

Toxicity and behavioural response of fingerlings of *Clarias gariepinus* (Burchell: 1822) to four inorganic fertilizers

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Abstract

The toxicity and behavioural response of fingerlings of *Clarias gariepinus* (Burchell: 1822) to four inorganic fertilizers (Urea, NPK, Calcium Ammonium Nitrate and Single Superphosphate) was studied under laboratory conditions. One hundred fingerlings of *C. gariepinus* were used for the short term static Bioassay. The concentration of the four inorganic fertilizers used for the study were prepared after a range find test to contain 28 mg/l, 48 mg/l, 68 mg/l, 88 mg/l and 108 mg/l of the fertilizers. There were four treatments T₁ Aquaria containing fish with non-chlorinated water treated with Urea-N-46; T₂ Aquaria containing fish with non-chlorinated water treated with NPK 20:10:10; T₃ Aquaria containing fish with non-chlorinated water treated with Calcium Ammonium Nitrate and T₄ which comprised Aquaria containing the experimental fish with non-chlorinated water treated with Single Superphosphate. Each treatment was replicated twice and the control. The effects of the four inorganic fertilizers on the test organism were monitored and records taken with respect to toxicity and behavioural response of the test organism. Percentage mortality of fish was calculated to obtain the media lethal time (LT₅₀). The LC₅₀ was obtained lethal times against concentrations. One-Way Analysis of Variance (ANOVA) revealed significant different ($p < 0.05$) in the levels of toxicity of inorganic fertilizers to the experimental fish. Probit analysis also revealed positive correlation between mortality rate of test organism and concentration of the inorganic fertilizer. Emerging results from the present study show that all four fertilizers were toxic to the experimental fish and also elicited varying behavioural responses. The fertilizers should be used with caution. In the alternative the use of less hazardous and ecofriendly fertilizers (Organic fertilizers) could be used as a viable option.

INTRODUCTION

Chemicals such as inorganic fertilizers, herbicides and pesticides are used mainly in agricultural activities. These chemicals are discharged into rivers and stream systems, it pollutes the water bodies and alter ecological balance (Osibanjo, 2002; Rand and Petrocelli, 2005). 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 (Odieta, 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 food organisms (Nwadukwe, 1995; Okpako, 2010). Inorganic fertilizers are, however, used in aquaculture to boost plankton 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 altering watershed processes 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.

The world is focused on increasing agricultural productivity, and as a result, fertilizer is being used in most countries where agriculture is now well developed (Chukwu *et al.*, 2009). Organic and inorganic fertilizers used in agricultural activities are rich in nutrients such as nitrates, phosphate, calcium, potassium and organic nutrients which could lead to excessive enrichment of water bodies (Mason, 1992; Angela, 2005 and Ademoroti, 2006), resulting in high biological oxygen demand, depletion of oxygen and ultimately death of aquatic organisms (Odiete, 2000). Moreover, it is known that diseases, pollution and presence of agricultural chemicals in water causes alterations in blood cells of fish resulting in losses in aquaculture (Smil, 2005).

Urea-N-46% fertilizer, also popularly called forty-six zero zero (46-0-0), is a simple or straight (single element) fertilizer that supplies the major essential element nitrogen in ammoniac form (NH_4^+). The positively charged ammonium ion (NH_4^+) is a non volatile and is one of the two forms of nitrogen that can be absorbed by plants, the other being nitrate (NO_3^-). Urea is the richest source of nitrogen among the common dry fertilizers. However, excessive urea fertilizer poses a serious threat to aquatic organisms particularly fish. There is therefore the need to determine the tolerance limit of urea fertilizer (Osibanjo, 2002).

NPK 20:10:10 is an inorganic fertilizer which comprises nitrogen, phosphorus and potassium in the ratio of 20:10:10. It is the commonest inorganic fertilizer used 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).

Calcium ammonium nitrate also known as nitro-limestone is widely used as inorganic fertilizers, accounting for 4% of all nitrogen fertilizer used worldwide (Smil, 2012). Calcium ammonium nitrate is hygroscopic. Its dissolution in water is endothermic, leading to its use in some instant packs.

