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Bioaccumulation levels of Lead (Pb) in Selected Organs (gill, liver and skin) of *Clarias gariepinus* in Odo-Ona River, Southwestern Nigeria

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Abstract: In this study, the bioaccumulation levels of Lead (Pb) in selected organs (gill, liver and skin) of *Clarias gariepinus* in Odo-Ona River were determined. Water and fish samples were collected from five different sampling stations along the effluent discharge points of some strategically selected industries. The physicochemical analysis done on the water samples include; pH, Alkalinity, Dissolved Oxygen (DO) and Biochemical Oxygen Demand (BOD). The digested water and fish samples were analyzed for Pb concentrations using the Perkin Elmer (A Analyst 200) version 6.0 Atomic Absorption Spectrometry (AAS). The water pH was moderately alkaline in the range of; 6.78 – 7.30. Alkalinity (95.29 $\pm$ 11.2 – 120.80 $\pm$ 12.0 mg/L) was considered high this was similar to high BOD levels with range of 442.50 $\pm$ 9.6 to 581.5 $\pm$ 5.6 mg/L. Pb concentration of the water ranged at 0.21 – 0.54 ppm. Trend in Pb uptake in the tissues according to this study is; Liver > Skin > Gill. The accumulation Factor also confirmed the trend. Generally, accumulations of trace elements are organ specific in *Clarias gariepinus*, however the level of Pb uptake is of a serious concern; knowing that it is a cumulative poison.
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INTRODUCTION

Environmental contamination is hazardous to aquatic biota, since the pollutants in both land and air eventually accumulate in aquatic environments. Among these pollutants are heavy metals which pose serious environmental problems due to their persistence, toxicity and tendency to be accumulated in organism[1,2,3]. Recently, heavy metal concentration of aquatic habitats has increased due to dense anthropogenic activities. The increased exposure of aquatic organisms to elevated levels of metals has threatened the health of aquatic organisms as well as human[4].

Lead (Pb) exists in natural waters as cations in many salts and oxides which are associated to particulate matter, or complexed to organic molecules as organometallic compounds. The free cationic form is more toxic to aquatic biota than complexed forms; the toxicity is higher at low pH and in soft waters[1]. Pb is present in trace amounts in soils, water and food[2]. There is no evidence that Pb is an essential trace metal and its tendency to be bioaccumulated is perhaps one of its most interesting biological properties[3]. In fishes, bioaccumulation of Pb has been reported in gills, liver, kidney and fins[5].

Pb is used in paints because of its luster and durability. Pb dusts are produced when these paints are old or chipped, thus the environment becomes contaminated with the dust. Lead based industries, such as lead-smelting, lead-refining, and battery manufacturing, constitute another major environmental source of lead poisoning[4]. Children may also ingest lead paint chips that taste sweet[5]. Vapour, fumes and powders generated by these industries contaminate the soil, food and water supply of the communities surrounding them. Vehicle exhaust may be a significant environmental source of Pb in countries that continue to use Pb as an anti-knock agent in their gasoline.

Pb accumulates slowly in the body and even low doses can eventually lead to poisoning. 95 % of Pb accumulated in the body is deposited in the bones and teeth while 99 % of Pb in blood is associated with erythrocytes[6]. Pb interferes with many enzyme systems in the body, thereby affecting the functions of virtually every organ.

It has been reported that low doses of Pb exposure can harm a child's mental development[7]. The health problems get worse as the level of Pb in the blood gets higher. Lead is much more harmful to children than adults because it can affect children's developing nerves and brains. The younger the child, the more harmful Pb can be. Unborn children are the most vulnerable[8]. Hence, the objective of this work is to determine the level of Pb in Odo-Ona River and its bioaccumulation in selected tissues of *Clarias gariepinus* in the river. The pH range of the water sample was moderate 6.78 – 7.30

