Growth Response and Survival Rate of *Clarias gariepinus* Fingerlings Exposed to Varying Metal Concentrations.

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ABSTRACT

Study on the effects of metals on growth rate and survival of *Clarias gariepinus* fingerlings bred in tanks containing water concentrated with detergents was conducted. 300 fingerlings of *C. gariepinus* (Mean±SD 7.5±0.24g) was stocked into different 5000m^3 capacity water tanks and fed with formulated diet for 8 weeks. The tanks were treated with 0ml, 50ml, 100ml, 150ml, 200ml, and 250 ml of detergents, respectively. Metal content in the fish was determined by wet acid digestion and extraction procedure using Atomic Absorption Spectrophotometer with digital read out and a standard single slot burner head. Samples of fish carcass were analyzed for proximate composition, while growth performance was evaluated using weight gain (WG) index. Means of parameters were subjected to analysis of variance (ANOVA) and levels of significance determined at 5% confidence limit using Duncan Multiple Range Test. A range of 0.08mg/g, 0.9 mg/g, 3.98 mg/g, 5.32 mg/g, 0.052 mg/g, 2.79 mg/g, 0.14 mg/g, 6.41 mg/g and 4.24 mg/g were obtained for Cu, Zn, Mg, P, Cd, Pb, Mn, K, and Na, respectively. These results were below the Federal Environmental Protection Agency (FEPA) limits. The survival rate recorded ranged from 88% to 0%. The results obtained indicated that 50ml of detergent is the tolerable limits in terms of growth and survival, for culturing *C. gariepinus* fingerlings in 5000m^3 of water.

(Keywords: metals, growth rate, survival, *C. gariepinus*, fingerlings)

INTRODUCTION

As the world population increases geometrically, there is a consequent demand for more food production, employment, goods and services. Increasing urbanization and industrialization has culminated in a dramatic growth in the volume of municipal wastewater produced worldwide which has resulted into the release of more waste materials of various kinds into the immediate environment, especially aquatic environment (Olowu *et al.,* 2012a; Okoye, 1991).

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 from industries and communities into water bodies even though no case of severe metal pollution has been reported. Concern has been expressed in some circles about the role and fate of the trace metals derived from these sources (Olowu *et al.,* 2012a; Oyewo, 1998; Kotze *et al*., 1999). Furthermore, factors such as high population growth accompanied by intensive urbanization and higher exploitation of natural resources including cultivable land have caused pollution increase (Olowu *et al.,* 2010; Ajao, 1996).

Mc Cormac (1971) defined pollution as release or disposal of materials and energy into the environment in such quantities that can cause deleterious effects to living resources and structures whose preservation is desirable.

Pollutants may be derived from point source and non-point source. Point sources of pollution occur when harmful substances such as effluent from sewage or wastes from factories are emitted directly into a body of water (Olowu *et al.,* 2012b; Ajao, 1996; Dickman *et al.,* 1998; Patel, 2000; Merlini, 1980). Alternatively, sources of pollution maybe diffuse, entering water sources from run-off and land drainage. An example of this type of water pollution is when fertilizer from a field is carried into a stream by rain in the form of run-off which in turn affects aquatic life (Olowu *et al.,* 2012a; Akinola *et al*., 1981).

Trace element and heavy metals such as Cd, Pb, Hg, As, Cr, Cu, Ni, Zn, B, Se, and Mo have been reported to bioaccumulate and cause adverse effects in human when ingested (Olowu *et al.,* 2012a; Sehgal *et al*., 1986). Nitrogen and Phosphorus in both organic and inorganic forms may also be present (Olowu *et al.,* 2012b; Goldstein *et al*., 1970). Many causes of pollution including fertilizer contain nutrients such as nitrates and phosphates. In excess levels, nutrients over stimulate the growth of aquatic plant and algae (Adey *et al*., 1993). 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. On the other hand pollution may be acute.

Heavy metals are important sources of pollution not just because they are toxic above relatively low concentration, but because they are persistent in the environments long after the source of pollution has been removed (Olowu *et al.,* 2012a; Prosi, 1981; Forstner *et al.,* 1983). The occurrence of heavy metals such as Pb and Cd in the environment has been a source of concerned to environmentalists, government agencies, and health practitioners and this is mainly due to their health implications since they are non-essential metal and of no benefit to human (Tovide *et al*., 2011; Taylor, 1997; Witeska, 2003).

