

Full Length Research Paper

Bioconcentration of metals in the body muscle and gut of *Clarias gariepinus* exposed to sublethal concentrations of soap and detergent effluent

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The toxicity of Sublethal concentrations of effluents from a soap and detergent industry were investigated on African catfish *Clarias gariepinus* using a renewable static bioassay. The trend of bioconcentration of metals in the muscle and gut of the test organisms differs significantly ($p < 0.05$) and it followed the order, gut > muscle. The result revealed that the muscle had the least concentration of manganese at 0.1×10^{-3} mg/kg and 10.7×10^{-3} mg/kg recorded for zinc as the highest. While the highest iron concentration of 15.80×10^{-3} mg/kg was recorded in the gut tissues of *C. gariepinus*, but mercury had the least concentration of 1.00×10^{-3} mg/kg. It was revealed that fish can bioaccumulate heavy metals from a polluted environment, which may result in impairment of natural population size; thus consumption of fish from such polluted environment should be discouraged.

Key words: Bioconcentration, *Clarias gariepinus*, effluent, toxicity.

INTRODUCTION

Metal contamination of aquatic ecosystems has long been recognized as a serious pollution problem. When fish are exposed to elevated levels of metal in a polluted aquatic ecosystem, they tend to take these metals up from their direct environment. (Dupreez and Steyn, 1992; Seymore, 1994). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi et al., 2007; Vosyliene and Jankaite, 2006; Ashraj, 2005).

Transport of metals in fish occurs through the blood where the ions are usually bound to proteins. The metals are brought into contact with the organs and tissues of the fish and consequently accumulated to a different extent in different organs or tissues of the fish. Most heavy metals released into the environment find their way into the aquatic environment as a result of direct input, atmospheric deposition and erosion due to rainwater; therefore aquatic animals may be exposed to elevated levels of heavy metals due to their wide use for anthropo-

genic purposes, (Kalay and Canli, 2000).

Contamination of a river with heavy metals may cause devastating effects on the ecological balance of the aquatic environment, and the diversity of aquatic organisms becomes limited with the extent of contamination (Suziki et al., 1988).

Once heavy metals are accumulated by an aquatic organism, they can be transferred through the upper classes of the food chain. Carnivores at the top of the food chain including humans, obtain most of their heavy metal burden from the aquatic ecosystem by way of their food, especially where fish are present so there exist the potential for considerable biomagnifications (Cumbie, 1975; Mance, 1987; Langston, 1990).

However all the metals taken up by fish are not accumulated because fishes have the ability to regulate their body metal concentration to some extent (Romanenko et al., 1986). Heath (1987) showed that excretion of metals could occur through the gills, bile (via faeces), kidney and skin. There are five potential routes for a pollutant to enter a fish. These routes are through the food, non-food particles, gills, oral consumption of water and the skin. Once the pollutants are absorbed, they are transported by the blood to either a storage point (that is, bone) or

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to the liver for transformation and storage (Oronsaye and Ogbebo, 1995). If the pollutants are transformed by the liver, they may be stored there or excreted in the bile or passed back into the blood for possible excretion by the gills or kidneys, or stored in fat, which is an extra hepatic tissue (Heath, 1987).

The effect of a pollutant on a target organism may either be lethal or sublethal (Manson, 1991). Lethal effect occurs quickly and death is usually the criterion, sublethal concentration of toxic pollutants may affect the behaviour of organisms that may lead to reduction in the fitness of the natural population.

Heavy metals are non-biodegradable and once they enter the environment, bioconcentration occurs in the fish tissue in the case of aquatic environment, by means of metabolic and biosorption processes (Hodson, 1988; Carpena et al., 1990; Wicklund-Glynn, 1991).

