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The effects of some agricultural fertilizers on fingerlings of *heterobranchus bidorsalis*

Abstract

The toxicity of NPK (15.15.15) and Urea fertilizers on *Heterobranchus bidorsalis* fingerlings (mean total length, 6.98 ± 0.30cm SD; mean body weight 2.04 ± 0.35g SD) was investigated in the present study. The fingerlings were exposed to increasing concentrations of NPK (0.00g/l - 6.25g/l), and Urea (0.00g/l - 16.25g/l) fertilizers 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. Examinations of water quality showed a reduction in the dissolved oxygen content and increase in total hardness and alkalinity as the concentrations of fertilizers were increased. After 96 hours of exposure, LC_{50}, associated confidence limits and safe concentration values for NPK and Urea fertilizers were (1.09, 0.29-3.86, and 0.11g/l) and (17.84, 11.88- 13.67, and 1.78g/l) respectively. Mortality rate was noted to be 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. Comparative study on the two fertilizers showed no significant difference between their effects on the fingerlings of *H. bidorsalis* ($P>0.05$). The findings from this study show that both Urea and NPK fertilizers could be classified as toxic and highly toxic respectively, to *H. bidorsalis* fingerlings at certain concentrations.

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Introduction

Water quality is one of the major environmental issues. Day by day the concern is growing about water quality suitable for use by humans and animals.[1] Daily activities of man by 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 different types of agricultural fertilizers, pesticides, insecticides and industrial effluents, pollute the water bodies and alter ecological balance.[2] A fertilizer is any organic or inorganic, natural or synthetic material which supplies to plants necessary nutrients for growth and optimum yield.[3]

Recently, farmers add fertilizers into water for increasing the production of natural fish food organisms.[4] Inorganic fertilizers are mined from mineral deposits with little processing e.g. lime, potash, or phosphate rock while chemical industrially manufactured through chemical processes e.g. NPK and Urea.[5] 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.[6] Fertilizer analysis can be categorized into organic and inorganic chemical varieties.[7]

Environmental pollution by chemical fertilizers had been reported by Lioyd R.[8] The sources of input of fertilizers into the aquatic environment as reported by Long DF,[9] include superphosphate production effluent, run-off, and discharge from industrial effluents. Increased interest in the use of chemical fertilizers (Urea and NPK) in aquaculture and agriculture in general, necessitated an investigation on the toxic effects these fertilizers on aquatic organisms. Thus, the aim of the present study was to evaluate the toxic effects of NPK (15:15:15) and Urea fertilizers on important cultivable species *Heterobranchus bidorsalis* fingerlings.

Description of test organism

The species, *H. bidorsalis* belongs to the family *Clariidae* and is one of the most widely cultured catfish in Nigeria.[10] It has ability to grow on a wide range of artificial and natural foods, with good feed conversion efficiency. *H. bidorsalis* has capacity to grow on adverse conditions like in low dissolve oxygen or hard water conditions. They have dark to brownish olive (sometimes with blotches) with flattened head, and strongly granulated as well as depressed body with four pairs of barbels, which are usually grey and white at their bases, with wide mouth and upper lip reddish.[11] *H. bidorsalis* has smooth and scale-less skin as well as accessory breathing organs on the head which help these fishes to survive out from the water for long time. Like Clarias, they can walk off from the pond if the conditions are unfavorable.[11-13] This fish species have high market value, rapid growth, and are propagated in Africa (mainly, South Africa and Nigeria), parts of Europe and Asia.[14,15] They are most commonly used as experimental fish.[16-18]

Materials and methods

Source of experimental fish

Five hundred fingerlings of *H. bidorsalis* were obtained from Premier Fisheries Limited, Akai Ubium, Nsit Ubium Local Government Area, Akwa Ibom State, Nigeria. The mean body weight (g) and total length (cm) of the species were 2.04 ± 0.35 and 6.98 ± 0.29 respectively.

