Toxicology Digest

Acute Toxicity of Inorganic Fertilizer, NPK (15:15:15) on Some Biological Parameters of African Catfish (*Clarias gariepinus)* Burchell, 1822

Daniel, U.I and Ezon-Ebi, E.

Fisheries and Hydrobiology Unit, Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.
Email: utibe.daniel@uniport.edu.ng

Abstract

Acute toxicity effects of inorganic fertilizer, NPK (15:15:15) on African Catfish *(Clarias gariepinus)* Burchell, 1822, was carried out under laboratory conditions. One hundred and fifty fingerlings of the fish of weight and length 4.5g and 3.9cm respectively, were acclimatized for 14 days. The test fish were divided into five groups (1-5) with 10 fish per group in triplicates. The concentrations were 0.00g/l, 5.00g/l, 15.00g/l, and 25.00g/l of NPK (15:15:15) fertilizer after a preliminary range-finding test. Results showed that the mortality rate increased in concentration and duration of exposure. After 96 hours, mortality was not recorded in the control. 5g/l concentration recorded 10% mortality but increased to 50% in the 10g/l, while the 15g/l and 25g/l recorded 90% mortality. There was a significant difference at $p<0.05$ in the 5g/l concentration as compared to the 10g/l and 15g/l concentration. The biological effects of the fertilizer in the exposed fish gills were concentration-dependent. It is therefore recommended that excess inorganic fertilizer, NPK (15:15:15) should not be used for pond fertilization to avoid deleterious effects on the fish.

Keywords: *Clarias gariepinus, inorganic fertilizer, mortality, biological effects.*

Introduction:

The introduction of most chemicals such as the inorganic fertilizers (NPK 15:15:15) into the aquatic environment like ponds, etc. directly or indirectly may be extremely hazardous to fish (Vander Oost *et al*, 2003). This chemical causes stress in fish which contributed to fish diseases, low production and mortality of fish (Rottman and Francis-Floyd, 1992). In the light of the above there is need to study and understand the toxicity and effects of the inorganic fertilizer, (NPK 15:15:15) on the aquatic organism using African Catfish (*Clarias gariepinus*) fingerlings as test organism.

The main objective in toxicity testing, according to Van der Merve (1993) is to predict, in combination with other environmental factors, with known accuracy, a concentration of a specific toxicant that will not harm the entire system. Normal physiological processes are affected long before the death of any organism hence, the need for physiological and biochemical indications of health (Van der Merve, 1993; Nussey, 1994).

Fish has been the most popular choice as a test organism because they are presumably the best understood organisms in the aquatic environment (Brungs, 1973) and the prominence of fish as food has grown with rapid expansion in the food industry as a result of increasing population awareness and demand for quality food (Jaffee and Henson, 2004). Aquatic pollution influence humans directly through the ingestion of fish due to bioaccumulation (Geyer *et al*., 2000). It is therefore expedient to evaluate pollution effects on fish for both environmental protection and socio-economic reasons (Atkins *et al*., 2011; Benton and Redclift, 2013).

Fertilizer supplies one or more elements required for the growth and productivity of plants. They are chemical compounds applied either through soil (for uptake by plants roots) or by foliar application (for uptake through the leaves). Fertilizers can be grouped into two; organic and inorganic fertilizers. Organic fertilizers are composed of decayed plants and animal matter. They are naturally occurring compounds manufactured through natural processes (such as composting) or naturally occurring mineral deposits. Organic fertilizers include barnyard manure, compost and green manure. Manure contains nitrogen and phosphorus. Organic manure adds nearly all of the nutritive substances necessary for the biological cycle. It also has favourable action on the structure of the soil. It also aids multiplication of bacteria in suspension in water which has favourable action on the development of zooplankton. Other examples of organic manure are poultry droppings, wastes like blood from slaughter houses, pig manures, human faeces and cow dungs. Organic manure leads to an increase in the biological oxygen demand (BOD) resulting in deoxygenation which can lead to death of fish if used excessively.

