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Lead biosorption and histological changes in gills and liver of juvenile african catfish (*Clarias gariepinus*) treated with neem (*Azadirachta indica*) and mango leaves (*Mangifera indica*)

Abstract

Lead (Pb) is non-biodegradable in nature, thus accumulate in the environment especially the aquaculture. This study was aimed at investigating lead biosorption and histological changes in gills and liver of juvenile African catfish (*Clarias gariepinus*) treated with neem (*Azadirachta indica*) and mango leaves (*Mangifera indica*). A total of 180 healthy juveniles of *Clarias gariepinus* were used for the period of six (6) weeks of this experiment. They were divided into two groups of pulverized neem and mango leaves with ninety (90) in each. They were further divided into three groups of thirty (30) with each comprising of two experimental groups of 10mg/L and 15mg/L of Pb and control. Experiment of biosorption was conducted in muscle, gills and liver while the histology was conducted in the gills and liver only. The results of pulverized neem showed a significant difference ($P<0.0001$) of biosorption of 15mg/L Pb in muscle, gills and liver with higher value (0.0017) in muscle while in pulverized mango, both 10mg/L and 15mg/L appeared statistically significant ($P<0.0001$) with liver (0.003) and gills (0.003) respectively had higher statistical values. There was irreversible histological damages of the gills in PNL and 15mg/L of Pb and PNL and 10mg/L Pb and a reversible histological damage in PML and 15mg/L Pb and PML and 10mg/L Pb groups. The histology of the liver appeared irreversibly damage in groups PNL and 15mg/L of Pb and PML and 15mg/L and reversibly damage in PNL and 10mg/L Pb and PML and 10mg/L Shows fatty degenerated parenchyma with focal necrosis with slight sinusoidal distortion. In conclusion, biosorption of lead is more effective with PML since the damage to the tissues are minimal and reversible.

Keywords: Lead, Biosorption, Histology, Neem, Mango, Fish.

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Introduction

Heavy metals contamination is of great toxic impact on the environment especially in aquaculture.[1] Lead as one of the heavy metals get introduced into streams by various industrial activities or exploitation of this metal.[2] It is non-biodegradable in nature, thus makes their threat multiplied by their accumulation in the environment through food chain.[3] In water bodies, it is absorbed by fish through the skin, gills, digestive tracts and some key metabolic organs resulting in sub lethal or lethal tendency, depending on the concentration of the accumulated metal in the fish.[4]

However, heavy metals in water were successfully removed through conventional methods which include chemical precipitation and oxidation, ion exchange, membrane separation, reverse osmosis and electro dialysis. These methods have been reported to be costly, not very effective, and require high energy input. The search for alternative new technologies for the removal of toxic metals or heavy metals from waste water has focused attention on biosorption.[4] Biosorption is very effective, easiest, safest and of low cost method for the treatment of waste effluents containing heavy metals.[5,6] Recent studies have shown that lead (Pb) can be removed using plant materials such as *Ficus benghalensis L.*,[7] rape seed,[8] *Ralstonia solanacearum*.[9] However, there are paucity of reports on lead biosorption and histological changes on gills and liver of the juvenile African catfish (*Clarias gariepinus*) treated with neem (*Azadirachta indica*) and mango leaves (*Mangifera indica*).

Materials and methods

Experimental site and location

The experiment was conducted from 30th September to 13th November, 2020 in the Fisheries Department, University of Agriculture, Makurdi, located in the Southern Guinea Savannah zone of Nigeria.

Preparation of pulverized *Azadirachta indica* and *Mangifera indica* leaves:

Neem (*Azadirachta indica*) and mango (*Mangifera indica*) leaves were obtained from the University of Agriculture, Research Farm. The leaves were chopped, washed and allowed to dry at laboratory temperature at 28 ± 3˚C. It was oven dried at 70˚C for 30hrs and 200˚C for 6hrs. The dried leaves were pulverized into fine powder and sieved with 0.25-0.5mm pore size and preserved in Ambian Bottles for use in subsequent experiment.

