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The effects of detergent and fertilizer toxicity on growth rate, nutrient utilization and survival of *Clarias gariepinus* (Burchell) fingerlings reared under controlled condition

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Abstract
Study on the effects of metals on growth rate and survival of *Clarias gariepinus* (Burchell 1822) fingerlings bred in tanks containing water concentrated with inorganic fertilizer and detergent was conducted. 300 fingerlings of *C. gariepinus* (7.5±0.24) were stocked into different tanks of 5000m^3 capacity water and fed with formulated diet for 8 weeks. The tanks were treated with 0ml, 50ml, 100ml, 150ml, 200ml and 250 ml of inorganic detergent and fertilizer respectively. The metal content in the fish was determined through wet acid digestion and extraction procedure using Atomic Absorption Spectrophotometer, while growth was determined using indices method. The values obtained for Cu, Zn, Mg, P, Cd, Pb, Mn, K and Na respectively were generally below the Federal Environmental Protection Agency (FEPA) limits. The survival rate recorded for fingerlings treated with fertilizer was 99% for all concentrations but a range between 88% to 0% survival was recorded for fingerlings stocked in detergent treated water. Toxicity behavior response observed in this study were, slow movement, gulping for atmospheric oxygen, darting and erratic swimming. The results obtained indicated that concentration of 50ml detergent and 100ml of fertilizer were the suitable and tolerable limits in terms of growth and survival for culturing *C. gariepinus* fingerlings.

Keywords: Growth rate, *C. gariepinus*, concentration, detergent, fertilizer, fingerlings, metals, survival

1. Introduction
Globally, most coastal nations are undergoing a rapid industrial revolution [1, 2], these developmental changes may lead to several environmental deterioration such as land, air and aquatic pollution [3, 4]. Aquatic pollution and over exploitation of coastal fish stock are detrimental to sustainable fish production. Therefore, an alternative source of fish production through aquaculture for a sustainable animal protein [5], best available cultural practices, bodes well for the aquaculture industry and ever increasing population [6]. Yet these aquatic resources provides livelihoods for about 3 billion people and about 10–12% of the world population engaged in the fisheries and aquaculture [7-9]. The population of the world is about 7.5 billion and requires renewable resources for sustainable existence. Of which over 1.3 billion people still suffer from hunger, this comprises the poor, especially those in rural areas, who lives below one dollar and 150g (50–60%) of protein required of adult per day) of protein per day [10]. Although 53% marine fishery resources of most fishing territories and water bodies have reached their maximum potential for fisheries production, while 32% is overfished, depleted, or recovering from depletion [11-14]. This is due to the fact that several millions of people around the world derived a source of income and livelihood in the coast and fisheries sector. This fish stock recruitment problem is characterized with increase in capture of large exotic species, negative aquaculture practices and other detrimental effects like pollution/eutrophication, climate change on fisheries production [15].
Aquaculture remains one of the fastest-growing agro-industrial activities in the last four decades and projected to outpace population growth. As the world population increases geometrically, there is a consequent demand for more food production, employment and goods and services [16]. These gave rise to an increase in industrialization, which has resulted into the release of more waste materials of various kinds into the immediate environment, especially

aquatic environment [17]. The problems of bioaccumulation of heavy metals in aquatic organisms, which ultimately serve as food to man therefore, deserve an urgent attention. In Africa generally and Nigeria in particular, it has been found that there is unregulated discharge of effluents [18] from agro-industrial activities and communities into water [19, 20]. Concern has been expressed in some circles about the role and fate of the trace metals derived from these sources [21].

Many causes of pollution including fertilizer contain nutrients such as nitrates and phosphates [22]. In excess levels, nutrients over stimulate the growth of aquatic renewable resources. However, excessive growth of these types of organism consequently clogs the water ways, use un-dissolved oxygen as they decompose, in turn, and prove very harmful to aquatic organism as it affects the respiration ability of fish [23]. On the other hand metallic pollution may be acute sources of pollution not just because they are toxic above relatively low concentration [24], but because they are persistent in the environments long after the primary source of pollutant has been removed [35]. Effluents from industries and agro-allied facilities have been identified as one of those introducing heavy metals along with other pollutants into the Nigeria aquatic environment [24, 26]. In addition, the effect of pollutants at the cellular level can accumulate and eventually alter the functional integrity of an organ or tissue system, which potentially affect life [27].

