OXIDATIVE STRESS RESPONSES IN *Oreochromis niloticus* (LINNAEUS, 1758) EXPOSED TO AQUEOUS SOLUTIONS OF TWO COMMON POND FERTILIZERS IN CULTURE MEDIA

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ABSTRACT
A bioassay was conducted to determine oxidative stress responses in *Oreochromis niloticus* exposed to aqueous solutions of NPK 15-15-15 and layer manure (LM) fertilizers, simulating concentrations resulting from the recommended application rates (RARs) of 120kg/ha/2weeks and 224kg ha/week respectively, on a 1ha pond of 0.8m depth. These RARs were serially diluted to yield 0.0mg/l, 15mg/l of NPK 15-15-15 and 28mg/l LM solutions, designated as control, treatments 1 and 2, respectively. Experimental units were rearing troughs of dimensions 1m×1m×0.5m filled to the 250-litre mark. Post-yolk sac larvae of *O. niloticus* were raised in the fertilizer solutions for a period of six months at a stocking density of 100 larvae/rearing trough. The levels of reactive oxygen species (ROS), superoxide dismutase (SOD), and total protein (TP) of *O. niloticus* were determined before exposure (initial samples), at 4, 84 and 168 days of exposure, following standard procedures. Temperature, dissolved oxygen, pH, total water hardness, nitrate and phosphate were monitored. Levels of TP, SOD, and ROS in initial and control were significantly different ($p<0.05$) from levels in fish challenged with N-P-K 15-15-15, and LM at 4, 84, and 168 days of exposure. TP had significant negative correlations with ROS (-0.985, $p<0.0001$) and SOD (-0.999, $p<0.0001$). ROS and SOD had significant correlations ($r = 0.988$, $p<0.0001$). All the oxidative stress indices and the physicochemical variables, had high regression coefficients with fertilizer solutions ($r=0.724 – 1.000$, $p<0.0001$), except pH and PO_4. Since fish are often used as bio-indicators in aquatic toxicity tests, the findings suggest that fertilizer runoffs could be causing widespread damage to inland water ecosystems, potentially impacting their productivity.

Keywords: Layer manure, NPK 15-15-15, oxidative stress, reactive oxygen species, superoxide dismutase, total protein,

INTRODUCTION

The escalating global demand for food production, driven by population growth, urbanization, and shifting consumption patterns, has led to the intensification of agricultural and aquacultural practices. Aquaculture, a vital component of global food security, plays a significant role in meeting this demand, providing a substantial source of protein for millions of people worldwide (FAO, 2024). However, the intensification of aquacultural practices often relies heavily on the use of fertilizers to enhance primary productivity, and increase fish production (Ofor and Oke, 2018; Senthilkumar and Sivasubramanian, 2018; Oke *et al.,* 2025). Fertilization of a fish pond increases the production of beneficial phytoplankton, free-floating algae that act as the basis of the food chain in an aquatic ecosystem. The primary nutrients; nitrogen, phosphorus and potassium (NPK) in fertilizers are essential for algal growth (Ofor and Oke, 2018; Oke *et al.,* 2025).

NPK 15-15-15 and layer manure (LM) are two commonly used fertilizers in aquaculture, each with distinct properties and effects on aquatic ecosystems. NPK fertilizers provide essential nutrients for phytoplankton growth, which serves as a food source for fish and other aquatic organisms (Singh and Craswell, 2021). LM, rich in nutrients, can stimulate the growth of aquatic plants and animals, enhancing overall productivity and biodiversity (Ofor *et al.,* 2015).

Fertilizers also contain mineral contaminants like heavy metals (Gillick *et al.,* 2007; Mustafa *et al.,* 2024) which can impact fish reproduction (Musa, 2010; USEPA, 2013; Ofor *et al.,* 2015; Oke and Ofor, 2015; Mansour *et al.,* 2018). Heavy metals in LM originate mainly from water and feed (Ghosh *et al.,* 2012; USEPA, 2013). In locally manufactured NPK 15-15-15, heavy metals are also present (Chibueze *et al.,* 2016). They originate from a variety of sources including parent rocks materials (Kpomblekou and Tabatabai, 1994), blending materials, impurities, and additives (Ofor and Oke, 2018). Fertilizers used in agricultural lands, run offs into water bodies, posing a major threat to the aquatic ecosystem (Manik and Biswapati, 2008; Omoregie *et al.,* 2009; Divya and Belagali, 2012). The use of these fertilizers can have unintended environmental consequences, including water pollution, eutrophication, and decreased water quality (Mustafa *et al.,* 2024). Excess nutrients from fertilizers can lead to algal blooms, deplete dissolved oxygen levels, and alter the natural balance of aquatic ecosystems. This threat is expected to increase as global consumption of NPK fertilizers is forecast to grow annually by 1.5, 2.2 and 2.4%, respectively from 2019 to 2022 (FAO, 2019).

