TOXICITY OF UREA FERTILIZER TO FINGERLINGS OF *Clarias gariepinus* AND ASSOCIATED BEHAVIOURAL CHANGES

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ABSTRACT
Clarias gariepinus fingerlings (mean TL, 3.20 ± 1.80 cm; mean weight 1.30 ± 0.50 g) were exposed in a daily renewal bioassay to different concentrations of urea fertilizer (0.00, 25.0, 26.0, 27.0, 28.0, 29.0 and 30.0 g/L), for 96 h to assess its toxicity and behavioural response. The opercular beat, tail fin beat frequencies and mortality were monitored during the exposure period. The pattern of response of opercular beat and tail fin beat frequencies at the various concentrations of the fertilizer generally increased with duration, peaking at the 24th hour after which it began to decline. Other observed behavioural reactions of the exposed fish before mortality were uncoordinated swimming, restlessness, frequent attempts at jumping out of the tank and quietness. Mortality of exposed fish in all toxicant concentrations increased with concentration and duration of exposure. 96-h LC_{50} of the toxicant was 26.54 g/L with 25.99 g/L and 27.00 g/L as the lower and upper limits respectively. Urea fertilizer caused acute behavioural changes and mortality of C. gariepinus fingerlings.
Keywords: composite fertilizer, NPK, African catfish, toxicants

INTRODUCTION

Composite fertilizers (NPK) are not classified as hazardous material according to EEC Directive 67/548/EEC (EPC, 1999). As these fertilizers contain phosphates they may cause adverse environmental impact such as eutrophication in confined surface water. NPK, without urea has low potential for bioaccumulation and low toxicity to aquatic life (EIFAC, 1973; EFMA, 2005). Clarkson *et al*. (1986) reported that ammonium is the main inorganic form of nitrogen.

Urea fertilizer, also known as carbamide, is the most important nitrogenous fertilizer, containing about 46 percent nitrogen. The environmental effects of urea relate to the degradation of urea to ammonia or other products of nitrogen (Bob-Manuel *et al*., 2006; Capkin *et al*., 2010). Ammonia occurs in natural water in un-ionized (NH_3) and ionized (NH_4^+) forms and can be a serious toxicant to fishes and other aquatic species (El-Shafai *et al.,* 2004). It enters water systems from several sources including industrial wastes, sewage effluent, agricultural input and animal feedlots. It is also a metabolic by-product of fish (Abbas, 2006). The accumulation of ammonia in water used for intensive fish culture is a potential problem because it is toxic to fish. Most nitrogen in feeds and fertilizers that is not converted to fish flesh enters the water as ammonia, either by direct excretion from fish or by bacterial action on wastes (Boyd and Massaut, 1999). Ammonia concentrations can increase rapidly when water exchange rates are low (Alabaster and Lloyd, 1980; Harry and Boyd, 1987).

The toxicity of ammonia to different fish species has been extensively investigated (Boyd and Massaut, 1999; Wicks and Randall, 2002; Chew *et al.,* 2003; Capkin *et al*., 2010). Ammonia toxicity depends principally upon the presence of NH_3, which can readily diffuse across the gill membrane due to its lipid solubility and lack of charge, whereas the ionized form cannot readily pass through the hydrophobic micropores in the gill membrane (Sheehan and Lewis, 1986). However, NH^+, is excreted across the gill only via a carrier mediated process in exchange for Na' and may also show considerable toxicity at low pH (Chew *et al*., 2003).

Ammonia is toxic, not only to fish but also to all aquatic animals (El-Shafai *et al.,* 2004), especially in pond aquaculture at low concentrations of dissolved oxygen (Boyd and Massaut, 1999). The toxic levels of un-ionized ammonia for short-term exposure usually are reported to lie between 0.6 and 2 mg/L, while some consider the maximum tolerable concentration to be 0.1 mg/L (Pillay, 1992). Feed efficiency and body composition of fish are negatively affected by ambient ammonia concentration. The main change in the body composition includes an increase in the water content (El-Shafai *et al.,* 2004). Sangeetha *et al*. (2011) investigated the acute toxicity of ammonium sulphate, urea, NPK-1 to fingerlings of fresh water fish *Catla catla* and observed that the 96- h LC_{50} values and behavioral responses of the fish among the three fertilizers, was in this order of toxic effect: ammonium sulphate> NPK-1> urea.

