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Full Length Research Paper

The assessment of acute toxicity of urea fertilizer against *Heterobranchus bidorsalis* fingerlings

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Abstract

The toxicity Urea fertilizer against *Heterobranchus bidorsalis* fingerlings (mean total length, 6.98 ± 0.30cm SD; mean body weight 2.04 ± 0.35g SD) was investigated. The fingerlings were exposed to increasing concentrations of Urea (0.00g/l, 8.75g/l, 10.00g/l, 11.25g/l, 12.50g/l, 13.75g/l, 15.00g/l and 16.25g/l) fertilizer in the static renewal bioassay for 96 hours. Exposed fish showed initial stress responses such as erratic swimming, restlessness, loss of balance, 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. The 96hours LC_{50}, associated confidence limits and safe concentrations for the fertilizer were (17.84, 11.88-13.67, and 1.78g/l respectively). 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 Urea fertilizer could be classified as toxic to *Heterobranchus bidorsalis* fingerlings at certain concentrations.

Keywords: Toxicity, acute, concentration, mortality, *Heterobranchus bidorsalis*

INTRODUCTION

One of the major environmental issues of our time is the growing concern about water quality suitable for use by humans and animals (Calamari and Naeve, 1994). The daily activities of man in one way or the other affect the aquatic environment negatively. These activities, which include the discharge into streams and river systems of various pollutants, such as agricultural fertilizers of different types, pesticides, insecticides and industrial effluents, pollute the water bodies and alter ecological balance (Osibanjo, 2002).These 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, 1999). It is a known fact that water quality conditions are constantly being threatened by pollution. The rivers and coastal water bodies are presently exposed to increasing quantities and concentrations of both natural as well as anthropogenically derived contaminants (Ezeka, 2004). Environmental concern about intensive agricultural practices and excessive or inappropriate use of chemical fertilizers calls for some global action among environmentally conscious individuals and other stakeholders (Nychas, 1990).

A fertilizer is any material organic or inorganic, natural or synthetic, that supplies plants with the necessary nutrients for growth and optimum yield (Addiscott*et al.,* 1991) or a substance added to water to increase the production of natural fish food organisms (Nwadukwe, 1995). Inorganic (or chemical) fertilizers are fertilizers mined from mineral deposits with little processing for

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example, lime, potash, or phosphate rock or industrially manufactured through chemical processes, for example NPK and Urea (Amadi, 1991). Inorganic fertilizers vary in appearance depending on the process of manufacture. The particles can be of many different sizes and shapes (crystals, pellets, granules or dust) and the fertilizer grades can include straight fertilizers (containing one nutrient element only), compound fertilizers (containing two or more nutrients usually combined in a homogeneous mixture by chemical interaction) and fertilizer blends (formed by physically blending mineral fertilizers to obtain desired nutrient rates (Alexander, 1996).Fertilizers are carried through surface run-off from cultivated agricultural farm lands and they enter into the aquatic environment in either soluble or particulate forms and consequently, deliver soluble phosphorus, nitrogen and carbon for uptake and growth of plants (Cooke, 1975). Fertilizer analysis can be categorized into organic and inorganic chemical varieties (Ball, 1949). The use of chemical fertilizers in agricultural production is highly indispensable and is widely acceptable by an ever increasing number of farmers, not because fertilizers help condition the fragmented and nutrient-depleted soil for further production and boast soil resistance to erosion, but also that it encourages vegetal cover (Almazan and Boyd, 1978).

