations of copper studied (50, 60, 70, 80, 100, and 120 mg/l) due to toxicity. Copper was significantly (no overlap in 95% C.L of 96 hrs LC_{50} values) more toxic to Oreochromis niloticus than the catfish. 96 hrs LC_{50} values for Oreochromis niloticus and Clarias gariepinus were revealed to be 58.837 and 70.135 mg/l, respectively. Behavioral changes, mostly locomotor responses (avoidance) were observed among the test animals on exposure to the different concentrations of copper sulphate. There is need to control the use of copper because of its observed toxicity and fish avoidance test shows to be an important predictive and sensitive biomarker in aquatic monitoring and pollution management.*

Keywords: *Heavy metal toxicity, 96 hrs LC_{50}, biomarker, dose-response effect.*

INTRODUCTION

Copper is highly toxic in aquatic environments and has effects in fish, invertebrates, and amphibians, with all three groups equally sensitive to chronic toxicity (U.S EPA, 1993; Horne and Dunson, 1995). Copper will bio concentrate in many different organs in fish and mollusks. While mammals are not as sensitive to copper toxicity as aquatic organisms, biomagnifications play critical role in their toxicity. Toxicity in mammals include a wide range of animals and effects such as liver cirrhosis, necrosis in kidneys and the brain, gastrointestinal distress, lesions, low blood pressure and fetal mortality (ATSDR, 1990; Kabata-Pendias and Pendias, 1992; Ware, 1983; Vymazal, 1995).

Khangarot et al. (1982) demonstrated that Hg^{2+} was the most toxic (96 hrs LC_{50} -0.023 mg/L) followed by Cu^{2+} (0.034 mg/L), Cr^{6+} (5.97 mg/L), and Zn^{2+} (10.49 mg/L) when the metallic compounds were tested singly against the freshwater Pulmonate snail, *Lumnaea acuminate* in Indonesia. Khunyakari et al. (2001) investigated toxicity of nickel, copper, and zinc in *Poecilia reticulata.* Heavy metal exposure caused increased mucus like secretion over gills, excessive excretion, anorexia and increased fin movement. Copper was found to be the most toxic followed by zinc and nickel.

Oyewo (1998) also tested some prominent metals found in the industrial effluents against five animal species namely; Cypris sp., Mugil sp. Tilapia sp, *Nerita senegalensis*, and *Clibanarius africanus* that normally inhabit the Lagos Lagoon. The author reported that the values on the general order of toxicity of the test metals was Hg, Cu, Mn, and Fe when tested separately against each of the Lagos lagoon species listed above. Consequently, in Nigeria, Oyewo (1998) reported that when heavy metals such as Fe, Mn, Cu, and Hg were tested against some estuarine macrofauna, the order of tolerance of the species were Cypris sp. followed by Mugil sp. Tilapia sp. *Clibanarius afrinuas*, *Nerita senegalensis* and *Tympamotomus fusatus* as the most tolerant species tested in a descending order of sensitivity.

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Behavioral changes represent a higher organizational level of biomarker than any considered so far (Walker *et al.*, 2003). One of the early proponents of the value of behavioral toxicology stated that 'the behaviour of an organism represents the final integrated result of a diversity of biochemical and physiological processes. Thus, a single behavioural parameter is generally more comprehensive than a physiological or biochemical parameter (Walker et al., 2003). Behavioral test that are most advanced are those involving fish. The fish avoidance test is well established in the laboratory as a means of showing effects well below the lethal range. Recent studies include many on the effects of heavy metals. If one compares the lowest observed effect concentration (LOEC) obtained from behavioural studies (avoidance, attractance and fish ventilation) with chronic toxicity studies, one finds that some of the behaviour tests are more sensitive than life cycle or early stage tests (Walker et al., 2003). The perception of motion is important for the survival and reproduction of many animals including fish (Albensi and Powell, 1998). In the laboratory, support for this idea comes from the observation that any fish show a tendency to follow a series of stripes revolving around a circular aquarium (Albensi and Powell, 1998).

