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## Original Article

# The effects of exposure to multiple stressors of Lead (Pb) and Cypermethrin on biochemical profiles of African catfish, *Clarias gariepinus*

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**ABSTRACT:** The African catfish (*Clarias gariepinus*) is a widely cultured fish species in many African countries because of its rich nutritional quality. In this study, the impacts of exposure to common environmental contaminants; a heavy metal (Lead) and a pyrethroid insecticide (Cypermethrin) on the biochemical contents of *C. gariepinus* was investigated. Juveniles of *C. gariepinus* were exposed to borehole water (control), 2 mg/L Pb, 0.5 µg/L cypermethrin or 2 mg/L Pb + 0.5 µg/L cypermethrin for 96 h after which the total protein levels, glycogen contents and total cholesterol in the liver and muscle were determined. Fish exposure to cypermethrin and a mixture of cypermethrin and Pb resulted in a significant decrease in glycogen and protein levels but a significant increase in the cholesterol levels in liver and muscle ( $p < 0.05$ ). There was no significant difference between the control and the group exposed to 2 mg/L Pb. The glycogen and total cholesterol levels were significantly higher in the liver in groups exposed to cypermethrin and the mixture of cypermethrin and Pb ( $p < 0.05$ ) while the total protein levels were higher in the muscle, although this difference was not statistically significant. The results of this study suggest that environmental pollution of aquatic environments have adverse effects on the health of resident fish as well as other aquatic life.

**KEYWORDS:** Environmental contamination; African catfish, *Clarias gariepinus*; glycogen content; total protein; cholesterol level.

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## INTRODUCTION

The aquatic ecosystem is constantly being threatened by the release of various environmental contaminants into the environments (Islam and Tanaka, 2004; Zhou *et al.*, 2008). Various human activities such as agriculture and mining have contributed to the release of different forms of pollutants into the environments. For example, agricultural pesticides and insecticides are routinely applied to agricultural crops and planting fields in order to control pests and unwanted weeds (Anyim, 2004). There are also reports of anthropogenic release of various heavy metals into the environments (Samecka-Cymerman and Kempers, 2001). Lead is one of such heavy metals that are very common in various ecosystems; land, water and air. Lead is released into the

environment through mining activities and wastes from different industrial and commercial usage e.g. in lead batteries, lead pipes, electrical cables, tennis rackets, glazing bar for stained glass etc. While most of these chemicals are most of the times not directly applied into the aquatic environments, however natural phenomenon such as leaching and erosion gets them into aquatic environments; aquatic environments serve as sinks and they can then be accumulated to levels where they interfere with the physiology of resident organisms. Fish being one the most important occupants of the aquatic environments provides human with rich nutritive diet. While various indices such as oxidative stress biomarkers, changes in plasma ions, changes in hormonal levels, changes in biochemical

enzymes etc. have been employed to show the impacts of environmental contamination on the physiology and biochemistry of fish inhabiting polluted sites, only few of these indices links the physiological status of stressed fish to the nutritional values that could be derived when such contaminated fish are consumed. However, change in biochemical profiles such as glycogen, protein and lipid levels serves these purposes.

There are reports of toxic effects of Pb and cypermethrin on aquatic organisms. Cypermethrin results in mortality in the freshwater fish, *Poecilia reticulata* (Gautam and Gupta, 2008), disruption of glycogen metabolism in the estuarine clam, *Marcia opima* (Tendulka and Kulkarni, 2012), reduced growth and abnormal changes in gonadal development in *Odontesthes bonariensis* (Carrizuri et al., 2009). Pb is neurotoxic and there are reports of Pb resulting in behavioral impairment in adult zebrafish (Chen et al., 2012), hematological changes in African catfish (Adeyemo, 2009) etc. Despite the vast documentation of these toxicity data, not much has been done to show the likely implications of exposure to mixture of lead and cypermethrin on the alteration of biochemical profiles in fish. This present study is therefore designed to investigate the effects of exposure to either cypermethrin or Pb on the biochemical contents of *C. gariepinus*, as well as the effects of exposure to the mixture of cypermethrin and Pb. We also showed the tissue-specific patterns in the alteration of biochemical profiles in contaminated *C. gariepinus*.