Source of water:

Water used was collected from the Borehole of Fisheries Department, University of Agriculture Makurdi, Benue State Nigeria. Temperature and pH were determined at the beginning and the end of the experiment using Hanna Multi- Parameters water tester Model HI 98129. The dissolved oxygen (DO) was measured using VWR Metre Model L89023

Experimental design:

A total of 180 healthy juveniles of *Clarias gariepinus* were used for this study. They were divided into two groups of pulverized neem and mango leaves with ninety (90) in each. They were further divided into three groups of thirty (30) with each comprising of two experimental groups of 10mg/L and 15mg/L of Pb and control. Each group was in an aquarium with dimension of 100×50cm containing 50 litres of water. Each treatment was replicated into three (3) and arranged in a completely randomized design at temperature of 25±3°C. (PNL). The experiment lasted for six (6) weeks.

Preparation of samples for analysis of lead (Pb) in fish

Approximately 10grams of the harvested samples (muscle, liver and gills) were homogenized. 1gram of the homogenate was used to achieve digestion. The 1gram was placed in a 100ml flask and mineralized under reflux using the mixture of 6ml, 2ml and 4ml of HNO_3, $HClO_4$ and H_2O_2 respectively. The procedure took 6 hour until a clear solution was obtained. The procedure was prepared in triplicate and carefully transferred to a flask. The digest were then analyzed for Pb using an atomic absorption spectrophotometer (Model: AAS Buck Scientific 205) with aqueous calibration standards prepared from the stock standard solutions of the respective elements as described by.[10]

Histology

The fish were sacrificed to remove gills and liver for histological examination. The removed samples were immediately fixed in 10% neutral buffered formalin for at least 24 hours. The formalin-fixed samples were then subjected to dehydration in grades of ascending alcohol concentrations (70%–100%), cleared in xylene, embedded in paraffin, and sectioned at 5 µm. The sections were then stained with hematoxylin-eosin and viewed with light microscope.

Statistical analysis

All data was analyzed using the JMP version 13 (2016).[11] It was analyzed ($P<0.05$) using a one way analysis of variance (ANOVA). Significant differences in least square means were separated using a Turkey post-hoc test.

Result

Physicochemical analysis of the aquarium

The physicochemical analysis of the aquarium before the commencement of the experiment showed that, temperature 27.1°C, pH 7.80, dissolved oxygen (DO) 6.15mg/L, total dissolved solid (TDS) 221mg/L and electricity conductivity (EC) 223µS/cm. After the experiment, data showed were temperature 27.7°C, pH 7.80, DO 6.70mg/L, TDS 440mg/L and EC 1661µS/cm (Table 1).

Table 1 Physicochemical analysis of the aquarium before and after the experiment

Temperature (0C)		DO (mg/L)	TDS (mg/L)	EC (µS/cm)
Before experiment	27.1	6.15	221	223
After experiment	27.7	6.7	440	1661

Biosorption:

The results of Pb biosorption was observed in muscle, gills and liver using pulverized neem and mango leaves were recorded in Tables 1 and 2. There was no significant difference in Pb biosorption of muscle, gills and liver at 10mg/L Pb using pulverized neem (Table 2). At 15mg/L Pb, the result showed a significant difference ($P<0.0001$) in muscle, gills and liver with higher value (0.0017) in muscle using pulverized neem (Table 2). In pulverized mango, both 10mg/L Pb and 15mg/L Pb were statistically significant ($P<0.0001$). The liver (0.003) and gills (0.003) respectively had higher statistical values (Table 3).

Table 2 Lead biosorption of tissues of juvenile *Clarias gariepinus* in aquarium treated with pulverised neem leaves

Least square mean concentration of lead			
Tissues	10 mg/L	15 mg/L	Control
Muscle	0.001	0.0017a	0
Gills	0.001	0.0010b	0
Liver	0.003	0.0010b	0
SEM	0	$4.55e^{-5}$	0
P value	-	0.0001*	0

Means with different superscript (a-b) in the same column are significantly different. Standard Error of Mean (SEM).