However, the increasing application of fertilizers has threatened the human environment and aquatic ecosystems with deleterious consequences (Enger and Smith, 1991). These harmful effects if unchecked can negate to quite some extent the benefits derived from increasing usage of fertilizer (Maxwell, 1973).Fertilizers and pesticides are often associated with pollution problems because they could be toxic to the fish and other aquatic organisms over a range of concentrations under varied environmental conditions (Rand, 1995).The concern about pesticides has always been present because of their chemical nature, whereas that of fertilizer use has appeared only relatively recently mainly because of the measurable effects of nitrate groundwater pollution and increase of nitrate in drinking water which pose serious health problems to the consumer and the public in general (Kolenbrader, 1985).Other problems include contamination of aquatic environment by heavymetals such as cadmium, through run-off containing phosphate fertilizers (Shaivon *et al.,* 1995) and nutrient enrichment, leading to eutrophication and deterioration of water quality (Kofoed, 1981). The objective of this study is therefore to determine the acute toxicity i.e. lethal concentrations (LC_{50}) and effects of urea fertilizer against the *H. bidorsalis* fingerlings.

MATERIALS AND METHODS

Description of Test Organism

The species, *Heterobranchus bidorsalis* belong to the family clariidae and is one of the most widely cultured catfish in Nigeria (Marioghae, 1991). They have the ability to grow on a wide range of artificial and natural foods, with good feed conversion efficiency. They are hardy and tolerant to low dissolved oxygen and other adverse culture conditions. They are dark to brownish olive (sometimes with blotches) with flattened head, and strongly granulated as well as depressed body. They have typically four pairs of barbels, usually grey and white at their bases, with wide mouth and upper lip reddish. *Heterobranchus bidorsalis* has smooth and scale-less skin as well as accessory breathing organs on the head and can survive out of water for a long time. Like *Clarias,* they can walk off from the pond if the conditions are unfavourable (Teugels *et al.*, 1998; Huisman and Richter, 1987; Haylor, 1993). Theyare important species with a high market value, rapid growth, and are propagated in Africa (mainly, South Africa and Nigeria), parts of Europe and Asia (Teugels, 1984; Viveen *et al.*, 1985). They are most commonly used as experimental fish (Robert, 1988; Clay, 1989, Okaeme; 1989).

Source of Experimental Fish

Five hundred fingerlings of *Heterobranchus bidorsalis* were obtained from Premier Fisheries Ltd., Akai Ubium, Nsit Ubium Local Government Area, Akwa Ibom State, Nigeria. They were transported in a fifty litre jerry-can by car to the Fisheries and Aquaculture hatchery, Department of Fisheries, University of Uyo, Nigeria. The mean body weight (g) and total length (cm) of the species were 2.04 ± 0.35 SD and 6.98 ± 0.29 SD, respectively.

Acclimation

The fish were acclimated for fourteen days, in groups of fifty fish per plastic container in thirty litres of dechlorinated tap water. The containers were aerated during this period, while the water was renewed daily to discard faecal material as well as left-over food. The species were fed twice daily with a 45% crude protein diet at 1% of their body weight, half at 08:00 and 16:00 hours GMT, respectively. During this period, dead and abnormal individuals were immediately removed. Mortality recorded during the acclimation period was less than 2% (OECD, 1992; APHA *et al.,* 2005). It was from the acclimated population that healthy individuals used as test fish in this study were carefully selected.

Preparationof Test Media and Exploratory Test

The whole experimental procedures used in this study were based on the guidelines in OECD (1992); ASTM, (2004a) and APHA *et al*., (2005). To obtain the ranges of concentrations as used in the experiment, five (5) fish each were exposed to four (4) litres of dechlorinated 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 two replicates (Akindele, 2004; Ogbeibu, 2005).Exposure concentrations of the fertilizer were: 0.00g/l (control), 8.75g/l, 10.00g/l, 11.25g/l, 12.50g/l, 13.75g/l, 15.00g/l,16.25g/l respectively.Sixteen plastic containers ($0.002m^3$) were randomly labeled and each filled with dechlorinated tap water to 8litres mark for each treatment. The different concentrations were prepared by dissolving directly different weights of the fertilizers in the 8 litres of dechlorinated tap water, to obtain the desired concentration (APHA *et al.*, 2005).The solution was stirred with a glass rod to obtain a homogenous mixture. Within an hour, the containers were randomly stocked with ten fish each using a scoop net.The test fish were not fed twenty-four hours (24hours) prior to the test (experiment) and during the ninety-six hours (96hours) exposure period. Test solution in each tank was drained completely each morning and the fish removed carefully with a scoop net and kept in a thirty litre (30litre) plastic container. Fresh solutions were prepared and the fish were carefully put back. Test solutions were renewed daily.