## MATERIALS AND METHODS

### Experimental organisms and contaminant exposure

Juvenile catfish (N=80) of average length  $10 \pm 2.5$  cm were collected from a commercial fish hatchery located in Abere, Osun State, Nigeria (07°43'55N; 004°31'07E). They were then transported live in plastic containers to the Research Laboratory of the Department of Biological Sciences of the Osun State University, Osogbo, Nigeria. Fish were acclimatized to laboratory conditions for seven days prior to commencement of experiments. During acclimatization, water was exchanged every 24 h and fish were fed with commercial fish pellets twice daily. At the end of the seven-day acclimatization period, fish were divided into four groups of about twenty fish per group; control, 2 mg/L Pb, 0.5 µg/L cypermethrin and 2 mg/L Pb + 0.5 µg/L. Experiments were conducted in 7.5 L plastic buckets and contaminant exposure lasted for 96 h. Three separate buckets containing 6–7 fish each were used for each treatment.

### Sample preparation for biochemical analyses

After the 96 h period of contaminant exposure, 6 fish were sampled from each treatment group (at least 2 fish from each of the three buckets in the treatment group) and were

ethanized with an overdose of benzocaine (200 mg/L), and the liver and muscle were carefully dissected out for biochemical analyses.

### Glycogen contents determination

Liver and muscle glycogen content was determined using the anthrone reagent in a method developed by Nicholas et al. (1956) and modified by Reddy et al. (1994). Briefly, liver tissue was homogenized in 10% trichloroacetic acid (TCA). A volume of 0.2 ml of the homogenate was then transferred into a microcentrifuge tube and precipitated by adding 1 ml of absolute ethanol. The solution was then incubated for 3 h at 37 °C. After the 3 h incubation, the solution was centrifuged at 3,000 g for 15 min. After this step, 0.2 ml of the supernatant was transferred into another tube for glucose measurement. A 1-ml volume of freshly prepared anthrone reagent was added to the sample and the mixture boiled in a waterbath for 15 min, after which it was allowed to cool to room temperature. The absorbance was then measured at 620 nm using an UV/Vis spectrophotometer (Jenway, Model 6400), and liver and muscle glycogen was expressed as mg glycogen per mg tissue wet weight, using a standard curve obtained with glucose solutions.

### Total protein determination

The total protein levels in the liver and muscle were quantitatively determined using the Bradford assay (Bradford, 1976). Bovine serum albumin (BSA) was used as the standard.

### Total cholesterol determination

The total cholesterol levels in the liver and muscle samples of *C. gariepinus* were determined colorimetrically using Liebermann-Burchard method reported in Burke et al. (1974). This reaction is based on reaction between acetic anhydride and cholesterol in chloroform solution to form a characteristic blue colour. Briefly, samples were homogenized in ice-cold phosphate buffer (pH, 7.4). Then, 0.2 ml of the homogenate was transferred into centrifuge tubes that contain about 5 ml of alcohol-acetone solvent solution. The tube was then immersed into a boiling water bath until the solvent began to boil, after which the tubes were removed from the water bath and allowed to cool to room temperature. The content of the tube was then centrifuged lightly, and the supernatant was discarded while the pellet was allowed to evaporate to dryness in a boiling water bath. The residue was then reconstituted by adding 2 ml of chloroform, followed by addition of 2 ml of acetic anhydride/concentrated sulphuric acid mixture. The tubes were left in the dark for about 10 min, after which the absorbance was read at 620 nm. The total cholesterol levels in liver and muscle was expressed as mg total cholesterol per mg tissue wet weight, using a standard curve obtained with cholesterol solutions.

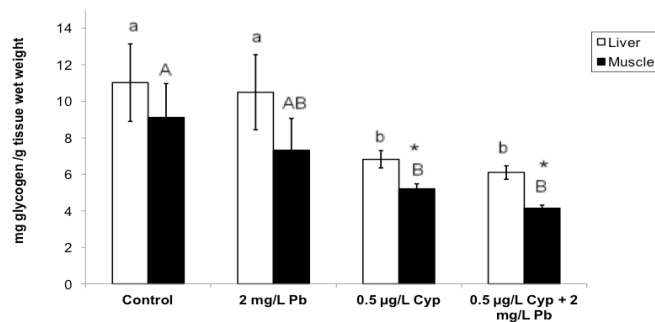
**Statistical analysis**

Data were parametrically analyzed since there was no significant deviation from normal distribution. Data were first subjected to a two-way mixed model analysis of variance, factors being the treatment group and experimental buckets (since multiple buckets were used per treatment group). Because the experimental bucket effect was not significant, this factor was eliminated from the model, and the data were subsequently analyzed using a one-way analysis of variance. For each tissue samples, the difference in mean glycogen contents, protein levels and total cholesterol was detected using one-way analysis of variance. This was followed by Tukey’s multiple comparison tests whenever there was a significant difference. We also performed a student’s t- test in order to show the difference in the tissue glycogen, protein and cholesterol levels between the liver and the muscle. All statistics were performed using JMP version 9.0 software (SAS Inc., 2010). For reporting purposes, data were expressed as mean ± standard error and statistical significance was assumed at  $P \leq 0.05$ .

