Investigating the Acute Toxic Effects of Urea Fertilizer on Juvenile Nile Tilapia (*Oreochromis niloticus*)

ABSTRACT

The research aimed to assess the acute toxicity of urea fertilizer on juveniles of *Oreochromis niloticus*. The findings indicated a correlation between increasing concentrations of urea fertilizer and higher mortality rates among the test organisms, with an LC_{50} value determined at 500 mg/l. Additionally, a rise in total dissolved solids was observed with higher concentrations of urea. Parameters such as temperature, electrical conductivity, dissolved oxygen and pH showed statistically significant ($P < 0.05$) changes as the concentrations of urea increases. Behavioural responses during the exposure phase included heightened excitability, vigorous swimming behaviours, loss of equilibrium, culminating in mortality. These results unequivocally demonstrate that urea fertilizer at acute concentrations poses significant toxic risks to juvenile *Oreochromis niloticus*.

Keywords: Acute toxicity, Urea, Juveniles, Organic fertilizer, *Oreochromis niloticus*

1.0 INTRODUCTION

The acute toxicity of fertilizers on aquatic organisms such as the Nile tilapia (*Oreochromis niloticus*) is a significant concern in aquatic ecology and environmental management. The use of fertilizersin agriculture has been instrumental in boosting crop yields and meeting the demands of a growing population. However, the widespread application of fertilizers has raised concerns about its impacts on water quality and aquatic species. The intricate relationship between fertilizers, water quality and aquatic species revolves around the complex dynamics of nutrients enrichment, ecosystem responses and ecological consequences. Fertilizers primarily containing nitrogen and phosphorous are essential for promoting plant growth and agricultural productivity.

However, when these nutrients are applied excessively or mismanaged, they can infiltrate water bodies via surface run-off, leaching or direct application in aquaculture ponds. As a results aquatic organisms are exposed to elevated concentrations fertilizers derived chemicals, which can have adverse effects on their health behaviour and survival (George and Atakpa, 2015a, b; George and Effiom, 2018;Jonah and George, 2019; Jonah *et. al*., 2019; George, *et. al*., 2020a, b; Jonah *et al*., 2020).

Once in aquatic ecosystem, these nutrients fuel the rapid growth of algae and other aquatic plants leading to a phenomenon known as eutrophication. Algal blooms, fueled by nutrient-rich run-off can blanket water surfaces, blocking sunlight and depleting oxygen levels as they decompose (George, *et. al*., 2020a, b; Jonah *et al*., 2020). This oxygen depletion known as hypoxia or anoxia creates dead zones where aquatic life struggle to survive.

The impacts of eutrophication extend beyond oxygen depletionas it is accompaniedwith harmful algal blooms, often dominated by toxin-producing species pose risks to human health, aquatic organisms and entire ecosystems (Jonah *et. al*., 2019; George, *et. al*., 2020a, b; Jonah *et al*., 2020). Additionally, the proliferation of algae can alter water chemistry, pH levels and light penetration, further disrupting the balance of aquatic ecosystem(George and Effiom, 2018).

Aquatic species ranging from fish to invertebrates and aquatic plants face numerous challenges in eutrophic environmentswhichmay lead to massive mortality among fish and other organisms (Effiong, *et. al*., 2021), while changes in water chemistry and habitat structure can affect reproductive success, growth rates and species diversity (Akpan, *et. al*., 2022 a, b).

The relationship between fertilizers, water quality and aquatic species is multifaceted and dynamic. While fertilizers play a crucial role in agriculture production, their improper use can result in detrimental effects on aquatic ecosystems. Addressing this challenge requires a comprehensive approach, including improved nutrients management practices, watershed management strategies and conservation effort aimed at restoring and preserving the health of aquatic environments.

O. niloticus is a widely cultured freshwater fish species known for its economic importance and ecological role. By examining the impacts of fertilizers on Nile tilapia at acute exposure level, we aim to elucidate the potential risk posed by ureafertilizer contamination to aquatic ecosystems and highlight the importance ofunderstanding and mitigating these effects for sustainable aquatic resource management.

2.0 Materials and Methods

2.1 Source of Experimental Fish

Juveniles of *O. niloticus* with mean weight (7.65 ± 0.42g) were obtained from Myra fish farm Gora, Nasarawa State. Nigeria. Fish were transported in aerated plastic containers to the hatchery of the Department of Fisheries and Aquaculture, Joseph Sarwuan Tarka University, Makurdi, Benue state. The fish were stocked in 11 plastic containersof 45-litercapacity with well aerated dechlorinated municipal tap water.

