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EFFECT OF SINGLE SUPERPHOSPHATE FERTILIZER ON SURVIVAL AND RESPIRATORY DYNAMICS OF NILE TILAPIA, *OREOCHROMIS NILOTICUS* (ACTINOPTERYGII: PERCIFORMES: CICHLIDAE)

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Background. Increasing global usage of inorganic fertilizers, including phosphate-based fertilizers has its negative consequences on the aquatic environment. Effects of single superphosphate fertilizer (SPF) remain unknown, particularly its influence on the respiratory dynamics of fish under continuous exposure. We investigated the effects of single SPF on the survival and respiratory dynamics of Nile tilapia, *Oreochromis niloticus*, under laboratory conditions.

Materials and Methods. Nile tilapia fingerlings (of mixed sex) (5.40 ± 0.03 g) were exposed to various concentrations of the fertilizer in five treatment regimes (in triplicate): 0.88, 1.75, 3.50, 7.00, 14.00 g · L^{-1} (and 0.00 g · L^{-1} for control). Each replicate was carried out in a 30-L circular plastic tank based on 20 fingerlings. The study involved: the mortality estimation, the oxygen consumption, the histopathological effects on fish gills, and the activities of lactate dehydrogenase (LDH), alcohol dehydrogenase (ADH) in liver of fish exposed to sublethal concentrations (0.44, 0.22, 0.11, 0.06, and 0.03 g · L^{-1}) of single SPF for eight weeks under laboratory conditions.

Results. Acute concentrations of SPF had serious adverse effects on mortality, oxygen consumption and opercular ventilation rates of exposed fish. All variables showed a dose-dependency. A mean value of 96-h LC_{50} of the SPF to the test fish was calculated to be 3.76 g · L^{-1}. At various acute concentrations, oedema and hyperplasia of gill lamellae were observed in exposed fish. Exposure of the fish to sublethal concentrations of the SPF resulted in reduction in the levels of lactate dehydrogenase and alcohol dehydrogenase activities in liver.

Conclusion. Concentrations of SPF in natural water bodies are deleterious to aquatic fauna. With rapid global economic development and need for more food production, pollution from agricultural fertilizers remains a major threat to the aquatic ecosystem. Therefore it is ultimately important that a balance is struck between achieving economic excellence and environmental protection through good pollution management strategies.

Keywords: *Oreochromis niloticus*, single superphosphate, dose-dependency, toxicity, respiratory distress

INTRODUCTION

With greater involvement of numerous countries in green revolution, phosphate-based fertilizers along with other types of fertilizers are commonly used in agriculture, entering ponds and other bodies of water through runoff associated with agriculture, industrial and human wastes (Fenn et al. 2003, Holland et al. 2005, Burgett et al. 2007). The amount of these fertilizers entering aquatic ecosystems is likely to remain the same, or even increase in the future (Tilman et al. 2001, Palanivelu et al. 2005). Fertilizers in the water bodies stimulate growth of phytoplankton and water weeds, which in turn provide food for the fish, however, at certain concentrations of these fertilizers, algae and waterweeds grow wildly clogging the water ways and result in the depletion of dissolved oxygen present in the system. For these reasons, surviving fish species and other aquatic organisms encounter physiological problems associated with the depleted oxygen levels. Studies of effects of fertilizers on aquatic animals, including fish as noted by Palanivelu et al. (2005) are gen-

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erally concerned with survival investigations. Fish species in shallow water-logged areas surrounding agricultural lands have to face respiratory problems resulting from inputs of fertilizer into the water bodies.

Respiratory distress experienced by fish in polluted water bodies has been reported by several authors. Banerjee (2007) noted that congestion of blood capillaries, periodic lifting and sloughing of respiratory epithelia of the secondary lamellae and haemorrhages are the major main damages observed on fish gills in polluted water bodies. This author also noted the extensive fusion of secondary lamellae and hyperplasia of the respiratory epithelia due to uncontrolled regeneration are the major causes leading to asphyxiation, and eventually death of the fish if exposure is prolonged excessively.