Single superphosphate (SSP) is one of the cheapest forms of phosphate. It supplies sulphate sulphur and calcium. The ratio of phosphorus and sulphur suits many crops and pasture needs. Both the phosphorus and sulphur are in readily available forms. It can be blended with other fertilizer products (except Urea and Diammonium phosphate). It can be stored easily for long periods without taking up moisture. It contains calcium and sulphur (mostly gypsum) and helps keep soil in good shape by maintaining soil structure. Single superphosphate provides a balance of phosphorus, calcium and sulphur (P, S, Ca) that mimics pasture growth requirements and is the best balanced P, S and Ca fertilizer for Australian pastures (Brummit, 2013).

The toxicity study of these fertilizers to fingerlings is essential to understand the environmental impacts of inorganic fertilizers. Tests measure endpoints such as survival, growth, reproduction are measured at each standardized acute and chronic toxicity tests, along with a control test (US Environmental Protection Agency, 2001). It was not until around the 1930's that the use of acute toxicity testing, especially on fish was established; Toxicity tests were used to provide qualitative and quantitative data on deleterious effects of toxicants on organisms. These tests can be performed in the field or laboratory (Brummit, 2013).

The present study was designed to determine the medial lethal concentration (LC_{50}) value and acute toxicity effects of Urea-N-46% fertilizer, NPK 20:10:10, Calcium Ammonium Nitrate and Single Superphosphate 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.

The present study was designed to determine the

the toxic effects of four inorganic fertilizers (Urea – N-46%, NPK 20:10:10, Calcium Ammonium Nitrate and Single Superphosphate) on fingerlings of the African Sharptooth catfish, *Clarias gariepinus*

To determine the levels of toxicity of the four inorganic fertilizers on the fingerlings of *Clarias gariepinus*. It is a known fact that the inorganic fertilizers boost production of phytoplankton and zooplankton but excess of it will reduce fish yield. More work on the toxicity of inorganic fertilizers still has to be done to elucidate the harmful effects of these chemicals on aquatic organism such as fishes. This forms the main thrust of the present study.

In aquaculture, urea-N-46% aids the growth of phytoplankton and algae which aid oxygen circulation in ponds during photosynthesis. Ionized ammonium (NH_4^+) is nontoxic, while high concentrations of unionized ammonia in plants tends to block oxygen transfer from gills of the blood and can cause both immediate and long term gill damage (Norm, 2000). Fish suffering from ammonia poisoning usually appear sluggish, often at the surface as if gasping for air (Okpako, 2010).

NPK fertilizer is primarily composed of three main elements. Nitrogen (N), Phosphorus (P) and Potassium (K), each of these being essential in plant nutrition. NPK 20:10:10 is an inorganic fertilizer of the NPK family. It is a multi-nutrient fertilizer providing nitrogen, phosphorus and potassium in the ratio of 20:10:10. It is hygroscopic in nature and it is the commonest inorganic fertilizer used in agriculture to enrich plants. Nitrogen helps plants grow quickly, while also increasing the production of seed and fruit, and bettering the quality of leaf and forage crops (Ayuba *et al.*, 2014).

Nitrogen is also a component of chlorophyll. Phosphorus is a key player in the photosynthetic process, and plays a vital role in a variety of the things needed by plants. Potassium, the third world essential nutrient plants demand, assists in photosynthesis, fruit quality, the building of proteins and the reduction of diseases. In aquaculture, NPK 20:10:10 aids the growth of plankton but poses threat to the environment when used excessively (Das and Jana, 2006; Okpako, 2010).

Calcium Ammonium Nitrate

Calcium ammonium nitrate also known as nitro-limestone is widely used as inorganic fertilizers,

accounting for 4% of all nitrogen fertilizer used worldwide (Smil, 2012). The term calcium ammonium nitrate is applied to multiple different, but closely related formulations.

MATERIALS AND METHODS

A total number of 100 fingerlings of fish with a mean standard length of 3 cm fingerlings was obtained from Udoka's farm in Owerri, Imo State and was transported to experimental site at Uli Ihiala Local Government Area Anambra State.

The experiment was carried out using twenty-four plastic Aquaria measuring 60cm x 50cm x 9cm, containing dechlorinated Water.

Management and Acclimatization of the Experimental Fish

The fingerlings of *Clarias gariepinus* were purchased from Udoka's farm in Owerri, Imo state. The fishes were randomly divided into the twenty-four plastic Aquaria and fed with the commercial fish feed (Coppens fish concentrate) for 12 days before the experiment commenced. The period of acclimatization was extended beyond one week to ascertain the condition of the fish. During the period of acclimatization, the fish were fed and water was changed daily in the morning to remove faeces and unconsumed feed. Disease conditions and general fitness were inspected. Feeding was discontinued 24 hours before commencement of the 96 hours Bioassay.