2.2 Acclimatization of Fish

Fish were acclimated to laboratory conditions for 7 days during which period they were fed twice daily (08.00am and 04.00pm) at five percent body weight with a commercial feed (vital feed). During this period, dead and abnormal fish were removed. The purpose of the acclimation was to enable them recover from collection and transportation stresses and stabilized to the experimental environment. Water was changed daily to discard faecal material and left-over food. Fish were not fed 24 hours prior to and during the exposure period. Mortality during the acclimation period was less than two percent. It was from the acclimated population that healthy individuals used as test fish in this study were carefully selected.

2.3 Preparation of test media and exploratory test

To obtain the ranges of concentrations as used in the experiment, five fishes were selected and each were exposed to 10 liters of dechlorinated tap water containing different weights of the fertilizer and used for the preliminary runs for twenty-four hours, until suitable concentration that resulted in 100% mortality was derived. The fish were not fed twenty-four hours before and during these trials. The ranges of concentration values used in this study were determined from the 100% mortality obtained from the trials.

2.4 Experimental Procedures

The exposure concentrations of urea fertilizer gotten from the trial include the following 400, 450, 500, 550and 600 mg/L with dechlorinated municipal water without fertilizer (0.00 mg/L) which serves as the control. Ten fish were exposed to each of the five concentrationsand eachconcentration had a replicate to ensure accuracy. The exposure period lasted 96 hours. The effect of the acute concentrations of urea fertilizer on opercula ventilation, tail beat rate and other behavioural characteristics were also observed. Dead fish were removed immediately to avoid fouling the test media and recorded against the concentration.

2.5 Monitoring of Specimen for Mortality

The effects of the various concentration of urea fertilizer on the juveniles was monitored on a 24 hours' basis for 96 hours as recommended by Udo *et. al.*, (2006) and Ekanem and Ekpo (2008).

2.5.1 Determination of Mortality and Survival Rates of *O. niloticus* Juveniles

The percentage mortality and survival rates of the juveniles in the different concentrations of urea fertilizer during the period of study was determine using the formula;

% mortality =n/N × 100 (Chan, 1977).

Where;

n = number of dead fish per aquarium per concentration

N = Total Individual Stocked

The difference between dead fish and survivors will give the percentage survival of the juveniles at the end of the experiment (96 hours) (Udo *et. al.*, 2006).

2.5.2 Determination of Mortality Lethal Median Concentration (96 hours LC_{50})

The effects of the various concentrations of urea fertilizer on the juveniles of *O. niloticus* was determined by graphical method (Probit Level Determination as recommended by Omoregie (2002), Omoregie and Ufodike (2000), Ekanem and Ekpo (2008) and Udo *et.al*. (2006). At Lethal Median Concentration LC_{50}, after 96 hours of test, the number of fingerlings that are expected to die was determined from the graph. Similarly, the concentration that will kill 50% of the stocked fingerlings at the end of the test (96 hours) was determined at the probit level (Omoregie, (2002) Omoregie and Ufodike (2000), Udo *et. al*., (2006); Ekanem and Ekpo (2008).

2.6 Water Quality Parameters

Water quality parameters that were measure include; Temperature, dissolved oxygen (DO), pH, electrical conductivity (EC) and total dissolved solid (TDS). Dissolve oxygen was measured using DO meter model L933246 while other parameters were monitored every 24 hours using a multi parameter water quality tester (Hanna meter, model Hi 98127), where the probes were immersed directly into the water and allowed to stabilize then readings were taken as displayed on the screen.

2.7 Data Analysis

Data collected were subjected to one way analysis of variance with Duncan's new multiple range post hoc-test for significance difference ($P < 0.05$).

3. RESULTS

3.1 Initial Water Quality Parameters

The initial water quality parameters prior to commencement of the toxicity studies are shown in Table 1. The values of dissolved oxygen (4.9 mg/l), temperature (29.5 ^{0}C) and pH (6.80) observed were within the acceptable range for aquaculture operations.

Table 1: Initial Physico-chemical parameters of the test water prior to stocking of test organism

Fish Species	Initial physico-chemical parameters prior to stocking		
	DO (mg/l)	**Temp (^{o}C)**	**pH**
Oreochromis niloticus	4.9	29.5	6.80

3.2 Physico-chemical parameters

The results of the water physico-chemical parameters of the experimental media (Table 2) shows there was an increase in the total dissolved solid content as the urea concentrations increased. There was no significant difference in temperature, there were however significant differences in the values of electrical conductivity, dissolve oxygen and pH ($P < 0.05$).

Table 2: Mean Water Quality Parameters of the Bioassay water for acute toxicity experiment with Urea Fertilizer on *Oreochromis niloticus*juveniles.