Aquatic organisms, particularly fish, are sensitive to changes in their environment and can exhibit stress responses on exposure to toxic substances, including changes in physiological, biochemical, and behavioral parameters (Ofor *et al.,* 2025; Oke *et al.,* 2025).

Attention on toxicity of fertilizers to fish has focused on relatively advanced life stages (fingerlings and juvenile) while interest on environmental impact of recommended field application rates of fertilizers has focused on toxicity in the terrestrial environment (Ofor and Oke, 2018). There is paucity of information on the impact of recommended field application rates of NPK 15-15-15 and LM on oxidative stress responses in *Oreochromis niloticus*.

O. niloticus, a widely cultured fish species, is potentially susceptible to the toxic effects of NPK 15-15-15 and LM. Exposure to these fertilizers can lead to oxidative stress, DNA damage, and altered gene expression, ultimately affecting fish growth, survival, and reproduction (Ofor *et al.,* 2025; Oke *et al.,* 2025). Understanding the oxidative stress responses of *O. niloticus* to these fertilizers is crucial for developing sustainable aquacultural practices that minimize harm to aquatic ecosystems and promote eco-friendly practices in the industry.

This study aims to investigate the oxidative stress responses of *O. niloticus* exposed to aqueous solutions of NPK 15-15-15 and LM in culture media and also, to determine the water quality parameters of the culture media. By examining the effects of these fertilizers on *O. niloticus*, this study provides insights into the potential environmental impacts of these commonly used fertilizers, and contributes to the development of more sustainable aquacultural practices. The findings of this study will inform strategies for minimizing the environmental footprint of aquaculture, promoting eco-friendly practices, and ensuring the long-term sustainability of aquatic ecosystems.

MATERIALS AND METHODS

Experimental Location

The study was carried out in the Grow-out section of the Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture, Umudike (MOUAU) Abia State, fish farm. Umudike, lies between latitude 5^{0}29'41.34″N and longitude 7^{0}32'25.73″E. The average temperature of the area is 26°C, maximum being 32°C and the minimum 22°C. Umudike is 122m (400ft) above sea level. It has an average rainfall (21698.8mm) which is obtained within 148-155 days. The relative humidity is 50-95%. The area is within the humid – rainforest zone characterized by long duration (7-12 months) of rainfall and short period of dry season (NRCRI, 2013).

Experimental Procedure

The study was conducted in two concurrent experiments. Experiment 1 was NPK 15-15-15, an inorganic (compound) fertilizer while experiment 2 was layer manure, an organic fertilizer. These two fertilizers were targeted because they are the two most commonly used in fertilization in both aquaculture and terrestrial agriculture. They were used in bio-assay to determine the level of reactive oxygen species (ROS), superoxide dismutase (SOD), and total protein (TP) of *O. niloticus,* challenged with both fertilizers. These indices were used as biomarkers, to determine the impacts of the fertilizers as environmental stressors on the fish.

Procurement and Acclimation of Brood Stock

To minimize environmental effect, thirty (30) farm-raised sexually mature *O. niloticus* were used. They were procured from a reputable fish farm and transported to the Grow-out Section of the MOUAU fish farm for acclimation. They were maintained on artificial commercial feed (35% crude protein) and were fed to satiation/day, undergoing acclimation in four (1m x 1m x 1m) concrete tanks with borehole water for two (2) weeks.

Pairing of Brood Stock

Semi-natural spawning method was adopted. Following acclimation, the male and female *O. niloticus* were paired at a ratio 1:3 (El-Sayed, 2020) in concrete tanks (1m x 1m x 1m) with 5 to 10 cm deep soft sandy bottom with water height of 30-60cm (FAO, 1990). Being maternal mouth brooders, *O. niloticus* lay eggs on a substrate, which are then fertilized. The female then incubates the eggs in her mouth until hatching.