MATERIALS AND METHODS

The study area is Odo-Ona River which flows through Oluyole Industrial Estate in Ibadan South-West Local Government of Oyo State, Nigeria. The river receives effluents from industries and residential areas. Industries located along the river's course include; food processing, carbonated drinks bottling, sawmill, furniture, plastics, paints, steel-works, printing, brick-making and chemical factories. Human activities at the river include; fishing, bathing, swimming, car-washing and cloth-washing especially at the banks. Also some sections of the river are used as dumping sites for refuse by communities along the river banks[9]. Five different sampling stations were strategically selected for collection of water and fish samples. Surface water temperature was taken *insitu* using 0-50 ^{o}C mercury-in-glass thermometer (Adrich) held at a depth of approximately 10 cm beneath water surface for 2-3 minutes. The pH was also assessed *insitu* using pocket pH meter (pH 1 model). The turbidity was assessed using *Hach* spectophotometer by adjusting its wavelength to 750 nm and then filling a 25 ml curvette with the water sample to the 25 ml mark before the reading. Dissolved oxygen (DO) was measured *in situ* with DO meter (Model DO–5509) and the water sample was taken to the laboratory, incubated for five days at 20 ^{o}C, after five days the reading was taken. The first day DO minus the fifth day DO (DO_1- DO_5) gave the BOD. Heavy metals analysis was determined by digesting 250 ml of water samples with 10 ml analytical grade nitric acid to acidify it, the solution was evaporated on a crucible to approximately 25 ml then filtered into a standard flask and diluted with distill water. The mixture was gently heated in a water bath until the acid was bleached. The digested water samples were analyzed for Pb using the Perkin Elmer (A Analyst 200) version 6.0 Atomic Absorption spectrometry (AAS).

Fishing were carried out using baited hooks, surface gillnets and traps. Traps and gillnets were left overnight while the baited hooks were used in the morning. All fish samples collected were identified to specify their species by means of taxonomic tools and description of Fischer[10]. Standard length and wet weight of each species were taken. Fish samples were dried whole to a constant weight at 105^{o} C in an oven and the dry weight recorded using a Mehtler PE electric balance. The perchloric acid-nitric acid-sulphuric acid digestion method of Sreedevi[11] was used. 1 g of milled whole fish was mixed in a conical flask containing 70 % perchloric acid ($HClO_4$), concentrated nitric acid (HNO_3) and concentrated sulphuric acid (H_2SO_4) in the ratio of 1:5:1. The conical flask and content was then transferred to a hot plate in a fume chamber and allowed to boil at 105 $\pm$ 2^{o} C [10]. During the digestion when the mixture turned dark-brown, more concentrated HNO_3 was added and heated further. Complete digestion was indicated by a clear solution. At this stage, the conical flask was brought down from the hot plate and allowed to cool. 1 – 2 ml of distilled water was added to the digested sample and this was allowed to boil for a minute on the hot plate before it was made to 20 ml with distilled water and allowed to cool and settle.

In the case of the selected organs of the fish; the freshly collected fishes were placed on plastic sheet and the organs and muscles were separated by dissected plastic knife in the laboratory. The dried samples were mechanically crushed with glass rod and homogenized. The digestion procedure was done as stated above. The digested samples were analyzed for Pb using the Perkin Elmer (A Analyst 200) version 6.0 Atomic Absorption spectrometry (AAS).

Data obtained was subjected to factor analysis such as Principal Components Analysis (PCA) which was used to summarize the major patterns of distribution and variation within the physico-chemical data using Predictive Analytics Software (PASW), version 20.

RESULTS AND DISCUSSION

The morphometric data of freshwater catfish (*Clarias gariepinus*) that were collected in the five sampling stations (Table 1) depicted mean weight ranging from 26.66 $\pm$ 3.08 to 32.13$\pm$ 3.24 g while freshly caught from the river and mean length ranges from 11.50 $\pm$ 2.88 to 15.88$\pm$2.17 cm. This typifies the standard size of adult African catfish free-ranging in its natural habitat[10]. Adult *Clarias gariepinus* has its organs all developed and have tendency to live for more 3 years. It has strong adaptive features which include hibernating in the water-bed during unfavourable conditions. In the regards, it would have ingest and bioacumulate contaminants during the hibernation session. *Clarias gariepinus* are voracious omninores and this is evident in their sizes. A healthy catfish in an intensive fish-pond can weigh as much as 5 kg[11].