**RESULTS**

**Liver and muscle glycogen contents**

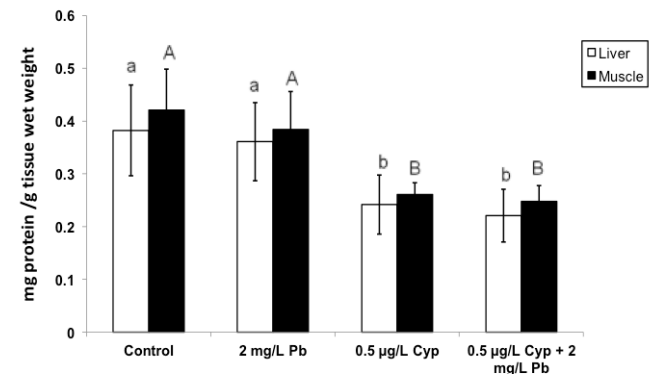
There was a significant difference in the glycogen content in both the liver ( $F_{3, 20} = 7.5208, p = 0.0029$ ) and muscle samples ( $F_{3, 20} = 6.3306, p = 0.0046$ ) among the groups. Fish exposed to cypermethrin and a mixture of cypermethrin and lead had a significantly reduced level of tissue glycogen compared to the control and those exposed to 2 mg/L Pb. The Liver glycogen level is higher in the liver than in the muscle, although this difference was only significant in the groups exposed to cypermethrin and the mixture of cypermethrin and Pb (Figure 1).



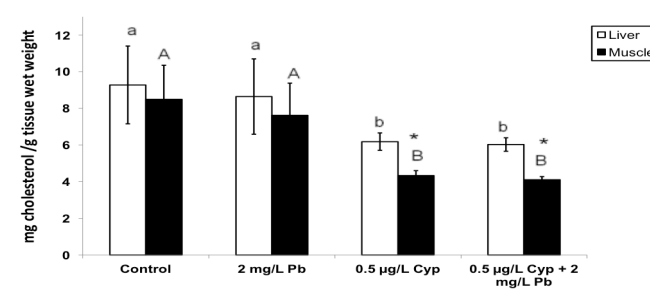
**Figure 1: The tissue glycogen contents in *C. gariepinus* exposed to either borehole water (control), 2 mg/L Pb, 0.5µg/L cypermethrin or 0.5µg/L cypermethrin + 2 mg/L Pb. Each bar is mean ± standard deviation (n = 6). Bars with different letters are significantly different (upper case = muscle, lower case = liver). The asterisk indicates a significant difference between the liver and muscle levels for a particular treatment**

**Liver and muscle protein level**

There was a significant reduction in the total protein level in both the liver ( $F_{3, 20} = 8.7402, p = 0.0046$ ) and muscle samples ( $F_{3, 20} = 9.8306, p = 0.0032$ ) after contaminant exposure. As in glycogen levels, total protein decreased significantly in fish exposed to either 0.5 µg/L cypermethrin or those exposed to the mixture of cypermethrin and Pb. However, unlike in glycogen content data where the values were higher in the liver, the total protein levels in the muscle were higher in all the treatment groups but the difference was not significant (Figure 2).



**Figure 2: The tissue total protein levels in *C. gariepinus* exposed to either borehole water (control), 2 mg/L Pb, 0.5µg/L cypermethrin or 0.5µg/L cypermethrin + 2 mg/L Pb. Each bar is mean ± standard deviation (n = 6). Bars with different letters are significantly different (upper case = muscle, lower case = liver)**



**Figure 3: The tissue total cholesterol levels in *C. gariepinus* exposed to either borehole water (control), 2 mg/L Pb, 0.5µg/L cypermethrin or 0.5µg/L cypermethrin + 2 mg/L Pb. Each bar is mean ± standard deviation (n = 6). Bars with different letters are significantly different (upper case = muscle, lower case = liver). The asterisk indicates a significant difference between the liver and muscle levels for a particular treatment**

### Liver and muscle total cholesterol level

The total cholesterol level was significantly higher in the liver ( $F_{3, 20} = 5.6404$ ,  $p = 0.0146$ ) and in the muscle ( $F_{3, 20} = 6.5402$ ,  $p = 0.0096$ ) in the groups exposed to 0.5  $\mu\text{g/L}$  cypermethrin and those exposed to the mixture of cypermethrin and lead compared to the control and the group exposed to just 2 mg/L Pb. The total cholesterol levels in the liver were significantly higher than the levels in the muscle in the groups exposed to cypermethrin and the mixture of cypermethrin and Pb (Figure 3).