Table 3 Lead biosorption of tissues of juvenile *Clarias gariepinus* in aquarium treated with pulverised mango leaves

Least square mean concentration of lead			
Tissues	10 mg/L	15 mg/L	Control
Muscle	0.0011b	0.0011b	0
Gills	0.0011b	0.0030a	0
Liver	0.0030a	0.0010b	0
SEM	$7.36e^{-6}$	$5.17e^{-6}$	0
P value	0.0001*	0.0001*	-

Means with different superscript (a-b) in the same column are significantly different. Standard Error of Mean (SEM).

Histopathology of the gills and liver

The gills of juvenile Clarias gariepinus exposed to graded dose of lead (Pb), pulverized neem leaves (PNL) and mango leaves (PML) were observed varied histological changes (Figure 1). At PNL and 15mg/L of Pb it was observed congested gill filament vessel, partially obliterated secondary lamellae. The result obtained in PML and 15mg/L Pb was typically a depletion of primary gill filaments. At PML and 10mg/L Pb, it was observed congested gill filament vessel while at PNL and 10mg/L Pb the primary gill filaments were congested vessel and secondary lamellae appeared obliterated (Figure 1).

The liver of juvenile Clarias gariepinus exposed to PNL and 15mg/L of Pb was observed depletion of hepatic parenchyma and infiltration lymphocytic cells into the parenchyma (Figure 2). The hepatic parenchyma appeared depleted with fatty degeneration in PML and 15mg/L Pb (Figure 2). The result of PNL and 10mg/L Pb appeared to have fatty degenerated parenchyma with focal necrosis while PML and 10mg/L Pb was observed to have fatty degenerated parenchyma with sinusoidal distortion (Figure 2).

Discussion

The neem and mango extracts have been reported to have antiparasitic and antibacterial properties. At higher concentration, the extracts can be slightly toxic/lethal in fish.[12-16] Higher concentrations of the extracts results in convulsion, somersaulting, difficulty in swimming, immobilization and loss of balance, which will subsequently lead to death.[14,17] Low concentration of Pb in tissues observed indicates the potency of pulverized neem and mango leaves. This appeared even much lower than the required standard concentration of Pb in

tissues.[18] With low concentration of Pb, biosorption appeared to be more effective. Similar findings observed decrease uptake of CU (II) with increased biosorbent dosage.[19,20]

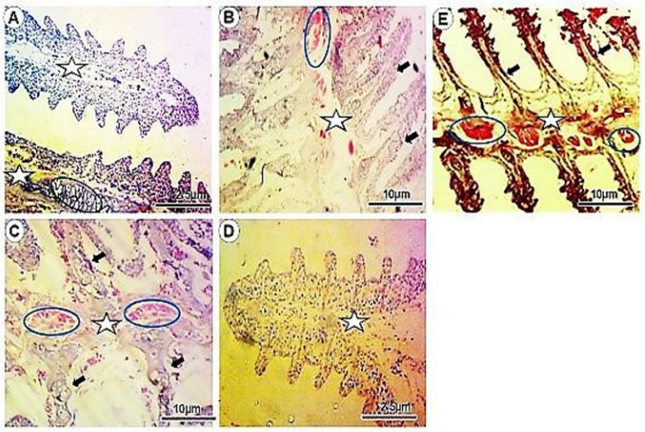

Figure 1 Photomicrograph of the gills of juvenile *Clarias gariepinus* exposed to graded dose of lead (Pb), pulverized neem leaves (PNL) and mango leaves (PML). (A) Control: Shows primary gill filaments (star) with lateral extension kwon as secondary lamellae. Gill filaments have a central cartilaginous support (black oval surrounding the cartilage), H and E. Scale bar, 2.5µm. (B) PNL and 15mg/L of Pb: Shows congested gill filament vessel (blue oval), partially obliterated secondary lamellae. (C) PNL and 10mg/L Pb: Shows primary gill filaments (star) with congested vessel (blue oval), and obliterated secondary lamellae (D) PML and 15mg/L Pb: Shows primary gill filaments (star) with depleted cells. (E) PML and 10mg/L Pb: Shows congested gill filament vessel (blue oval).

