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ACUTE TOXICITY OF NPK 20:10:10 TO FINGERLINGS OF *CLARIAS GARIEPINUS*

DOI: 10.7904/2068–4738–V(9)–34

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Abstract. Fertilizers application might positively or negatively affect the aquatic environment to the benefit or detriment of aquatic organisms including fish. Inorganic fertilizer are used in pond for fertilization purposes, however, beyond a tolerance limit it could be potentially dangerous to fish causing stress as well as death, this study therefore seek to determine the acute toxicity of NPK 20:10:10 fertilizer to Fingerlings of African catfish *Clarias gariepinus* using a static bioassay test. African catfish were obtained from a homogeneous source and acclimatized for two weeks. The 96hour LC_{50} of NPK 20:10:10 to *Clarias gariepinus* was observed to be 3.299g/l with lower and upper limits of 3.232g/l and 3.337g/l respectively, toxicity of Nitrogen in NPK 20:10:10 was estimated to be 0.659 with lower limits of 0.6463 and upper limits of 0.675 while toxicity of potassium/phosphate in NPK 20:10:10 were obtained to be 0.329 with lower and upper limits of 0.3232 and 0.3378 respectively. Toxicosis symptoms observed includes loss of balance, respiratory distress, vertical and erratic movement and death. Observation of toxicosis symptoms in experimental fish began on the second day of the study with it peak at about the 62nd hour. Precautious use of NPK in pond fertilization is advised.

***Keywords*:** African Catfish, fertilizer toxicity, stress pattern, Agricultural fertilizer.

Introduction

Inorganic nitrogen (NH^-_4, NO^-_2 and NO^-_3) may be present naturally in aquatic ecosystems as a result of atmospheric deposition, surface and groundwater runoff, dissolution of nitrogen–rich geological deposits, N_2 fixation by certain prokaryotes cyanobacteria (particularly), and biological degradation of organic matter [GLEICK, 1993; WETZEL, 2001; RABALAIS, 2002].

However, activities of human species have substantially altered global nitrogen cycle, increasing both availability and mobility of nitrogen over large regions of the Earth [VITOUSEK *et al.*, 1997; CARPENTER *et al.*, 1998; GALLOWAY and COWLING, 2002].

Inorganic nitrogen (NH^-_4, NO^-_2, and NO^-_3) enters aquatic ecosystems via anthropogenic sources such as animal farming, urban and agricultural runoff, industrial wastes, and sewage effluents [SMITH *et al.*, 1999; WETZEL, 2001; RABALAIS, 2002].

Also, the atmospheric deposition of inorganic nitrogen (mainly in the form of NO^-_3) has dramatically increased because of the extensive use of nitrogen fertilisers and huge combustion of fossil fuels [CARPENTER *et al.*, 1998; MOOMAW, 2002; BOUMANS *et al.*, 2004].

As a result, concentrations of nitrate in ground and surface waters are increasing around the world, causing one of most prevalent environmental problems responsible for water quality degradation on a worldwide scale [WETZEL, 2001; RABALAIS, 2002; SMITH, 2003].

The use of fertilizer in aquaculture is important for pond fertilization as it increase fish production as a result of nutrient availability for primary production.

In aquaculture, fertilizers have been used in various forms and quantities to enhance fish production for greater abundance of fish food organisms but excessive uses of fertilizer may have adverse effect on water quality and also causes gill damage [HAYGARTH and JARVIS, 2002].

Organic and inorganic fertilizers used in agricultural processes could lead to excessive enrichment of water bodies resulting in high biological oxygen demand, depletion of oxygen [HAYGARTH and JARVIS, 2002; VIDAL *et al.*, 2000], decreased growth [THURSTON and RUSSO, 1983; PALANICHAMY *et al.*, 1985], changes in fish behavior [RANI *et al.*, 1997; WICKS

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et al., 2002], increased vulnerability to disease [THURSTON et al., 1984] and ultimately death of aquatic organisms [HAYGARTH and JARVIS, 2002; VIDAL et al., 2000].

Although composite fertilizers (NPK) are not classified as hazardous material according to EEC Directive 67/548/EEC [EPC, 1999] and Concentrations of 0.3 mg inorganic–N/L have been identified as levels above which environmental problems can occur, however, it is important to elucidate toxicity affects of fertilizer on aquatic organisms, since most aquacultures' employ its use in pond fertilization and uncontrollable amount periodically gets into the aquatic system through runoffs.