Many researchers have been concerned with the physiological effects and bioconcentration patterns of individual metals in aquatic ecosystems. Senthil et al. (2008) reported that bioconcentration of zinc followed the order of liver > kidney > intestine > muscle of *Chana punctatus* after chronic exposure to zinc. Trace metals like copper, iron and zinc are readily concentrated in different fish tissues (Villegas-Navarro and Villarreal-Trevino, 1989; Grobler van Heerden et al., 1991; Mohan and Choudhary, 1991; Peres and Pihan, 1991; Pelgrom et al., 1995; Odiete, 1999; Adewoye et al., 2005). It has further been shown that uptake of sublethal concentrations of these metals leads to altered physiological processes which reduces the normal functioning of the organism (Grobler et al., 1989; Wepener et al., 1992). Trace metals, copper and cadmium, and the organochlorine pesticide, dieldrins have all been shown to alter metabolism in fish (Mc Geer et al., 2000; Rajotte and Coutre, 2002).

This study focuses on the bioaccumulation of metals in the body muscle and gut of African catfish *Clarias gariepinus* exposed to sublethal concentrations of soap and detergent effluents.

MATERIALS AND METHODS

The waste water (effluent) used for the toxicity test was collected from Global soap and detergent industry Ilorin, Kwara State. The collections were made daily between 8:00 am to 9:00 am for two weeks. The wastewaters were then pooled together to avoid variability in concentration. The samples were then kept in the refrigerator to avoid further activities of microorganisms.

The test organisms used (*C. gariepinus*) ranged between 20 - 22 cm in length, 120 - 150 g in weight and they were collected from the Ministry of Agriculture, Fishery Division, Ogbomoso, Oyo State. The fish specimens were then transported to the laboratory in two large plastic containers that have been previously rinsed with 5% potassium trioxonitrate to remove any adhered metals. The test organisms were then kept in two large plastic baths containing aerated borehole water. They were then acclimated for fourteen days during which they were fed with 2 g of 35% crude protein pellet feed once daily.

Renewal bioassay test was employed in the experimental set up. Range finding tests were carried out in order to determine the concen-

trations of the effluent used. Ten *C. gariepinus* each was placed in six different plastic containers containing well aerated bore-hole water. Fish were then exposed to detergent effluent at sublethal concentrations of 0.0 (control), 0.002, 0.003, 0.004, 0.005 and 0.006 mgL⁻¹ of concentration for 4 weeks. All the experiments were set up in two replicates.

Careful observations were then made to note the number of mortalities of the test organisms. The specimens were dissected to remove the whole body muscle and gut, which were then kept in the freezer prior to analysis. The dissected parts were oven dried at 70 - 73°C until constant weight was obtained. The specimens were then grounded to fine powder and stored in desiccators in order to avoid moisture accumulation before digestion. The digestion procedure was then carried out as described by (Kotze et al., 2006).

The heavy metals were analyzed using PYE UNICAM Atomic Absorption Spectrophotometer (AAS). The obtained data were statistically analyzed by using one way analysis of variance (ANOVA) followed by Duncan multiple range tests as a post-hoc test, with the aid of SPSS 10 computer statistical software package.

RESULTS

Physico-chemical analysis

Table 1 show the results of the physico-chemical characteristics of the detergent effluent used and the values obtained shows some level of deviations FEPA (1991) standard for effluent discharge into any category of water bodies.

Observed fish behaviour

Two hours after the introduction of the effluent into the plastics containing the test organisms, test organisms were observed to exhibit erratic swimming, frequent movement to water surface gasping for air, with their opercula and mouth moving rapidly. The fish were observed to later exhibit reduced activities (swimming and feeding). Colouration change from black to pale black was also observed, the fish later became weak with thick mucus covering the entire fish body and their barbells became reddened.

Mortality response

Figure 1 shows the mortality response of the test organisms to the detergent effluent used.

Table 2 shows the bioaccumulation of metals in the body tissue of the test organisms at sublethal level. There were significant differences ($p < 0.05$) among the levels of concentration. Zinc had the highest concentration of $10.7 \times 10^{-3} \text{ mg kg}^{-1}$ and the least one was recorded for manganese at concentration of $0.10 \times 10^{-3} \text{ mg kg}^{-1}$.

