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Research Article

Pulverised Neem and Mango Leaves Biosorption Effect on Hematology of *Clarias gariepinus* (African Catfish) Cultured in Lead-Contaminated Aquarium

Koplamma Nenchini Bala[1]*, Chinwe Christy Isitua[1], Inya Joel Odo[2], Gosomji Innocent Jonah[3], Chioma Bertha Ehis-Eriakha[1,4] and Bardi I Juliet[1]

[1]Department of Biology and Forensic Science, Admiralty University of Nigeria, Ibusa, Delta State, Nigeria

[2]Department of Fisheries and Aquaculture, Joseph Sarwuan Takar University Makurdi, Benue State, Nigeria

[3]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Jos, Plateau State, Nigeria

[4]Department of Microbiology, Edo University Iyamho, Uzairue, Edo State, Nigeria

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***Corresponding author:** Koplamma Nenchini Bala, Lecturer, Biology and Forensic Science, Admiralty University of Nigeria, Ibusa, Delta State, Nigeria, E-mail: nenchinik@gmail.com

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Abstract

Lead lethal and sub-lethal impact on aquaculture affect the environment, physiology and haematological indices in fish. The research investigated the biosorption effect of pulverized neem and mango leaves on lead-contaminated aquarium cultured with *Clarias gariepinus*. A total of 180 healthy juveniles of *Clarias gariepinus* were used for the period of the experiment. They were further divided into two groups of pulverized neem and mango leaves with ninety (90) in each. This was further divided into three groups of thirty (30) with each comprising the experimental groups of 15 mg/L and 10 mg/L of Pb and control. Biosorption was conducted on environment and blood parameters status was investigated. The results of pulverized neem and mango leaves showed no significant difference ($p < 0.05$) of biosorption in the environment and physicochemical properties while blood parameters showed significant differences ($p < 0.05$). For Pulverized Neem Leaf (PNL) and 10 mg/L had the higher statistical values in Haemoglobin count (HCT) 8.327%, White Blood Corpuscle (WBC) 64.160fL, Haemoglobin (Hb) 8.070fL, Platelet total (PLT) 326.000% while Pulverized Mango Leaf (PML) with 15 mg/L of lead appeared statistically significant in Mean Corpuscular Volume (MCV) 111.500fL and corpuscular haemoglobin count (MCHC) 94.000fL. The effect of PNL and PML on physicochemical properties and blood parameters was positive remediation in contaminated environment and physiological function in aquaculture.

Introduction

Lead contamination has harmful impact on aquaculture all over the world [1,2]. Toxic heavy metal ions get introduced into streams by various industrial activities and their products during mining, refining ores, tanneries, batteries recycling, discarded phones batteries, paper making and paint production among others and these pose serious threat to water bodies or the environment [3]. Heavy metals also have a devastating effect on ecological balance of the recipient environment, the diversity of aquatic organisms [4]. According to United States Agency for Toxic Substances and Disease Registry (ATSDR) [5] ranking, lead was rated in toxicity first and in frequency and severity respectively.

This heavy metal is absorbed by fish and other organisms in the food chain and amplified by animals that feed on fish (biomagnification). Lead is non-biodegradable in nature makes their threat multiplied by their accumulation in the environment through food chain [6].

WHO [7] gave the permissible level of lead and mercury in water as 0.01 mg/L. [8] described lead and mercury as metals

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that can cause neurological disorders in humans and unhealthy environment when they get into water and food or water bodies by direct discharge of effluents and water runoff.

Biosorption is a physicochemical process that bind heavy metal ions on biosorbents biomass surface. It is said to be effective and economical, because of its relatively low cost. Also, is the easiest, safest and most cost-effective method for the treatment of waste effluents containing heavy metals [9-11]. Pulverised neem and mango leaves biosorption is through surface area, pore sizes, cellular structure, chemical compounds and the presence of functional groups and ion exchange [12,13].