Due to the economic value and resistance nature of C. *gariepinus* as a potential culturable fish species in Nigeria, the aims and objectives of this research was to investigate the best conditions for cultivating *C. gariepinus*. Also to determine the effects of inorganic fertilizer and detergent as pollutants in the culture medium with resultant effects on growth performance and survival of *Clarias gariepinus* fingerlings.

2. Materials and Methods

2.1 Experimental fish samples

A total of three hundred (300) fingerlings were obtained from the Aquaculture/fisheries unit of the Agricultural Science department, Adeniran Ogunsanya College of Education, Oto/Ijanikin, Lagos State, and transported in aerated plastic bags to the *In-situ* hatchery facilities of Fisheries department, Lagos State University, Ojo, Lagos for the experiment (Figure 1). The weight of the fingerlings collected ranged between average mean weights of 7.5 ± 0.24g, and were four weeks old at the time of collection. They were acclimatized for 24 hours prior to the commencement of the experiment.

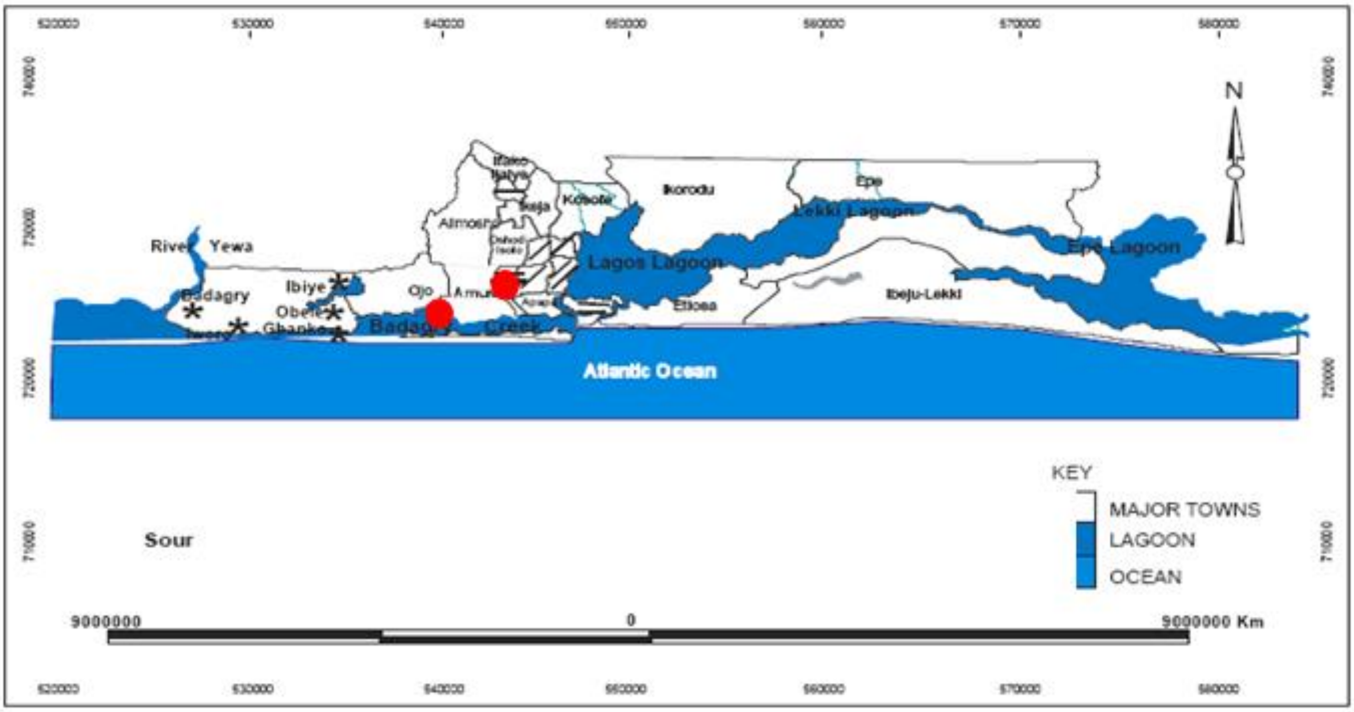

Fig 1: Locations of the fish collection site and *In-situ* experimental research facility.