Concentration (mg/L)	pH	DO	Temperature	TDS	EC
0.00	8.10 ± 0.10^{b}	4.90 ± 0.05^{a}	28.15 ± 0.05^{a}	263.15 ± 0.15^{d}	527.20 ± 0.20^{d}
400.00	6.60 ± 0.10^{d}	3.90 ± 0.10^{b}	28.10 ± 0.10^{a}	664.05 ± 0.05^{a}	1434.20 ± 0.25^{a}
450.00	7.15 ± 0.15^{c}	3.40 ± 0.10^{c}	28.20 ± 0.10^{a}	629.15 ± 0.15^{b}	1266.20 ± 0.15^{b}
500.00	7.45 ± 0.05^{c}	3.20 ± 0.10^{c}	28.25 ± 0.25^{a}	446.15 ± 0.15^{c}	904.25 ± 0.25^{c}
550.00	8.80 ± 0.00^{a}	3.95 ± 0.05^{b}	27.40 ± 0.10^{b}		
600.00	9.20 ± 0.10^{a}	4.80 ± 0.10^{a}	28.15 ± 0.15^{a}		
P-Value	0.00	0.00	0.04	0.00	0.00

**Means in the same column with different superscripts differ significantly ($P<0.05$)*

3.3 Mean Mortality of Juveniles Exposed to Urea Fertilizer for 96 hours

The percentage mortality and survivors of *O. niloticus*juveniles at the end of the test period in each of the concentrations are shown in Table 3 for the two batches of the experiment.

At concentrations of 600.00 mg/l, 90% mortality was recorded at the end of 96 hours of test (Table 3). At concentrations of 550.00, 500, 450 and 400 mg/l, the mortality recorded were 80, 50, 30 and 20% respectively at the end of 96 hours of test. No mortality was recorded in the control (0.00 mg/l) (Table 3) in both batches.There was a positive correlation between fertilizer concentration versus mortality which indicates that as concentration of fertilizer increased, mortality rate also increased.Statistical Analysis using one-way Anova (SPSS 20.0) showed that there was no significant difference ($p>0.05$) in mortality between the two batches.

Table 3: **Percentage Mortality of** *Oreochromis niloticus*juveniles**exposed to acute concentrations of Urea Fertilizer for 96hours**

Concentration (mg/L)	6 Hrs. Mortality (%)	12 Hrs. Mortality (%)	24 Hrs. Mortality (%)	72 Hrs. Mortality (%)	96 Hrs. Mortality (%)	Total Mortality (%)
0.00	0.00	0.00	0.00	0.00	0.00	0.00
400.00	0.00	10.00	0.00	10.00	0.00	20.00
450.00	10.00	0.00	10.00	10.00	0.00	30.00
500.00	20.00	20.00	10.00	0.00	0.00	50.00
550.00	30.00	20.00	10.00	20.00	0.00	80.00
600.00	30.00	30.00	20.00	10.00	0.00	90.00

3.4 Determination of 96 Hours LC_{50}

The 96 hours LC_{50} for *O. niloticus*juveniles exposed to the different concentrations of urea fertilizerwas determine using probit analysis. The concentrations were first transformed into log for the probit analysis (Table 4). The 96 hours LC_{50} is given at 500.00 mg/l representing a log transformed concentration of 2.74 mg/l a point where 50 % of the test organisms would be killed at the end of the experiment.

Table 4:Mortality Record of ***O. niloticus*****juveniles****exposed to acute concentrations of Urea Fertilizer for 96hours.**

Concentration (mg/l)	Log_{10} Conc	Total Number of Test Fish	Mortality Rate after 96 Hours	Percentage Mortality (%)
0.00	0	10	0	0.00
400.00	2.60	10	2	20.00
450.00	2.65	10	3	30.00
500.00	2.70	10	5	50.00
550.00	2.74	10	8	80.00
600.00	2.78	10	9	90.00

3.3 General behavioural changes

Behavioural changes occurred in the fish treated with Urea fertilizer at different concentrations. The abnormal behaviours observed in fish exposed to concentrations of Urea fertilizers were characterized by restlessness, gulping of air, attempts to jump out of the tank and erratic swimming before death. These behavioural changes showed by the exposed fish in response to the effect of the fertilizer was more pronounced in tanks containing higher concentrations, but decreased with increase in time of exposure. There were no obvious changes in fish behaviour in the lower concentrations (400mg/l) for the first 24 h of exposure. However, fish in the control group of the treatment did not exhibit any abnormal behavior.

4.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 DISCUSSION

Urea is a widely used nitrogen fertilizer in agriculture due to its high nitrogen content and cost-effectiveness. Despite its agricultural benefits, urea can pose environmental and health risks, particularly when used inappropriately or in excessive amounts. In fish farms, chemical fertilizers are often applied before stocking the pond to stimulate the production of organisms that may serve as first food for many species of fish and also increase survival and growth (Ofujekwu, *et al*., 2008 a). Such applications may not be harmful if enough time is allowed for the degradation of these fertilizers by the micro flora. In the context of fish nursery management, it would seem prudent to avoid situations where chemical fertilizers are added intermittently to the ponds, because such subsequent additions may result in total juveniles' mortality, if the concentrations exceed the established LC_{50} reported in this study.