Heavy metals in water can be removed successfully 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 not economical, not very effective, and require high energy input. The search for ecofriendly new technologies for the removal of toxic metals or heavy metals from waste water has focused attention on biosorption [13].

According to [14], fish were exposed to two sub-lethal concentrations (5 and 10 mg Pb/L) for 96-hour. Blood was sampled after metals exposure to check the alterations in hematological as well as serum biochemical parameters. The analysis of hematological parameters and serum biochemical parameters showed that high concentration of Pb affects more the count of red blood cells, white blood cells, hemoglobin, MCV, MCHC, MCH, total Cholesterol, Glucose, total protein and albumin than low dose which, in turn, affect physiological condition of fish.

A sublethal research carried out by Stanley and [15] reported ninety *Clarias gariepinus* juveniles (mean Length 35.0 ± 2.0 cm and mean weight 1.50 ± 5.20 g) divided into 3 groups of thirty fish each wee use for the study. The fish were exposed to zero (0) sublehal concentrations (0.0, 0.1 and 0.4 mg/L^{-1} of lead as lead chloride. The changes in the haematological parameters of the fish were assayed every seven (7) days. When compared with control, the haemoglobin concentration decreased significantly ($p < 0.05$) with increasing lead concentration and with the duration. Erythropoisesis increased significantly ($p < 0.05$) with lead concentration. The erythrocyte count differed significantly ($p < 0.05$) in the between treatment groups. There was significant leucocyte as the test concentration increased and with MCV, MCH and MCHC decreased as the test concentration increased except on day 28 when the MCHC increased with concentration ($p < 0.05$).

This research work exposed cats fish into a lethal environment with biosorption remediation (pulverized neem and mango leaves) and the effects were investigated on haematology of *clarias gariepinus* (african cat fish) and cultured in lead-contaminated aquarium. However, there were paucity of reports on effects of biosorption on haematology of fish and other animals.

Materials and methods

Experimental site and location

The experiment was conducted from 30th September to 13th November, 2023 in the Fisheries Department, University of Agriculture, Makurdi, located on latitude 7°48'24"N and longitude 8° 37'03"E.

Sampling technique

Experimental design: A total of 180 healthy juveniles of *Clarias gariepinus*, 75 mg/L of Pb and 1000g of pulverised neem and mango leaves were used for the experiment respectively. 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 10 mg/L and 15 mg/L of Pb with dosage of 55.55g of pulverized neem and mango leaves and control without PNL and PML graded dosage. 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 ℃. (PNL). The experiment lasted for six (6) weeks.

Preparation of Neem Leaves (PNL) for analysis

Azadirachta indica leaves were obtained from the University of Agriculture Makurdi Research Farm and identified in the Department of Botany Herbarium. They were taken to the Advanced Biology Laboratory where they were washed repeatedly with distilled water to remove dust and impurities. The washed leaves were allowed to dry at Laboratory temperature at 28 ± 3 ˚C. The leaves were oven dried at 70 ˚C for 30 hrs till the leaves became dry and crispy with Gallen Kamp oven model 605 England according to [16]. The dried leaves were pulverised into fine powder by using pulveriser. The powdered material was sieved with sieve of 0.25-0.5 mm pore size (NLP) and preserved in Ambian Bottles for use in subsequent experiment, according to the methods of [17,18].

Preparation of Pulverised Mango Leaf (P ML)

Mango leaves were collected from University of Agriculture Makurdi Research Farm as identified Botany Department Herbarium. The leaves were washed and sun dried for five (5) days and thereafter, oven dried at 200 ˚C for six hours in a Gallen Kamp oven Model 605 England. Dried leaves were ground with pestle and mortar and washed several times with distilled water or tap water till the entire coloured impurities were removed, again dried in hot air oven at 200 ˚C for 8 hours. The powdered leaves were sieved with sieve of desired pore size of (0.25-0.5 mm) and preserved in Ambian Bottles, according to method of [19].

Experimental water

Water used for the experiment was collected from Fisheries Department Borehole University of Agriculture Makurdi Benue State Nigeria. The following physicochemical properties such as pH, temperature before the experiment. Same physicochemical properties were also gauged at the end of the experiment.

