

## Ionoregulatory Impairment in African Catfish (*Clarias gariepinus*) Exposed to Combined Stressors of Lead and Cypermethrin

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### Abstract

The contamination of aquatic ecosystem with heavy metals and pesticides has increased dramatically over the years, and this has generated a lot of attention by environmental experts. There are reports on impacts of different single toxicants on aquatic organisms but not much has been done to investigate the impacts of mixtures of toxicants on aquatic life. In this study we investigated the effects of multiple stressors of Pb and cypermethrin on ionoregulation in the African catfish (*Clarias gariepinus*). Adults *C. gariepinus* were exposed to either borehole water (control), 2 mg/L Pb, 0.5 µg/L cypermethrin or a mixture of 2 mg/L Pb and 0.5 µg/L cypermethrin for 96 h, after which the levels of plasma Na<sup>+</sup>, Cl<sup>-</sup> and K<sup>+</sup> were determined as a measure of branchial ionoregulatory disturbance. The exposure of *C. gariepinus* to 0.5 µg/L cypermethrin resulted in a significant reduction in plasma Na<sup>+</sup> (43% decrease) and Cl<sup>-</sup> (31% decrease) but a 2-fold increase in plasma K<sup>+</sup> concentrations. Exposure to a mixture of cypermethrin and Pb resulted in 53% decrease in plasma Na<sup>+</sup>, 40% decrease in plasma Cl<sup>-</sup> and a 3-fold increase in plasma K<sup>+</sup>. There was no significant difference between the control and those exposed to just only 2 mg/L Pb for all the parameters measured. The results of this study indicated that exposure of aquatic organisms to combined stressors of heavy metals pollution as well as pesticide contamination could have a serious effect on the physiology especially on ionic regulation in the exposed fish.

**Keywords:** fish, ionoregulation, multiple stressors, plasma electrolytes

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### Introduction

The rate of toxic substances released in the environment has increased rapidly due to various human activities (Chakkaravarthy, 2003). The advancement in technology and increased global industrialization has increased the production of toxic heavy metals e.g. lead, and the aquatic environments often serve as the sink for these substances (Sobha *et al.*, 2007). In the recent times, there have been a noticeable increase in the usage of agricultural pesticides, this could be in response to increasing global population which have exerted more pressure on human to respond to the attendant problem of food insecurity arising from global population explosion (Pimentel, 2001). There is the lure to overuse these agricultural chemicals such that they may bioaccumulate at toxic levels in the environments. While pesticides and other agricultural chemicals are not often applied directly into the aquatic environment, they may eventually get into water bodies through leaching and run-offs, thus posing serious risks to resident aquatic organisms (Forstner and Prosi, 1979).

The gill is the major site of ionoregulation in fish but

unfortunately it serves as the primary target for the acute toxic action of contaminants (Evans *et al.*, 2005). For example, the exposure of a freshwater fish, rainbow trout to copper impaired branchial ionoregulation by both inhibiting the active transport of Na<sup>+</sup> and Cl<sup>-</sup>, and increasing the ionic permeability of the gills which results in a net loss of these ions (Lauren and McDonald, 1985; Lauren and McDonald, 1986). Lead is a non-biodegradable naturally occurring heavy metal and may potentially become accumulated in various tissues of aquatic organisms. There are reports on the toxicity of Pb in fish; alteration of biochemical and haematological parameters in the rainbow trout (Ates *et al.*, 2008), oxidative stress in *Clarias batrachus* (Maiti *et al.*, 2010), histological changes in the gills of neotropical fish, *Prochilodus lineatus* (Martinez *et al.*, 2004) and behavioural alteration in the rainbow trout (Weber *et al.*, 1997). Cypermethrin is a synthetic pyrethroid insecticides, which have widespread application in the control of insects and pest in agriculture, in homes, and in the garden. It has been proposed as possible alternatives to some organochlorine insecticides, that have been prohibited in many countries (Jee *et al.*, 2005).