Effluents from textile industries have been identified as one of those introducing heavy metals along with other pollutants into the Nigeria aquatic environment (Bhalerato and Adeeku, 1987; Ayodele *et al*., 1991; Chyne *et al*., 1987; Calamari *et al*., 1994). 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.

The aim of this present study is geared towards investigating the effects of metals (i.e., Cu, Mg, Cd, K, P, Na, Zn, and Mn) using detergent, on growth performance and survival of *Clarias gariepinus* fingerlings.

MATERIAL AND METHODS

A total of three hundred (300) fingerlings were obtained from a commercial fish farm at Ojo Lagos State, and transported to a hatchery for the experiment. At the time of collection the fish were four weeks old with an average weight of 7.5 ± 0.24 g. The fish were acclimatized for 24 hours prior to the commencement of the experiment.

Experimental Design/Procedure

The fingerlings were grouped into different plastic tanks of 5000 m^3 water. 50 g each of detergent was separately weighed and dissolved in water and left for 48 hours. The solution was measured into the different tanks labeled as D1 to D5 respectively with D as a control. The specimens were introduced to various already prepared concentrations of detergent (0 m1, 50 ml, 100 ml, 150 ml, 200 ml and 250 ml) after 24 hours acclimatization and fed to satiation using formulated feed. The experiment lasted for 8 weeks and the weight changes of the fish were measured weekly.

Feed Formulation

Fish feed of 30%CP was formulated using the Pearson square methods while the preparation of the diet was based on the description of Garling and Wilson (1977).

Determination of Growth Response

The weight gain was calculated using growth index method as described by Sveier *et al.* (2000); Khaled (2002); and Fashina-Bombata and Hammed (2010).

Chemical Evaluation of Experimental Fish

Samples of experimental diets and fingerlings were analyzed for proximate composition according to the methods of A.O.A.C. (1995).

Physicochemical Parameters

Water quality indicators were monitored closely by determining pH, temperature, dissolved oxygen and total ammonia levels in the water as described by Boyd (1998).

Survival

Percentage mortality was calculated using the formula:

% Survivability = number of fish stocked – number of mortality x 100

Determination of Wet Acid Digestion of P, Na, Mg, Cd, Mn, Pb, Zn, and Cu

0.5g of grinded sample (*Clarias gariepinus*) material (oven dry 60^0C) was weighed into a 125 ml Erlenmeyer flask washed with acid and distilled water. Perchloric acid of about 4 ml, 25 ml conc. HNO_3 and 2 ml conc. H_2SO_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 40-50 ml 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 was filter completely with a wash bottle into a 100 ml Pyrex volumetric flask and stored in acid washed plastic bottles. The elements P, K, Na, Mg, Cd, Mn, Pb, Zn and Cu were measured by atomic absorption spectrophotometry (AAS), using a Varian Spectra atomic absorption spectrophotometer, Buck Scientific 210 GVP model. All determination was done in triplicates and a spike sample was used to verify the accuracy of the procedure (AOAC 1995, Olowu *et al.,* 2012b).

Statistical Analysis

Means of parameters were subjected to analysis of variance [ANOVA] while levels of significance were determined at 5% confidence limit using Duncan Multiple Range Test (Duncan 1955).

RESULTS AND DISCUSSION

Proximate and gross compositions of the diet with different ingredients used are presented in Table 1, summary of the growth, metals concentration, water quality parameters and percentage survival are presented in Tables 2, 3, 4, and 5, respectively.

Table 1: % Proximate and Gross Composition of Feed Ingredient at 30% C.P.

Proximate Composition		**Gross Composition of Feed Ingredient**	
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		

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: Concentration of Metals in *Clarias gariepinus* treated with Detergent.

Conc. (ml)	Metals (mg/g)								
	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 4: Water Quality Parameters of Fish in Detergent Solution.

Concentration (ml)	**D 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	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	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	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 5: Percentage Survival of Fish in Detergent Solution.