The impact of ammonium chloride fertilizer on the physiology and biochemistry of a freshwater teleost, Mozambique tilapia, *Oreochromis mossambicus*, was reported by Baskaran and Palanichamy (1990) who noted that feeding energetic parameters and the rate of oxygen consumption of exposed *Oreochromis mossambicus* decreased, whereas the opercular beats increased with increasing concentrations of the fertilizer. Equally, protein content of tissue such as muscle, liver, gill, and intestine decreased as the feeding rate decreased (Rani et al. 1998). The inhibition of dehydrogenase in fish exposed to polluted water medium as reported by Svoboda et al. (2001) resulted in accumulation of metabolic intermediates and decreased the formation of acetyl-CoA leading to accumulation of lactate in liver which culminated to physiological stress and death of the fish.

Oxygen consumption rate and respiratory enzyme activity are commonly used in assessment of physiological stress, hence in this study, we have investigated the effects of varying sublethal concentrations of single superphosphate fertilizer on the survival and respiratory dynamics of Nile tilapia, *Oreochromis niloticus*, under laboratory conditions.

MATERIALS AND METHODS

This investigation was conducted at the University of Jos Fisheries Research Unit. Fingerlings (mixed sex) of the Nile tilapia, *Oreochromis niloticus*, mean weight ± SE (5.40 ± 0.03 g), were collected from Panyam Fish Farm, Nigeria. They were transported to University of Jos Fisheries Research Laboratory in well aerated oxygen bags. The fish were held in plastic tanks and acclimated to laboratory conditions for a period of two weeks. During the process of acclimation and the exposure period, fish were fed with laboratory-formulated feed.

In the first experiment, the oxygen consumption rate and survival were investigated; exposure period was for 24 and 96 h, respectively to lethal concentrations of the SPF of 14.00, 7.00, 3.50, 1.75, and 0.88 g · L^{-1}. While in the second experiment respiratory enzyme activities were investigated; sublethal concentrations used were 0.44, 0.22, 0.11, 0.06, and 0.03 g · L^{-1}. In the sublethal experiments, exposure lasted for a period of 8 weeks. The concentrations were obtained after preliminary investigations. The control was an experimental medium with no fertilizer in it (0.00 g · L^{-1}). For each set of experiments, eighteen circular plastic tanks of 30-L capacity were used as each concentration was in triplicates. De-chlorinated and well aerated municipal tap water was used throughout the exposure period. Aeration of the experimental tanks was achieved using laboratory aerators. Each tank was stocked with 20 fish and the fish were fed twice daily (0800 and 1600 h) *ad libitum* at 3% of their body weight. Faecal matter and remnant of unconsumed feed were siphoned out daily while the water in each tank was changed every four days. During the exposure period, each tank was aerated using laboratory aerators. The SPF used was obtained from the Plateau Agricultural Development Programme, Jos, Nigeria and composed of 12% P_2O_5.

Mortality of exposed fingerlings to lethal concentrations was monitored every 6 h and dead fish were immediately removed and examined for abnormal colouration of the gills and skin. During exposure, the opercular ventilation rate (OVR) per minute of exposed fish was estimated as described in Omoregie (2002) by visual observation of individual fish in the experimental tank. Oxygen consumption rate by fish was estimated both in the control and exposed fishes by placing individual fish in a simple airtight respiratory chamber (1-L capacity) containing the test medium for 24 h. The concentration of dissolved oxygen in the medium before introduction of test fish and at hourly intervals thereafter was estimated by the 'Winklers' volumetric method (Anonymous 1995) and the results were expressed as oxygen consumed per test fish for 1 h as mg · h^{-1} per fish.

For the estimation of lactate dehydrogenase (LDH) and alcohol dehydrogenase (ADH), the experimental period was for eight weeks. Two experimental fish were sacrificed from each test medium on weekly basis and the liver removed and quickly ground in a chilled mortar placed in a round plastic container containing ice blocks. Thereafter the activity of LDH and ADH in liver of experimental fish was assessed by method described by Bergmeryer (1967).