Acclimatization

The fish were acclimatized for 14 days, in groups of fifty fish per plastic container in thirty litres of dechlorinated tap water. The containers were aerated during this period, and water was renewed daily to discard fecal 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, respectively. During this period, dead and abnormal individuals were immediately removed. Mortality recorded during the acclimation period was less than 2%.[19,20] It was from the acclimated population that healthy individuals used as test fish in this study were carefully selected.

Preparation of test media and exploratory test

To obtain the ranges of concentrations as used in the experiment, five fishes were selected and each were exposed to four litres dechlorinated tap water containing different weights of the fertilizers and 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.[21]

Experimental procedures

Exposure concentrations of NPK and Urea fertilizers were: 0.00g/l, 2.50g/l, 3.13g/l, 3.75g/l, 4.38g/l, 5.00g/l, 5.63g/l, 6.25g/l and 0.00g/l, 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 upto 8 litres mark for each treatment. The different concentrations were prepared by dissolving directly different weights of the fertilizers in the dechlorinated tap water.[20] 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 prior to the experiment and during the ninety-six hours exposure period. Test solution from each tank was drained out completely every morning and the fish removed carefully with a scoop net and kept in a thirty litre 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 96 hours intervals during the experimental period.[20,21]

Temperature

The temperature was monitored with thermometer. The thermometer was inserted into the container and the corresponding readings were taken and recorded.

Dissolved oxygen

The dissolved oxygen content was assessed with a Dissolved Oxygen Meter.

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 stopper 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.[20,21]

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 solochrome 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.[20,21]

Data collection

Water quality parameters were determined at fixed intervals of 24, 46, 72 and 96 hours respectively. Mortality of the fish species in each tank was observed and recorded at fixed intervals of 24, 48, 72 and 96 hours, respectively. Dead fish were removed immediately from the test media, which it helped in preventing pollution in 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, and restlessness, jumping out of the tank and gasping for air.

Data analysis

Each set of results obtained from these experiments was analyzed at 5% probability level, and the T-test was used to test for significant difference ($P<0.05$) between the two treatments.[23-26] 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 NPK and Urea fertilizers were determined by Probit Analysis using Statistical Package for the Social Sciences (SPSS) Data Editor version 10.0.[23,24,27] Safe concentrations 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.[22-24]

Results

Physico-chemical parameters

The result of the physico-chemical characteristics of the experimental media (water along with various fertilizers concentration) for both NPK and Urea fertilizers showed that there was a significant reduction in the mean values of dissolved oxygen. Conversely, alkalinity and total hardness values increased as both fertilizer concentrations were increased, compared to their control groups ($P<0.05$). However, there was no significant difference between the various mean values of temperature and pH ($P>0.05$), (Tables 1 & 2).

General behavioural changes

Behavioural changes occurred in the fish treated with NPK and Urea fertilizers at different concentrations. The abnormal behaviours such as restlessness, gulping of air and erratic swimming before death were observed in fish exposed to concentrations of NPK fertilizers. Similarly, abnormal behavior was reported by the fish exposed to various concentrations of Urea fertilizer but it is slightly differed as compared to those which were exposed by NPK fertilizers. In Urea 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 fish to Urea fertilizer concentrations before death were uncoordinated swimming, frequent attempts at jumping out of the tank and quietness.

These behavioural changes showed by the fish in response to the effect of toxicants and were more pronounced in tanks containing higher concentrations of fertilizers, but decreased with increase in time of exposure because of the gradual reduction in the concentrations of fertilizers. There were no obvious changes in fish behaviour in the lower concentrations less than 3.13g/l (NPK) and 10.00g/l (Urea) for the first 24 hours of exposure. However, fish in the control groups for NPK and Urea fertilizers did not exhibit any abnormal behaviour.

