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ACUTE TOXICITY OF FERTILIZER (NPK, 20:10:10) ON Clarias gariepinus FINGERLINGS

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Abstract

The toxicity and behavioural response of fingerlings of Clarias gariepinus (mean total length, 5.04 ± 0.7 cm; mean body weight 4.05 ± 0.3 g) to inorganic fertilizers (NPK, 20:10:10) was studied under laboratory conditions to determine the acute toxicity of NPK, 20:10:10 fertilizer to fingerlings of Clarias gariepinus using a static bioassay test. The fingerlings were exposed to increasing concentrations of NPK, 20:10:10 (0.00g/l, 8.00g/l, 12.00g/l, 16.00g/l and 20.00g/l,) fertilizer in the static renewal bioassay for 96 hours. Exposed fish showed behavioural responses such as agitated swimming, air gulping, restlessness, loss of balance, caudal, frequent attempts at jumping out of the tank and quietness. Water quality examinations showed a decrease in the dissolved oxygen content and increase in total hardness and alkalinity as the concentration of fertilizer was increased. Clarias gariepinus fingerlings were more susceptible to fertilizer NPK, 20:10:10 at higher concentrations as indicated by the percentage of death in the results and the LC_{50} = 12g/L, safety limit =1.20g/L. The concentrations of the toxicants were dose dependent and caused changes in skin colouration of the fish, mortality was highest in the highest concentration of fertilizer and no mortality in the control. Mortality rates were concentration-dependent and death rate in the highest concentrations were significantly higher (P<0.05) than the others. Mortality rate was influenced by both concentration and time. The findings from this study show that NPK, 20:10:10 fertilizer could be classified as toxic to Clarias gariepinus fingerlings at certain concentrations.

Key Words: Strain, Fertilizer, Fingerlings, Toxicosis, Clarias gariepinus

Introduction

Chemicals such as inorganic fertilizers, herbicides and pesticides are used mainly in agricultural activities. These chemicals are discharged into rivers and stream systems, they can pollutes the water bodies and alter ecological balance (Petrocelli, 1985; Osibanjo, 2002; Rand and Petrocelli, 1985 and Muojieje *et al.*, 2021). Pollutants in their effects influence

the quality of these water bodies which is of high importance in the aquatic ecosystem balance and consequently, affect the survival of aquatic organisms inhabiting such environments (Odiete, 2000). Environmental concern about intensive agricultural practices and excessive or inappropriate use of chemical fertilizers call for some global action among environmentally conscious individuals and stake holders (Nyachas, 2000).

A fertilizer is any material, organic or inorganic, natural or synthetic, that supplies plant with the necessary nutrients of growth and optimum yield (Addiscott et al., 2000) or a substance added to water to increase the production of natural fish organisms (Nwadukwe, Okpako, 2010; Muojieje et al., 2021). Inorganic fertilizers are, however, used in aquaculture to boost phytoplankton production. There has been overtime increase in fish production in Nigeria, but land use practices such as forestry, grazing, agriculture, urbanization and mining disrupt aquatic ecosystems by watershed processes altering that ultimately influence the attributes of streams, lakes and estuaries (Chukwu et al., 2009). In aquaculture, fertilizers have been used in various forms and quantities to enhance fish production for greater abundance of fish food organisms, but excessive use of fertilizers has been shown to have adverse effects on water quality (Hunt and Boyd, 1985; Chukwu and Okpe, 2006), and also causes organ damage to fishes. NPK 20:10:10 is an inorganic fertilizer which comprises nitrogen, phosphorus and potassium in the ratio of 20:10:10. It is frequently used inorganic fertilizer for gardening, Suit orchards, vineyard, plantation crops, vegetables and many other crops. It is also of high importance in aquaculture to enhance fish growth. Nevertheless, when used in large quantities, they contribute to high biological oxygen demand and ultimately death of these aquatic organisms (Okpako, 2010).

Fish production can be increased with accurate application of fertilizers and supplementary feeds in aquaculture system. The main purpose of pond fertilization is to augment the production of plankton which serves as natural food of the fishes; because fertilization stimulates both the autotrophic and heterotrophic levels which increase fish production. The use of fertilizer in aquaculture is important for 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).