Collection of Larvae

The experiment was conducted under ambient water quality parameters. Following successful hatching of eggs and absorption of yolk sac, post-yolk sac larvae were collected, 20 days after hatching (DAH), from the breeding tanks. Initial length (cm) and weight (g) of the larvae were determined with a digital caliper meter-scale and a digital sensitive weighing balance (M-METLAR Analytical Balance M311L 0.01g) respectively. The initial mean length of the larvae was 2.25±0.19cm while the initial mean weight was 0.44±0.06g.

Test Concentration Media

The NPK 15-15-15 fertilizer was procured from National Root Crops Research Institute Umudike, Abia State, Nigeria. LM was obtained fresh from battery cage system of the poultry unit of Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, Nigeria. The birds were raised following standard industry practices. The LM was air-dried to a constant weight. Serial dilutions of the fertilizers were made, using the recommended pond application rates (RAR) for NPK 15-15-15; 0.3 kg/25m^{2}/2weeks (120kg/ha/2weeks) (Kolo *et al.*, 2003) and LM 224kg ha/week (Ita, 1980). The volume-converted concentration representing the RAR of NPK 15-15-15 (15mg/l) (Oke and Ofor, 2015) and LM, 28mg/l (Ofor *et al.,* 2015; Ofor and Oke, 2018) on a pond of dimension 100m x 100m x 0.8m, were used in this study. The treatments (fertilizers concentrations), control (CR) (0.0mg/l), 15mg/l for NPK; Treatment 1 and 28mg/l for LM; Treatment 2 were replicated thrice. The control was water without fertilizer (a placebo). A total number of nine (9) rearing troughs with 1m×1m×0.5m dimension (500l filled to the 250l mark) were used for the experiments. The experiments ran for a period of six months, with fortnightly renewal of the media and constant aeration of the set up.

Exposure of Post-yolk-sac Larvae to Test Media

Five post-yolk sac larvae of *O. niloticus* were randomly sampled for determination of the initial levels of ROS, SOD, and TP, before the commencement of stocking into the experimental troughs. Post-yolk sac larvae of *O. niloticus* were raised in the fertilizer solutions. The stocking density was 100 larvae/rearing trough. Larvae were fed to satiation with 0.5-0.8mm commercial feed with 45% crude protein. *O. niloticus* samples were collected from treatments and control rearing troughs at 4, 84, and 168 days after exposure. Samples were homogenized and analyzed to detect ROS fluorometrically, while spectrophotometric methods were used for the quantification of antioxidant enzymes (Pasciu *et al.,* 2023). For ROS, the modified oxygen radical absorption capacity (ORAC) method of Huang *et al.* (2002) was used. Superoxide dismutase activity was assayed by the inhibition of autoxidation of epinephrine according to Sun and Zigman (1978). Absorbance was read at intervals of 1 minute for 3 minutes at 480nm. The Biuret method was used for total protein determination. This is based on the principle of peptide bonds of proteins and polypeptides containing at least two peptide bonds producing a violet-coloured complex as followed:

$Cu^{++} + 2(R\text{-}CO\text{-}NH\text{-})\ OH \rightarrow [Cu{+}2(RCONH)2] + 2H2O + 2e\text{-}$

A sample/A standard × C standard = C sample (mg/dL).

The absorbance of the complex at 546 nm is directly proportional to the concentration of protein in the sample (mg/dL).

Water Quality Parameters

Temperature (^{o}C), Dissolved Oxygen (DO) (mg/l), pH and total water hardness (mg/l) were monitored with EXTECH® Instruments SDL 150 DO metre (Model: Q598945) made in Taiwan, combined with thermometer for temperature readings, pH meter; HANNA® Instruments (Model: HI 991300) made in Europe (Romania) and AQUASOL® Code: AE 201 total hardness test kit respectively while nitrate and phosphate were monitored with HANNA Nitrate Test Kit (Model: H13874) and Phosphate Test Kit (Model: H13833) respectively.

Data Collection

Data on ROS, SOD, and TP content of homogenized samples as well as water quality parameters were collected for analysis.

Data Analyses

One-way Analysis of Variance (ANOVA) (Gomez and Gomez, 1984) was done to compare the values of ROS, SOD and TP in the sampling periods, for significant differences between the initial, control and the RAR of the two fertilizers, using general linear model as contained in the Minitab® software version 21.0. Significant differences among treatment means were separated using the Tukey's HSD at 5% level of probability. Correlation and regression analyses of the oxidative stress indices and physicochemical parameters of the culture media of *O. niloticus* exposed to NPK and LM solutions were done with IBM SPSS Statistics 23 package.