Results obtained from this research revealed that the 96 h LC_{50} value of urea fertilizer for *Oreochromis niloticus* was 500.00 mg/L representing a log transform concentration of 2.74 mg/l. The result obtained was at variance with the 96 h LC_{50} of 1.78mg/l reported by Essien-Ibok *et.al*., (2014) when *Heterobranchus birdosalis* was exposed to acute concentrations of urea fertilizer. Also, the present findingsdo not align with LC_{50} reported by Asuquo, *et. al*., (2016) when reporting on the effects of agricultural fertilizers on fingerlings of *Heterobranchus birdosalis.*The difference may be due to difference in fish species, levels of concentration used and environmental conditions. Inorganic fertilizers produce intermediate products that may result in stress, fatigue, nervous disorder and death. This could be understandable as the toxicity of chemicals depend on the type, composition, technical grade of preparations and the susceptibility of the exposed organisms (Aguigwo, 2002). The results of the toxicity of urea fertilizer are indeed concentration dependent, meaning its harmful effects increase as the concentration of urea increases. The results of the present study agree favourably with the findings of previous authors (George *et. al*., 2023 a, b, c, d; George *et. al*., 2024 a, b, c; Essien-Ibok *et. al*., 2024) who reported similar scenario of mortality in a concentration-dependent pattern.

Water quality showed some variations during the test period. There were significant differences in the mean values of some parameters measured (P > 0.05). The air gulping and the attempt to jump out of the tank observed in the exposed fish is an indication of insufficient amount of dissolved oxygen in the experimental media. The variation observed in the water quality is as a result of the addition of urea fertilizer as no variation was observed in the control tank. Similar results have been documented by (George *et. al*., 2013 a, b; George *et. al*., 2014 a, b; George *et. al*., 2015 a, b, c).

Behavioural responses of fish to most toxicants and differences in reaction times have been observed to be due to the effect of the chemical, their concentrations, species, size and specific environmental conditions (George, *et. al*., *2023* a, b). The behavioural responses reported for the test fish in this study are similar to those reported by other authors for clarrids under various stress conditions (Onusiriuka and Ufodike, 2000; Nwanna, *et al*., 2000; Auta *et al*., 2004). George *et. al*., (2024 a, b) identified four main phases in the exposure time on behavioural responses of fish to toxicants. These are the contact phase (brief period of high excitability), exertion (visible avoidance characterized by fast swimming, leaping and attempts to jump out of the toxicant), loss of equilibrium, followed by (death) phase, when opercular movement and responses to tactile stimuli cease completely. In-spite of the numerous advantages of chemical fertilizers to improve fish production, they have a startling number of adverse effects on aquatic life in water bodies that receive run-off from farmlands or from excess direct application in the aquatic environment (FAO, 2000).

The stressful behaviours exhibited by the fish as established in the study, suggest that they suffered respiratory impairment, due to the effect of the toxicant on the gill and general metabolism. These behavioural responses are indications of processes leading gradually to death due to nervous disorder and insufficient oxygen supply. This result agrees with the findings of other authors, who studied the effects of inorganic fertilizers and fertilizer effluents at their acute concentrations on fish fingerlings (Ekweozor *et al*., 2001; Bobmanuel *et al*., 2006; Nwani *et al*., 2008; Ofojekwu *et al*., 2008a, b).

4.2 Conclusion

The toxicity of urea fertilizer recorded increasing mortality rates with rising concentrations of urea, clearly demonstrate a strong concentration-dependent lethality. Specifically, mortalities of 20, 30, 50, 80 ad 90 percent were observed at concentrations of 400, 450, 500, 550 and 600 mg/l, respectively. These findings highlight the potential acute toxicity of urea when organisms are exposed to high concentrations, which is an important consideration for environmental and health safety regulations. The observed behavioral abnormalities at higher concentrations further indicate that urea can have sub-lethal, yet harmfuleffects on living organisms before reaching lethal thresholds. Such abnormalities may include changes in motor activity and general distress, which could precede death and signal significant distress within affected populations. The results suggest that urea, while beneficial as a fertilizer due to its high nitrogen content, poses serious risks when concentrations in the environment exceed safe levels. This underscores the necessity for careful management and regulation of urea use in agriculture settings to prevent environmental contamination and protect aquatic and terrestrial life. Additionally, these findings should guide future research and policy making to established more precise guidelines for the safe application of urea, ensuring that its agricultural benefits do not come at the expense of ecological and public health.

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