Determination of the physicochemical properties of the aquarium water

Temperature, pH and electricity Conductivity were measured using Hanna Multi- Parameters water tester Model

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HI 98129. This was done by selecting or pressing the Mode Key and selecting the corresponding reading or figure displayed on LCD of the meter. While the DO was measured using VWR Metre Model L89023.

Blood samples collection

After the experiment, blood samples were collected from 180 juveniles cat fish from each experimental group for haematological analysis, in a complete randomised method, thirty (30) cats fish were sampled each from the two experimental group and control. 2ml of blood was also collected after six weeks of experiment (6 weeks) from the caudal peduncle of fish in each experimental unit and control. Blood collected were dispensed into tubes (Lithium heparin) containing an anticoagulant and taken to the haematology unit [20], Federal Medical Centre Makurdi for determination of Packed Cell Volume (PCV), Platelet (PLT) Haemoglobin Count Total (HCT) Mean Cell Volume (MCV) Haemoglobin (Hb), White Blood Cells (WBC) and Red Blood Cells (RBC).

Statistical method

All blood parameters, tissues and physicochemical parameters data were subjected to one way ANOVA analysis with means separated according to Fisher Least Significance Differences (F-LSDs). Significant differences in least square means were separated using a Turkey post-hoc test for tissues [21].

Results

The physicochemical analysis of the aquarium before the commencement of the experiment showed that, temperature 27.1 ℃, pH 7.80, dissolved oxygen (DO) 6.15 mg/L, Total Dissolved Solid (TDS) 221 mg/L and electricity conductivity (EC) 223 µS/cm. After the experiment, data showed were temperature 27.7 ℃, pH 7.80, DO 6.70 mg/L, TDS 440 mg/L and EC 1661 µS/cm. The (Table 1). Other physicochemical properties were not considered before and after the experiment according to Aquaculture Fisheries Department Aquarists Advice of Joseph Sarwuan Takar University Makurdi, Benue State, Nigeria. The source of water was reliable and free from Biochemical Oxygen Demand (BOD5), Chemical Oxygen Demand (COD), chloride iron (CI-) Concentration, Total Hardness (TH), turbidity and total suspended solids (TSS) but the alkalinity was within range as gauged with VWR Metre Model L89023.

Temperature in Table 2 showed there were no significant differences among the means. The above result was similarly so for pH. Dissolved Oxygen (DO) showed that, there were significant differences among the treatment means and it were in this order of decreasing magnitude: 10 mg/L, 15 mg/L and control. For Dissolved Total Solid (TDS), there were significant differences and it was in this order of magnitude: 10 mg/L, 15 mg/L and control. Electricity Conductivity (EC) means showed significant differences and the order of decreasing magnitude was similar to the above result.

Physicochemical parameters in Table 3 for temperature showed significant differences among the mean values. The differences in the temperature were in this decreasing order of lead concentration: 10 mg/L, 15 mg/L and control. pH means showed no significant differences among treatment means. For Dissolved Oxygen (DO), there were significant differences among the treatment means. The differences in the DO were in this decreasing order of magnitude: control, 10 mg/L and 15 mg/L. Total Dissolved Solid (TDS) showed that, there were significant differences among the mean values. The TDS differences were in this decreasing order of magnitude: 10 mg/L, 15 mg/L and control. Electricity Conductivity (EC) showed significant differences among the treatment means. The differences in the EC were in this decreasing order of concentrations: 10 mg/L, 15 mg/L and control.