Test involving a variety of locomotor behaviors have been insufficiently studied especially with respect to heavy metal lethality to enable a judgment of their sensitivity or utility. In response to the above fact, the present work investigates the lethality (LC_{50}) of the varying concentrations of copper sulphate ($CuSO_4.5H_2O$) and the behavioural locomotor response and changes of the two fish species (*Oreochromis niloticus* and *Clarias gariepinus*) exposed as the most sensitive indication of potential toxic effects.

MATERIALS AND METHODS

Healthy adult fish species (*Oreochromis niloticus* and *Clarias gariepinus*) were obtained from a commercial hatchery and brought to the laboratory within in plastic bags with sufficient air. The plastic bags were placed into the maintenance aquarium for 30-35 minutes for acclimatization. Then the bags were cut open and the fish were allowed to swim into the aquarium water. The aquaria were aerated with a central system for a period of 48 hours and the fish were exposed to 15 days conditioning period at room temperature. The fish were fed with commercial feed diet and minced liver trice a day during this period. Care was taken to keep the mortality rate of fish not more than 5% in the last four days before the experiment was started.

Chemically pure salt of zinc sulphate ($CuSo_4.5H_2O$) dissolved in distilled water, was used as toxicant. The test organisms were subjected to different concentrations (50, 60, 70, 80, 100 and 120 mg/l) of the copper sulphate ($CuSO_4.5H2O$). For the acute bioassay tests, 20 fish were used per concentration. The containers were not aerated at the dosing time. The amount of copper sulphate to be added in each aquarium was calculated after the volume of each aquarium was accurately determined.

There was a simultaneous control group together with the actual experiments. The control group was kept in experimental water without adding the copper sulphate; keeping all other conditions constant. Water quality parameters (temperature, dissolved oxygen (DO), $CaCO_3$ hardness, and pH) used in the aquaria were periodically determined before the bioassay tests. The water temperature was kept 27 ± 2.0 ^{0}C. In addition, the experimental medium was aerated in order to keep the amount of oxygen not less than 6 mg/l.

All experiments were carried out for a period of 96 hrs period. The number of dead fish were counted every 24 hours and removed from the aquarium as soon as possible. The mortality rate was determined at the end of the 96th hour. No food was given to the fish during the experiments.

Toxicological dose-response data involving quantal response (mortality) following toxicity of copper on the test species, *Oreochromis niloticus* and *Clarias gariepinus* were determined by the use of Finney's Probit Analysis LC_{50} Determination Method (Finney, 1971). Mortality response of the fish species was taken to be when the animals sank to the bottom of the containers and became motionless. The rate of response determined at the end of the 96th hours. The index for toxicity measurement was LC_{50} and deductions were based on the 96 hours LC_{50};

TF (Toxicity factor) = this is used to measure the relative potency ratios =

$$\frac{LC_{50} \text{ of a compound X}}{LC_{50} \text{ of another compound Y}}$$

Significance in 95% confidence limit of the detected 96 hrs LC_{50} values were determined using the Chi-Square technique. The limit of significance was 0.05.

RESULTS AND DISCUSSION

Copper was found toxic to the test fish species with *Oreochromis niloticus* responding higher than *Clarias gariepinus*. Table 1 shows the 96 hours acute toxicity of copper sulphate to *Oreochromis niloticus* and *Clarias gariepinus*, respectively with Figures 1 and 2 displaying the Probit line graphs of the toxicity data for the test freshwater fish species. Various authors in different parts of the world including Nigeria (Khangarot and Ray, 1989; Mackie, 1989; Oyewo, 1998; Khunyakari et al., 2001) have similarly observed and recorded differential toxicity of heavy metal compounds against different test animals. The observed differences in the acting metal (copper) might be due to the physicochemical characteristics of the test medium (Cusimano et al., 1986; Solbe, 1984), species and ages of fishes used and their susceptibility rates to the test chemical, which resulted in their subsequent

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toxicity values. Consequently, the observed toxicity values of copper were shown to be less than those reported by Khangarot et al. (1982).