Bioaccumulation of the metals in the gut of the test organisms is shown in Table 3. The bioaccumulation level also differed significantly ($p < 0.05$). Iron had the highest bioaccumulation level at $15.8 \times 10^{-3} \text{ mg kg}^{-1}$ and the least was recorded for mercury at $1.00 \times 10^{-3} \text{ mg kg}^{-1}$.

Table 1. Physicochemical characteristics of detergent effluent.

Parameters	Detergent Effluent (mg l ⁻¹)	F.E.P.A 1991 Specification (mg l ⁻¹)
p ^H	8.7	6.9
DO	3.0	5.0
BOD	1.2	5.0
Total suspended solids	56	30
Oil and grease	5.0	10.0
Alkalinity	55.0	45.0
Iron	0.5	1.0
Cadmium	0.06	<1.0
Chromium	ND	<1.0
Sulphide	ND	0.2
Nitrate	8.5	20
Cyanide	0.0	20
Lead	0.015	<1.0
Copper	0.0	<1.0
Zinc	1.2	<1.0

KEY
ND: NOT DETECTED

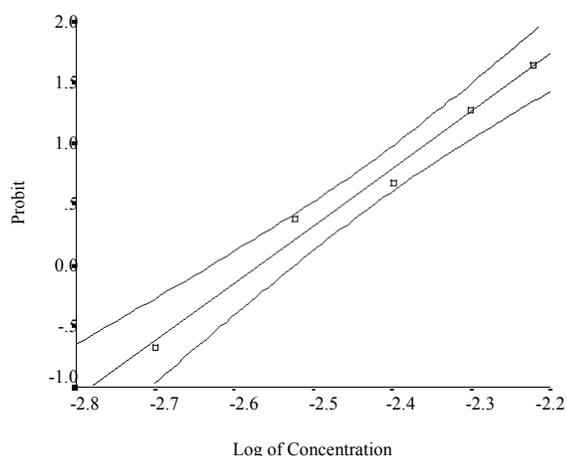


Figure 1. Probability plot for deaths response of the test organism.

DISCUSSION

The physico-chemical characteristics of the effluent revealed that there were high total suspended solids, high pH level and low dissolve oxygen content. This might have resulted from the organic loads in the effluent, which serves as a suitable medium for microorganisms that competes with the test organisms for the utility of the limited available oxygen. Most of the parameters investigated in the physico-chemical characteristics of the effluent showed deviation from the Federal Environmental Protection Agency (1991) safe limit for waste discharge into water bodies, thus the discharge of the effluent into water bodies over time might lead to the

bioaccumulation of metals in fish tissues and organisms in such water bodies.

In this study, the fish exposed to detergent effluent were observed to be highly irritable and they displayed erratic swimming, they were seen at the water surface frequently with their opercula and mouths moving rapidly. Toxic effect of the effluent causes the depletion of the oxygen content of the medium. This might have led to the reactions of the test organisms like erratic swimming and frequent surfacing. Activities of test organisms like swimming and feeding reduced drastically and they became very weak since they could no longer feed well. Colour changes from black to pale black with mucus covering the entire body in response to the toxic effect of the effluent. The mucus covering the entire body of the test organisms might have resulted from the excretion of some accumulated metals in their tissues. Khalaf et al. (1985) supported this observation; he reported that the skin is an important excretory organ for heavy metals; presumably by means of mucus (Heath, 1987). Varanasi and Markey (1978) reported that sloughing off of mucus from the fish skin surface assists in reducing the bioaccumulation of metals.

The 4-week LC₅₀ recorded in this study indicated that at 0.003 mgL⁻¹ concentration of the effluent in an aquatic habitat, half the natural population of such habitat would have died or impaired. In this study, it was observed that mortality response of the test organism increases as concentration of the effluent increases as can be seen in Figure 1. Also accumulation of metals in the body muscle and gut of the test organisms increased as the concentration of the detergent effluent increases (p < 0.05). This result was confirmed by Adewoye et al. (2005) who found that the accumulation of metals in muscles of *C. gariepinus* exposed to cassava waste water was

Table 2. Levels (\pm se) of bioaccumulated metals in the body muscle of *C. gariepinus* exposed to sublethal concentrations of detergent effluent for 4weeks.