Inorganic fertilizers are composed of simple chemicals and minerals. They are manufactured through chemical processes such as Haber process and also using naturally occurring deposits, while chemically altering them (for example, concentrated super phosphate). Inorganic fertilizers have three main elements which are nitrogen, phosphorus and potassium (Verhoeven and Schmitz, 1991). The secondary elements are boron, sulphur, magnesium, calcium, manganese, iron, zinc, copper and molybdenum. The most common inorganic fertilizers include ammonium fertilizer (82% nitrogen); nitrogen, phosphorus and potassium combination in the ratio (15:15:15); urea

(46% nitrogen), super phosphate, monobasic and dibasic ammonium phosphate (contains nitrogen and phosphorus), calcium nitrate and phosphorus hydroxide chloride (muriate of potash).

Fertilizers have beneficial effect in the ecosystem such as promotion of primary production as well as detrimental such as causing fish mortality (Barg, 1992). The rate of application of fertilizer is taken into consideration because high application of inorganic fertilizer leads to increased leaching of nitrates into ground water. Eventually, nitrogen enriched ground water makes its way into lakes, bays and oceans where it accelerates the growth of algae, disrupt the normal functioning of the water ecosystem and kills fish in a process called eutrophication (which may cause water to become cloudy and coloured green, yellow, brown or red).

Storage and application of some nitrogenous fertilizers in some weather soil conditions can cause emissions of greenhouse gas, nitrogen oxide. Ammonia gas is emitted following application of inorganic fertilizer. Besides supplying nitrogen, ammonia can increase soil acidity. Excessive nitrogen fertilizer application can also lead to pest problems by increasing the birth rate, longevity and overall fitness of certain pests (Snyder *et al*., 2009; Siavoshi and Laware, 2011).

Phosphate fertilizers have an effective action in increasing the production of 50-125% inorganic fertilizer. Nitrogen fertilizers can give extra production of about 50% when used alone or in combined form (Jensen and Hauggaard-Nielsen, 2003).

Toxic fertilizers are recycled industrial wastes that introduce several classes of toxic materials to farmlands, garden soil and water streams. The most common toxic elements in this type of fertilizer are mercury, lead and arsenic (Dudka and Miller, 1999), leading to a major environmental problem due to the fact that toxic waste is being disseminated terrestrial or aquatic ecosystem on our land and in our water

Nitrogen-rich compounds found in fertilizer run-off are the primary sources or causes of serious depletion of oxygen in parts of the water body, especially the coastal zone. These nitrogen and oxygen molecules that crops need to grow eventually make their way into rivers, lakes and oceans, fertilizing blooms of algae that deplete oxygen and leaving vast areas in their waste also referred to as “dead zones” (Edwards, 2008). In this dead zone, no fish or other lives survive.

Materials and Methods:

The study was aimed at determining the toxicity of the inorganic fertilizer (NPK 15:15:15) on some biological parameters of *Clarias gariepinus* (Burchell, 1822) fingerlings. The objectives were to determine the median lethal concentration (LC_{50}) of the fertilizer, the effects of the fertilizer on the tissues and behavioural responses of the fish.

The study was carried out using a non-renewable bioassay method under laboratory temperature (Health, 1995; Sprague, 1971; Brungs, 1973). The concentrations of the fertilizer were 0.0g/l, 5g/l, 10g/l and the 15g/l. The test organism in the different test solutions of the fertilizer were monitored for 96hrs.

The experiment was done randomly with four treatments and a control replicated three times. A 96-hour non-renewal static bioassay method was adopted for the experiment. The treatments consisted of different concentration levels of NPK (15:15:15) fertilizer to evaluate its ability to cause toxic effects on fingerlings of *Clarias gariepinus* and also its acute toxicity effect on the same organism. The test organisms were stocked in each container at 10 fish per 5 litres of water. After the 96-hour experiment, histopathological studies were carried out with the fish that died from the acute toxicity test.