Table 1 - 96 hours acute toxicity of copper to fish species

Test Animal	96 hrs LC_{50} (mg/l)	96 hrs LC_5 (mg/l)	96 hrs LC_{95} (mg/l)	S.E	T.F
Oreochromis niloticus	58.837	19.627	176.375	1.424	1
Clarias gariepinus	70.135 (58.023-84.771)	21.481 (9.166-31.224	228.989 (157.545-536.575)	1.241	1.19

TF: Toxicity Factor

Probit Transformed Responses

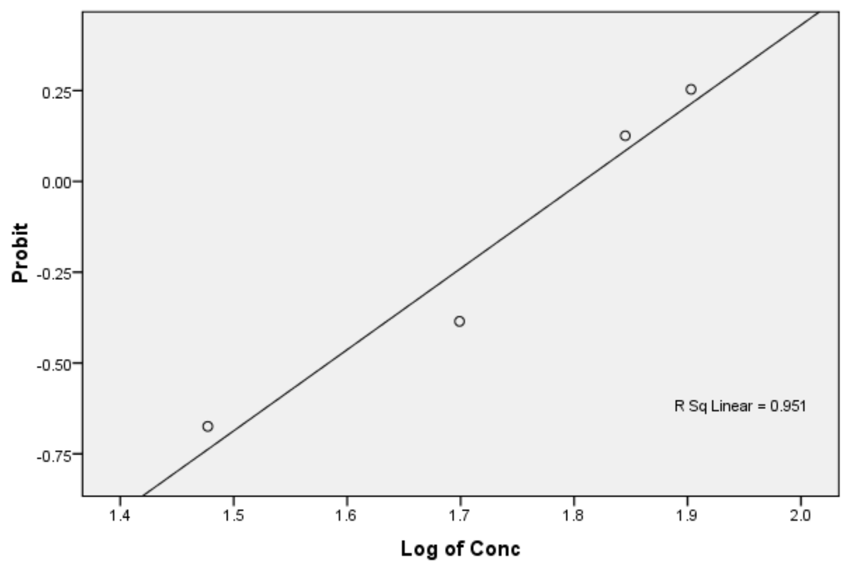

Fig. 1 - Probit line graph of acute toxicity of copper sulphate to tilapia *(Oreochromis niloticus)*

Probit Transformed Responses

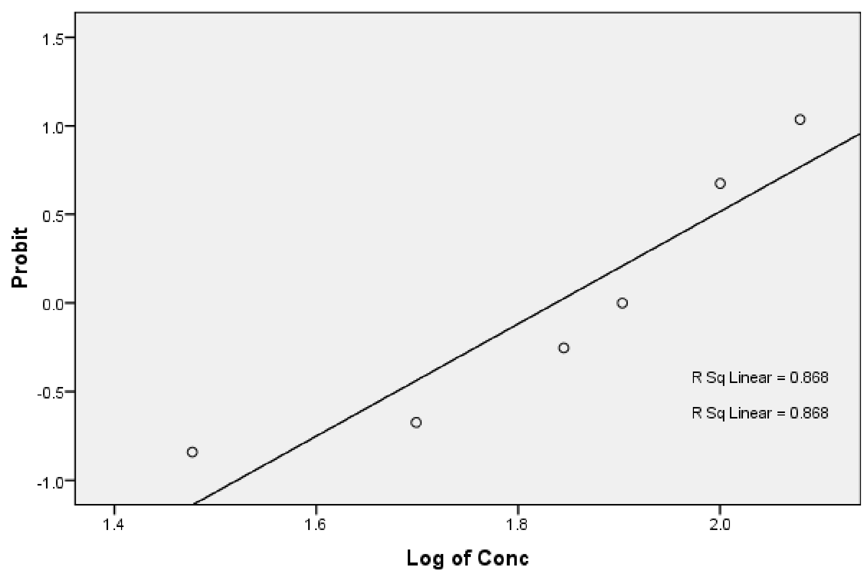

Fig. 2 - Probit line graph of acute toxicity of copper sulphate on catfish (*Clarias gariepinus*)