C. gariepinus is an important fish for aquaculture in Nigeria because it meets up one partial solution for the increasing demand for protein. It has been artificially reproduced and cultured under various Nigerian aquaculture systems. Comparatively urea fertilizer production cost is low and it is commonly used for all types of crops and soils in Nigeria. In view of urea fertilizer's decomposition even at room temperatures, particularly in humid atmospheres releasing ammonia and carbon dioxide and scanty of information on its toxicity on fish species, the present study was undertaken to measure the acute toxicity of urea to *C. gariepinus.*

MATERIALS AND METHODS

Fingerlings of *C. gariepinus* (mean ± SD TL, 3.2 ± 1.80 cm; mean ± SD weight 1.40 ± 0.5 g) were obtained and transported from Bagiwa fish farm, Funtua, Kastina State in cold boxes with ample water to the Department of Biological Sciences' Fisheries & Hydrobiology Laboratory, Ahmadu Bello University, Zaria, Kaduna State. They were acclimated for two weeks in 4 oval/rectangular shaped bath tubs, separately containing water of about 150 liters. The fish were fed twice daily at 3% biomass on a 30% crude protein diet. Holding tanks were cleaned daily and water was changed every 24-hour.

Five kilogrammes (5.00 kg) of urea fertilizer was obtained from the Kaduna State Ministry of Agriculture, Zaria office. Standard methods (APHA, 1998) with few modifications were employed in carrying out the experiment. A range finding test was performed by exposing five fish each to five different concentrations of solutions of the urea fertilizer (25.0, 26.0, 27.0, 28.0, 29.0 and 30.0 g/L). After the range finding test, ten fish was exposed to 25.0, 26.0, 27.0, 28.0, and 29.0 g/L and a control (0.0 g/L). The bioassay test were carried out in 11 glass tanks, each of size 30.5 x 30.5 by 92.5 cm, into which approximate weight of urea fertilizer and dilution water were taken and mixed, to give a final volume of 25.0 L. The fish were starved 24 hours before commencement and during the bioassay trails. The solutions were stirred for homogenous mixing before each aquarium was randomly stocked in duplicates with ten fish. The test solutions and control were renewed daily.

Opercular and tail beat frequencies of two fishes/each aquarium were recorded at 0, 24, 48, 72 and 96 hours. A fish was considered dead when there was lack of response to gentle prodding with a glass rod and dead fish removed with an aquarium net. The data obtained were subjected to one ANOVA. The 96-h LC_{50} concentration of the toxicant was computed by probit method using SPSS version 17 soft ware®. In each tank, at the start and at the end of the 96-h bioassay, temperature, total dissolved solids (TDS), electrical conductivity, pH and dissolved oxygen of the test water were determined using standard methods (APHA, 1998).

RESULTS

The mean physicochemical characteristics of the test solution are: Temperature (26.70 ± 1.02 °C), pH (8.01 ± 0.13), TDS (143 ± 1.5 mg/L), DO (6.41 ± 2.3 mg/L) and electrical conductivity (277.30 ± 2.01 µS/cm). *C. gariepinus* fingerlings exposed to various concentrations of urea fertilizer showed an initial rapid opercular and tail fin movements, accompanied by incessant gulping for air, loss of balance, and restlessness. The increase in these activities declined after 24 hours of contact with the toxicant. The intensity of the behavioural activities of the fish decreased with increasing concentration and duration of exposure. However, fish in the control maintained close to normal behavior within the 96 hours of the experiment.