The use of fertilizer in aquaculture is important for pond fertilization as it increase fish production as a result of nutrient availability. It has 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. 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 decreased growth changes in fish behaviour increased vulnerability to disease and

ultimately death of aquatic organisms (Vidal *et al.*, 2000).

The study was designed to determine the lethal concentration (LC₅₀) value and acute toxicity effects of fertilizer, NPK 20:10:10 on fingerlings of *Clarias gariepinus* as well as the behavioural effects of these inorganic fertilizers on the fingerlings. The effects of poison on fish, however, depends on a number of factors such as type of fish, species used, exposure time, concentration of the poison used, type of toxicant used and lifecycle stage of the fish exposed.

Materials and Method Study of Area

The experimental site was located in Adamawa state of Nigeria, in Fisheries Modibbo Laboratory, Adama University, Yola. Adamawa state located in the northern eastern part of Nigeria with a population of 3,737,223 people and land Mass of 36,917m2 Yola. Adamawa state lies between latitudes 7-11N of the Equator and longitudes 11-14E of the Greenwich Meridian. The research were carried out at the dry laboratory, Department of fisheries, Modibbo Adama University, Yola (Adebayo and Tukur, 1999).

Description of Clarias gariepinus

Clarias gariepinus is a specie of catfish of the family clariidae, the air breathing catfishes. It is a large, eel – like fish, usually of dark gray or black coloration on the back, fading to a white belly. It also comprises of a slender body, flat bony heads, notably flatter than in the genus Silurus, and broad, terminal mouths with four pairs of barbells. It also has large accessory breathing organs composed of modified gill arches. Also only the pectoral fins have spines. Catfish has no

scales, their bodies are naked and the skin secret mucus (Britz and Pienaar, 1992). The male and female of *Clarias gariepinus* can be easily recognized as the male has a distinct sexual papilla, located just behind the anus. This sexual papilla is absent in females (Groenewald, 1964).

Source of Experimental Fish

Two hundred and fifty (250) fingerlings of *Clarias gariepinus* of mean weight $4.05 \pm 0.3g$ and total length $5.04 \pm 0.7cm$ were obtain from Gaji Fish farm Ltd, Yola, Adamawa state. They were transported in a fifty litre jerry-can to the laboratory, Department of fisheries, Modibbo Adama University, Yola.

Acclimation

They were acclimatizing for two weeks in the plastic tank containing water of about 250 fish before the exposure period using plastic tank. The containers were aerated during this period, while the water was renewed daily to discard faecal material as well as left-over food. The fish were fed twice daily at 35% crude protein diet. During this period, dead and abnormal individuals were immediately removed. Mortality recorded during the acclimation period was less than 1% (OECD, 1992; APHA et al., 2005). It was from the acclimatized population that healthy individuals used as test fish in this study were carefully selected.

Preparation of Test Media and Exploratory Test

The whole experimental procedures used in this study were based on the guidelines in APHA *et al.* (2005). To obtain the ranges of concentrations as used in the experiment, ten (10) fish each were exposed to twenty (20) litres of water containing different weights of the fertilizer. It was 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.

Experimental Design and Procedures

The experiment is a Completely Randomized Design. The treatment levels had four replicates (Akindele, 2004; Ogbeibu, 2005). The methods of acute tests as described by APHA (1995) were employed. 10 fingerlings of Clarias gariepinus fingerlings were exposed to four different concentrations of the toxicants in each test plastic tank were triplicate and control .The concentrations used are 0.0g/L, 8g/L, 12g/L, 16g/L, and 20g/Fingerlings of fairly equal weight, total length and standard length were selected randomly, weighed and distribute into 15 plastic tanks containing definitive concentration of NPK, 20:10:10 and the bioassay test was taken and to give a final volume of 20.0 L. The test fish were not fed twelve hours (12hours) prior to the test (experiment) and during the ninety-six hours (96hours) exposure period. The solutions were stirred for homogeneous mixing before each tank were randomly stocked in triplicate with 10 fingerlings of fish while the test solutions and control were renewed daily.