Histopathological examination of the gills after exposure period was done using methods described by Buck and Wallington (1972).

The OVR results were analyzed with repeated measure ANOVA with time as within-subjects factor ($P \leq 0.05$). Mortality data were analysed using Toxcalc^{TM} (Tidepool Scientific Software, McKinleyville, California). Upper and lower confidence limits of 96 h LC_{50} was estimated by the criterion of non-overlapping 95% confidence intervals (CI) using the method for acute toxicity tests as recommended by UNEP (Anonymous 1989). The statistical significance of differences between LDH and ADH values was determined by Student's *t*-test.

RESULTS

No mortality was observed in control group as well as in lowest SPF concentration 0.88 g · L^{-1}, however, mortality gradually increased with increasing concentrations of SPF in water, showing a dose-response relation (Fig. 1). 100% mortality was observed in the groups of fish exposed to 14.00 g · L^{-1}. The first 24-h of exposure was critical for the fish's survival, as most of the deaths occurred during this period. No fish survived longer than 48 h during exposure to 3.50 g · L^{-1} concentration and above. A weak r^2 value (0.362) was observed from dose-response relation plot. The mean value of 96-h LC_{50} of the fertilizer to the test fish was calculated to be 3.76 g · L^{-1} with lower and upper confidence limits of 2.99 and 4.01 g · L^{-1} respectively.

During the exposure period of *Oreochromis niloticus* to varying lethal concentrations of SPF for 24 h, the amount of oxygen consumed by test fish decreased with increases in concentrations (Table 1). The oxygen consumed by the groups of fish exposed to 14.00, 7.00, 3.85, and 1.75 g · L^{-1} was significantly lower than the amount consumed by the control groups. All groups of the test fish exposed to varying lethal concentrations of SPF for 96 h exhibited significantly lower OVR compared to the values obtained for the control groups during exposure period (Table 2). Repeated measure ANOVA analysis indicated

Table 1

Oxygen consumption rate* in *Oreochromis niloticus* exposed to varying concentrations of SPF for 24 h

Concentration [g· L^{-1}]	Oxygen consumption rate per fish [mg· h^{-1}]
14.00	3.00 ± 0.15 ($P < 0.05$)
7.00	3.22 ± 0.09 ($P < 0.05$)
3.5	3.85 ± 0.12 ($P < 0.05$)
1.75	4.10 ± 0.11 ($P < 0.05$)
0.88	5.08 ± 0.18
0.00 (Control)	5.44 ± 0.04

*(mean ± SD) each value represents the average of four individual estimations per each experimental tank.

Table 2

Opercular ventilation rate per minute* in *Oreochromis niloticus* exposed to varying concentrations of SPF for 96 h

Concentration [g· L^{-1}]	Exposure period [h]				
	start (0)	24	48	72	96
14.00	130 ± 3.76^a	100 ± 2.11^b	68 ± 2.91^c		
7.00	130 ± 3.01^a	110 ± 4.71^b	95 ± 2.11^c	90 ± 5.11^c	60 ± 1.00^d
3.50	135 ± 1.09^a	130 ± 1.98^a	120 ± 5.01^b	100 ± 1.01^c	80 ± 1.05^d
1.75	130 ± 2.98^a	137 ± 1.00^a	130 ± 6.11^a	120 ± 0.09^c	100 ± 1.26^d
0.88	140 ± 0.94^a	144 ± 1.01^a	138 ± 3.23^a	120 ± 0.12^b	100 ± 0.00^c
0.00	150 ± 0.02^a	150 ± 0.05^a	145 ± 0.95^a	140 ± 0.11^a	150 ± 0.01^a

*(mean ± SD) each value represents the average of four individual estimations per each experimental tank at each time point; Values within same row with different superscripts are significantly different ($P < 0.05$).