The opercular ventilation rates of fishes exposed to the fertilizer and the control fishes peaked at the 24^{th} and 48^{th} hours respectively (Fig. 1a). Beyond this point ventilation rates became more variable declining with exposure time. However, ventilation rates of the control fishes rose again slightly after the 72^{nd} hour. The tail fin beat followed a similar trend as the opercular beats. The tail fin beats of the control fishes were less variable than those of the fishes exposed to the toxicant solution (Fig. 1b). The tail fin beats were generally less variable than the opercular beats especially at the 48^{th} hour. Analysis of variance revealed that the average opercular beat and tail fin beat per minute differ significantly between the treatments ($P < 0.05$).

Mortality in the exposure concentrations increased with duration becoming more variable with time. None of the fertilizer concentrations caused 100% mortality of the fish. Mortality was recorded in all the tanks except that of the control and the rate of mortality was dependent upon concentration (Table 1). The computed LC_{50} of urea fertilizer for *C. gariepinus* is 26.54 g/L with 25.99 g/L and 27.00 g/L as the lower and upper limits respectively. Dead fishes were observed to be covered with slimy materials on their body. The skins of the fishes were observed to peel off in the test aquaria.

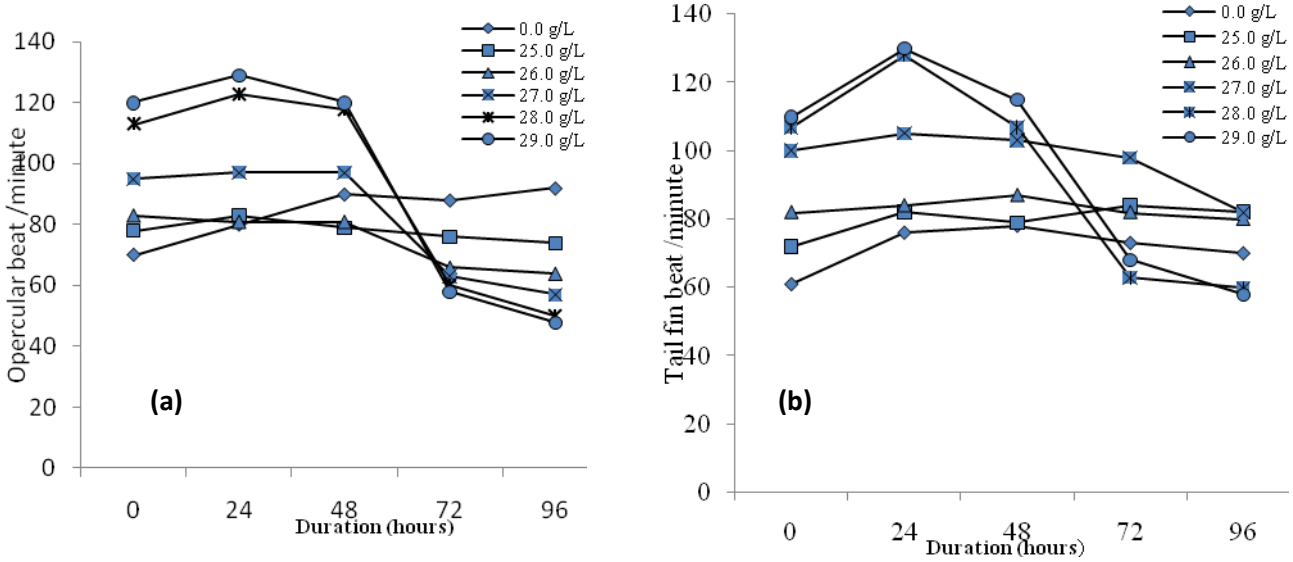

Fig 1: Acute effects of urea fertilizer on: (a) opercular ventilation rate and (b) tail fin beat frequencies of *Clarias gariepinus*

Table 1: 96-h mortality and effects of urea fertilizer on *Clarias gariepinus*

Conc. tanks (g/L)	Log of Conc.	96 -h mortality Replicate A	Replicate B	Total observed mortality	Total expected mortality	Residual	Probability
Control	0.00	0	0	0	0	0	0
25.0	1.398	2	0	2	2.646	-0.646	0.132
26.0	1.415	5	3	8	6.394	1.606	0.320
27.0	1.431	4	6	10	11.225	-1.225	0.561
28.0	1.447	8	8	16	15.493	0.507	0.775
29.0	1.462	10	8	18	18.175	-0.175	0.909