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Table 1 Physicochemical characteristics of water exposed by NPK fertilizer and *H. bidorsalis* fingerlings during 96 hours exposure

Fertilizer concentration (g/l)								
Parameters	0	2.5	3.13	3.75	4.38	5	5.63	6.25
(a) Temperature(0C)	27.56±0.41	27.38±0.16	27.47±0.34	27.39±0.34	27.36±0.29	27.38±0.29	27.31±0.33	27.42±0.35
(b) Dissolved Oxygen (mg/l)	7.00±0.006	6.46±0.39	5.16±0.14	4.27±0.37	3.08±0.11	2.26±0.38	2.24±0.34	2.05±0.08
(c) Total hardness	28.31±0.37	31.08±0.75	31.58±0.50	32.57±0.08	33.51±0.35	34.42±0.42	35.88±0.73	36.34±0.79
(d) pH	6.06±0.13	6.35±0.13	6.19±0.12	6.15±0.12	6.17±0.15	6.13±0.12	6.15±0.12	6.21±0.16
(e) Alkalinity (mg/l)	30.6±0.24	35.45±0.49	40.72±0.57	45.53±0.39	53.63±0.21	57.66±0.70	61.29±0.92	68.79±1.11

Value given in tables are Mean of 3 replicates per treatment level, ± SD (n = 40)

Table 2 Physicochemical characteristics of water exposed by Urea fertilizer and *H. bidorsalis* fingerlings during 96 hours exposure

Fertilizer concentration (g/l)								
Parameters	0	8.57	10	11.25	12.5	13.75	15	16.25
(a) Temperature (0C)	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
(b) 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
(c) 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
(d) 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
(e) 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

Value given in tables are Mean of 3 replicates per treatment level, ± SD (n = 40).

Mortality of fingerlings exposed to NPK and urea fertilizers

The mean mortality (Figures 1 & 2) of the fingerlings exposed to various concentrations of NPK and Urea fertilizers indicated that, the different concentrations caused significant (P<0.05) but variable death rate in the exposed fish. Mortality rate was directly correlated with the concentration gradients t, at highest concentrations highest death rates was reported and it was significantly higher than the others concentrations. No mortality was recorded in the control groups of fish for both fertilizers. The lethal effects of NPK and Urea fertilizers for 24, 48, 72 and 96 hours exposures were expressed by LC_{50} value and their associated 95% confidence limits (Tables 3 & 4). Results of the study showed that the range of LC_{50} value for 24 hours exposure was between the 7.48g/l and 26.36g/l, while for the 96 hour this value was 1.09g/l and 17.84g/l respectively for NPK and Urea fertilizers. A safe concentration at LC_{50} was 0.75g/l & 2.64g/l for 24hours exposures while 0.11g/l & 1.78g/l for 96hrs exposure; for NPK and Urea, respectively. The LC_{50} values were decreased with increase the time of exposure from 24 to 96 hours. At 24 hours, the LC_{50} values were greater than the 96 hours LC_{50} values.

Table 3 LC_{50} values and associated confidence limits along with safe concentrations of NPK fertilizers to *H. bidorsalis* for 24, 48, 72 and 96 hours exposure

		Confidence Limits (g/l)		
Time (hrs)	LC_{50} (g/l)	Lower	Upper	Safe Conc. (g/l)
24	7.48	9.64	15.86	0.75
48	5.65	7.23	9.65	0.57
72	3.88	2.96	4.79	0.39
96	1.09	0.29	3.86	0.11

Table 4 LC_{50} values and associated confidence limits along with safe concentrations of NPK fertilizers to *H. bidorsalis* for 24, 48, 72 and 96 hours exposure

		Confidence Limits (g/l)		
Time (hrs)	LC_{50}(g/l)	Lower	Upper	Safe Conc. (g/l)
24	26.36	17.65	21.84	2.64
48	21.2	15.44	18.57	2.12
72	19.33	13.23	15.46	1.53
96	17.84	11.88	13.67	1.78