Table 3

Summary of repeated measures ANOVA results for the opercular ventilation movements of *Oreochromis niloticus* (time as within subject factor)

Effect	SS	dF	MS	*F*	*P*
SPF	67.40	5	13.48	714.14	<0.01
Error	227	12	19		
TIME	36.39	4	9.10	771.33	<0.01
TIME*SPF	25.69	20	1.28	108.92	<0.01
Error	556	48	12		

that OVR decreased with the SPF concentration and time elapsed since the experiment began (Table 3 and Fig. 2).

The results of the rate of change in activity levels of LDH and ADH in the liver of *Oreochromis niloticus* exposed to the various concentrations of SPF are represented graphically in Figs. 3 and 4, respectively. Activity levels of these enzymes decreased as exposure period increased, and the decrease was directly proportional to the increase in SPF concentrations. Statistical analysis showed a significance difference ($P < 0.05$) between the values recorded in the exposed groups of fish compared to recorded values for the control groups.

Histopathological examination of the gill section of test fish that survived the exposure period revealed degeneration of the gill filaments and various pathological disruptions (Figs. 5–7). The most commonly notice pathological damage on the gill hemibranch of exposed fish included oedema and the thickening of the secondary lamellae base as a result of hyperplasia due to proliferation of cells within the lamellae.

DISCUSSION

In our study fingerlings of Nile tilapia, *Oreochromis niloticus*, exposed to various concentrations of single

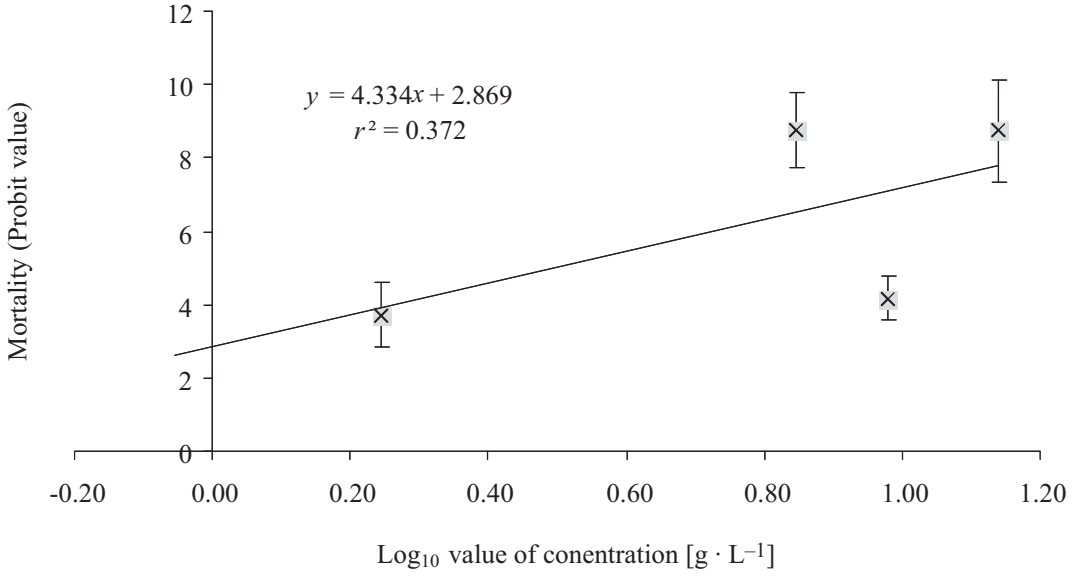

Fig. 1. Linear relation between $\log_{10}$ value of concentration of SPF and probit value of mortality of *Oreochromis niloticus* as a function of 96-h exposure under laboratory conditions