RESULTS

Oxidative Stress Responses of *Oreochromis niloticus* Challenged with Aqueous Solutions of NPK 15-15-15 and Layer Manure Recommended Rates of Application

The results of the analysis of oxidative stress responses of *O. niloticus* challenged with NPK 15-15-15 and LM fertilizers in culture media are shown in Figures 1, 2, 3 and Table 1. Levels of TP, SOD, and ROS in initial and control were significantly different from levels in fish challenged with N-P-K 15-15-15, and LM at 4, 84, and 168 days of exposure ($p<0.05$). There were significant differences ($p<0.05$) in TP level in control (CR) (22.41g/L), NPK 15-15-15 (15.82g/L) and LM (14.59g/L) fertilizers respectively. Also, SOD levels increased with increase in ROS in initial, control (CR) (0.84 µmol/min/g protein, 0.31 IU/ml) and fish samples challenged with NPK 15-15-15 (3.00µmol/min/g protein, 2.00 IU/ml) and LM (3.48µmol/min/g protein, 2.94 IU/ml) fertilizers respectively. TP levels in fish samples challenged with NPK 15-15-15 and LM fertilizers, decreased with increased levels of ROS and SOD in the two fertilizers, compared with those in the initial and control groups. TP had significant negative correlations with ROS (-0.985, $p<0.0001$) and SOD (-0.999, $p<0.0001$). ROS and SOD had a high significant positive correlation ($r = 0.988$; $p<0.0001$) (Table 2). All the oxidative stress indices and the physico-chemical variables, had high regression coefficients with fertilizer solutions ($r=0.724 – 1.000$, $p<0.0001$), except pH and PO_4(Table 3).

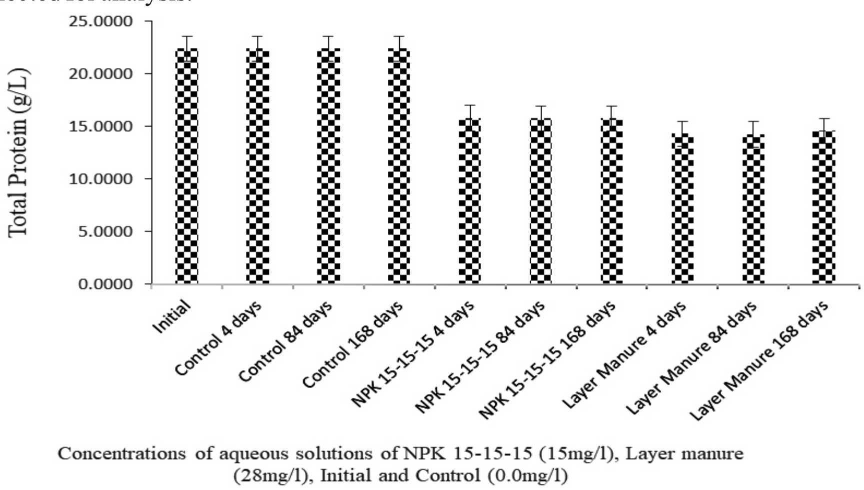

Figure 1: Total protein level of *O. niloticus* challenged with NPK 15-15-15 and Layer manure fertilizers recommended application rates in culture media. Data points are mean values (±SEM) of three replicates.

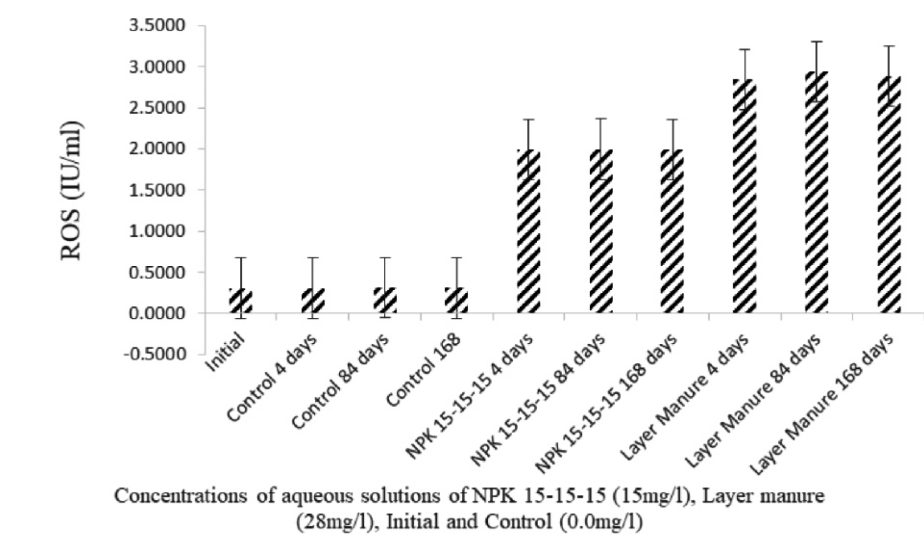

Figure 2: Reactive oxygen species (ROS) level of *O. niloticus* challenged with NPK 15-15-15 and Layer manure fertilizers recommended application rates in culture media. Data points are mean values (±SEM) of three replicates.