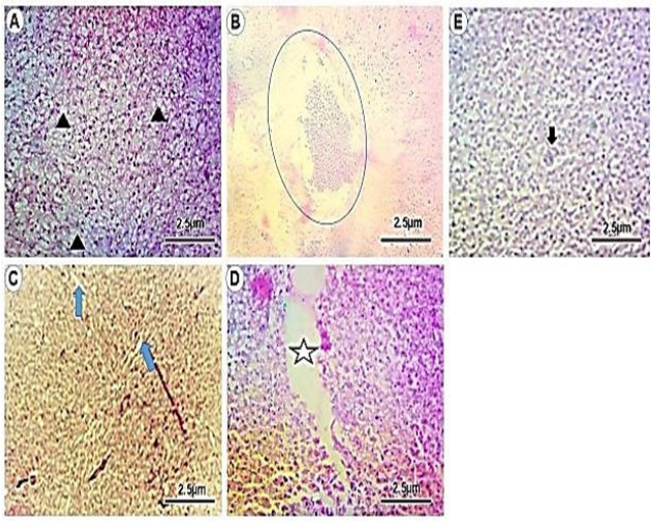

Figure 2 Photomicrograph of the liver of juvenile *Clarias gariepinus* Fig. Photomicrograph of the livers of juvenile *Clarias gariepinus* exposed to graded dose of lead (Pb), pulverized neem leaves (PNL) and mango leaves (PML). (A) Control: Shows the parenchyma composed of polyhedral hepatocytes typically with central nuclei. The nuclei of these cells are elongated and protrude into the sinusoidal lumen. (B) PNL and 15mg/L of Pb: Shows the depletion of hepatic parenchyma and infiltration lymphocytic cells into the parenchyma. (C) PNL and 10mg/L Pb: Shows fatty degenerated parenchyma with focal necrosis (black arrow). (D) PML and 15mg/L Pb: Shows the depletion of hepatic parenchyma (star) with fatty degeneration. (E) PML and 10mg/L PbShows fatty degenerated parenchyma with sinusoidal distortion (blue arrow).

Though biosorption was higher with higher PNL and PML concentration, the organs appeared reversibly and irreversibly damaged depending on the concentration of Pb. Histopathological examination revealed congested gill filament vessel, partially obliterated secondary lamellae, primary gill filaments with congested vessel (blue oval), and obliterated secondary lamellae, gill filaments with depleted cells. The extracts at concentrations of 5 and 7.5 gL^{-1} damaged the gills, but not influenced the osmoregulatory capacity of the *Prochilodus lineatus* (Winkaler et al., 2007). Slight pathological changes at the 30 mg/l concentration of NeemAzal T/S and significant changes at the highest concentration of 60 mg/l with morphological changes were found in the gill such as oedema of gill lamellae, focal separation of gill lamellar surface and aggregates of mononuclear cells.[21]

In this study, the histological damages in the gills observed in PNL and 15mg/L of Pb and PNL and 10mg/L Pb appeared irreversible due congestion and obliteration of the primary gill filament vessel, obliteration of secondary lamellae. While lesions observed in PML and 15mg/L Pb and PML and 10mg/L Pb groups appeared reversible. Similar findings were reported in the liver of heteroclarias juveniles exposed to lethal and sub-lethal concentrations of chlorpyrifos.[22] Irreversible (B) PNL and 15mg/L of Pb and PML and 15mg/L Pb depletion of hepatic parenchyma and infiltration lymphocytic cells into the parenchyma. The depletion of hepatic parenchyma with fatty degeneration. Reversible PNL and 10mg/L Pb and PML and 10mg/L Shows fatty degenerated parenchyma with focal necrosis with slight sinusoidal distortion. Similar findings were obtained in freshwater cyprinid when examining lead and other metals in the liver.[23]

Conclusion

The effective decrease of Pb concentration in tissues used in this study has proven that PNL and PML are good biosorption agents. These can be effectively used in aquaculture environment with the tendency of Pb poisoning. Though PNL and PML are effective, PML seems to be more effective since the damage to the tissues are minimal and reversible.

Conflict of interest

All authors attest that there is no conflict of interest.

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