### DISCUSSION

Fish are consumed for their rich nutritive value and affordability. Fish, being one of the most abundant groups in the aquatic ecosystem are sometimes exposed to all sorts of environmental assaults such as heavy metal pollution and agricultural chemicals contamination, which can consequently have a serious effect on the nutritive quality of fish inhabiting the contaminated aquatic environments. In Nigeria, the African catfish is probably the most cultured fish species owing to its numerous desirable characteristics; high tolerance of extreme environmental conditions, ease of cultivation, high quality flesh, distinctive taste and texture, relatively low fat and absence of intramuscular spines (El-Shebly, 2006; Khwuanjai *et al.*, 1997; Luckhoff, 2005).

The results of this study indicate that environmental pollution with heavy metals and run-offs of agricultural chemicals into aquatic environments have adverse effects on the biochemical contents of fish inhabiting contaminated sites. *C. gariepinus* that were exposed to cypermethrin and lead showed a significant decrease in glycogen contents compared to the control. This result is consistent with the findings of Tendulka and Kulkarni (2012), which reported disruption of glycogen metabolism in the estuarine clam, *Marcia opima* exposed to cypermethrin. The decreased glycogen levels in the exposed fish could be due to increased glycogenolysis in order to make more glucose available in response to a higher energy demand associated with contaminant exposure (Hargreaves *et al.*, 1995). In addition, the low glycogen levels in stressed fish could be due to contaminants' interference with the mechanisms involved in glycogen synthesis since there are reports of heavy metals and organic pesticides directly modulating the processes involved in glycogen biosynthesis (Misra *et al.*, 1991). The higher liver glycogen contents in comparison to the lower level in the muscle could be due to the fact that liver is principally involved in numerous metabolic activities and more importantly, contaminant detoxification occurs in the liver (Sahi *et al.*, 2010). These processes are energetically expensive, so a higher quantity of energy substrate molecules in the liver is quite understandable.

The tissue protein level in *C. gariepinus* decreased significantly after exposure to cypermethrin and a mixture of

cypermethrin and lead. The reduced protein level in the liver and muscle of exposed fish could infer a possible use of protein as additional energy substrate molecule so as to augment the available glycogen. There are reports that in circumstances of low glucose or glycogen levels, other molecules could be used by the cells to produce energy required for essential metabolic processes (Brooks, 1987). It is also possible that the reduction in protein level is actually due to more utilization of protein in the repair of damaged tissue as a result of contaminant exposure or possibly a reduction in the rate of synthesis of protein. (Reichert *et al.*, 1998). The total protein level in the muscle is higher than the level measured in the liver. This may be an indication that the muscle of *C. gariepinus* is richer in protein than the liver, although one cannot also overrule the possibility that the lower protein level in the liver is due to the fact that more energy demanding metabolic activities occurs in the liver, which could have used some of the available protein as an alternative source of energy. In contrast to the reduced glycogen and total protein levels in the liver and muscle of exposed fish, exposure to cypermethrin and lead resulted in an increase in the levels of total cholesterol in the liver and muscle. This may be an indication that contaminant exposure could disrupt the catabolism of cholesterol thus enhancing the build-up of cholesterol in the muscle and liver (Dutta and Haghghi, 1986). This may have a direct negative impact on the physiology of exposed fish, and in particular to man when such fish with high cholesterol levels are consumed.

In conclusion, we show in this study that the alteration in tissue biochemical profiles is a reliable index of stress in *C. gariepinus*. While there was no direct evidence that exposure to 2 mg/L Pb has a significant adverse effect on the biochemical profile of *C. gariepinus*, however there are some evidence of synergistic effects in this study since alteration in the biochemical profile was more significant in fish exposed to a mixture of cypermethrin and Pb, in comparison to those exposed to just only cypermethrin.

### ACKNOWLEDGEMENTS

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