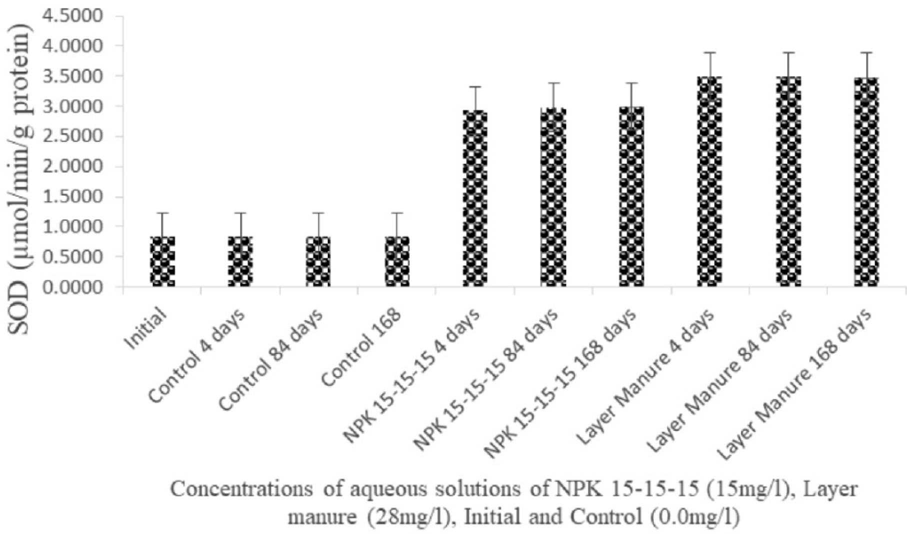

Figure 3: Superoxide dismutase (SOD) level of *O. niloticus* challenged with NPK 15-15-15 and Layer manure fertilizers recommended application rates in culture media. Data points are mean values (±SEM) of three replicates.

Table 1: Oxidative stress indices of *Oreochromis niloticus* exposed to NPK 15-15-15 and Layer manure fertilizers recommended application rates in culture media

Treatments	Total Protein g/L	SOD µmol/min/g protein	ROS (IU/ml)
Initial	22.41±0.00[a]	0.84±0.00[d]	0.31±0.00[d]
Control (CR) 4 days	22.41±0.00[a]	0.84±0.16[d]	0.31±0.00[d]
Control (CR) 84 days	22.41±0.00[a]	0.84±0.00[d]	0.31±0.00[d]
Control (CR) 168 days	22.41±0.00[a]	0.84±0.00[d]	0.31±0.00[d]
NPK 15-15-15 4 days	15.82±0.04[b]	2.92±0.05[c]	1.99±0.01[c]
NPK 15-15-15 84 days	15.77±0.01[b]	2.98±0.00[b]	2.00±0.00[c]
NPK 15-15-15 168 days	15.78±0.00[b]	3.00±0.00[b]	1.99±0.01[c]
Layer Manure 4 days	14.32±0.32[c]	3.48±0.01[a]	2.84±0.05[b]
Layer Manure 84 days	14.27±0.38[c]	3.49±0.01[a]	2.94±0.05[a]
Layer Manure 168 days	14.59±0.05[c]	3.48±0.01[a]	2.89±0.00[ab]
SEM	0.09	0.01	0.01
P value	<0.0001	<0.0001	<0.0001

Data points are mean values (±SD) of three replicates. Means within the same column with different superscripts are significantly different ($p < 0.05$). SEM = Standard Error of Mean. SD = Standard Deviation. Significant differences among treatment means were separated using the Tukey's HSD test.