As a result of its numerous applications, the potential of its discharge into the aquatic environment has increased and there are reports that cypermethrin is extremely toxic to fish (David *et al.*, 2004).

Most studies on the effects of environmental pollutants are limited to reporting the effects of either pesticide treatment or metal exposure individually, and only few reports are available on the effects of multiple stressors of pesticides contamination and heavy metal pollution on aquatic organisms. Studying the effects of mixture of contaminants on organisms is particularly necessary because aquatic organisms are exposed to combinations of stressors in their environment. This study is therefore designed to investigate the joint effects of a pyrethroid insecticide, cypermethrin and a heavy metal, lead (Pb) on ionoregulation in the African catfish, *Clarias gariepinus*.

## Materials and methods

### Experimental organisms and contaminant exposure

Adult catfish ( $n = 80$ ) of average weight  $300 \pm 10$  g were collected from a commercial fish hatchery located in Abere, Osun State, Nigeria ( $07^{\circ}43'55\text{N}$ ;  $004^{\circ}31'07\text{E}$ ). They were transported live in plastic containers to the Biology Laboratory of the Department of Biological Sciences of the Osun State University, Osogbo, Nigeria. In the laboratory, fish were divided into four groups of about twenty fish per group; control, 2 mg/L Pb, 0.5  $\mu\text{g/L}$  cypermethrin and 2mg/L Pb + 0.5  $\mu\text{g/L}$  cypermethrin. 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 exposed to either borehole water (control), 2 mg/L Pb, 0.5 $\mu\text{g/L}$  cypermethrin or 2mg/L Pb + 0.5  $\mu\text{g/L}$ . The choice of the concentrations stated above for each of this compound was due to the available toxicity data that have been reported for a similar fish species in literature (Olaifa *et al.*, 2003; Stephenson, 1982). Experiments were conducted in 7.5 L plastic buckets, and there were three replicates for each treatment group of about 6-7 adult fish per bucket. Contaminant exposure followed an acute exposure and lasted for 96 h.

### Blood collection and plasma ions determination

At the end of the 96 h exposure to contaminants, fish were mildly anaesthetized with benzocaine, and blood samples were collected from the caudal vein of each fish. The blood was collected in anticoagulant centrifuge tubes. Plasma was obtained by centrifugation of blood at 3,000 rpm for 10 min. The plasma samples were then stored at  $-20^{\circ}\text{C}$  until analysis. The levels of plasma  $\text{Na}^+$  and  $\text{K}^+$  were determined by flame photometry (Jenway PFP clinical flame photometer) while the plasma  $\text{Cl}^-$  ion level was measured spectrophotometrically using the method reported by Yokoi (2002).

### Statistical analyses

The data were checked for normality using the Shapiro-

Wilk test and there was no significant deviation from normal distribution. The differences in the mean plasma  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  levels among the groups were detected using a one-way analysis of variance (ANOVA) test, this was followed by Tukey's multiple comparison tests whenever there was a significant difference. All statistics were performed using the SPSS version 10.0 statistical software (SPSS Inc., Chicago, IL, USA). Differences were considered significant at  $p \leq 0.05$ . Data were presented as mean  $\pm$  standard deviation.