2.2 Experimental Design

The fingerlings were grouped into different tanks with 5000m^3 of water, same age group were used in each of three treatments {(Control (C) $_{1,2,3}$, Fertilizer (F) $_{1,2,3}$ and Detergent, (D) $_{1,2,3}$ } and three replicates were weighed and stocked in 5000 m^3 culturing bowls. 50g each of fertilizer and detergent were separately weighed and dissolved in 5 litres of water and allowed to stay for 48 hours to dissolve completely. The solution of the fertilizer (F) and detergent (D) were measured into the different tanks labeled as $F1_{1,2,3}$ to $F5_{1,2,3}$ and $D1_{1,2,3}$ to $D5_{1,2,3}$ while F_0 and D_0 are control for fertilizer and detergent respectively. The fishes were introduced into various already prepared concentrations of fertilizer and detergent (Om1, 50ml, 100ml, 150ml, 200ml and 250ml) after 24 hours acclimatization.

2.3 Feed Formulation & Determination of Growth and Nutrient Utilization

Fish feed of 30% crude protein level (fish meal, soybean meal, white maize, palm oil, Ca_2So_4 and premix) was formulated using the Pearson's square method as described in [28] and reported by [23], while the preparations of the diet were based on the description by [29]. The feed was administered twice (per day at 08:30 and 17: 30 hr) daily at 4% body weight for 8 weeks. Weight changes were measured every three days and the feeding rate adjusted accordingly to accommodate the change [30]. The growth rate was carried out using growth indices method as described by [31, 32].

2.4 Physicochemical Parameters and Chemical Evaluation of Experimental fish

Samples of experimental diets and fingerlings were analyzed for proximate composition, according to the methods of [33]. Water quality indicators were monitored closely by determining pH, temperature, dissolved oxygen and total ammonia levels in the culture medium as described by [34, 35].

2.5 Survival

Percentage mortality was calculated using this formula

$$\% \text{ Survivability} = \frac{\text{Number of fish stocked} - \text{Number of mortality x 10}}{\text{Number of fish stocked.}}$$

2.6 Determination of wet acid digestion of P, Na, Mg, Cd, Mn, Pb, Zn and Cu

0.5g of grinded fish sample material (oven dry 60 °C) was weighed into a 125ml Erlenmeyer flask which has been previously washed with distilled water. Perchloric acid of about 4ml, 25ml conc. $HN0_3$ and 2ml conc. H_2S0_4 was added under a fume hood. The contents were mixed and heated gently at low to medium heat on a hot plate under perchloric acid fume hood. The sample was allowed to cool and 40-50ml of distilled water was added. Boil for another half a minute on the same plate at medium heat. The sample was cooled and the solution is filter completely with a wash bottle into a 100ml Pyrex volumetric flask. The solution was stored P and Fe, determinations of Zn, Na by calorimetric and Mg was determined by atomic absorption spectrometry (AAS) [36].

2.7 Statistical analysis

All data collected were analyzed for significant differences (p<0.05) (ANOVA) on Graph Pad Prism 5. The results were expressed as mean X ± SD (standard deviation) and pictorial representation. Determined differences among treatments were partitioned by the Least Significant Difference (LSD) and the Duncan's New Multiple Range Test (DNMRT) [37].

3. Results

Proximate and gross compositions of the diet with different ingredients used are presented in Table 1, weight gain of fish treated in detergent and fertilizer were showed in Table 2 and 3 respectively. While metals concentration in both fish treated detergent and fertilizer were presented in Table 4 and 5. The water quality parameters of both culture medium detergent and fertilizer are showed in Table 6 and 7 respectively. The percentage survival of fish and toxicity behavior respond in detergent solution are presented in Table 8. Table 1: Shows the proximate and gross composition of feed ingredient formulated at 30% crude protein, with the gross composition of the diet is high for carbohydrate, with soybean and fishmeal contributed 22.4% each of the diet.