D Control 0 ml	D_1 50 ml	D_2 100 ml	D_3 150 ml	D_4 200 ml	D_5 250 ml
100%	80%	28%	16%	0%	0%

Experimental investigations generally revealed the presence of zinc (Zn), Lead (Pb), Copper (Cu), Cadmium (Cd), and minerals such as; Sodium (Na), Potassium (K), Magnesium (Mg), and Phosphorus (P) in the samples analyzed. 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.

Mean value of Zn varies between 0.75 mg/g and 1.08 mg/g (Table 3). The value was by far higher than the reported value of 0.02 mg/g from rivers of South Carolina (Koli *et al.,* 1978) as shown in Figure 1 but lower than those of Kaduna River, Nigeria (Nwaedozie, 1998). Alabaster and Lloyd (1980) 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 levels of Cd (0.03-0.05 mg/g) recorded not significant, however, the values was higher than the reported values of <0.01 mg/g in rivers of South Carolina (Koli, *et al.,* 1978). The highest Cd level recorded is an indication of anthropogenic input. Cadmium has been known to have contributed to pulmonary disease, reduced glucose tolerance, severe kidney and liver damage and death in human beings (Voogt, *et al,* 1985).

Also, levels of Pb varied between 1.94 mg/g and 2.50 mg/g in the fish sample. The mean values recorded were statistically significantly ($p<0.05$) compared with reported mean value of 0.56 mg/g from Kaduna River, Nigeria (Nwaedozie, 1998). Lead is known as a deadly and cumulative poison even when consumed in small quantities and is capable of deadening nerve receptors in man (Bodansky and Latner, 1987, Nwadinigwe 1999).

The mean levels of Mn recorded ranges from 0.11 to 0.14 mg/g, these levels are below the range set by FEPA. The range value of copper (0.064-0.081 mg/g) recorded in sampled fish was statistically significant ($p<0.05$) from 0.11-11.9 mg/g observed by Oyewo (1998) when he tested the toxicity of this metal on some pelagic and benthic fish species. Copper levels were statistically significant ($p<0.05$) compared with other metals investigated in this study. Potassium levels in fish bred in fertilizer is high compare to fish in detergent. This simply explains that the level of potassium in the environment greatly affect the level of potassium in the fish. This is in line with the findings of Taylor (1997) and Okoye (1991) who opined that fishes are susceptible to physical and chemical in the environment and may reflect in blood composition. There was slight variation in Phosphorus level. This variation was explained by Santosh et *al.,* (2007) 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, the level fall within the tolerable limits, except for ammonia and dissolved oxygen. It has been report earlier that the amount of oxygen in water is not as constant as in air but fluctuates markedly depending on depth (Olowu *et al*., 2010). The mean dissolved oxygen values obtained ranges from 0.4 ± 0.1 to 4.00 ± 0.4, these values are relatively lower than the range considered desirable for fish survival at 30˚ C (Boyd, 1992).

The values of the pH is good for fish growth and survival as this falls within the range considered good for fish survival by Boyd (1992). The temperature and salinity values are characteristic of a tropical freshwater ecosystem (Adeniyi, 1986). The values of carbon dioxide are within the FEPA (1991) guidelines, but the ammonia was relatively higher than the limits set by FEPA (1991).

The above may imply an increased toxicity with the raised values of the physcio- chemical parameters. Powell and Fielder (1982) 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