## Results

The plasma  $\text{Na}^+$  levels differed significantly among the groups ( $F_{3,16}=54.76$ ;  $p=0.0006$ ). Fish exposure to 0.5  $\mu\text{g/L}$  cypermethrin resulted in a significant decrease (43%) in plasma  $\text{Na}^+$  level compared to the control ( $p < 0.001$ ). The decrease was even more pronounced in the group exposed to the mixture of cypermethrin and 2 mg/L Pb (53%). There was no significant difference in plasma  $\text{Na}^+$  levels between the group exposed to 2 mg/L Pb and the control (Fig. 1). Again, we observed a significant difference in plasma  $\text{Cl}^-$  levels among the exposure groups ( $F_{3,16}=8.7649$ ;  $p=0.0046$ ). The plasma  $\text{Cl}^-$  levels followed a similar trend observed for the plasma  $\text{Na}^+$  levels. Contaminant exposure caused a significant reduction in plasma  $\text{Cl}^-$ . There was approximately 31% decrease in plasma  $\text{Cl}^-$  level in the group exposed to only 0.5  $\mu\text{g/L}$  cypermethrin whereas exposure to a mixture of cypermethrin and Pb resulted in 40% decrease in the plasma  $\text{Cl}^-$  levels. Exposure to 2 mg/L Pb resulted in approximately 11% decrease in the plasma  $\text{Cl}^-$  levels (Fig. 2).

While contaminant exposure resulted in a decrease in the levels of plasma  $\text{Na}^+$  and  $\text{Cl}^-$  however exposure tended to significantly increase the levels of plasma  $\text{K}^+$  levels in *C. gariepinus* ( $F_{3,16}=5.6274$ ;  $p=0.0096$ ). Exposure to 5  $\mu\text{g/L}$  cypermethrin resulted in approximately 2-fold increase in the plasma  $\text{K}^+$  levels while exposure to a mixture of cypermethrin and 2 mg/L Pb resulted in a 3-fold increase in the plasma  $\text{K}^+$  levels. There was no significant change in the plasma  $\text{K}^+$  levels between the group exposed to 2 mg/L Pb and the control (Fig. 3).

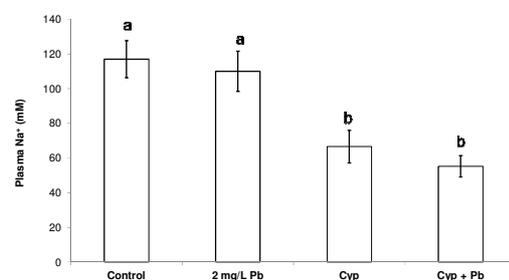


Fig. 1. Mean ( $\pm$ SE,  $n=6$ ) plasma  $\text{Na}^+$  in *C. gariepinus* exposed to either borehole water (control), 2 mg/L Pb, 0.5 $\mu\text{g/L}$  cypermethrin or 0.5 $\mu\text{g/L}$  cypermethrin + 2 mg/L Pb. ( $n=6$ ). Bars with different letters are significantly different

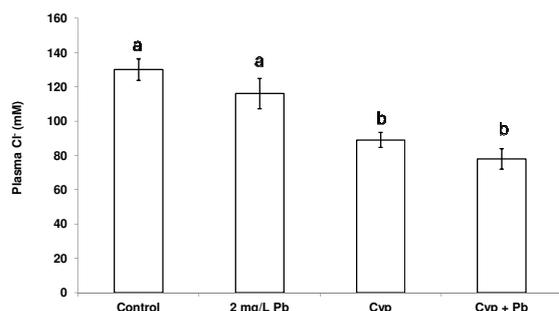


Fig. 2. Mean ( $\pm$ SE, n=6) plasma Cl<sup>-</sup> in *C. gariepinus* exposed to either borehole water (control), 2 mg/L Pb, 0.5 $\mu$ g/L cypermethrin or 0.5 $\mu$ g/L cypermethrin + 2 mg/L Pb.. Bars with different letters are significantly different

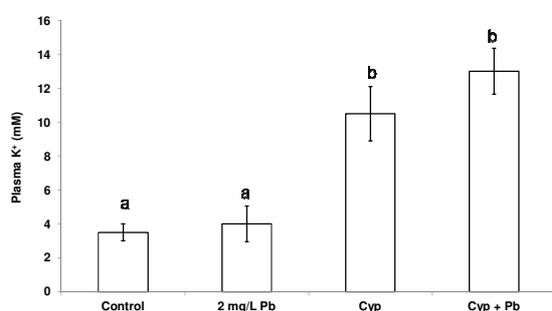


Fig. 3. Mean ( $\pm$ SE, n=6) plasma K<sup>+</sup> in *C. gariepinus* exposed to either borehole water (control), 2 mg/L Pb, 0.5 $\mu$ g/L cypermethrin or 0.5 $\mu$ g/L cypermethrin + 2 mg/L Pb. Bars with different letters are significantly different