Table 1: % Proximate and gross composition of feed ingredients

Proximate composition 30% C.P		Gross composition of feed ingredient 30% C.P	
Moisture content	19.30	Fishmeal	22.40
Dry matter content	80.87	Soybean	22.40
Fat content	4.04	Maize	48.60
Ash content	9.53	Vitamin Premix	1.00
Crude fibre content	10.32	Oil	5.00
Crude protein content	7.62	Dicalcium phosphate	0.50
Nitrogen free extract	49.36		

The summary of the growth in Table 3: Shows the biweekly weight gain of fish in detergent tank. All concentration and control are not different in initial weight but the final weight gain are significantly different with D1 having highest weight gain. No weight gain recorded for D4 and D5 beyond week 2 due to death of the fish.

Table 2: Biweekly weight gain (WTG) of fish treated with detergent

Days	Concentrations of detergent (ml)					
	Control	50	100	150	200	250
14	0.35 ± 0.30^a	0.25 ± 0.07^b	0.30 ± 0.14^b	0.30 ± 0.14^b	0.35 ± 0.07^c	0.45 ± 0.07^d
28	0.40 ± 0.00^a	0.10 ± 0.00^b	0.10 ± 0.00^b	0.40 ± 0.28^c	-	-
42	0.85 ± 0.49^a	0.80 ± 0.14^b	0.70 ± 0.14^c	0.55 ± 0.21^c	-	-
56	1.10 ± 0.14^a	1.25 ± 0.90^b	0.85 ± 0.00^b	-0.25 ± 0.21^c	-	-

Figures in the same horizontal row having the same superscript are not significant different (p > 0.05)

Table 3: Biweekly weight gain (WTG) of Fish treated with Fertilizer

Days	Concentrations of in-organic fertilizer (ml)					
	Control	50	100	150	200	250
14	0.30 ± 0.14^a	0.30 ± 0.00^a	0.30 ± 0.14^a	0.25 ± 0.07^b	0.20 ± 0.00^b	0.35 ± 0.07^c
28	0.30 ± 0.14^a	0.35 ± 0.21^b	0.30 ± 0.28^a	0.60 ± 0.14^c	0.45 ± 0.35^c	0.35 ± 0.21^b
42	1.05 ± 0.78^a	0.80 ± 0.56^b	0.80 ± 0.48^b	0.80 ± 0.57^b	0.65 ± 0.49^c	0.70 ± 0.50^c
56	1.20 ± 0.42^a	0.85 ± 0.21^b	1.50 ± 0.70^c	1.45 ± 0.07^c	1.30 ± 0.57^d	1.25 ± 0.35^d

Figures in the same horizontal row having the same superscript are not significant different (p > 0.05)

Table 4: Concentration of metals in *Clarias gariepinus* treated with detergent

Conc. (ml)	Metals ($mg.g^{-1}$)								
	Na	K	P	Mg	Cu	Zn	Cd	Pb	Mn
Control	4.08±0.01	6.05±0.07	4.57±0.07	3.24±0.20	0.08±0.05	0.87±0.01	0.04±0.03	2.21±0.02	0.13±0.07
50	3.82±0.03	5.61±0.01	3.68±0.05	2.88±0.01	0.06±0.01	0.83±0.04	0.05±0.02	2.09±0.04	0.13±0.03
100	1.05±0.04	1.71±0.01	3.47±0.01	3.77±0.07	0.07±0.00	0.75±0.02	0.04±0.01	2.29±0.03	0.12±0.02
150	4.24±0.01	6.41±0.40	4.2±0.01	3.62±0.04	0.06±0.01	0.93±0.01	0.04±0.01	2.46±0.01	0.13±0.00
200	3.11±0.07	5.40±0.14	4.90±0.12	3.72±0.02	0.08±0.02	1.08±0.01	0.05±0.01	2.19±0.02	0.14±0.01
250	2.85±0.02	5.20±0.07	5.0±0.06	3.98±0.06	0.07±0.00	0.88±0.05	0.03±0.01	1.94±0.01	0.11±0.02

Table above shows the summary of heavy metals, Cu, Zn, Cd, Pb, Mn and nutrient, Na, P, K in *Clarias gariepinus* cultured in detergent solution with K having the highest value 6.41 ± 0.14 $mg.g^{-1}$ while Cd has the lowest value 0.03 ± 0.01 $mg.g^{-1}$.