Experiment Procedures

The concentration of Urea-N-46%, NPK 20:10:10, Calcium ammonium nitrate and Single Superphosphate used for the toxicity test were determined by a preliminary test as described by Solbe (2005) and Ayuba *et al.*, (2014).

After a range finding test, the concentrations prepared for the experiment were 28mg/l, 48mg/l, 68mg/l, 88mg/l and 108mg/l with two replicates as described by Iroegbu *et al.*, (2008). There were four treatments, namely T1, T2, T3 and T4. T1 comprised the experimental fish exposed to Urea-N-46% T2 comprised the experimental fish exposed to NPK 20:10:10. T3 comprised the experimental fish exposed to Calcium ammonium nitrate while T4 comprised the experimental fish exposed to Single superphosphate. The desired concentrations of the fertilizers were measured and dissolved into the appropriate plastic Aquaria and marked accordingly. Water was kept up at 8 litre level. The control Aquaria also contained twenty fish with no fertilizer treatment.

The fishes were not fed during the period of the experiment. Mortality of the fish was recorded for up to 96 hours of exposure. Fish were presumed dead when there were neither opercula nor body movement upon gentle prodding. Dead fish were removed and time of mortality of individual fish was recorded.

Statistical Analysis

Data collected was analyzed statistically by arithmetic means, percentages and standard deviation using one-way Analysis of Variance (ANOVA) and testing at 5 % level of significance. Mortality of fish against time of death was plotted to obtain the median lethal time (LT₅₀) and defined as the time at which mortality is recorded for 50% of the exposed fish to each of the organic fertilizers. The LC₅₀ was obtained from the regression of median lethal times against concentrations of Urea-N-46%, NPK 20:10:10, Calcium ammonium nitrate

and Single Superphosphate. The physico-chemical parameters were determined using standard method by APHA (1998). Probit analysis was done using Finney (1971).

RESULTS AND DISCUSSION

Parameters test media mean values of water quality parameters of the four test media (28mg/l, 48mg/l, 68mg/l, 88mg/l and 108mg/l) did not vary significantly (p>0.05)

Acute Toxicity Test

The results of the acute toxicity test are presented in Tables 1, 2, 3, 4, 5, and Figure 1 – 4. The LC₅₀ values based on probit analysis as shown in Tables 6, 7, 8 and 9 were found to be 48mg/l for Urea, 68mg/l for NPK 28mg/l for Calcium ammonium nitrate and 38mg/l for Single Superphosphate. There was no mortality in the control experiment throughout the duration of the static Bioassay.

Table 1: Physico-Chemical Parameters of the Test Media

	pH	DO (mg/l)	Tem (°C)	H-NO ³ (mg/l)	P-PO ₄ (mg/l)
28mg/l	7.68±0.5	6.70±0.02	26.57±0.30	42.25±0.02	43.02±0.02
48mg/l	7.64±0.4	6.50±0.05	26.55±0.31	42.24±0.02	43.50±0.03
68mg/l	7.62±0.3	6.45±0.02	26.50±0.30	41.23±0.03	43.05±0.04
88mg/l	7.58±0.2	61.35±0.04	26.48±0.25	41.20±0.01	44.05±0.06
108mg/l	7.50±0.3	6.30±0.04	27.07±0.30	40.25±0.02	44.06±0.50

Table 2: Mortality rate of post fingerlings of *Clarias gariepinus* on exposure to Urea-N-46%

Concentration	24 hours	48 hours	72 hours	96 hours	Mortality
Control	0	0	0	0	0
28mg/l	0	0	2	2	4
48 mg/l	0	1	2	2	5
68 mg/l	1	2	3	1	7
88 mg/l	2	2	3	1	8
108 mg/l	2	3	4	0	9

Table 3: Mortality rate of post fingerlings of *Clarias gariepinus* on exposure to NPK 20:10:10

Concentration	24 hours	48 hours	72 hours	96 hours	Mortality
Control	0	0	0	0	0
28mg/l	0	0	1	2	3
48 mg/l	0	0	2	2	4
68 mg/l	0	1	2	2	5
88 mg/l	1	1	2	3	7
108 mg/l	2	1	2	3	8

Table 4: Mortality rate of post fingerlings of *Clarias gariepinus* on exposure to Calcium Ammonium Nitrate