METALS mg/kg ¹ Conc. mg l ⁻¹	LEAD	MERCURY	COPPER	IRON	MANGANESE	ZINC	CADMIUM
	(Pb) (x 10 ⁻³)	(Hg) (x 10 ⁻³)	(Cu) (x 10 ⁻³)	(Fe) (x 10 ⁻³)	(Mn) (x 10 ⁻³)	(Zn) (x 10 ⁻³)	(Cd) (x 10 ⁻³)
0.0 00	1.37 ^a \pm 0.01	0.00 ^a \pm 0.00	1.00 ^a \pm 0.00	0.00 ^a \pm 0.00	0.00 ^a \pm 0.00	0.80 ^a \pm 0.00	0.15 ^a \pm 0.00
0.002	3.51 ^b \pm 0.01	0.00 ^a \pm 0.00	2.00 ^a \pm 0.00	4.10 ^b \pm 0.00	0.00 ^a \pm 0.00	1.65 ^b \pm 0.00	0.50 ^b \pm 0.01
0.003	3.60 ^c \pm 0.01	0.00 ^a \pm 0.00	4.00 ^b \pm 0.00	7.35 ^c \pm 0.00	0.10 ^b \pm 0.00	2.85 ^c \pm 0.00	1.22 ^c \pm 0.00
0.004	3.73 ^d \pm 0.01	0.00 ^a \pm 0.00	4.50 ^{bc} \pm 0.00	8.80 ^d \pm 0.00	0.20 ^c \pm 0.00	6.00 ^d \pm 0.00	1.50 ^d \pm 0.00
0.005	3.81 ^e \pm 0.01	0.00 ^a \pm 0.00	4.50 ^{bc} \pm 0.00	9.25 ^e \pm 0.00	1.90 ^d \pm 0.00	6.95 ^e \pm 0.00	1.62 ^e \pm 0.01

Table 3. Levels of bioaccumulated metals in the gut of *C. gariepinus* exposed to sublethal concentration of detergent effluent.

METALS mg/kg ⁻¹ Conc. mg l ⁻¹	LEAD	MERCURY	COPPER	IRON	MANGANESE	ZINC	CADMIUM
	(Pb) (x 10 ⁻³)	(Hg)(x 10 ⁻³)	(Cu)(x 10 ⁻³)	(Fe)(x 10 ⁻³)	(Mn) (x 10 ⁻³)	(Zn) (x 10 ⁻³)	(Cd) (x 10 ⁻³)
0.000	0.10 ^a \pm 0.00	0.00 ^a \pm 0.00	0.00 ^a \pm 0.00	0.00 ^a \pm 0.00	0.00 ^a \pm 0.00	2.00 ^a \pm 0.00	0.53 ^a \pm 0.01
0.002	2.92 ^b \pm 0.01	0.00 ^a \pm 0.00	0.00 ^a \pm 0.00	5.25 ^b \pm 0.00	0.00 ^a \pm 0.00	2.00 ^a \pm 0.00	1.10 ^b \pm 0.00
0.003	3.72 ^c \pm 0.01	0.00 ^a \pm 0.00	2.50 ^b \pm 0.00	5.85 ^{cd} \pm 0.00	1.90 ^b \pm 0.00	2.75 ^b \pm 0.00	1.12 ^c \pm 0.00
0.004	4.59 ^d \pm 0.01	0.00 ^a \pm 0.00	4.00 ^c \pm 0.00	5.80 ^c \pm 0.00	2.70 ^c \pm 0.00	5.45 ^c \pm 0.00	1.31 ^d \pm 0.00
0.005	4.82 ^e \pm 0.01	1.00 ^b \pm 0.00	5.00 ^c \pm 0.00	5.95 ^d \pm 0.00	3.20 ^d \pm 0.00	6.25 ^d \pm 0.00	1.69 ^e \pm 0.01
0.006	4.99 ^f \pm 0.01	3.50 ^c \pm 0.00	4.50 ^c \pm 0.00	15.80 ^e \pm 0.00	3.45 ^e \pm 0.00	6.40 ^e \pm 0.00	1.91 ^f \pm 0.00

Means within the same column having the same alphabet (s) are not significantly different ($P > 0.05$) S.E = standard error.

concentration dependent.