Table 5: Concentration of metals ($mg.g^{-1}$) in *Clarias gariepinus* treated with fertilizer

Conc.(ml)	Na	K	P	Mg	Cu	Zn	Cd	Pb	Mn
50	4.03± 0.07	5.95± 0.07	4.72 ± 0.02	3.11±0.07	0.07± 0.02	0.95± 0.02	0.04 ± 0.01	2.00± 0.01	0.12 ± 0.02
100	4.17± 0.01	6.1 ± 0.14	5.06 ± 0.01	3.85±0.07	0.07± 0.07	0.93± 0.01	0.03 ± 0.00	2.50± 0.01	0.14 ± 0.01
150	3.95± 0.01	5.61± 0.05	3.89 ± 0.02	3.86± 0.00	0.07± 0.01	0.88± 0.03	0.04 ± 0.00	2.79± 0.03	0.12 ± 0.01
200	4.01± 0.01	5.7 ± 0.14	5.32 ± 0.03	3.87± 0.01	0.08± 0.01	0.93± 0.02	0.04 ± 0.01	2.44± 0.04	0.14 ± 0.02
250	3.81± 0.01	5.3 ± 0.12	4.88 ± 0.03	3.78± 0.03	0.06± 0.02	0.83± 0.01	0.04 ± 0.01	2.11± 0.02	0.12 ± 0.01

Table shows the summary of heavy metals, Cu, Zn, Cd, Pb, Mn and nutrient, Na, P, K in *Clarias gariepinus* cultured in fertilizer solution with K having the highest value 6.1±0.14 $mg.g^{-1}$ while Cd has the lowest value 0.03±0.00 $mg.g^{-1}$.

Table 6: Water quality parameters of culture medium with detergent solution

Concentration (ml)	D_0 control 0 ml	D_1 50 ml	D_2 100 ml	D_3 150 ml	D_4 200 ml	D_5 250 ml
Temperature °C	26.10 ± 0.07 ^{a}	26.20 ± 0.31^a	26.4 ± 0.12^a	25.6 ± 1.03^a	26.0 ± 0.05^b	24.4 ± 1.93^c
pH	7.1 ± 0.1 ^{a}	6.9 ± 0.1 ^{b}	6.9 ± 0.1 ^{b}	7.0 ± 0.1 ^{b}	7.0 ± 0.1 ^{b}	7.1 ± 0.1 ^{b}
Salinity %	0.2 ± 0.0 ^{a}	0.2 ± 0.0 ^{b}	0.2 ± 0.0 ^{b}	0.2 ± 0.0 ^{b}	0.2 ± 0.0 ^{b}	0.2 ± 0.0 ^{b}
(CO_2) $mg.l^{-1}$	3.0 ± 1.7 ^{a}	1.6 ± 0.1 ^{b}	1.1 ± 0.2 ^{b}	0.8 ± 0.4 ^{b}	0.8 ± 0.6 ^{b}	1.4 ± 0.6 ^{b}
(NH_3) $mg.l^{-1}$	0.10 ± 0.11^a	0.66 ± 0.09 ^{b}	0.45 ± 0.09 ^{b}	0.31 ± 0.12^b	0.45 ± 0.09 ^{b}	0.49 ± 0.04 ^{b}
(DO) $mg.l^{-1}$	4.00 ± 0.4 ^{a}	1.8 ± 0.0^b	1.8 ± 0.8 ^{b}	0.5 ± 0.3^c	1.6 ± 0.6^b	0.4 ± 0.1 ^{c}

Values in the same row having the same superscript are not significantly different ($p > 0.05$).

Table 7 shows the values of water quality parameters of detergent solution, all the value of each parameters fall within the range set by FEPA except the values for DO which is lower and NH_3 which is high.