## Discussion

The results of this study showed that exposure of *C. gariepinus* to either 0.5  $\mu$ g/L cypermethrin or a mixture of cypermethrin and Pb resulted in ionic disturbance, revealed by imbalance in the levels of important plasma ions. Current understanding holds that the osmo/ionoregulatory disturbances induced by pesticides and metals were associated with an increased epithelial permeability and inhibition of active ion uptake, which may result in a significant decrease in the number of active chloride cells (De Boeck *et al.*, 2001; Monteiro *et al.* 2005). There are reports that exposure of fish to certain heavy metals competitively inhibited the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase, a tetrameric protein that is located in the basolateral region of the gills and that have been shown to be actively involved in ionic regulation in teleosts (Adeyemi *et al.*, 2012; Atli and Canli, 2011). Fish exposure to Pb or cypermethrin caused a drastic loss of both plasma Na<sup>+</sup> and Cl<sup>-</sup>. This is particularly as expected since it has been reported that plasma Na<sup>+</sup> and Cl<sup>-</sup> tend to be similarly affected by waterborne toxicants (McDonald *et al.*, 1989). The ionoregulatory disturbance was more pronounced in the group that was exposed to the mixture of cypermethrin and Pb, thus ascertaining the complex effects of exposure to multiple stressors in an

aquatic ecosystem. This therefore necessitates the need for development of more models that could be used to extrapolate the effects of exposure to multiple stressors in ecological risk assessment, since most of the available models concentrates on the effects of a single stressor which is very far from the situation in most aquatic ecosystems where organisms are exposed to a wide array of xenobiotics simultaneously.

There was no evidence that exposure of *C. gariepinus* to 2 mg/L Pb resulted in a significant decrease in the levels of plasma ions in this study. This may be an indication that this exposure concentration (2 mg/L Pb) is too low to elicit such physiological disturbance in this fish. Firat *et al.* (2011) showed that exposure of Nile Tilapia to even a lower concentration of Pb (0.05 mg/L Pb) did cause a significant reduction in the levels of plasma Na<sup>+</sup> and Cl<sup>-</sup> although this effect was observed during longer exposure periods. It may therefore be inferred that *C. gariepinus* is not as sensitive to environmental contamination as other surrogate fish species. However, caution must be taken before generalizing that *C. gariepinus* is not as sensitive to environmental contamination as other surrogate fish species since the current study followed an acute exposure; the result could be quite different if the experiment followed a chronic exposure.

The plasma K<sup>+</sup> levels increased significantly in groups that were exposed to either cypermethrin alone or a mixture of cypermethrin and Pb. This is consistent with the findings of Bernard and Grazyna (1999) and Firat *et al.* (2011) whose studies showed that plasma K<sup>+</sup> levels actually increased in fish following exposure to heavy metals and pesticides. The excessive accumulation of K<sup>+</sup> in the plasma of exposed fish could be an indication of renal disturbance in this fish, since K<sup>+</sup> is normally excreted by the kidneys.

In conclusion, exposure of *C. gariepinus* to either cypermethrin or a mixture of cypermethrin and Pb at concentrations tested in this study was found to cause impairment of ionoregulation. The ionic disturbance was not detected in *C. gariepinus* that were exposed to only 2 mg/L Pb. The additive effects of exposure to mixture of cypermethrin and Pb in this study re-emphasizes the need to direct more attention towards including more data on mixture toxicity as a first step towards a more robust risk assessment.

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