Collection of Test Organism (*Clarias gariepinus*)

The fish were purchased from African Regional Aquaculture Centre (ARAC), Aluu, Rivers State, and were transported in the early hours of the day with a plastic container to the Animal House Laboratory of the Department of Animal and Environmental Biology, University of Port Harcourt, Choba Campus, Port Harcourt. The fish were kept for one week to acclimatization. During this period, the fish were fed using commercial feed pellets at least twice a day. Water was changed every day during the period of acclimatization.

The average length of the fish was determined by selecting thirty fish randomly from the total population and measuring with a transparent 30cm ruler. The average length was found to be 3.9cm. The average weight of the fish was obtained by selecting forty fish randomly from the total population and the selected fish were weighed with Furi 600g capacity sensitive weighing balance and divided by forty. The average weight was found to be 4.5g. A total of 150 *Clarias gariepinus* fingerlings were used for the experiment.

Preparation of the Toxicant NPK (15:15:15) Fertilizer

The fertilizer was purchased at the Agricultural Development Programme

(ADP) Office, Rumudomaya, Port Harcourt, Rivers State.

Results and Discussions:

Observed Behavioural Patterns

The result on Table 1shows the responses of *Clarias gariepinus* exposed to acute toxicity of NPK (15:15:15) fertilizer. After the initial 6 hours, there were no observable behavioural changes of the test organisms in the 5g/l, 10g/l and the 15g/l concentrations of NPK (15:15:15). However, there was a drastic change in behavior in the 25g/l concentration instantly. The fish were gasping for air, swimming irregularly and restless. After 6 hours duration, there was loss of pigmentation observed on the fish. The fish died at this 6 hours period and the skin surface contained slime.

As the exposure time increased, behavioural changes were observed in the different concentrations except the 5g/l and the control, before the occurrence of death. The different behavioural changes observed at the different levels of treatment were caused by the toxic effects of the inorganic fertilizer (NPK 15:15:15). (Figure 1).

Table 1: Responses of *Clarias gariepinus* exposed to acute toxicity of NPK (15:15:15) fertilizer.

Parameters	**Observations**
Body movement	Restlessness and irritations
Buoyancy	Loss of balance and equilibrium
Death	No opercula movement, lying ventral side upwards

The concentration of the test solutions that caused 50% of the *Clarias gariepinus* fingerlings (LC_{50}) was found to be 9.72g/l from the results. Increase in the concentration of the fertilizer caused increase in mortality in the experiment. Meanwhile there was no mortality in the control tank.