Haemoglobin Count Total (HCT) presented in Table 4 showed significant differences among the treatment means. The differences in HCT were recorded in this decreasing order of lead concentration magnitude: 10 mg/L less than control and 15 mg/L. White Blood Corpuscle (WBC) showed significant differences among the treatment means and the differences in the WBC were in increasing order of lead concentration: 10 mg/L and 15 mg/L. For Red Blood Corpuscle (RBC), there were significant differences among the mean values and it was in this order of decreasing magnitude of lead: control less than 10 mg/L and 15 mg/L. Haemoglobin (Hb) showed significant differences among the mean values. The differences in Hb were in this decreasing order of lead concentration: control, 10 mg/L and 15 mg/L. Platelet Total (PLT) showed significant differences among treatment means with decreasing order of

Table 1: Physicochemical Analysis of the Aquarium Before and after the Experiment.

	Tempt. °C	DO (mg/L)	TDS (mg/L)	EC (µS/cm)
Before experiment	27.1	6.15	221	223
After experiment	27.4	6.5	441	1661

Table 2: Physicochemical Characteristics of the Water in the Aquarium with Lead and Pulverised Neem Leaf (PNL) after the Six Weeks of the Experiment.

Parameters	Concentrations			SD	LSD
	15 mg/L	10 mg/L	Control		
Temperature℃	28.350^{a}	27.650^{a}	27.200^{a}	27.25	1.023
pH	7.850^{a}	7.910^{a}	7.750^{a}	0.090	0.225
DO(mg/L)	5.450^{c}	6.250^{b}	6.750^{a}	0.589	0.225
TDS(mg/L)	378.000^{b}	416.000^{a}	111.000^{c}	148.000	6.364
EC(µS/cm)	753.500	834.000^{a}	221.000^{c}	298.000	0.319

Values are triplicate means. Means with different superscript in the same horizontal row are significantly different at $p < 0.05$ according to LSD.
DO: Dissolved Oxygen; TDS: Total Dissolved Solid; EC: Electricity Conductivity

Table 3: Physicochemical Characteristics of the Water in the Aquarium with Lead and Pulverised Mango Leaf (PNL) after Six Weeks of the Experiment

Parameters	Concentrations			SD	LSD
	15 mg/L	10 mg/L	Control		
Temperature℃	27.500^{b}	28.200^{a}	27.550^{b}	0.356	0.290
pH	7.775^{a}	8.000^{a}	7.850^{a}	0.125	0.297
DO(mg/L)	3.885^{c}	4.400^{b}	6.550^{a}	1.266	0.293
TDS(mg/L)	513.500^{b}	705.000^{a}	101.500^{c}	276.00	8.009
EC(µS/cm)	1028.50^{b}	1417.500^{a}	204.000^{c}	554.000	8.009

Values are triplicate means. Means with different superscript in the same horizontal row are significantly different at p <0.05 according to LSD.
DO: Dissolved Oxygen; TDS: Total Dissolved Solid; EC: Electricity Conductivity

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lead magnitude: 10 mg/L and 15 mg/L. Packed Cell Total (PCT) showed there were no significant differences among the mean values. Mean Corpuscular Volume (MCV) showed significant differences among the mean values. The differences in the MCV were in this decreasing order of lead concentration: 10 mg/L and 15 mg/L. Mean Corpuscular Haemoglobin Concentration (MCHC) showed there was significant differences among the treatment means and it was in this order of decreasing lead concentration magnitude: 15 mg/L, 10 mg/L and control (Table 4).

The Haemoglobin Count Total (HCT) showed no significant differences among the treatment means in Table 5. White Blood Corpuscle (WBC) showed there was significant differences among the mean values. The differences in the WBC were in this order of lead concentration: control, 10 mg/L and 15 mg/L. For Red Blood Corpuscle (RBC), there was significant differences among the mean values and it was in this order of decreasing magnitude of lead: control, 10 mg/L and 15 mg/L. Haemoglobin (Hb) showed significant differences among the treatment means. The differences in Hb were in this decreasing order of lead concentration: control, 15 mg/L and 10 mg/L. Platelet Total (PLT) showed significant differences in mean values and it were in this decreasing order of lead magnitude: control, 15 mg/L and 10 mg/L. Packed Cell Total (PCT) showed no significant differences among the mean values. Mean Corpuscular Volume (MCV) showed there were significant differences among the treatment means. The differences were in this decreasing order of lead concentration 15 mg/L, control and 10 mg/L. Mean Corpuscular Haemoglobin Concentration (MCHC) showed significant differences among the treatment means and it was in decreasing order of magnitude: 15 mg/L, 10 mg/L and control (Table 5).