Behavioral Studies

After exposure of the fish to various concentrations of fertilizer NPK, 20:10:10, observations were carried out on the behavioral and morphological responses of the fish at 12, 24, 48, 72and 96 hours (Drummond *et al.*,1986). Fingerlings in control were also monitor along with those in test concentrations to provide a reference for assessing any

behavioral or morphological changes. The behavioural and morphological characteristics that were monitor are erratic swimming, loss of equilibrium, general activity, increased excitability, mortality, vertical suspension, mucous secretions, startle response, deformities and hemorrhage. Each test tank was observed for 10 to 15 minutes which allowed sufficient time for an accurate evaluation of each fish.

Mortality Rate of LC₅₀

Observations to determine mortality rate of Clarias gariepinus fingerlings were made at 12, 24, 48, 72 and 96 hours. Clarias gariepinus fingerlings were considered dead when there is no sign of opercula movement or no response to gentle prodding or floating on the water. The numbers of dead fingerlings in each group were record against the time of their death in a tabular (Stephan, 1977). The fingerlings were removed immediately to avoid fouling.

Water Quality Parameters

Some water quality parameters such as Temperature, dissolved oxygen, pH, total alkalinity, Ammonia (NH₃) and total hardness of the control and various test media were determined at 12, 24, 48, 72 and 96hours intervals during the experimental period (ASTM, 2004a; APHA *et al.*, 2005; Adakole, 2000).

Hydrogen ion concentration (pH)

The hydrogen ion concentration (pH) of the water sample was determined in the hatchery using pH meter (Verma *et al.*, 2012).

Dissolved oxygen (DO)

Dissolved oxygen (DO) of the experimental water was determined directly by using DO meter (Smitha, 1980).

Total Alkalinity

The total alkalinity of the water in each replicate was determined directly by using water parameter starter kit 2013 (Dickson, 1992).

Ammonia (NH₃)

Ammonia (NH₃) of the experimental water was determined using a bulb pipette, 10 mL aliquot of the cleaning solution sample was quantitatively transferred to a 100 mL volumetric flask.

Hardness

Water hardness of the experimental was determine.

Temperature

The temperature was determined using mercury in-glass Thermometer and recorded.

Data Analysis

Mean of data generated was analyzed for significance differences using one-way Analysis of Variance. Probit Analysis was used to determine Probit value, from the mortality percentages. Regression analysis was used to determine the LC₅₀ of NPK, 20:10:10 concentrations.

Result

Water Quality Parameters

The water quality parameters of the experiment water monitored during the experiment are given in Table 2. The result of the physico-chemical parameters of the experiment for the fertilizer showed that there was a significant reduction in the mean values of dissolved oxygen. Conversely, temperature, total alkalinity and total hardness values increased as the fertilizer concentrations were increased, compared to the control group (P<0.05). However, there was no significant difference between the various mean values of pH and ammonia (P>0.05).

Behavioural Responses of Clarias gariepinus Fingerlings to NPK, 20:10:10 Fertilizer

Table 1 presents the behavioural responses of catfish exposed to NPK fertilizer. Behavioural responses occurred in the fish treated with NPK, 20:10:10 fertilizer at different concentrations. Number code '1-6' represents different behavioral reactions in order of their appearances. Agitated movement and loss of equilibrium occur across all the concentrations from 12uL⁻¹ to 20uL⁻¹.The behavioral responses of air gulping occurred under doses of 8.0uL⁻¹ to 20.00uL⁻¹, while sign of weakness and skin colouration occurred from the dose of 12.00uL⁻¹ to 20.0uL⁻¹. There were no obvious changes or responses in fish behaviour in the lower concentrations less than 12.00g/l for the first 96 hours of exposure. However, fish in the control group did not exhibit any abnormal behaviour.