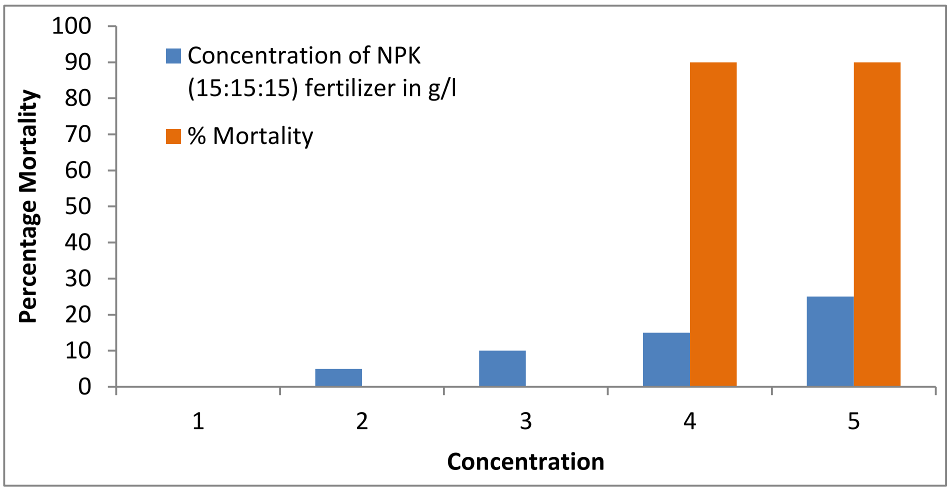

Fig. 1: A graph of % mortality against concentration of NPK 15:15:15 fertilizer

The results of the study showed that the inorganic fertilizer (NPK 15:15:15) caused some histopathological changes in the fish gills and subsequent death of the exposed fish. Moreover, the severity and types of changes observed on the gills were concentration-dependent. Therefore, it is recommended that the inorganic fertilizer (NPK 15:15:15) when applied to the pond should be done at the appropriate dosage, as excess dosage could be harmful to the cultured fish or result in eutrophication.

Increase in concentration of the inorganic fertilizer, NPK (15:15:15) caused increase in mortality in the non-renewal static bioassay, though no mortality was observed in the control tank.

The pH measured throughout the test period was 4.67 – 7.57. There were pulses and fluctuations in the water quality parameters such as temperature, salinity and pH. Therefore, the temperature variation obtained in the entire bioassay media was between 22.2^{O}C – 27.8^{o}C. The dissolved oxygen (DO) range was 6.27 – 8.67. High dissolved oxygen (DO) promotes feeding and growth rates in fish more especially in fingerlings in the absence of the inorganic fertilizer (Alabaster and Boyd, 1980).

The 90% mortality recorded in this acute toxicity test does not mean that both concentrations had the same intoxicating strength. It could imply that the dose that could kill 90% of a population lies within 15.00g/l – 25.0g/l, since at these concentrations all the test organisms died. The resulting mortality was subjected to statistical analysis using the one-way Analysis of Variance to test for levels of significance between the concentrations.

From the results, it was observed that fifty percent of *Clarias gariepinus* died within 96 hours of exposure to 9.72g/l level of NPK (15:15:15). This is the LC_{50} value.

A high influx of fertilizer – rich effluents in high concentrations will expose the *Clarias gariepinus* to high degree of impact in a short time and could lead to massive fish mortality.

The effect on the gills is a pointer to its sensitivity as a site of respiration and osmoregulation in aquatic organisms and this makes it a good indicator for pollution monitoring. Iroegbu *et al*. (2008) emphasized that the gill is a good indicator parameter in assessing environmental toxicity.

In toxicology, an organism gets impacted by poison either by inhalation through the gills, by ingestion through food or drinking, or by absorption through the skin surfaces (Altug, 2002). This makes the skin/body tissue a good working tool in aquatic toxicology because these organisms are in direct contact with water.

In conclusion, the different levels of concentration; 10.0g/l, 15.0g/l and 25.0g/l of inorganic fertilizer, NPK (15:15:15) was toxic to the exposed *Clarias gariepinus* as evidenced by the recorded mortality in some of the exposed fish including the observed histopathological lesions in the gills. Therefore, the inorganic fertilizer, NPK (15:15:15) should not be used in excess for the purpose of fertilizing pond water because it is hazardous to fish (Briggs and Fvnge-Smith 1994).

References

Alabaster, J.S. and Lloyd, R. (1980). Water quality criteria for fresh water fish. *Agriculture, Ecosystems and Environment*, 74(1), pp. 19-31.

Altug, T. (2002). Introduction to toxicology and food. (CRC Press). Water pollution and human health in China. *Environmental Health Perspectives*, 107(4), p251.

Atkins, J.P., Burdon, D., Elliott, M., and Gregory, A.J. (2011). Management of the marine environment: integrating ecosystem services and societal benefits with the DPSIR framework in a systems approach. *Marine Pollution Bulletin*, 62(2), 215-226.

Barg, U.C. (1992). Guidelines for the promotion of environmental management of coastal aquaculture development (No. 328). Food and Agriculture Organization.