Water Quality Parameters

Temperature, dissolved oxygen, pH, alkalinity and total hardness of the control and various test media were determined at 24, 48, 72 and 96hours intervals during the experimental period (ASTM, 2004a; APHA *et al.,* 2005).

Temperature

The temperature was monitored with mercury in-glass thermometer. The thermometer was inserted into the test water and the corresponding readings were taken and recorded.

Dissolved Oxygen

The dissolved oxygen content was assessed by Winkler's method (ASTM, 2004a). The procedures involved filing oxygen bottles (125ml) with water samples from each tank and fixing immediately with 2ml of manganous sulphate and 2ml of potassium iodine solution. A brown precipitate was formed. 2ml of concentrated sulphuric acid solution was added to the bottle and shaken to further dissolve the precipitate. 10ml of this solution was pipetted into a 25ml conical flask and titrated with sodium thiosulphate solution (0.25N) using starch as an indicator until a colourless endpoint was reached.

pH

The pH was determined with a digital pH meter (Hannah product Portugal, Model HA 989).

Alkalinity

The procedure involved the collection of water samples from each tank in stoppered bottles. 25ml of the sample was pipetted into a conical flask and 5 drops of methyl red indicator and bromocresol green was added and titrated with standard HCl acid (0.01N) from a 10ml burette, with continuous shaking until the color changed from blue to pale pink. The endpoint of pH was read with a pH meter.

Total Hardness

The procedure involved the collection of 25ml of water samples from each tank into a 100ml conical flask. 1ml of diluted buffer solution of borax was added and a measure of solo-chrome black indicator added also, with constant shaking. This was then titrated with 1.00g of disodium salt of ethylene diamine-tetra acetic acid (EDTA) solution, from a 2ml burette until the wine red colour changed sharply to blue.

Data Collection

Water quality parameters were determined at fixed intervals of 24, 46, 72 and 96hours respectively. Mortality of the fish species in each tank was observed and recorded at fixed intervals of 24, 48, 72 and 96hours, respectively. Dead fish were removed immediately to prevent polluting the test media. A fish was considered dead, when there was lack of movement and reaction to gentle prodding with a glass rod. Other unusual signs of stress were equally monitored, such as uncoordinated and irregular swimming pattern, vertical erection, overturning, restlessness, jumping out of the tank and gasping for air.

Data Analysis

Each set of results obtained from these experiments was analyzed in Completely Randomized Design (CRD) at 5% probability level, then the Student's t-test was used to test for significant difference ($P<0.05$) in the treatment (Irwin 1953; Finney 1978,1979; Akindele, 2004; Ogbeibu, 2005). Analysis of the lethal concentration (LC_{50}) values for the 24, 48, 72 and 96 hours with associated confidence intervals for the various concentrations of the fertilizer were determined by Probit Analysis using Statistical Package for the Social Sciences (SPSS) Data Editor version 10.0 (Finney, 1978; 1979; Stephan, 1977).

Table 1: Mean water quality parameters during 96hours exposure of *Heterobranchus bidorsalis* fingerlings to acute concentrations of Urea fertilizer, mean ± SD (n = 40, 2 replicates per treatment level).

	Fertilizer Concentration (g/l)							
Parameters	0.00	8.57	10.00	11.25	12.50	13.75	15.00	16.25
Temperature(^{0}C)	27.47±0.37	27.34±0.26	27.17±0.36	27.34±0.13	27.48±0.16	27.40±0.30	27.56±0.33	27.28±0.26
Dissolved oxygen (mg/l)	7.16±0.32	6.33±0.32	6.07±0.07	5.50±0.46	5. 41±0.35	5.37±0.40	4.77±0.13	4.36±0.38
Total hardness	16.47±0.38	16.58±0.41	17.34±0.86	18.58±0.34	18.76±0.17	19.48±0.21	20.54±0.52	21.37±0.21
pH	6.27±0.16	6.31±0.25	6.25±0.14	6.30±0.13	6.36±0.09	6.26±0.24	6.43±0.11	6.51±0.03
Alkalinity (mg/l)	27.38±0.79	31.10±0.60	32.12±0.75	32.74±0.37	34.57±0.38	36.80±0.80	36.50±0.87	38.22±0.62