Table 7: Water quality parameters of culture medium with fertilizer solution

Concentration	F_0 Control 0ml	F_1 50ml	F_2 100ml	F_3 150ml	F_4 200ml	F_5 250ml
Temperature °C	26.1 ± 1.93 ^{a}	25.2 ± 0.10^a	25.1 ± 1.13 ^{a}	25.1 ± 0.15 ^{b}	26.1 ± 0.08 ^{b}	27.1 ± 1.13 ^{b}
pH	7.0 ± 0.1 ^{a}	6.9 ± 0.1 ^{b}	7.0 ± 0.1 ^{b}	6.9 ± 0.5 ^{b}	6.9 ± 0.0 ^{b}	6.9 ± 0.0 ^{b}
Salinity %	0.2 ± 0.0 ^{a}	0.2 ± 0.0 ^{a}	0.2 ± 0.0 ^{a}	0.2 ± 0.0 ^{b}	0.2 ± 0.0 ^{b}	0.2 ± 0.0 ^{b}
(CO_2) $mg.l^{-1}$	3.0 ± 1.7 ^{a}	2.1 ± 1.4 ^{b}	3.1 ± 1.7 ^{b}	2.5 ± 1.3 ^{b}	2.7 ± 1.2 ^{b}	2.9 ± 1.3 ^{b}
(NH_3) $mg.l^{-1}$	0.10 ± 0.12^a	0.66 ± 0.55 ^{b}	0.69 ± 0.23 ^{b}	0.71 ± 0.06 ^{b}	0.67 ± 0.12 ^{b}	0.73 ± 0.04 ^{b}
(DO) $mg.l^{-1}$	4.00 ± 0.10^a	0.70 ± 0.00^b	0.65 ± 0.5 ^{b}	2.85 ± 1.2 ^{c}	2.45 ± 1.8 ^{c}	0.90 ± 0.1 ^{b}

Values in the same row having the same superscript are not significantly different ($p > 0.05$).

Table 8 shows the values of water quality parameters of fertilizer solution, all the value of each parameters fall within the range set by FEPA except the values for DO which is lower and NH_3 which is high.

Table 8: Percentage survival rate of fish and toxicity behavior response in detergent solution

	Concentrations					
	D_0 control 0ml	D_1 50ml	D_2 100ml	D_3 150ml	D_4 200ml	D_5 250ml
Percentage Toxicity	100%	80%	28%	16%	0%	0%
Fish Behavior Response	Normal	Slow movement	Reduce feed intake and gulping for atmospheric oxygen	Gulping, Stop feeding and erratic swimming	Stop feeding, darting and erratic swimming	Stop feeding, darting and erratic swimming

4. Discussion

As illustrated in Table 2 shows the final body weight gain of *Clarias gariepinus* treated with detergent. It indicates that the feed was utilized for body growth, but as the concentration of the detergent increases, survival rate reduced. The final weight gain of fish treated with fertilizer in Table 3 shows that the feed were fully utilized for body growth. This is in line with the observation of [38] who reported that the fertilization of water allows for adequate phytoplankton production which enhances a better growth and survival of the fish.