Materials and Methods

Fingerlings of African catfish *Clarias gariepinus* of the same breeding history (mean weight, 1.2±0.5 g and mean total length, 3.0±1.2 cm), were obtained from the University of Agriculture Makurdi Fisheries Farm and transported to the Department of Fisheries and Aquaculture Hatchery University of Agriculture Makurdi Nigeria where they were acclimated for two weeks.

The fish were fed twice daily at 3% of their biomass on a commercial diet of 44% crude protein. NPK 20:10:10 fertilizer was obtained from Wadata market in Makurdi Metropolis.

The concentration of NPK 20:10:10 used for the acute toxicity test were determined by a preliminary test as described by [SOLBE, 1995].

Twenty five Fingerlings were randomly selected and transferred from the holding plastic bowls into 5 replicate test plastic bowls of 60 litters capacity, this was filled to 40 litters mark for purpose of the study, NPK concentrations decided were measured and dissolved into the appropriate plastic bowl and marked accordingly.

The control tanks also had ten fish with no fertilizer treatment.

Mortality of the fish was recorded for up to 96 hours of exposure.

Fish were considered dead when gill movement ceased and no response observed upon gentle prodding.

The physic–chemical parameters (temperature, hydrogen ion concentration (pH), dissolved oxygen (DO), total alkalinity and free carbon–dioxide) were determined using standard method by APHA (1985).

Descriptive Statistical Analysis, as well as Analysis of variance of results was done with a computer Programme Gen stat® discovery edition 4, probit graph was drawn with Microsoft excel 2007 while LC_{50}, lower and upper confident limits were determined with Mini Tab® 14 for NPK 20:10:10 and the equivalent concentration of Nitrogen, phosphate and potassium in the fertilizer ration.

Results and discussion

Physico–chemical parameters of water with NPK at different concentrations where observed not to be different from each other (P>0.05) during the period of the experiment (Table 1).

Table 1.
Physiochemical parameters of water

PARAMETERS	2.8 (gL^{-1})	3.0 (gL^{-1})	3.2 (gL^{-1})	3.4 (gL^{-1})	3.6 (gL^{-1})	P–value
Temp. (°C)	27.23±0.02	26.02±0.02	27.00±0.00	27.45±0.05	27.20±0.00	0.09
D. Oxygen (mg/L)	6.6±0.01	6.5 ± 0.02	6.50 ± 0.00	6.40 ± 0.00	6.32 ± 0.48	1.02
pH	6.63 ± 0.02	6.5 ± 0.03	6.45 ± 0.05	6.30 ± 0.10	6.25 ± 0.25	0.41

Symptoms of toxicosis observed included loss of balance, vertical and erratic movement, and respiratory distress such as gasping at the surface, rapid opercula and tail movements and finally death.

The degree of death occurrence increased with increase in concentration of the toxicant with highest concentration recording the most deaths while no death was recorded in the control (Table 2).

Table 2.

Mean Mortality, Probit and Log Concentration of NPK 20:10:10 exposed to *C. gariepinus* fingerlings for 96hours

Conc.(g/L) NPK	N (20%NPK)	P/K (10%NPK)	Log Conc	No. of fish per treatment	No. of Dead fish 96–hrs.	Mean Mortality	% Mortality	Probit
Control	–	–	–	50	0	0	0	–
2.8	0.56	0.28	0.45	50	5+5	5	10	3.72
3	0.60	0.30	0.48	50	8+12	10	20	4.16
3.2	0.64	0.32	0.51	50	20+20	20	40	4.75
3.4	0.68	0.34	0.53	50	26+32	28	56	5.15
3.6	0.72	0.36	0.57	50	34+46	40	80	5.84

Acute toxicity of NPK 20:10:10 as determined in probit curve (Figure 1) was 3.299g/L with lower and upper limits of 3.232g/L and 3.337g/L.