Differential effects of NPK and urea fertilizer concentrations on *H. bidorsalis* fingerlings

Mean mortality of the species at the various concentrations of NPK and Urea fertilizers were significantly different. Mortality rate was significantly influenced by interactions between concentration and time of exposure. In the NPK fertilizer, ANOVA showed that the treatment differences were significant (P<0.05) as there was significant difference (P<0.05) among treatment concentrations on the mortality of the fingerlings. In Urea, ANOVA showed that the treatment differences were not significant (P>0.05) and that there was no significant difference (P>0.05) among treatment concentrations on the mortality of fingerlings. ANOVA also showed that treatment effects were not equal among all the experimental units (NPK), while treatment effects were equal among all experimental units (Urea). However, the student's t-test further showed that there was no significant difference (P>0.05) between the effects of NPK and Urea fertilizers on mortality of *H. bidorsalis*.

Discussion

Water quality parameters

The water quality parameters of the experimental media for NPK and urea fertilizers showed a significant reduction in the mean values of dissolved oxygen content, while conversely, alkalinity, and total hardness values were increased as the fertilizer concentrations increased (P < 0.05), compared to those of their control groups. 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.[28,29] Results of the study are in agreement with the work of Ofojekwu, et al.,[30]

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Ofojekwu et al.[31] and Ufodike, et al.[32] 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 ($P>0.05$).

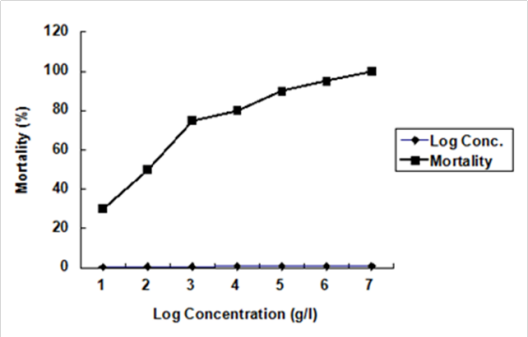

Figure 1 Mortality rate of *H. bidorsalis* exposed to different concentrations of NPK fertilizers for 96 hours.

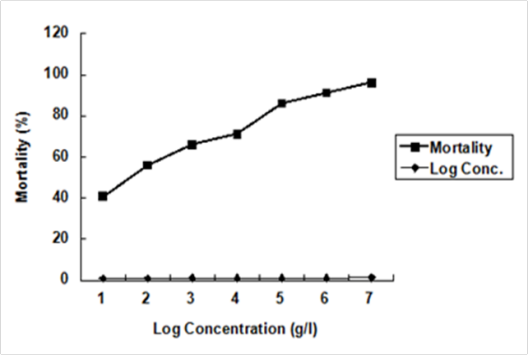

Figure 2 Mortality rate of *H. bidorsalis* exposed to different concentrations of Urea fertilizers for 96 hours.

The affected parameters may have contributed significantly to observed behaviors 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. The recorded mean values for temperature and pH in both NPK and Urea fertilizer test media were within the tolerance range for this tropical species and may not have contributed to the toxicity of the fertilizers on the behaviours and mortality of the exposed fishes.

General behavioural responses and lethal concentrations of the toxicants to the exposed fish species

Changed behavioural responses of fish have been observed to toxicants and on various time duration, probably it is due to the effect of chemicals, their concentrations, species, size and specific environmental conditions.[33] 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.[32,34-40]

Besch[41] 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), loss of equilibrium, followed by lethal (death) phase when opercular movement and responses to tactile stimuli cease completely. Despite 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.[34]