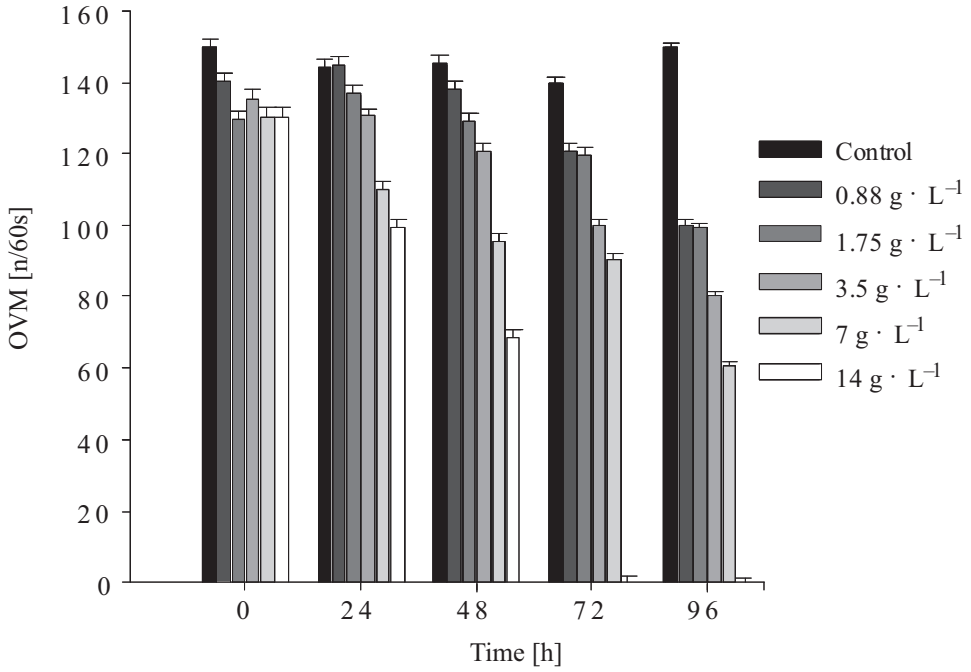

Fig. 2. Changes in opercular ventilation rate of *Oreochromis niloticus* (means; 95% confidence limits) in various SPF concentrations over a 96-h exposure period

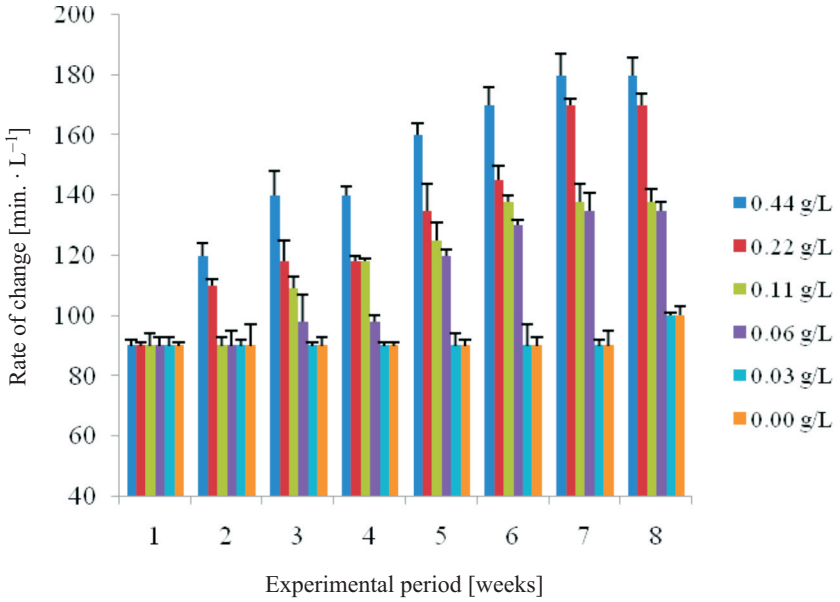

Fig. 3*. The rate of change in the level of lactic dehydrogenase of *Oreochromis niloticus* exposed to various sublethal concentrations of SPF fertilizer for 8 weeks