Table 2: Correlation analysis of oxidative stress indices in *Oreochromis niloticus* exposed to NPK 15-15-15 and Layer manure fertilizers recommended application rates in culture media

Parameters	TP	SOD	ROS
TP		-0.999***	-0.985***
SOD			0.988***
ROS			

Key: p<0.0001-***, un-indicated-no correlation, TP -total protein (g/L), ROS -reactive oxygen species (IU/ml), SOD - superoxide dismutase (µmol/min/g protein)

Table 3: Regression of oxidative stress indices and physicochemical parameters of culture media of *Oreochromis niloticus* exposed to NPK 15-15-15 and Layer manure fertilizers recommended application rates

Independent variable: Fertilizer solutions

	Dependent variables								
	Oxidative stress indices				Physico-chemical parameters				
	TP	ROS	SOD	Temperature	DO	Total hardness	pH	NO_3	PO_4
R^2	0.724	0.969	0.890	0.967	0.913	0.806	0.017	1.000	0.009
P	****	****	****	****	****	***		****	

Key: p<0.0001-****, p<0.001-*** un-indicated-no correlation. LM- Layer manure, TP -total protein (g/L), ROS -reactive oxygen species (IU/ml), SOD -superoxide dismutase (µmol/min/g protein), Temperature (^{0}C), DO –Dissolved oxygen (mg/L), Total Hardness (mg/l $CaCO_3$), NO_3 (mg/l), PO_4 (mg/l

Water Quality Parameters of the Culture Media of *O. niloticus* Challenged with Aqueous Solutions of NPK 15-15-15 and Layer Manure Recommended Application Rates

The results of the water quality parameters monitored are presented in Table 4. Fertilizers significantly affected (p<0.05) the physico-chemical variables of water. DO decreased from 6.57 mg/l in control, to 5.63 mg/l and 5.27 mg/l in the NPK and LM culture media respectively. Total hardness increased from 14.67 (mg/l CaCO3) in control, to 39.32 (mg/l CaCO3) and 340.85 (mg/l CaCO3) in NPK and LM culture media respectively. pH increased from 5.26 in control to 7.25 in the NPK culture media and decreased to 4.94 in the LM culture media. NO_3 increased from 22.17 mg/l in control to 66.47 mg/l and 110.77 mg/l in the NPK and LM culture media respectively. PO_4 increased from 2.20 mg/l in control to 7.87 mg/l in the NPK treatment while it decreased to 1.53 mg/l in the LM treatment. All the physico-chemical variables of water monitored, had high regression coefficients with fertilizer solutions (p<0.0001, r=0.806 – 1.000), except pH and PO_4 (Table 3). Temperature had a high significant positive correlation with TP (r = 0.934, p<0.0001) SOD (r = 0.739, p<0.05) and ROS (r = 0.814, p<0.001). DO negatively correlated with TP (-0.866, p<0.001) and SOD (-0.675, p<0.05) but did not correlate with ROS. Total hardness positively correlated with TP (r = 0.896, p<0.001), SOD (r = 0.684, p<0.05) and ROS (r = 0.825, p<0.001). pH did not correlate with any of the oxidative stress indices. NO_3 significantly influenced all the oxidative stress indices (TP; r = 0.946, p<0.0001, SOD; r = 0.734, p<0.05 and ROS; r = 0.760, p<0.05). PO_4 did not influence any of the oxidative stress indices. Temperature was negatively correlated with DO (-0.906, p<0.0001) and positively correlated with total hardness (r = 0.926, p<0.0001) and NO_3 (r = 0.984, p<0.0001). DO negatively correlated with total hardness (-0.752, p<0.05) and NO_3 (-0.956, p<0.0001) but did not correlate with pH and PO_4. Total hardness positively correlated with NO_3 (r = 0.898; p<0.001) but did not correlate with PO_4. pH did not correlate with any of the physico-chemical variables of water except PO_4(r = 0.999, p<0.0001) (Table 5).

Table 4: Water quality parameters of the culture media of *Oreochromis niloticus* exposed to NPK 15-15-15 and Layer manure fertilizers recommended application rates

Parameters	Control (CR)	Fertilizer NPK	Layer manure (LM)	SEM	P value
Temperature(°C)	27.83±0.06[c]	28.23±0.06[b]	28.80±0.10[a]	0.04	<0.0001
DO (mg/l)	6.57±0.12[a]	5.63±0.06[b]	5.27±0.12[c]	0.07	<0.0001
Total Hardness (mg/l $CaCO_3$)	14.67±0.01[c]	39.32±0.02[b]	340.85±0.33[a]	0.11	<0.0001
pH	5.26±0.01[b]	7.25±0.01[a]	4.94±0.01[c]	0.01	<0.0001
NO_3 (mg/l)	22.17±0.01[c]	66.47±0.03[b]	110.77±0.02[a]	0.01	<0.0001
PO_4 (mg/l)	2.20±0.10[b]	7.87±0.15[a]	1.53±0.06[c]	0.06	<0.0001

Data points are mean values (±SD) of three replicates. Means within the same row with different superscripts are significantly different ($p < 0.05$). SEM = Standard Error of Mean. SD = Standard Deviation. Significant differences among treatment means were separated using the Tukey's HSD test.