Safe concentrations (Table 2) at the various time intervals were obtained by multiplying the lethal concentration (LC_{50}) value by a factor of 0.1 or dividing by a factor of 10 (Irwin, 1953; Finney, 1978; 1979).

RESULTS

Physico-chemical Parameters

The result of the physico-chemical parameters of the experimental media for the fertilizer showed that there was a significant reduction in the mean values of dissolved oxygen. Conversely, 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 temperature and pH ($P>0.05$), (Table 1).

General Behavioural Changes of Exposed Fish

Behavioural changes occurred in the fish treated with Urea fertilizer at different concentrations. At different fertilizer concentrations, the fish stood in an upright position with their snouts above the water surface gasping for air. Other behavioral reactions exhibited by the exposed to the fertilizer concentrations before death were uncoordinated swimming, frequent attempts at jumping out of the tank and quietness. These changes showed by the exposed fish in response to the effect of the toxicant were more pronounced in tanks containing higher concentrations, but decreased with increase in time of exposure, though there was gradual reduction also at higher concentrations. There were no obvious changes in fish behaviour in the lower concentrations less than 10.00g/l for the first 24 hours of exposure. However, fish in the control group did not exhibit any abnormal behavior. Figure 1 shows mortality rate of *Heterobranchus bidorsalis* exposed to different concentrations of urea fertilizers for 96hours.

Mortality of Fingerlings Exposed to Urea Fertilizer for 96 Hours

Mortality of the fingerlings exposed to various concentrations (Figure 1) of Urea fertilizer indicated that, the different concentrations caused significant ($P<0.05$) but variable death rate in the exposed fish. Mortality rate was concentration-dependent, with highest concentrations having death rates significantly higher than the others. No mortality was recorded in the control group of the exposed fish.The lethal effects of Urea fertilizer on the fingerlings expressed as LC_{50} for 24, 48, 72 and 96hours and their associated 95% confidence

Figure 1: Mortality rate of *Heterobranchus bidorsalis* exposed to different concentrations of urea fertilizers for 96 hours

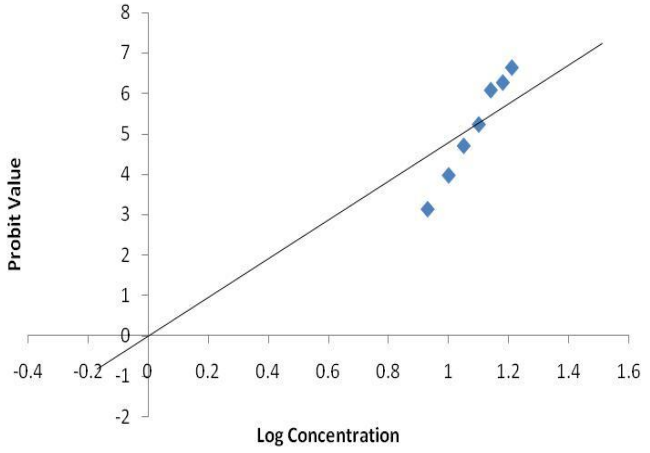

Table 2: 24, 48, 72 and 96hours LC_{50} values, associated confidence limits and safe concentrations of Urea fertilizer to H*eterobranchus bidorsalis*

Time (hrs)	LC_{50} (g/l)	Confidence Limits (g/l)		Safe Conc. (g/l)
		Lower	Upper	
24	26.36	17.65	21.84	2.64
48	21.20	15.44	18.57	2.12
72	19.33	13.23	15.46	1.53
96	17.84	11.88	13.67	1.78

limits (Table 2), showed that the range of values between the 24 hours LC_{50}; 26.36g/l, and 96hrLC_{50}; 17.84g/l of the toxicant was wide. A safe concentration at LC_{50}; 2.64g/l for 24hrs and 1.78g/l 96hrswas very low. The LC_{50} values of the concentrations decreased with increase in time of exposure from 24 to 96hours. At 24hrs, the LC_{50} values were greater than the 96hrs LC_{50} values.