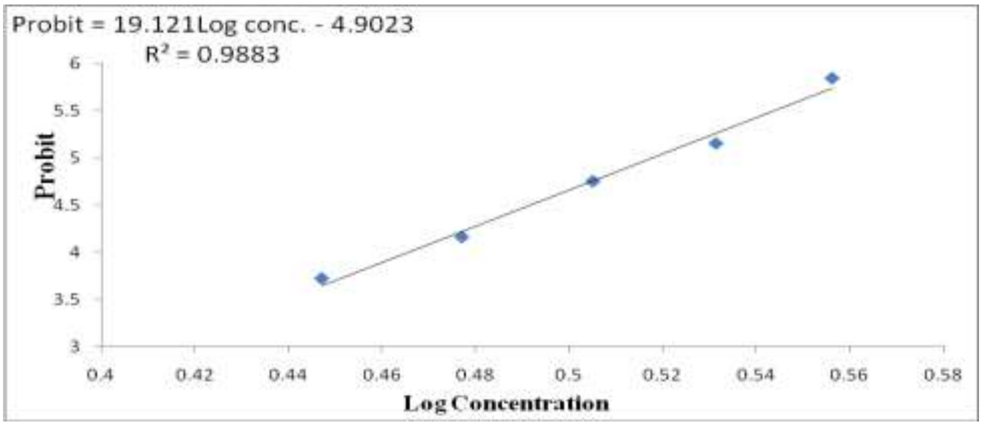

Figure 1. Linear relationship between probit mortality and log concentration of NPK 20:10:10 on *Clarias gariepinus* fingerlings for 96 hours

Respectively, toxicity of Nitrogen in NPK 20:10:10 was estimated to be 0.659 with lower limits of 0.6463 and upper limits of 0.675 (Figure 2)

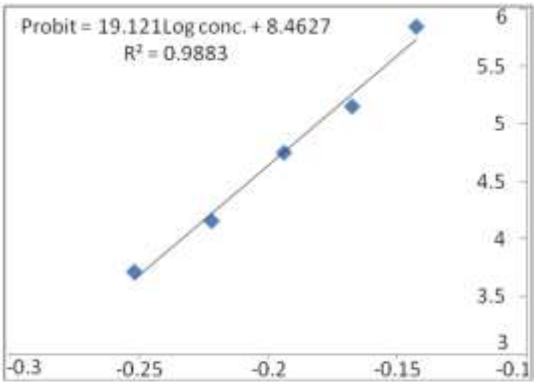

Figure 2. Linear relationship between probit mortality and log concentration of Nitrogen in NPK20:10:10 on *Clarias gariepinus* fingerlings for 96 hours

while toxicity of potassium/phosphate in NPK 20:10:10 were obtained to be 0.329 with lower and upper limits of 0.3232 and 0.3378 respectively (Figure 3).

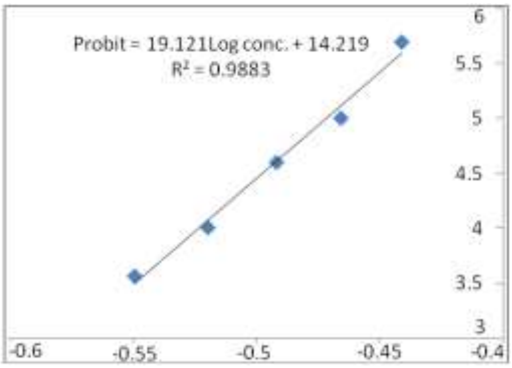

Figure 3. Linear relationship between probit mortality and log concentration of Phosphate/ Potasium in NPK 20:10:10 on *Clarias gariepinus* fingerlings for 96 hours

The physicochemical parameters measured were not significantly different among concentration and so were not thought to be cause of fish mortality as they were found to be within tolerance range as recommended by [MACKERETH, 1963].

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Also the water quality parameters were within recommended range for the culture of tropical fishes. [BOYD, 1979] recommended a pH range of 6.5–9 and temperature range of 25°C–32°C which were in range with present experiment.

However, it should be noted that fertilizer effect on physicochemical parameter of water is specific to individual kind of fertilizer used and may differ from one region to another.

Organic fertilizers are most likely to cause significant changes in dissolved oxygen levels due to biodegradation.

NPK 20:10:10 toxicity increased with increased concentration.

Behavioural responses of fish to most toxicants are most sensitive indicators of potential toxic effects [EIFAC, 1983].

The detection of abnormal activity in this study was based on the observations of fish under the control treatment compared with those with toxicant, [RICHMONDS and DUTTA, 1992] also stated that comparisons of the responses of exposed fish with activity measured during a baseline or pre–exposure period could give a strong indication of stress.

The Fingerlings exposed to high concentrations showed no initial signs of stress.

Disturbed swimming behaviour such as erratic movement, gasping at surface, rapid opercula and tail movements, began at about the 40th hour and increased with increasing concentration with it peak at about the 62nd hour.