Table 5: Correlation analysis of oxidative stress indices and physicochemical parameters of culture media of *Oreochromis niloticus* exposed to NPK 15-15-15 and Layer manure fertilizers recommended application rates

Parameters	TP	SOD	ROS	Temperature	DO	TH	pH	NO_3	PO_4
TP		-0.999***	-0.985***	0.934***	-0.866**	0.896**		0.946***	
SOD			0.988***	0.739*	-0.675*	0.684*		0.734*	
ROS				0.814**		0.825**		0.760*	
Temperature					-0.906**	0.926***		0.984***	
DO						-0.752*		-0.956***	
TH								0.898**	
pH									0.999***
NO_3									
PO_4									

Key: p<0.0001-***, p<0.001-**, p<0.05-* un-indicated-no correlation, LM- Layer manure, TP -total protein (g/L), ROS - reactive oxygen species (IU/ml), SOD -superoxide dismutase (µmol/min/g protein), Temperature (°C), DO –Dissolved oxygen (mg/L), TH-Total Hardness (mg/l $CaCO_3$), NO_3 (mg/l), PO_4 (mg/l)

DISCUSSION

This study examined the oxidative stress responses of *O. niloticus* exposed to aqueous solutions of NPK 15-15-15 and LM in culture media. Oxidative and antioxidant activities were detected in this study. Fertilizers in aquatic environments can cause environmental toxicity to fish and induce oxidative stress owing to an excessive production of ROS in fish bodies (Gaye-Siessegger *et al.,* 2023; Lee *et al.,* 2023; Oke *et al.,* 2025; Teixeira *et al.,* 2025; Yu *et al.,* 2025). Aquatic organisms including fish, have cellular strategies to counter oxidative stress, which depend on the scavenging effect of antioxidants and antioxidant enzymes (Diler *et al.,* 2022). The strategy employed, depends on the ROS present. ROS can be free radicals (hydroxyl-OH^-), reactive anions containing oxygen atoms (superoxide-O_2^-), or molecules containing oxygen atoms that can produce free radicals or are activated by them (hydrogen peroxide-H_2O_2, hydroperoxyl-HOO, and peroxyl radicals-ROO) (Menon *et al.,* 2023). The lifespan of ROS is reportedly short, because of which a more long-lasting effect; the effect on total protein was also monitored in this study.

Fish have developed various antioxidant systems to protect themselves from ROS; thus, a change in antioxidant responses in fish can be a criterion for evaluating oxidative stress resulting from fertilizer exposure. Because fertilizer exposure may be recognized as an exogenous substance by a fish body, it may lead to the stimulation or suppression of its immune system (Singh and Pandey, 2021; Lee *et al.,* 2023). Various immune responses can be assessed to evaluate fertilizer toxicity in fish. ROS is produced during aerobic metabolism. SOD, catalase and glutathione peroxidase are antioxidants that act as first order defence against free radicals, preventively or by scavenging. SOD is a group of metalloenzymes existing in all aerobic organisms (Khandare *et al.,* 2023). It catalyzes the dismutation of superoxide radical to hydrogen peroxide. To prevent the conversion of the H_2O_2 to harmful hydroxyl radical, catalase catalyzes the rapid conversion of the H_2O_2 into water and molecular oxygen. As catalase is absent in mitochondria, glutathione peroxidase performs this role. There are also second, third-, and fourth-line defence antioxidants. Antioxidants may act to repair radical-induced damage.

In the absence of toxicants or environmental stress, there is a balance between ROS and antioxidant defenses even during embryogenesis, which ensures the wellbeing of the organism (Ofor *et al.,* 2025). This seems to have happened in the initial and the control group in this study. SOD catalyses the dismutation of ROS (Khandare *et al.,* 2023), maintaining the balance between ROS and antioxidants in healthy organisms in the absence of pollutants. In this study, the antioxidant mechanism was overwhelmed. This was evident by the increasing ROS levels, notwithstanding the increase in the SOD expression and the decrease in TP levels in both fertilizers. It has been reported that ROS produced during stress, causes oxidation and direct and indirect modifications of proteins (Das and Roychoudhury, 2014). The effect of ROS can be mediated through the effect on protein coding for various physiological processes (Jomova *et al.,* 2023). Pollutants in the environment lead to the production of high levels of ROS and an impairment of their regulatory system (Menon *et al.,* 2023), leading to death of an organism.