Mortality of C. gariepinus Fingerlings Exposed to NPK, 20:10:10 Fertilizer for 96 Hours

Mortality of the fingerlings exposed to various concentrations (Figure 1 and Table 3) of NPK, 20:10:10 fertilizer. Mortality occurred in the entire culturing container except in the control (0.00gL⁻¹). The first mortalities of C. gariepinus fingerlings were observed in 12 hours for container containing 20.0µL-1 of NPK, 20:10:10 fertilizer. By 48 hrs mortalities were observed in all concentrations except 0.00 and 8.00g/L⁻¹. The highest percentage value of 95% was observed at the highest concentration of 20.00gL⁻¹ followed by 90% at 16gL⁻¹ Figure 1 showed the LC₅₀ of C. gariepinus to NPK, 20:10:10 to be 1.08 which is at 12g/L concentration of NPK, 20:10:10 fertilizer.

Table 1: Behavioural Responses of *Clarias gariepinus* Fingerlings to Different Doses of NPK, 20:10:10 Fertilizer at 96 hours

Behavioural	Doses of NPK, 20:10:10 Fertilizer in uL ^{-T}					
Responses						
	$T_0(0.00g)$	$T_1(8.0g)$	$T_2(12.0)$	$T_3(16.0g)$	T ₄ (20.0g)	
Agitated movement	0	0	1	1	1	
Loss of equilibrium	0	0	2	2	2	
Air gulping	0	3	3	3	3	
Sign of weakness	0	0	4	4	4	
Skin colouration	0	0	5	5	5	

^{1-6 =} Behavioural responses in different concentrations of NPK, 20:10:10

Key: Agitated movement = 1; Loss of equilibrium = 2; Air gulping =3; Sign of weakness = 4; Skin colouration= 5

Table 2: Water Quality Parameter

Conc.(uL/l)	Temp°C	Dissolved oxygen (mg/l)	рН	Water hardness (mg/l)	Alkalinity (mg/l)	NH ₃ (mg/L)
T (0g/L)	21±0.05 ^a	6.2±0.00°	5.22±0.11 ^a	100±1.50 ^a	1.2±0.00 ^a	0.025±0.00a
T1 (8g/L)	22 ± 0.10^{a}	4.2 ± 0.11^{b}	5.6 ± 0.04^{b}	150 ± 2.00^{b}	4.1 ± 0.05^{b}	0.101 ± 0.01^{a}
T2 (12g/L)	24 ± 0.55^{b}	3.2 ± 0.20^{a}	5.72 ± 0.33^{b}	170 ± 2.10^{c}	4.3±0.11 ^b	0.126 ± 0.10^{b}
T3 (16g/L)	23±0.01 ^b	3.1 ± 0.50^{a}	5.71 ± 0.40^{b}	200 ± 3.00^{d}	$5.6 \pm 0.50^{\circ}$	0.140 ± 0.02^{c}
T4 (20g/L)	25±0.22°	3.0 ± 0.00^{a}	5.89 ± 0.25^{c}	250±2.03e	6.3 ± 0.50^{d}	0.196 ± 0.01^{d}

Mean with different superscript are statistically different to each other (p>0.05)

Table 3: Probit Value of *Clarias gariepinus* Exposed Fingerlings to NPK, 20:10:10 Fertilizer for 96 hrs

Conc.(g/L)	Log conc.	Total fish	No. of death	% Death	Probit
		exposed			Value
T ₀ (0g/L)	0.00	10	0	0	0.00
T_1 (8g/L)	0.90	10	3	30	4.48
$T_2 (12g/L)$	1.08	10	8	80	5.84
$T_3 (16g/L)$	1.20	10	9.5	95	6.28
T ₄ (20g/L)	1.30	10	10	100	6.64

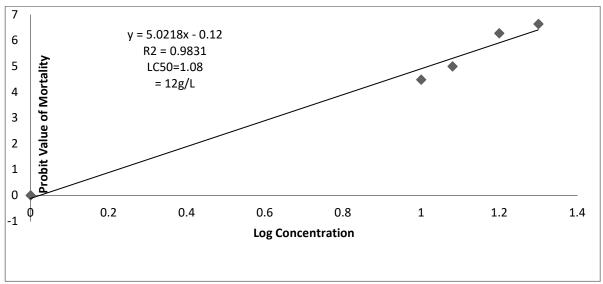


Fig. 1: Mortality of Clarias gariepinus Fingerlings Exposed to Varying Concentration (g/L) of NPK Fertilizer