Benton, T., and Redcliff, M. (2013). Social theory and the global environment. Routledge

Briggs, M.R.P., and Fvnge-Smith, S.J. (1994). A nutrient budget of some intensive marine shrimp ponds in Thailand. *Aquaculture Research*, 25(8), pp. 789-811.

Brungs, W.A (1973). Continuous flow bioassays with aquatic organism: procedures and applications. In: Biological methods for the assessment of water quality. *ASTM International*. pp. 117-126.

Dudka, S., and Miller, W.P. (1999). Accumulation of potentially toxic elements in plants and their transfer to human food chain. *Journal of Environmental Science and Health Part B,* 34(4), 681-708.

Edwards, M.R. (2008). Green Algae Strategy: End Biowar I and Engineer Sustainable Food and Biofuel. Lulu.com.

Francis-Floyd, P.A. (1992). Submitting a sample for fish kill investigation. Southern Regional Aquaculture Center Publication 472, Mississippi State Univ. MS.

Geyer, H.J., Rimkus, G.G., Scheunert, I., Kaune, A., Schramm, K.W., Kettrup, A., and Mackay, D. (2000). Bioaccumulation and occurrence of endocrine-disrupting chemicals (EDCs), persistent organic pollutants (POPs), and other organic compounds in fish and other organisms including humans. In: Bioaccumulation-New Aspects Developments. Springer Berlin Heidelberg pp. 1-166.

Health, A.G. (1995). Water Pollution and fish physiology. Second Edition. CRC Press Inc., Florida. pp. 359.

Iroegbu, B.C, Hart, A.I and Abowei, J.F.N. (2008). Survival response of Sarotherodon melanotheron (Ruppel, 1852) fingerlings to various concentration levels of perfekthion under laboratory condition. *Journal of Applied Science and Environmental Management (JASEM)* 12(12): 47-54.

Jafee, S., and Henson, S. (2004). Standards and Agro-food exports from developing countries: rebalancing the debate (Vol. 3348). World Bank Publications.

Jensen, E. S., and Hauggaard-Nielsen, H. (2003). How can increased use of biological N2 fixation in agriculture benefit the environment? *Plant and Soil*, 252(1), pp. 177-186.

Nussey, G. (1994). The effect of copper on blood coagulation. (Cichilidae). M.Sc. Thesis, Rand Afrikaans University, Johannesburg, South Africa.

Rottman, R.W., Francis-Floyd, R. (1992). The role of stress in fish diseases. Southern Regional Aquaculture Centre (SRAC) Publishing, p.474.

Siavoshi, M., and Laware, S.L. (2011). Effect of organic fertilizer on growth and yield components in rice (Oryza sativa L.). *Journal of Agricultural Science*, 3(3), pp. 217.

Snyder, C.S., Bruulsema, T.W., Jensen, T.L., and Fixen, P.E. (2009). Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agriculture, Ecosystems and Environment*, 133(3), 247-266.

Sprague, J.B. (1971). Measurement of pollutant toxicity to fish-III: Sub lethal effects and “safe” concentrations. *Water Research,* 5(6), 245-266.

Temilsina, G.R., and Shrestha, A. (2010). Biofuels: markets, targets and impacts. *World Bank Policy Research Working Paper Series,* 56(3), 123-231.

Van Der Merve, M., (1993). Aspects of heavy metal concentration in the Oilfants River, Kruger National Park and the effect of copper on the hematology of *Clarias gariepinus* (Claridae). M.Sc. Thesis, Rand Afrikaans University, South Africa.

Van der Oost, J., Beyer, J, and Vermeulen, N.P. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ Toxicol Pharmacol.* 13(2):15-149

Verhoeven, J.T.A., and Schmitz, M.B. (1991). Control of plant growth by nitrogen and phosphorus in mesotrophic fens. *Biogeochemistry,* 12(2), pp. 135-148.