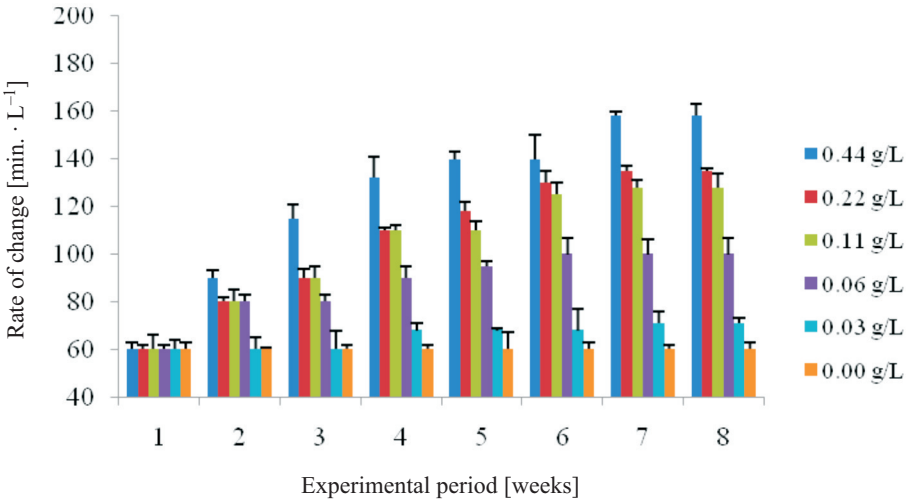

Fig. 4*. The rate of change in the level of alcohol dehydrogenase of *Oreochromis niloticus* exposed to various sublethal concentrations of SPF fertilizer for 8 weeks

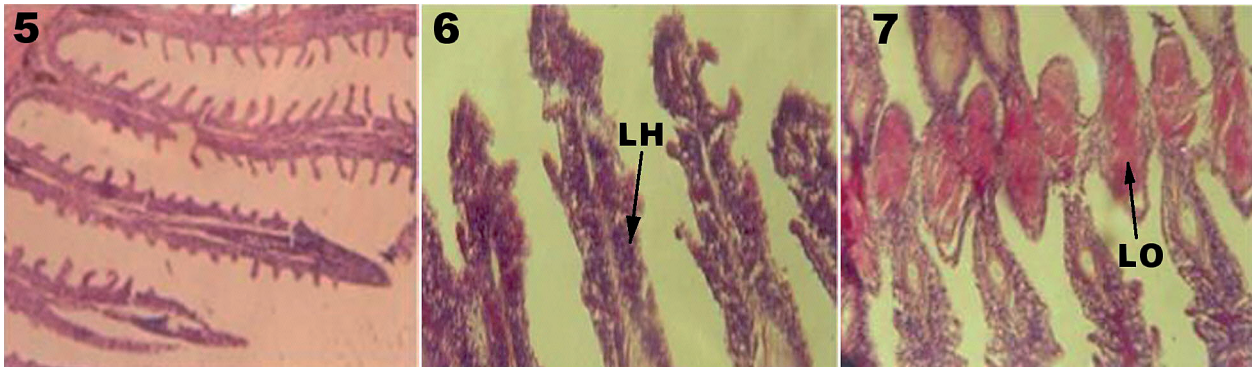

Fig. 5–7*. Histopathological section of the gill (×100) of *Oreochromis niloticus* exposed to lethal concentrations of SPF under laboratory conditions for 96 h; **Fig. 5.** Exposure to 1.7 [g · L^{-1}]; **Figs. 6, 7.** exposure to 14.00 [g · L^{-1}] (LH = lamellar hyperplasia, LO = lamellar oedema)

*The authors assume sole responsibility for the quality of photographs

superphosphate fertilizer displayed high sensitivity to the fertilizer under laboratory conditions. The mean value of 96-h LC_{50} of SPF to the test fish was 3.76 g · L^{-1} with lower and upper confidence limits of 2.99 and 4.01 g · L^{-1}, respectively. The observed mortality increased with increasing concentrations of SPF in water, showing a dose-response relation. Exposure to SPF at concentrations above 1.75 g· L^{-1} revealed a reduction of the fright reaction of the fish and, in general, test fish remained motionless. This observed flight reaction have been reported by several other researchers when they exposed different species of fish to different toxicants, examples include: Wedemeyer and Yasutake (1974), Okwuosa and Omoregie (1995), and Svecevičius (2007).