It could be observed that introduction of fertilizers to the culture media, significantly affected all the oxidative stress indices and physico-chemical variables, as evident by the high regression coefficients. The impact of the fertilizers on the oxidative stress indices of *O. niloticus,* may have been direct via the effects of the chemical constituents of the fertilizers, or indirect, through their effect on water physico-chemical parameters. NPK is a complex of chemical compounds. The seeming influence of individual chemical constituents of NPK and physico-chemical parameters of water on oxidative stress indices of *O. niloticus*, may not be attributable to any one chemical constituent or physico-chemical parameter. The nitrogen in NPK is in the form of Nitrate-nitrogen ($N-NO_3$), ammoniacal-nitrogen ($N-NH_4$) and ureic-nitrogen ($N-NH_2$), the phosphorus exists in the form P_2O_5 while potassium exists as K_2O (IPPC, 2007). Nitrates contribute to water hardness. With introduction of NPK to the culture media, the hardness increased and quantities of K^+ are also expected to rise. On the other hand, pH is made more alkaline by the ammonium and ions. These ions in the medium increased with introduction of fertilizer to the culture media.

LM contains mineral contaminants (Gillick *et al.*, 2007) that are eco-toxic (USEPA, 2013). Apart from the nutrient elements P and K, the most abundant mineral constituents of LM, Ca and Mg (Gillick *et al.,* 2007) contribute significantly to water hardness (Ingin *et al.,* 2024). The introduction of LM to the culture media, greatly influenced the total hardness level compared to those of NPK 15-15-15 and control media. This may have resulted from a variety of sources including calcium and magnesium in the diet of the laying birds. Other sources may have been ammonium salt, the major one of which is ammonium carbonate; and nitrate which is an essential component of poultry manure (Akporube *et al.,* 2023), and a major contaminant of surface water in intensively cultivated areas (Manik and Biswapati, 2008).

pH was highest in the NPK medium, lower in control medium and the least in the LM medium. The variation in pH can be explained by the composition of poultry manure. The major source of nitrogen in poultry manure is uric acid (Murakami *et al.,* 2011). Although not very soluble in water, enough of it must have dissolved to influence the pH of the culture media. It is also known that phosphate is lost to the sediments, mainly in the upper 5cm, irrespective of sediment type (Shrestha and Lin, 1996). This loss has also been reported in glass jars, in the absence of bottom soil and is aided by aerobic and acidic water (Hepher, 1958), which explains the similar trend in the phosphate level with the introduction of layers droppings to the culture media.

CONCLUSION AND RECOMMENDATION

The study has demonstrated that environmental conditions caused by the presence of NPK 15-15-15 and LM at levels corresponding to the recommended rates of application, induce oxidative stress in *O. niloticus*, compromising the welfare of the species in culture and in the wild. This could ultimately threaten the survival of the species. It signals a more pervasive damage to the aquatic environment and the necessity of care in the use of agrochemicals and other pollutants in aquatic and riparian habitats. Based on the findings of this study, levels of the chemicals through which these fertilizers (NPK and LM) exert their influence should be monitored in water. Application rates of fertilizers for arable farming should be monitored and regulated with a view to minimize run-offs and the potential negative consequences of inducing toxic stress on wild fish stocks. Legal application limits may be necessary, on the basis of the capacity of the fertilizers to induce oxidative stress on fish stocks.

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AUTHORS CONTRIBUTION

COO and AOO - Writing original draft, Writing – review & editing, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Funding acquisition. UDE - Writing – original draft, Writing – review & editing, Validation, Formal analysis. FEO – Methodology, Investigation, Data curation. GCI - Methodology, Investigation. All authors approved the manuscript for submission.

Ethical Approval

The research methods were certified by the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike Research Ethics Committee, to ensure compliance with ethical standards. The study entitled: “Development of Gonad of *Oreochromis niloticus* (Linnaeus, 1758) in Aqueous Solutions of NPK and Layer Manure Fertilizers”, underwent a thorough review and verification process of the methodology. The ethical approval number assigned to the work is: MOUAU/CVM/REC/202518.

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