Concentration	24 hours	48 hours	72 hours	96 hours	Mortality
Control	0	0	0	0	0
28mg/l	0	1	1	3	5
48 mg/l	0	2	1	3	6
68 mg/l	1	2	3	2	8
88 mg/l	2	3	2	3	10
108 mg/l	3	3	4	0	10

Table 5: Mortality rate of post fingerlings of *Clarias gariepinus* on exposure to Single Superphosphate

Concentration	24 hours	48 hours	72 hours	96 hours	Mortality
Control	0	0	0	0	0
28mg/l	0	0	0	2	2
48 mg/l	0	0	1	2	3
68 mg/l	0	0	2	2	4
88 mg/l	0	2	1	2	5
108 mg/l	2	1	2	1	6

Table 6: Calculated Logdose and Probit values for Urea – N – 46%

Concentration	Logdose	Mortality (%)	Probit
28mg/l	1.4472	40	4.75
48 mg/l	1.6812	50	5.60
68 mg/l	1.8325	70	5.25
88 mg/l	1.9445	80	5.84
108 mg/l	2.0334	90	6.28

Table 7: Calculated Logdose and Probit values for NKP 20:10:10

Concentration	Logdose	Mortality (%)	Probit
28mg/l	1.4472	30	4.48
48 mg/l	1.6812	40	4.76
68 mg/l	1.8325	50	5.00
88 mg/l	1.9445	70	5.52
108 mg/l	2.0334	80	5.84

Table 8: Calculated Logdose and Probit values for Calcium Ammonium Nitrate

Concentration	Logdose	Mortality (%)	Probit
28mg/l	1.4472	50	5.00
48 mg/l	1.6812	60	5.25
68 mg/l	1.8325	80	5.84
88 mg/l	1.9445	100	8.34
108 mg/l	2.0334	10	8.34

Table 9: Calculated Logdose and Probit values for Single Superphosphate

Concentration	Logdose	Mortality (%)	Probit
28mg/l	1.4472	20	4.16
48 mg/l	1.6812	30	5.48
68 mg/l	1.8325	40	4.76
88 mg/l	1.9445	50	5.00
108 mg/l	2.0334	60	5.25