Different LC_{50} values have been reported for different fish species exposed to various types of toxicants. In assessing contaminant sensitivity to endangered and threatened aquatic species, Dwyer et al. (2005) documented that outcome of results could be affected by the test procedures, hence the differences in documented results even for same species exposed to same toxicant. Variation in LC_{50} may also be due to the size, age, temperature, hardness, pH, oxygen level, etc. as reported by Palanivelu et al. (2005). The effect of age and size on toxicity of toxicants on aquatic animals as documented by Rand and Petrocelli (1985) is that small individuals of fish have a greater gill surface area for absorption of toxicant per amount of body mass.

Changes in oxygen consumption rate have been measured as a response to toxicant stress. Therefore, testing of oxygen consumption rate is one of the important methods of bioassay. In this investigation, exposed fish consumed significantly less oxygen compared to the control. Similarly, opercular ventilation of exposed fish decreased with increase in concentrations as well as increase in exposure period indicating reduced respiration rate. Though hyperventilation is often associated with toxic exposure, the hypoventilation response reported here is supported by the investigations of Wedemeyer (1971) when rainbow trout (*Oncorhynchus mykiss*) and Coho salmon (*Oncorhynchus kisutch*) were exposed to formalin. Such response they had attributed to exhaustion as exposure lengthens.

Respiratory irregularities noticed in exposed fish could have been caused by mucous precipitation on the gill epithelia in response to the toxicant which resulted in abnormal behaviour as earlier documented by Banerjee (2007). In addition, reduction in the activity levels of respiratory enzymes such as LDH and ADH and the depletion in the level of consumed oxygen could all have contributed to the observed behaviour and eventual mortality. This phenomenon was supported by the investigations of Lusková et al. (2002) and Das et al. (2004). Ceron et al. (1997) had earlier reported a reduction in lactate concentration when fish were exposed to Diazinon at acute concentrations. More so, the decrease in LDH activity indicated decrease metabolic activities of the exposed fish. The decrease in oxygen consumption rate observed could have implication on the subsequent reduced LDH activity. The inhibition of these enzymes would result in the accumulation of metabolic intermediates in the liver therefore causing physiological stress in fish, which eventually lead to mortality if exposure is prolonged as suggested by Das et al. (2004).

The sensitivity of *Oreochromis niloticus* exposed to SPF resulted in the degeneration of the gill lamellae, which according to Omoregie and Ufodike (1991), Banerjee and Chandra (2005), and Banerjee (2007) would lead to branchial malfunctioning. Results from this investigation have revealed that degenerated gill lamellae have far-reaching consequences for oxygen uptake by the fish and therefore homeostasis in the fish system. Even at low levels of SPF in natural water and aquaculture systems, the changes caused in the respiratory parameters may compromise the fish's performance and represent a serious problem. Ferreira da Costa et al. 2004 documented problems such as the low buffer capacity of ion-poor water associated with daily fluctuations in temperature and dissolved oxygen. Earlier, Peuranen et al. (1994) had noted that damage to gill lamellae could also lead to negative ion balance and changes in the haematocrit and mean cellular haemoglobin values of the blood causing disturbed gill respiration due to dilation of blood channels and severe congestion with greatly increased erythrocyte density.

The main aim of toxicological research is to assess the risk of environmental toxicants on the ecosystem in order to provide the framework to protect and maintain them. In general, fish are extremely sensitive to pollution, hence as noted by Dwyer et al. (2005) and Omoregie and Okunsebor (2005), they are good indicators of onset of pollution in the aquatic environment. With rapid economic development in several countries and the global need for more food production both for human consumption and biofuel production, pollution from agricultural fertilizers remains a major threat to the aquatic ecosystem. The value of fishery resources to global economy cannot be overemphasized. It is, therefore, important that a balance is struck between achieving economic excellence and environmental protection through good pollution management strategies. Without such a balance, the world risk losing the very resources that would ensure that we achieve a sustainable future. Apparently SPF, a widely used fertilizer in the tropics is harmful to fish as reported in research and so may reduce productivity and possibly lead to physiological dysfunction under prolonged exposure.

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