DISCUSSION

Water Quality Parameters

The water quality parameters of the experimental media for urea fertilizer showed that there was a significant reduction in the mean values of dissolved oxygen content, while conversely, alkalinity, and total hardness values increased as the fertilizer concentrations were increased ($P < 0.05$), compared to those of the control group. There was no significant difference between the various means values of temperature and pH ($p > 0.05$) as both were within the suggested tolerance ranges for warm water fish species (Boyd, 1979; Mackereth, 1963; Adeniji and Ovie, 1989). This result agrees with the work of Ofojekwu*et al.,*(2008a); Ofojekwu *et al.,* (2008b) and Ufodike and Onusiriuka (1990) who exposed *Tilapia zilli* and *Clarias gariepinus* fingerlings respectively to acute concentrations of inorganic fertilizers; NPK, urea, calcium hydroxide ($Ca(OH)_2$, potassium phosphate ($Na_3PO_4.12H_2O$) and sodium nitrate ($NaNO_3$) and reported of no significant difference between the various mean values of temperature 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 alkalinity and total hardness may imply an increased toxicity with the raised values of physico-chemical parameters (Table 1).

The toxicity of a chemical in water depends largely on the concentration and the physico-chemical properties of the medium. Svobodova *et al.,* (1993); Sprague (1969, 1970) and Klussmann *et al.,* (1969) stated that the piscicidal activity of chemicals vary with temperature, pH and other environmental factors. Osekita (2003) also documented that, the toxicity of a pollutant to fish usually increases with the physico-chemical properties of the medium 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.

General 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). The behavioural responses reported for the test fishes in this study are similar to those reported by other authors for clarrids under various stress conditions (Onusiriuka and Ufodike 1994; 1998; 2000; Ufodike and Onusiriuka 1990; 1992; Avoajah and Oti, 1997; Auta *et al*., 2004; Nwanna *et al*., 2000).

Besch (1975) 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), and loss of equilibrium, followed by lethal (death) phase when opercula movement and responses to tactile stimuli cease completely. Inspite 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).

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). However, the concentrations used in this investigation relatively corresponds with the values documented by Omoregie *et al.,* (2003) when they worked on the effects of sub-lethal concentrations of NPK (15.15.15) fertilizer on growth and feed utilization by the toothed carp (*Aphyosemion gardneri).*

At the concentrations used in this investigation, the fertilizer led to significant reduction in the dissolved oxygen and an increase in 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 earlier documented that insufficient amount of dissolved oxygen is one of the contributing factors to mortality in some fish species.

The results obtained from this research shows that the 96hr LC_{50} for the exposed fish species was 17.84g/l with lower and upper confidence limits of 11.32 and 14.88g/l respectively. The 17.84g/l 96hr LC_{50} obtained for *Heterobranchus bidorsalis* for urea in this study closely agrees with the 96hrLC_{50} (15.85g/l) reported by Ofojekwu *et al.,* (2008b) when they exposed *Tilapia zilli* 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 water surface gasping for air, uncoordinated swimming, restlessness, frequent attempts at jumping out of the tank and quietness reported in this study for urea fertilizer have been earlier documented by Omoregie and Ufodike (1991); Okwuosa and Omoregie (1995); Omoregie *et al*., (1997); Oti (1997; 2002); Adakole (2005) and Ayuba and Ofojekwu (2005) when they exposed fish fingerlings to acute concentrations of different toxicants. At concentration 16.25g/l, 95% mortality was recorded for fertilizer. Also, at concentrations 15.00g/l, 13.75g/l, 12.50g/l, 11.25g/l, 10.00g/l and 8.57g/l, cumulative mortality (%) recorded were 90, 85, 70, 65, 55 and 40 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 Omoregie and Ufodike (1991); Avoaja and Oti (1997); Omoregie (1998); Oti (2002) and; Ayuba and Ofojekwu (2005). This study also reveals that concentrations above 10.00g/l were lethally threat to the test fish within 96 hours as 55% mortality was recorded.