N = total number of fish exposed = 20

DISCUSSION

Behavioural responses of fish to most toxicants and differences in reaction times have been observed to be due to the effect of chemicals, their concentrations, species, size and specific environmental conditions (Adakole, 2005; Bobmanuel *et al*., 2006). The behavioral responses recorded for *C. gariepinus* in this study are similar to those reported by other authors for clariids under various stress conditions (Onusiriuka and Ufodike, 2000; Adakole, 2005; Bobmanuel *et al*., 2006). Besch (1975) identified four main phases in the responses of fish to toxicants. The contact phase (brief period of high excitability), exertion (visible avoidance characterized by fast swimming, leaping and attempt to jump out of the toxicant), loss of equilibrium followed by the lethal (death) phase when opercular movement and responses to tactile stimuli cease completely.

Changes in behavioural patterns exhibited by fish were possibly to counteract aquatic hypoxia condition (Kind *et al.*, 2002), possibly caused by the urea fertilizer. When there is impossibility of escape from hypoxic stress, physiological alternations may be evoked to compensate for low oxygen supply (Val *et al.*, 1998). The stressful behaviors of exposed fish such as erratic swimming, increased opercular beat and tail fin beat rates, regular visit to the surface to gulp in air, loss of balance, restlessness and finally mortality of fish in this study agrees with the findings of Omitoyin *et al.* (2006) in *C. gariepinus* exposed to paraquat and *Tilapia guineensis* treated with acute concentrations of NPK (Chukwu and Okpe, 2006). The disruption of the functioning of nervous system

of fish might be the cause of slow movement, lethargic and erratic swimming and loss of equilibrium (Pal and Konar, 1989). The sudden change in behaviour may be due to shock while the rise and subsequent decrease in opercular and tail fin beat rates may be due to fatigue resulting from suppressed metabolic rate and thus low oxygen demand (Jensen *et al*., 1993). According to Kind *et al*. (2002), air breathing (regular surfacing) provides access to a high and stable supply of oxygen to the gills which acts in tandem with increased haemoglobin-oxygen affinity to preserve oxygen uptake.

Nitrogen fertilizers such as urea fertilizer can increase ammonium concentrations in the water (Palanivelu *et al.*, 2005; Bob-Manuel *et al*., 2006). The results of this acute testing demonstrate that urea fertilizers exhibit acute lethal toxicity to *C. gariepinus*. Ufodike and Onusiriuka (2008) estimated that the 96-h LC_{50} value of composite fertilizers for African catfish (*C. gariepinus*) ranged from 33.9 mg/L for Ca $(OH)_2$ to 1.25 g/L for $NaNO_3$. In another study, *Ofojekwu et al. (2008) reported that* the 96 hr LC_{50} of urea fertilizer for *Tilapia zilli* fingerlings to be 15.85 g/L with lower and upper confidence limits being 8.85 and 28.46 g/L respectively. In the present study, the 96-h LC_{50} value of urea fertilizer for *C. gariepinus* is 26.54 g/L with 25.99 g/L and 27.00 g/L as the lower and upper limits respectively. This is higher than that reported by *Ofojekwu et al. (2008) for Tillapia zilli while similar to the report of* Ufodike and Onusiriuka (2008) for *C. gariepinus*. The difference between this current report and that of *Ofojekwu et al. (2008)* might be related to differences between the fish species. *Clarias* species is a well known hardy fish.

CONCLUSION

In summary, urea fertilizer is toxic to *Clarias gariepinus.* The toxicity increased with increasing of fertilizer concentration as well as exposure time. It is concluded that urea fertilizer may have toxic potentials in the aquatic bodies and therefore it should be carefully used in the areas close to water bodies.

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