These stress signs ceased on the 3rd day of exposure with the fish preferring to settle at the bottom of the bowl.

The initial reaction of the fish was to swim actively possibly due to the effect of the fertilizer on the nervous system [UFODIKE and ONUSIRIUKA, 2008]; the rapidity of swimming was directly proportional to the concentration of the NPK.

The stressful and erratic behaviour of the *Clarias gariepinus* fingerlings also tend to indicate respiratory impairment probably due to the effect on the gills.

Fish breathe by movement of water, dissolved oxygen and any water contaminants present, in and out through their gills, so gills are usually site of first contact of the internal organ.

This agrees with the finding of [UFODIKE and ONUSIRIUKA, 2008). AYUBA and OFOJEKWU, 2002], observed in *C. gariepinus* exposed to acute toxicity of *D. innoxia* leaf extract loss of balance, respiratory disorder gulping of air and erratic swimming before death, this is also in line with findings of [OMOREGIE *et al.*, 1998; TAWARI–FUFEYIN *et al.*, 2008; OLOLADE & OGINNI, 2010; OKOMODA *et al.*, 2010; OKOMODA & ATAGUBA, 2011; OKOMODA *et al.*, 2013], who made similar observations when they exposed *Clarias gariepinus* to different toxicants.

The fish that survived the 96 hrs bioassay test had pale skin color which increased with increasing concentration, with the fishes in highest concentration being very pale from their neck towards the caudal fin.

However, fish in the control maintained normal behavior within the 96 hours of the experiment.

Acute toxicity of NPK 20:10:10, Nitrogen and Phosphate/Potassium in NPK 20:10:10 as observed in this study was 3.299g/l, 0.659 and 0.329 respectively.

The concentrations of ammonium sulphate, calcium ammonium nitrate, NPK15:15:15 and NPK 25:5:10 that killed 50% of the rainbow trout within 96 h. was 0.149, 0.259, 0.258 and 0.408 g/L, respectively as reported by [CAPKIN *et al.*, 2010].

Fish mortality due to toxicant exposure mainly depends upon its sensitivity to the toxicant, its concentration and duration of exposure [RAM *et al.*, 2009; UFODIKE and ONUSIRIUKA, 2008] estimated the 96hr LC_{50} value of composite fertilizers for African catfish (*Clarias gariepinus*) to range from 33.9 mg/L for $Ca(OH)_2$ to 1.25 g/L for $NaNO_3$.

In another study [MAC KINLAY and BUDAY, 1997], 96hr LC_{50} values of NPK 28:0:0 and NPK 10:34:0 for the rainbow trout were found to be 0.585 g/L and 1.342 g/L, respectively.

The value reported in this study was higher than the values reported by [UFODIKE and ONUSIRIUKA, 2008; MAC KINLAY and BUDAY, 1997].

The difference might be related to fertilizer composition, fish and physicochemical characteristics of test water [SAHA *et al.*, 2002; PALANIVELU *et al.*, 2005]. [WHO, 1994] earlier stated that LC_{50} values depend on the toxicant, fish species and the test conditions.

Experiment by [SERVIZI *et al.*, 1989] on MONO818® a glyphosate formulation reveals LC_{50} of 2–3 mg/L for sockeye, rainbow, and coho fry while LC_{50} of Roundup® for bluegill sunfish and rainbow trout is only slightly higher at 6–14 mg/L and 8–26 mg/L, respectively.

However [OKOMODA and ATAGUBA, 2011] reported LC_{50} of 17.5 mg/L for African catfish exposed to acute concentrations of Sunsate®. 96 hour LC_{50} of formalin was reported by [OKOMODA *et al.*, 2010] to be 114.83µl/L for the African catfish, [ORONSAYE and OGBEBO, 1997] also reported LC_{50} of 0.4mg/l for *Clarias gariepinus* exposed to 96 hour of copper sulphate, [AYUBA and OFOJEKWU, 2002] also reported 204.17 mg/L for *Datura innoxia* root extracts.

Acute concentration of different toxicant differs with formulation and with environmental conditions.

Fertilizers might positively or negatively affect the ecosystem quality to the benefit or detriment of live aquatic organisms including fish [YARO *et al.*, 2005].

Conclusions

This study has revealed NPK 20:10:10 fertilizer to be acutely toxic at 3.31g/l with mortality increasing with increase in concentration, hence, it use in aquatic environments for pond fertilization is advised to be done with caution.

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Received: December 24, 2013
Accepted: February 23, 2014