Discussion

Water Quality Parameters

The result of the physico-chemical parameters of the experiment for the fertilizer showed that there was a significant reduction in the mean values of dissolved oxygen. Conversely, temperature, total alkalinity and total hardness values increased as the fertilizer concentrations were increased, compared to the control group (P<0.05). However, there was no significant difference between the various mean values of pH and ammonia (P>0.05) as both were within the suggested tolerance ranges for warm water fish species (Adeniji and Ovie, 1989). However, it should be noted that fertilizer effect on physiochemical parameters of water is specific to individual kind of fertilizer used and many differ from one region to another. This result agrees with the work of Ofojekwu et al. (2008) and Ufodike and Onusiriuka (1990) who exposed Tilapia zilli and Clarias gariepinus fingerlings respectively to acute concentrations of

inorganic fertilizers; NPK 20:10:10, urea, calcium hydroxide (Ca(OH)2, potassium phosphate (Na₃PO₄.12H₂O) and sodium nitrate (NaNO₃) and reported of no significant difference between the various mean values of ammonia and pH (P>0.05). The affected parameters may have contributed significantly to the observed behaviours and mortality of the test fish species exposed to these fertilizer concentrations. The increase in temperature, alkalinity and total hardness may imply an increased toxicity with the raised values of physico-chemical parameters.

The physio-chemical parameters of water fluctuated slightly during the bioassay. This fluctuation was not enough to have caused the mortality. Death of the test fish exposed to chemical fertilizer NPK, 20:10:10 may be attributed to the destruction of such organs as gills, liver, kidney, brain, blood system and pancreas. Ufodike and Onusiriuka, (2008) reported that fish breathe by the movement of water, and any water pollutants present, in

and out through their gills. Since gills are the primary site for osmo-regulation and reparations they are highly vulnerable to lesions due to their immediate contact with aquatic pollutants while some pollutants enter their body.

The toxicity of a chemical in water depends largely on the concentration and the physico chemical properties of the water. Osekita (2003) also documented that, the toxicity of a pollutant to fish usually increases with the physicochemical properties of the water which may be due to an increased uptake of toxin to added environmental stress e.g. reduced oxygen solubility (Ananthakrishman and Kutty, 1974). The recorded mean values for temperature and pH in the fertilizer test media were within the tolerance range for this tropical species and may not have contributed to the toxicity of the fertilizer on the behaviours and mortality of the exposed fishes.

Olollade and Oginni, (2010) also reported that toxicants affect gas exchange of fish and other aquatic organisms this might probably be the reason for the decline of dissolved oxygen concentration as the concentration of chemical fertilizer NPK, 20:10:10 increases. Because the water parameters had little variation, this can be evident that physical and chemical properties of water holding tanks were within the desirable range of fish culture (Boyd, 1979).

Behavioural Responses and Lethal Concentrations of the Toxicants to the Exposed Fish Species

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 (FAO, 1984; Essien-ibok, *et*

al., 2014). The fingerlings exposed to high concentration showed several abnormal disturbed swimming behaviors or behaviors such as erratic movement, gasping of air at surface, rapid opercula and tail movement, loss of equilibrium and resting motionless at the bottom of the glass tanks were observed. This is similar to the observation of Ufodike and Onusiriuka (2008), who also reported these abnormal behavioral responses in fingerlings Clarias gariepinus exposed to toxicants (Fertilizer NPK, 20:10:10) at different concentrations. The erratic swimming, restlessness gulping of air and resting motionless at the bottom of the glass tanks observed in this research study are indications that mortality of the exposed fish is not only due to impaired metabolism, but could in addition be due to nervous disorder. This is similar to the finding of Ufodike and Onusiriuka (2008), who also reported the rapidity of swimming was directly proportional to the concentrations of NPK, 20:10:10 on Clarias gariepinus fingerlings exposed to toxicants at 96 hours period.