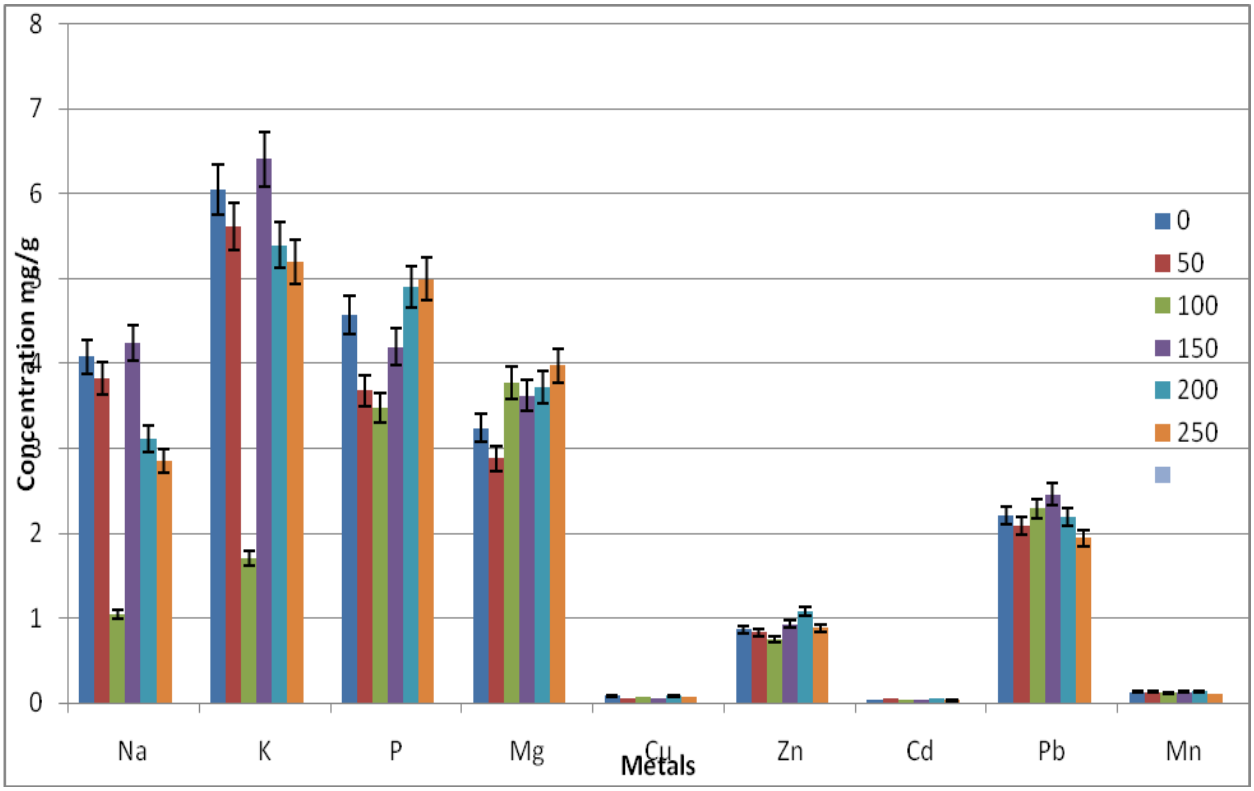

Figure 1: The Bar Chart Showing the Concentration Levels of Metals in the Sampled Fish Species.

e.g. reduced oxygen solubility (Ananthakrishnan and Kutty 1974).

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 Kuma and Krishnamoorthi (1983) who indicated ammonia as the main cause of toxicity of pollutant from industrial plant and where death occurs.

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 200 ml and 250 ml, survival was at a quick decline as none of the fish survive, meanwhile the fishes increased their activity to cope with rise in level of concentration, but the 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.

CONCLUSION

As a result of recent information on the state of productivity and pollution by human activities in coastal settlement, useful aquatic organisms are depreciated. This research conducted on fingerlings of *Clarias gariepinus* under cultured environment using detergent as an indicator relates the effect that these posed in the natural environment on the species. It is therefore concluded that 50ml of detergent in 5000m^3 of water are the safe concentration for *Clarias gariepinus* under cultured environment.

More so, in view of the various demand made on the environment under the disguise of exploiting the resource by man and industrial revolution, there is need to bear in mind the possible negative effect of these pollutants on the environment. Thus monitoring status of the water quality of water body for all seasons to ascertain its effect on the fisheries of the area should be ensured by the Environmental Protection Agency of the state. Environmental impact assessment should be carried out on all the industries found along the water body and the vessels used on the water for transportation and fishing. Also periodic Environmental auditing should be made mandatory, while installation of pollution facilities may be practicable and reasonable for existing industries as well as recycling units for waste from the industries and fish farms, meanwhile with monetary penalties and suspension of license of incorporation of defaulting industries.

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