Table 4: Effect of Pulverized Neem Leaf on the Lead Content and Quality of the Blood of *Clarias gariepinus*.

Parameters	Concentrations			SD	LSD
	10 mg/L	15 mg/L	Control		
HCT(%)	8.327^{a}	0.345^{c}	0.805^{b}	0.92	1.221
WBC(fL)	64.160^{a}	3.655^{c}	51.800^{b}	21.81	0.521
RBC(fL)	0.720^{b}	0.035^{c}	2.825^{a}	1.36	0.071
HB(g/dL)	8.070^{b}	4.240^{c}	10.025^{a}	2.20	0.373
PLT(%)	326.000^{a}	94.500^{c}	184.500^{b}	22.54	3.182
PCT(%)	$0.340h^{a}$	0.085^{a}	0.065^{a}	0.05	0.031
MCV(fL)	117.000^{a}	76.500^{c}	107.000^{b}	12.10	2.250
MCHC(g/dL)	96.200^{b}	98.310^{a}	32.775^{c}	14.66	2.368

Values are triplicate means. Means with different superscript in the same horizontal row are significantly different at $p < 0.05$ according to LSD.
HCT: Haemoglobin Count; WBC: White Blood Corpuscle; RBC: Red Blood Corpuscle; HB: Haemoglobin; PLT: Platelete; PCT: Packed Cells Total; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Haemoglobin Concentration

Table 5: Effect of Pulverized Mango Leaf on the Lead Content and Quality of the Blood of *Clarias gariepinus*.

Parameters	Concentrations			SD	LSD
	10 mg/L	15 mg/L	Control		
HCT(%)	0.570^{c}	1.205^{a}	0.805^{b}	0.28	1.221
WBC(fL)	2.740^{b}	0.155^{c}	51.800^{a}	26.00	0.521
RBC(fL)	0.045^{c}	0.120^{b}	2.825^{a}	1.41	0.071
HB(g//dL)	$4.100d^{c}$	5.370^{b}	10.025^{a}	2.79	0.373
PLT(%)	60.500^{c}	72.000^{b}	184.500^{a}	61.30	3.182
PCT(%)	0.045^{a}	0.065^{a}	0.065^{a}	0.011	0.031
MCV(fL)	104.000^{c}	111.500^{a}	107.000^{b}	3.53	2.250
MCHC(fL)	92.750^{b}	94.000^{a}	32.775^{c}	31.30	2.368

Values are triplicate means. Means with different superscript in the same horizontal row are significantly different at $p < 0.05$ according to LSD.
HCT: Haemoglobin Count; WBC: White Blood Corpuscle; RBC: Red Blood Corpuscle; HB: Haemoglobin; PLT: Platelete: PCT: Packed Cells Total; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Haemoglobin Concentration

Biosorbents and biosorption result analysis

The results of Pb biosorption as observed in muscle, gills and liver using pulverized neem and mango leaves were recorded. There was no significant difference in Pb biosorption of muscle, gills and liver at 10 mg/L Pb using pulverized neem. At 15 mg/L Pb, the result showed a significant difference ($p < 0.0001$) in muscle, gills and liver with higher value in muscle using pulverized neem. In pulverized mango, both 10 mg/L Pb and 15 mg/L Pb were statistically significant ($p < 0.0001$). The range values for biosorption reduction in PNL and PML experimental treatment were 0.0087 ppm and 0.0101 ppm while 99.98% and 99.86% recorded in percentage respectively. The bioaccumulation values in different tissues in both pulverized neem and mango leaves recorded were 0.0065 ppm and 0.0075 ppm.