Results obtained from this study reveals that for 96 hour LC_{50} for *H. bidorsalis* fingerlings exposed to NPK fertilizer was 1.09g/l, with lower and upper confidence limits of 0.29 - 3.86g/l respectively. This value is at variance with the values reported by Ufodike, et al.[32] when they exposed C. gariepinus to acute concentrations of some inorganic fertilizers. They reported that, the 96hrs LC_{50} for calcium hydroxide ($Ca(OH)_2$), NPK, sodium phosphate ($NaPO_4.12H_2O$) and sodium nitrate ($NaNO_3$) fertilizers were 33.9mg/l, 83.6mg/l, 748mg/l and 1258.9mg/l respectively. But results of present study are closely agrees with the values by Ofojekwu, et al.[30] when Tilapia zilli fingerlings were exposed to acute concentrations of NPK (15.15.15) fertilizer. These differences between these two findings maybe because of the difference in the fish species and levels of concentration used.[42] At the concentrations used in this investigation, the fertilizers led to significant reduction in the dissolved oxygen and an increase in alkalinity and total hardness of the water. 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 fertilizers. This result is in line with the report of Warren[43] 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.

In Urea fertilizer, the results obtained from this research revealed that the 96 hours exposure has 17.84g/l LC_{50} values with lower and upper confidence limits of 11.32 and 14.88g/l respectively. LC_{50} values for urea in present study are in agreement with the findings of Ofojekwu et al.[31] when they exposed Tilapia zilli fingerlings to acute concentrations of Urea fertilizer (LC_{50} value 15.85g/l). The slight difference may be due to the different in species used, fertilizer concentrations and other environmental factors, as different fish species respond to the effect of a pollutant differently.[19] 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, et al.,[45] Avoaja, eta l.,[46] Omoregie,[47] Oti,[48] Adakole,[49] Ayuba, et al.[50] when they exposed fish fingerlings to acute concentrations of different toxicants.

Results of the study further revealed that percentage mortality increases with increase in concentration of the toxicant, and in these findings are in agreement with the earlier reports documented by Omoregie, et al.[45] Avoaja, eta l.,[46] Omoregie,[47] Oti,[48] Ayuba, et al.[50] This study also reveals that concentrations above 10.00g/l (Urea) and 3.13g/l (NPK) were lethally threat to the test fish within 96 hours as 55% and 50% mortality was recorded respectively. Differences in mortality of *H. bidorsalis*[51] fingerlings may be due to the difference in chemical grade or composition of the two treatments and their different levels of concentrations. ANOVA showed that there was significant difference among treatment and concentration levels on mortality of the test fish for NPK fertilizer, while there was no significance difference among treatment and concentration levels on mortality of the test fish for Urea fertilizer. This further signifies that the treatment effects were not equal among the different experimental units ($P<0.05$) for NPK while treatment effects were equal among experimental units ($P>0.05$) for urea fertilizer.

The mortality rate of the test fish at 3.75g/l (NPK) and 11.25g/l (Urea) 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 both NPK and urea fertilizers are harmful to H.bidorsalis fingerlings at acute concentrations.[52] This confirms the findings of Nwani, et al.,[53] Ofojekwu, et al.,[30] Ofojekwu et al.[31] 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 both NPK and urea fertilizers; they are rated highly toxic and toxic r to *H. bidorsalis* fingerlings.[54] 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.

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.[55] Kormakik, et al.,[56] Kuma, et al.[57] reported that increased utilization of protein when fish is under the influence of a pollutant leads to stress. 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.[30,31,47,53,58,59]

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.[60] 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 NPK and Urea fertilizers are harmful to *H. bidorsalis* fingerlings.

It is thus recommended that the application of these fertilizers in aquatic ecosystems either in ponds, irrigations or farms should be carefully controlled or monitored, such that concentrations that are lethal to aquatic life could be avoided. There is also a great need to provide further baseline data on urea and NPK fertilizers. Such studies should be concerned with providing information on research such as, the effects of sub-lethal concentrations of Urea and NPK fertilizers on the haematology, serum/plasma enzymes, metabolites, hormones and tissues of *H. bidorsalis*.

Acknowledgments

None.

Conflicts of interest

None.

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