High concentration of copper and zinc were observed in the body muscle of the test organisms after four weeks of exposure of the effluent. This is in accordance with the observation made by Hughes and Flos (1978); Bezuidenhout et al. (1990); Seymore, (1994); Seymore et al. (1996); Oronsaye and Ogbebo (1995); Senthil et al. (2008) who stated that copper and zinc mainly accumulate in the skin, bone, liver, gill, heart, kidney and muscle tissue of fish. The recorded high level of manganese in the body muscle at higher concentration of the effluent agreed with the work of Coetzee (1996) who recorded high concentration of manganese in the flesh of *Labeo umbratus* and *C. gariepinus* from the Olifant River in South Africa.

It was observed from this study that the bioaccumulation of the analyzed metals was significantly higher in the gut at higher concentration than the whole body muscle ($P < 0.05$) when compared with the control group (gut > muscle) this agrees closely with the study done by kotze et al. (2006) and Senthil et al. 2008. The reason for this could be due to the fact that alimentary canal can be considered as the interface of the organisms and its ambience. It is the system which receives the metal directly from ambient source

(Matheissen and Brafield, 1975). This could also be attributed to the fact that metals may be ingested along with water and food into the stomach and finally into the gut where absorption of digested food materials takes place. It was observed in this study that accumulation of heavy metals in the body muscle followed the order Zn > Fe > Mn > Cu > Pb > Hg > Cd. Similarly, in the case of the gut, the order was Fe > Zn > Cu > Pb > Hg > Mn > Cd. In all the heavy metals analysed, the bioaccumulation of iron and zinc proportion was significantly increased in the gut and body muscle of *C. gariepinus*. The result conformed closely with the work done by Vinodhini and Narayanam (2008) where they carefully observed the trend of bioaccumulation of heavy metals in various organs of the fresh water fish *Cyprinus carpio* (common carp) exposed to heavy metal contaminated water system.

Cadmium and mercury was found in this study to have low levels of bioaccumulation in the gut and muscle tissues investigated. These metals can remain in the body like other heavy metals for long period of time and can bioaccumulate for many years after exposure to low levels (Ground work, 2002), thus if a fish contaminated with these metals finds its way into man's food chain biomagnifications of such heavy metal might occur and

this becomes harmful to man's health. The result indicates that the heavy metal contamination definitely affects the aquatic life of the fresh water fish. Hence, a scientific method of detoxification is essential to improve the health of these economic fish in any stressed environmental conditions.

However, the high concentration of the analyzed metals in the whole body muscle and gut investigated could be due to the storage role played by the gut and body muscle of fish. Fish in an ecosystem contaminated by trace metals are known to accumulate significantly more metals in edible muscle tissue than do fish in an uncontaminated ecosystem (Murphy et al., 1978; Du Preez et al., 1993).

The recorded significant differences in the bioconcentration of metals in the fish under study may be attributed to the physiological changes, behavioural and metabolic responses of the fish to the effluent.

Conclusion

It can be conclusively deduced from this study that fish has the tendency to bioaccumulate heavy metals in a polluted environment. Since virtually all metals investigated were found in higher concentrations except for cadmium and mercury, which may even be biomagnified over time, so Government should put up food quality assurance agency that will ascertain the quality of foods that enters the market for human consumption or the pre-existing agencies such as NAFDAC (National Agency for Food Drug Administration and Control) should be more empowered.

Therefore, Federal government should also enact laws that will ensure that industries make use of standard waste treatment plants for the treatment of their wastes. The government should not only enact laws on discharge of wastes, they should ensure compliance with these laws by enforcing the treatment of wastes by industries before they are being discharged into water bodies. Likewise, the general public should be given the awareness on the proper ways of handling and disposal of wastes and not to see water bodies as a sewer.

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