ANOVA showed that there was no significance difference among treatment and concentration levels on mortality of the test fish for the fertilizer. This further signifies that the treatment effects were equal among experimental units ($P>0.05$). The mortality rate of the test fish at 11.25g/l killing more than 50% of the test fish indicates that the higher the concentration, the higher the mortality rate at a given exposure time. This also clearly indicates that urea fertilizer is harmful to *Heterobranchus bidorsalis* fingerlings at acute concentrations. This confirms the findings of Nwani *et al.,* (2008) and Ofojekwu *et al.,* (2008 a/b) who exposed *Tilapia zilli* fingerlings to acute concentrations of different inorganic fertilizers.

Based on the 24, 48, 72 and 96hrs LC_{50} values determined from this study for urea fertilizer, it could be rated as toxic to *Heterobranchus bidorsalis* 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 LC_{50} reported in this investigation.The 96hrs LC_{50} and safe concentration of the fertilizer i.e. 17.84g/l (1.78g/l) respectively, to *Heterobranchus bidorsalis* fingerlings suggest that, this species is not tolerant to acute concentrations of this toxicant.

This study also establishes that, with prolonged exposure to the toxicant, the fish became fatigued and stressed. Substances involved in energy generation such as protein, carbohydrates and fat which play significant roles in body building and energy production in the fish may be negatively affected under environmental stress (Heath, 1989). Kormakik and Cameron (1981); Kuma and Krisnamoorthi (1983) reported that increased utilization of protein when fish is under the influence of a pollutant leads to stress. Exposure to increasing concentrations of toxicant was observed by Umminger (1970) and Saroj (1987) to cause fatigue due to utilization of the energy substances. The energy sources may not be simultaneously used, but if the principal and immediate source is depleted, the others show a proportional depletion as the metabolism of these substances are inter-linked through the common metabolic pathway-tricaboxylic acid cycle, hence a depletion in all the components of fish tissue, leading to stress and fatigue conditions (Robert, 1978).

The stressful behaviours exhibited by the fish as established in this study, suggests 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 as well as fertilizer effluents at their acute concentrations on fish fingerlings (Oti and Chude, 1997; Ekweozor *et al.,* 2001; Bobmanuel *et al.,* 2006; Nwani *et al.,* 2008; Ofojekwu *et al.,* 2008a/b).

CONCLUSION AND RECOMMENDATIONS

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 (Ludwig e*t al*., 1998). 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 fingerling mortality, if the concentrations exceed the established LC_{50} reported in this study.

The study clearly shows that acute concentrations of Urea fertilizer are harmful to *Heterobranchus bidorsalis* fingerlings. It is thus recommended that the application of this fertilizer in aquatic ecosystems either in ponds, irrigations or farms should be carefully controlled andmonitored, such that concentrations that are lethal to aquatic life could be avoided.

Based on the results obtained from this study, the following deductions can be drawn:

(i) Exposure to acute levels or concentrations of Urea fertilizer produced significant and adverse behavioural changes in *Heterobranchus bidorsalis* fingerlings.

(ii) The tolerance range of *Heterobranchus bidorsalis* fingerlings to Urea fertilizer were (11.88-13.67g/l),

96 hour LC_{50} (17.84g/l) and safe concentration (1.78g/l), respectively.

There is also a great need to provide further baseline data on urea fertilizer. Such studies should be concerned with providing information on research such as, the effects of sub-lethal concentrations of Urea fertilizer on the haematology, serum/plasma enzymes, metabolites, hormones and tissues of *Heterobranchus bidorsalis*.

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