Experimental investigations revealed the presence of zinc (Zn), Lead (Pb), Copper (Cu), Cadmium (Cd), and minerals such as; Sodium (Na), Potassium (k), Magnesium (Mg), Phosphorus (P) in the samples analyzed. The values obtained are quite lower when compared to those obtained by the Federal Environmental protection agency [39]. Table 4 and 5 shows the mean value of Zn varies between 0.75 $mg.g^{-1}$ and 1.08 $mg.g^{-1}$ in both pollutants. The value was by far higher than the reported value of 0.02 $mg.g^{-1}$ from rivers of South Carolina [40] but lower than those of Kaduna River, Nigeria [41, 42] posited that zinc has low toxicity to man, but relatively high toxicity to fish. The appreciable levels recorded in this study would therefore be a serious cause for concern. The mean levels of Cd (0.03-0.05 $mg.g^{-1}$) recorded in this study was low. However, the values was higher than the reported values of <0.01 $mg.g^{-1}$ in rivers of South Carolina [40]. The highest Cd level recorded is an indication of anthropogenic input [43]. Cadmium has been known to have contributed to pulmonary disease, reduced glucose tolerance, severe kidney and liver damage and death in human beings [44, 45]. Also the mean levels of Pb varied between 1.94 $mg.g^{-1}$ and 2.50 $mg.g^{-1}$ in the catfish samples and these values recorded were higher than the reported mean value of 0.56 mg/g from Kaduna River, Nigeria [41]. Lead is known as a deadly and cumulative poison even when consumed in small quantities and is capable of deadening nerve receptors in man [46, 47]. The mean levels of Mn ranges from 0.11 - 0.14 $mg.g^{-1}$ in the catfish sampled and are below the range set by [39]. The range value of copper (0.064 - 0.081 $mg.g^{-1}$) recorded in sampled fish were below the value (0.11-11.9 $mg.g^{-1}$) observed by [48] when he tested the toxicity of this metal on some pelagic and benthic fish species. However, copper levels were lower than the other metals investigated in this study. Potassium levels in fish bred in fertilizer is high compare to fish in detergent. This simply explain that the level of potassium in the environment of greatly affect the level of potassium in the fish, this is in line with the findings of [49] who opined that fishes are susceptible to physical and chemical in the environment and may reflect in blood composition. Phosphorus level in both pollutants varies differently; the variation of phosphorus was explained by [50] who gave knowledge on role of key nutrient in pathogenesis of skeletal fish with an emphasis on mineral.

There was no significant difference ($p > 0.05$) in the mean values of all the physico-chemical parameters in the treatments; they fall within the acceptable limits, except the values for ammonia and dissolved oxygen. [51] stated that the amount of oxygen in water is not as constant as in air but fluctuates markedly depending on depth. The mean dissolved oxygen values obtained in both treatments ranges from 0.4 ± 0.1 - 2.8 ±1.2, these values are relatively lower than the range considered desirable for fish survival at 30ºC [52]. The values of the pH is good for fish growth and survival as this falls within the range considered good for fish survival by [52]. The temperature and salinity values obtained in this study are characteristic of a tropical freshwater ecosystem [53]. The values of carbon dioxide are within the [39] guidelines standard for this parameter. But the ammonia was relatively higher than the limits set by [39]. The above may imply an increased toxicity with the raised values of the physcio- chemical parameters. [43] stated that toxicity of pollutant of fish usually increased with temperature which may be due to an increased uptake of toxin which added environmental stress e.g. reduced oxygen solubility [54]. The recorded values of water temperature were within tolerable range for the fish species and may not have affected the toxicity of the treatments. Ammonia (NH_3) appears to be the major source of toxicity in the experiment to which *Clarias gariepinus* was exposed. This is in line with the observations of [55] who indicated ammonia as the main cause of toxicity of pollutant from industrial plant and where death may occur in aquatic organisms.

Difference in mortality of *Clarias gariepinus* may be due to the differential toxicity of the stock solutions. As it was observed the test solution of detergent concentrate at 200ml and 250ml survival was at a quick decline as none of the fish survive, mean while the fishes increased their activity to cope with rise in level of concentration. This is similar to [56] that hyperactivity wave down with the depletion of fish energy eventually causing their death. However, the results indicate that survival time for fishes in the detergent test solution declined with increase in concentration and time of exposure. But no mortality was observed for the fertilizer test solution in this experiment because the fish were able to utilize the minerals from the fertilizer for their survivability, since fertilizers are known to boost pond phytoplankton use as natural food for fish. Meanwhile continued discharged of these effluents (i.e. detergent and fertilizer) over a long period could lead to uptake and bioaccumulation in the aquatic ecosystem.

5. Conclusion

It is concluded that 50ml of detergent and 100ml of inorganic fertilizer are the safe concentration for *Clarias gariepinus* under cultured environment. Thus it is recommended that: (i) Monitoring status of the water body for all seasons to ascertain its effect on the fisheries must be ensured by the concerned Federal and State Agencies. (ii) Environmental impact assessment and auditing should be carried out on all the industries found along the water body, and (iii) Installation of recycling facilities for aquatic waste, with stringent penalties on defaulting industries.

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