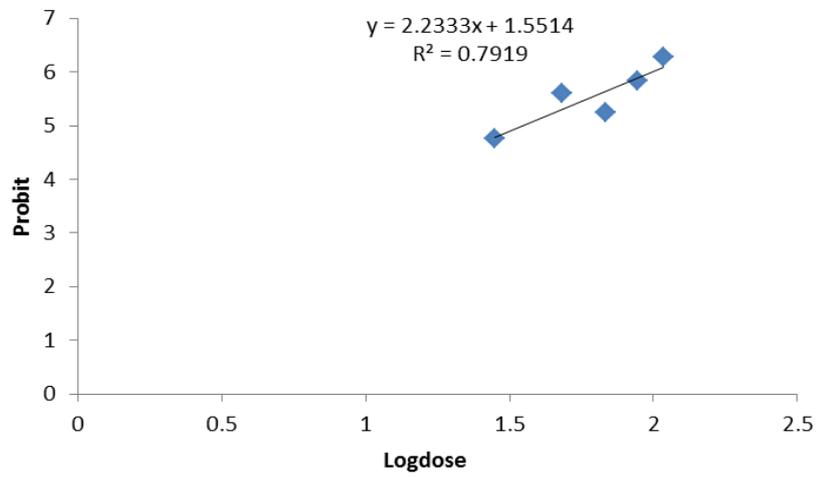


Fig. 1: Graph of Probit against Logdose concentration of Urea

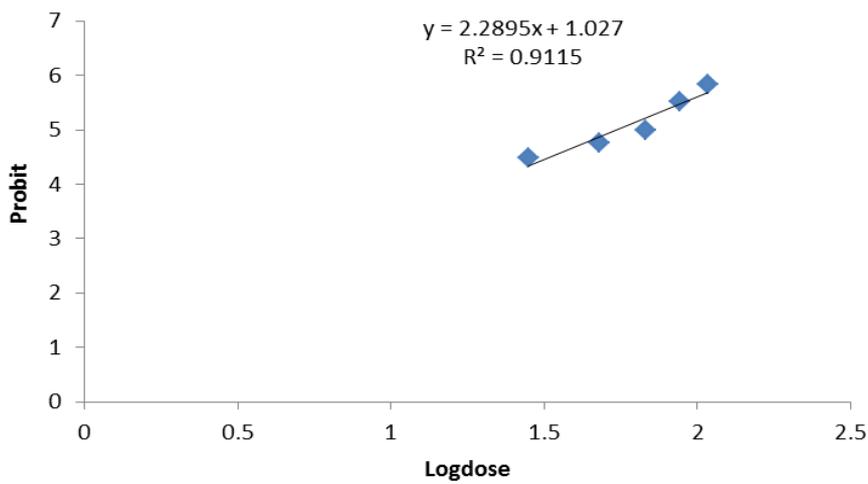


Fig. 2: Graph of Probit against Logdose concentration of NPK

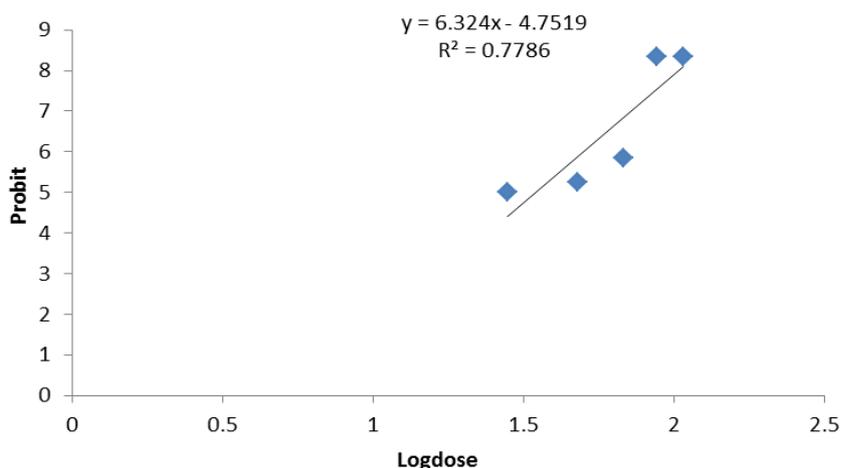


Fig 3: Graph of Probit against Logdose concentration of Calcium Ammonium Nitrate

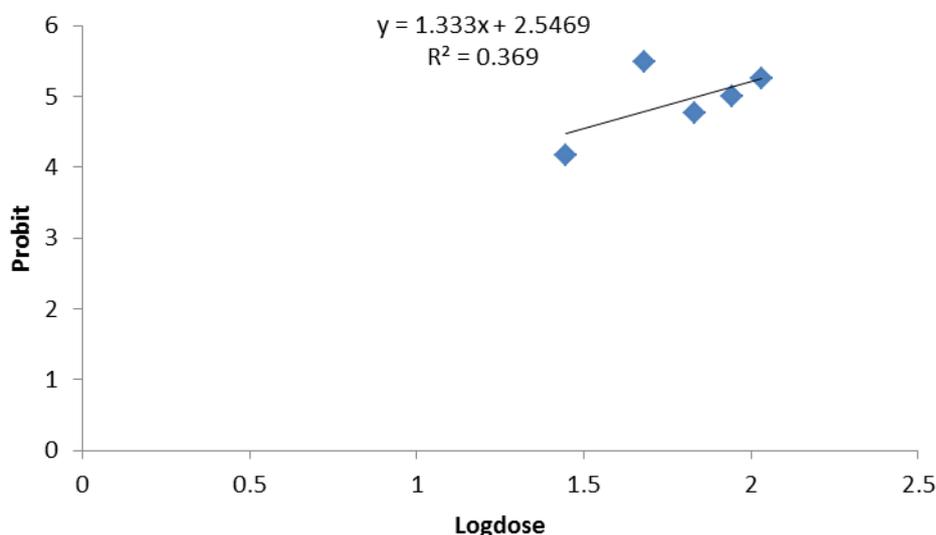


Fig 4 : Graph of Probit against Logdose concentration of Single Superphosphate

Discussion, Recommendation and Conclusion

The accumulation of fertilizers in aquatic habitats however leads to an increase in the level of concentration which is toxic to aquatic organisms and poses a serious threat to life in aquatic habitats. Stress signs were noticed at about 20th hour for Urea, 22nd hour/ for NPK, 18th hour for Calcium ammonium nitrate and 23rd hour for Single Superphosphate. The 96 hour LC₅₀ value for Urea was 48mg/l, 68mg/l for NPK, 28mg/l for Calcium ammonium nitrate and 88mg/l for Single Superphosphate. The median Lethal time (LT₅₀) for urea was not reached at concentration level of 28mg/l but occurred at the 90th hour for 48mg/l, 65th hour for 68mg/l, 60th hour for 88mg/l and 48th hour for 108mg/l. For NPK, the LT₅₀ was not reached at concentration level of 28mg/l and 48mg/l but