Similarly, the stressful and erratic behaviours of Clarias gariepinus fingerlings also tend to indicate respiratory impairment probably due to the effect on the gills. Fish breathe by movement of water, dissolve oxygen and any water contaminants present in and out through their gills so gills are usually site of first contact of the internal organ. Similarly to the observation of Ayuba and Ofojekwu, (2002), observed in Clarias gariepinus exposed to acute toxicity of fertilizer NPK, 20:10:10 loss of balance, respiratory disorder, gulping of air and erratic swimming before death, this is also in line with the findings of Omoregie et al. (1997) and Tawari–Fufeyin et al., (2008)

Who made similar observation when they exposed Clarias gariepinus to different toxicants. The fish that survived the 96hours bioassay test had pale skin colour which increased with increasing concentration, with the fishes in highest concentration being very pale from their neck towards their caudal fin Okomoda and Ataguba (2011). However, fish in the maintained their control normal behaviours within the 96hours of the experiment.

Besch (1975) identified four main in the exposure time phases behavioural responses of fish to toxicants. These are the contact phase (brief period of high excitability), exertion (visible avoidance characterized by swimming, leaping and attempts to jump out of the toxicant), and loss of equilibrium, followed by lethal (death) phase when opercula movement and responses to tactile stimuli 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 environment (FAO, 2000).

At the concentrations used in this investigation, the fertilizer led to significant reduction in the dissolved oxygen and an increase in temperature, total alkalinity and total hardness of the test media. The air gulping reported in the exposed fish in this study is an indication of insufficient amount of dissolved oxygen in the experimental media which may have been depleted by the fertilizer. This result is in line with the report of Warren (1977) who observed that, the introduction of a toxicant into an aquatic

system might decrease the dissolved oxygen content which in turn impairs respiration, thus leading to asphyxiation. Stickney (1977)had also documented that insufficient amount of dissolved oxygen is one of the contributing factors to mortality in some fish species.

The 12.00g/l 96hr LC₅₀ and applicable factor of empirical (Sprague, 1971), the safety level was 1.20g/l NPK, 20:10:10 concentrations on C. gariepinus in this study closely agrees with the 96hrLC₅₀ (17.84g/lg/l) reported by Essien-ibok, et al. (2014) when they Heterobranchus bidorsalis exposed fingerlings to acute concentrations of Urea fertilizer. The slight difference may be due to the different species used and their sizes at exposure, fertilizer concentrations and other environmental factors, as different fish species respond to the effect of a pollutant differently (OECD, 1992). The upright position with snouts above the surface gasping for uncoordinated swimming, restlessness, frequent attempts at jumping out of the tank and quietness reported in this study for NPK, 20:10:10 fertilizer have been earlier documented by Ayuba and Ofojekwu (2005) when they exposed fish fingerlings to acute concentrations of different toxicants.

At concentration 20.00g/l, 100% mortality was recorded for fertilizer. Also, at concentrations 16.00g/l, 12.00g/l and 8.00g/l, cumulative mortality (%) recorded were 95, 80 and 30, respectively. No mortality was however recorded in the control group of the treatment. This results show that percentage mortality increases with increase in concentration of the toxicant as earlier documented by Oti (2002) and; Ayuba and Ofojekwu (2005).

This study also reveals that concentrations 12.00g/l are lethally threat to the test fish within 96 hours as 80% mortality was recorded.

Based on the 12, 24, 48, 72 and 96hrs LC₅₀ values determined from this study for NPK, 20:10:10 fertilizer, it could be rated as toxic to *C. gariepinus* fingerlings (Helfrich et al., 1996). Thus, it would seem prudent to avoid situations where inorganic fertilizers are added intermittently to the ponds because such subsequent additions may result in total fingerling mortality, if the concentrations exceed the established LC50 reported in this investigation. The 96hrs LC₅₀ and safe concentration of the fertilizer i.e. 12.00g/l, 1.20g/l respectively, to C. gariepinus fingerlings suggest that, this species is not tolerant to acute concentrations of this toxicant.

Conclusion

The study clearly shows that acute concentrations of NPK, 20:10:10 fertilizer are harmful to *Clarias gariepinus* fingerlings. It is thus recommended that the application of this fertilizer in aquatic ecosystems either in ponds, irrigations or farms should be carefully controlled and monitored, such that concentrations that are lethal to aquatic life could be avoided.

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