Copper was significantly (no overlap in 95% C.L of 96 hrs LC_{50} values) more toxic on *Oreochromis niloticus* than the catfish. Tables 2 and 3 portray the parameteric estimates of the Probit analysis, and Chi-Square test for the acute toxicity of the copper sulphate to *Oreochromis niloticus* and *Clarias gariepinus*, respectively. Several workers such as Oyewo (1998) have demonstrated the relatively higher toxicity of the copper repeatedly and

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Otitoloju (2001) against local animal species of which the former author documented Tilapia sp. to be highly sensitive in relation to other aquatic animals employed in the study. Acute (LC_{50}) and sub lethal copper effects on adult fish physiological parameters or copper hazards to invertebrates have been extensively studied and reported by Hogstrand and wood (1996), Svecevicius and Vosyliene (1996), Khangarot (1989) and Eisler (1998). However, the reported LC_{50} values for this metal by the authors were lower than the values obtained in our study. Generally, there was corresponding increase in mortality response of the test fish species with increased exposure and time (Figures 1 and 2).

Table 2 - Parameter estimates of the Probit analyses for *Oreochromis niloticus* and *Clarias gariepinus*

Parameter		Estimate	Std. Error	Z	Sig.	95%Confidence Interval	
						Lower Bound	Upper Bound
Tilapia Species (*Oreochromis niloticus)*							
PROBIT[a]	Conc	3.637	0.796	4.570	0.000	2.077	5.196
	Intercept	-6.350	1.424	-4.460	0.000	-7.774	-4.926
Catfish Species (*Clarias gariepinus)*							
PROBIT[a]	Conc	3.201	0.669	4.783	0.000	1.889	4.513
	Intercept	-5.909	1.241	-4.759	0.000	-7.150	-4.667

[a] PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Table 3 - Chi-Square tests for 96 hrs LC_{50} values of *Oreochromis niloticus* and *Clarias gariepinus*

		Chi-Square	df[a]	Sig.
Tilapia Species (*Oreochromis niloticus)*				
PROBIT	Pearson Goodness-of-Fit Test	7.991	3	0.046[b]
Catfish Species (*Clarias gariepinus)*				
PROBIT	Pearson Goodness-of-Fit Test	3.941	4	0.414[c]

[a] Statistics based on individual cases differ from statistics based on aggregated cases; [b] Since the significance level is less than 0.050, a heterogeneity factor is used in the calculation of confidence limits; [c] Since the significance level is greater than .050, no heterogeneity factor is used in the calculation of confidence limits.

The behavioral changes observed among the fish species exposed to various concentrations of copper sulphate are as follow:

Experimental Groups

There was avoidance of the copper sulphate contaminated water through unsteady swimming pattern with jerky movements. Their fins became hard and stretched following high excitability. There was lost of balance and exhaustion. After period of stressful avoidance through various behavioral anomalies, fish remained suspended in vertical position with the mouth up, near the water surface and the tail pointing downward. Finally, they sank to the bottom of the water, became motionless, and did not respond to gentle probing.

Control Groups

There were no observable behavioral changes and death among the fish species during the bioassay. The theoretical spontaneous response rate was zero.

Obviously, the present investigation shows the behavioral anomalies and subsequent death of fish exposed to the heavy metal toxicant (copper sulphate). This could be explained by the fact that the toxic effect is mediated through the perturbed nervous systems, affecting almost all vital activities of the organisms, dopaminergic pathways, and related functions. The effects of pollution on behavior have been reviewed with primary reference to aquatic animals by Atchison et al. (1996), which should be consulted for further information. Neurological impairment has been observed in factory workers exposed to copper dust (ATSDR, 1990).

While emphasizing the importance and acute toxicity of copper to biological systems, there is indication that behavioral changes in fish are an adequate biomarker for pollution monitoring and management of aquatic environment. The employment of other fish species including invertebrates in different aquatic environments is highly recommended to determine their differential sensitivity and applicability in aquatic eco-toxicology and pollution management.

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