occurred at 90th hour for 68mg/l, 80th hour for 88mg/l and 72th hour for 108mg/l. For Calcium ammonium nitrate; LT₅₀ occurred 95th hour M 28mg/l; 80th hour at 48mg/l, 72th hour for 68mg/l, 48th hour for 88mg/l and 38th hours for 108mg/l. For Single Superphosphate, the LT₅₀ was not reached at concentration of 28mg/l, 48mg/l and 68mg/l but occurred at the 90th hours for 88mg/l, 70 hours for 108mg/l. These showed Calcium ammonium nitrate to be the most toxic to the experimental organisms than NPK, Urea and Single Superphosphate. The physico-chemical parameters measured were not significantly different (P >0.05) at the different levels of concentration and so were not thought to be the cause of fish mortality as they were found to be within tolerance range as recommended by Boyd (2009).

It should be noted that fertilizer effect on physico-chemical parameters of water is specific to individual kind of fertilizer used as seen in the case of Calcium ammonium nitrate being more toxic than Urea, NPK and Single Superphosphate, they may differ from one region to another. Organic fertilizers are most likely to cause significant changes in dissolved oxygen levels due to biodegradation (Ayuba *et al.*, 2014). Urea, NPK, Calcium ammonium nitrate and Single Superphosphate toxicity increased with increased concentration. The post fingerlings exposed to high concentrations showed no initial signs of stress but began to show signs at about the 20th hour for Urea, 22nd hour for NPK, 18th hour for Calcium ammonium nitrate and 23rd hour for Single Superphosphate. Disturbed swimming behaviour such as erratic movement, incessant jumping, gasping at the surface, rapid opercula movement are attributed to respiratory stress and asphyxiation as the gills were ruptured, lifted and eroded at death. This could be the reason why the fishes died with the mouth open as they gasped for air due to gas exchange failure at the gill surfaces. These findings are similar to observations made by Pascoe and Matthey (1977) and Iroegu *et al.*, (2008) after a study of Cadmium toxicity effect on *Gasterosteus aculeatus* L. and survival response of *Sarotheron melanotheran* fingerlings exposed to various concentration levels of Perfekthion under laboratory conditions. These stress signs ceased at the 96th hour of exposure with the fish preferring to settle at the bottom of the Aquaria This is in line with the findings of Ayuba *et al.*, (2014), who made similar observations when they exposed fingerlings of *Clarias gariepinus* to different toxicants.

The 96 hour LC₅₀ value for Urea was 68mg/l, 88mg/l for NPK, 48mg/l for Calcium ammonium nitrate and 108mg/l for Single Superphosphate. The median Lethal time (LT₅₀) for urea was not reached at concentration level of 28mg/l, it occurred at 90 hours for 48mg/l, 65 hours for 68mg/l, 60 hours for 88mg/l and 48 hours for 108mg/l. For NPK; LT₅₀ was not reached at concentration level of 28mg/l and 48mg/l but occurred at 90 hours for 68mg/l, 80 hours for 88mg/l and 72 hours for 108mg/l. For Calcium ammonium nitrate; LT₅₀ was 95hours at 28mg/l, 80 hours at 48mg/l, 72 hours for 68mg/l, 48 hours for 88mg/l and 38 hours for 108mg/l. For Single Superphosphate, the LT₅₀ was not reached at concentration of 28mg/l, 48mg/l and 68mg/l, it occurred at 90 hours for 88mg/l, 70 hours for

108mg/l. These showed Calcium ammonium nitrate to be the most toxic to the experimental organisms than NPK, Urea and Single Superphosphate. Logdose probit analysis revealed positive correlation between mortality rate of the test organisms and level of concentration for Urea, NPK, Calcium ammonium nitrate and Single Superphosphate fertilizers.

CONCLUSION

The result of the study revealed that Calcium ammonium nitrate was most toxic than Urea-N-46%, NPK 20:10:10 and Single Superphosphate fertilizers with mortality increasing with increase in concentration, hence its use on terrestrial environment for plant fertilization and in aquatic environment for pond fertilization is advised to be done moderately and with caution. The choice of fertilizers should be made with regard to the environment as revealed by the present study.

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