Discussion

Physicochemical properties for temperature and pH with pulverized neem leaf and 10 mg/L and 15 mg/L of lead showed no differences over control. This was due to efficiency and weather condition of the Laboratory [22] reported decline in Dissolved Oxygen (DO) and other physicochemical properties in exposed fish studied under laboratory conditions. For pH, Dissolved Oxygen, Total Dissolved Solid and Electricity Conductivity treated with pulverized neem and mango leaves and 15 mg/L and 10 mg/L of lead concentrations showed significant differences over control. This was due to biosorption materials, chemical, weather of laboratory and *Clarias gariepinus* juveniles introduced in the aquaria. This agrees with which suggests [22] that it may be possible that some chemicals make use of oxygen in decomposition process; this may probably be one of the reasons for decline in oxygen in treated aquaria. Temperature, pH, Dissolved Oxygen (DO), Total Dissolved Solids (TDS) and Electrical Conductivity (EC) increased was intewoven throughout from low concentration to high.

Haematological indices are important and sensitive index to monitor physiological and pathological changes in fishes. Standard ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology (Rambhaskar and [23,24]. For Haemoglobin Count Total (HCT), White Blood Corpuscle (WBC), Red Blood Corpuscle (RBC), Haemoglobin (Hb), Platelet Total (PLT), Packed Cell Total (PCT), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Count treated with pulverized neem leaves and 15 mg/L and 10 mg/L of lead showed significant decrease in HCT, RBC, Hb, PCT, MCV, MCH and MCHC while WBC and PLT recorded higher count at 10 mg/L of lead (64.160 and 326.000) over the control. It was reported same by [14]. This may be due

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to the *Clarias gariepinus* adaptation strategy. The population of exposed white blood cells which have been swiftly multiplied by previously surviving ones according to [1] is usually comprised of new or younger cells (because the old ones are killed) that would fight the invaders; they stated that the increase in the number of younger cells may be responsible for the differences in the white blood cell volume observed between exposed and control group. Therefore, sharp increase in white blood cells (WBC) count above that of the control group could be resistance to prevalent unwanted change and adaptability to the environment. This agrees with [25] investigation on efficacy of Neem leaf powder (NLP) as detoxifying agent and as adsorbent agent in removal of cadmium in water and *O. niloticus* tissues and as immunostimulants in improving the physiological and immunological status of *O. niloticus*. This disagrees with [22] which opined that haemoglobin reduction invariably contributes to the stress and anaemic state of organisms which further alters respiration, metabolism and induces morbidity and mortality. Hence, haemoglobin reduction in treated fish might have impaired oxygen supply to the various tissues, resulting to slow metabolic rate and low energy production which further explains the changes in their behaviours that included restlessness, gasping for air, low food consumption and subsequently loss of body weight amongst others. For similar concentration above but treated with pulverized mango leaves showed decrease in RBC, WBC, Hb, MCV and PLT at 10 mg/L and 15 mg/L) while high increase recorded in the count or concentration of HCT, MCH and MCHC at 15 mg/L and PCT was normal at 15 mg/L of Pb. This disagrees with [14,15,26] that reported RBC, Hb, HCT, MCHC, MCV and PCT while WBC is not affected counts. [27] submission aligns with [14,15] who reported increased in white blood cells (WBCs) and decreased Red Blood Cells (RBCs), lymphocytes, Hemoglobin (Hb), Platelets (Plt), and Hematocrit (Hct) in downstream with concentrated heavy metals contamination.

The antecedents of results from physicochemical properties and haematological status earlier discussed are evidence to prove the efficacy and efficiency. Pulverized neem and mango leaves were good biosorption agents.

Conclusion

The potency of pulverized neem and mango leaves were established significantly on the physicochemical properties quality and haematological parameters status that showed no significant difference while for PML and 10 mg/L of Pb showed insignificant difference. As such, the haematological indices and the environment was healthy for habitation by the cultured cat fish. PNL and PML are good biosorbents in closed aquaculture contaminated with lead. On general efficacy, efficiency and performance, PNL and PML are positive biosorbents for biosorption in lead-contaminated aquaculture.

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