Table 1: Morphometric Data of *Clarias gariepinus* from the five sampling stations

Sampling Stations	Mean Standard Length (cm)	Mean Standard Weight (g)	Mean Dry Weight (g)	Moisture Content (%)
1	14.38±3.96	29.25±2.53	9.59±2.08	67.21
2	15.88±2.17	32.13±3.24	13.18±2.51	58.98
3	13.55±3.12	29.55±2.88	9.43±3.2	68.02
4	14.76±4.2	30.54±4.3	9.77±3.42	68.01
5	11.50±2.88	26.66±3.08	10.58±2.21	60.32

Values are expressed as means + SD, n = 3 with significant difference ($p<0.05$)

The high concentrations of alkalinity (95.29 ± 11.2 – 120.80 ± 12.0 mg/L) in water samples from Odo Ona River may be due to moderate pH (6.78 – 7.30, Table 2). The pH level of a water-body is not expected to be too high or too low; this is important for aquatic organisms' optimum metabolic activities. The pH status also affects the solubility and toxicity of chemicals and heavy metals in the water[12]. As pH levels move away from this safe range (up or down) it can stress animal biological systems and reduce hatching and survival rates. The further alteration and stretch in the pH range beyond the optimum limits, the higher the tendency for high mortality rates. The more sensitive a species, the more affected it is by changes in pH. In addition to biological effects, extreme pH levels usually increase the solubility of elements and compounds, making toxic chemicals more "mobile" and increasing the risk of absorption and bioaccumulation of these toxic heavy metals by aquatic life[13]. This explains the relatively high concentration[14] of elemental Pb in Odo Ona river (0.21 – 0.54 ppm, Table 2). The levels of BOD recorded at the five sampling stations were very high with range of 442.50 ± 9.6 to 581.5 ± 5.6 mg/L. Water with BOD less than 4 mg/L is termed reasonably clean and unpolluted, while water with level greater than 10mg/L are considered polluted since they contain large amount of degradable organic materials[15]. The sampling station 2 recorded the highest BOD level in this study (581.5±12.3mg/L). This could be attributable to both clustered residential settlements and the industrial presence found about 500m away from this particular sampling station. The high level of BOD indicates that Odo-Ona River is under high organic pollution which makes it bad for drinking and water quality. High biochemical oxygen demand has been reported to be a good indication of organic pollution[14]. The high BOD recorded in this study could explain slightly low Dissolved Oxygen (DO) recorded in this study. This relationship between DO and BOD was also observed by in studies of Ogunpa River[15], which is also a major river in Ibadan metropolis. The Odo-Ona River would therefore be considered unsuitable for drinking.

Table 2: Physico and biochemical Parameters of the water sample from Odo-Ona River

Sampling Stations	**pH**	**Alkalinity (mg/L)**	**Dissolved Oxygen (mg/L)**	**BOD (mg/L)**	**Pb (ppm)**
1	7.15±1.06	95.29±11.2	2.58±0.4	442.50±9.6	0.21±0.042
2	7.30±1.13	96.16±20.0	2.60±0.6	581.50±5.6	0.26±0.037
3	6.87±1.43	120.80±12.0	2.63±0.3	496.80±8.2	0.27±0.092
4	6.78±1.13	111.23±16.2	1.64±0.4	499.44±6.5	0.54±0.022
5	6.88±0.56	100.24±9.3	1.38±0.4	465.33±3.1	0.34±0.073

The three tissues investigated showed comparatively[16] low Pb bioaccumulation (Table 3), with the liver having the highest Pb deposits of 0.01044 – 0.01134 µg/g. The general order of Pb intake in the tissues according to this study is; Liver > Skin > Gill. It has been reported that aquatic animals can take up trace metals both from water and food they ingest and also through the ingestion of inorganic particulate materials. The biological mechanism involved in heavy metal uptake and accumulation involves lysosomes, low molecular weight proteins such as metallothioneins and intracellular calcified concretions, which concentrate and possibly excrete metals in aquatic species[9]. An understanding of this mechanism is essential in order to elucidate the relationship, between metal uptake and toxicity. The mechanism of Pb bioaccumulation is likely to be through adsorption and complexing of free Pb ions from the water. Source of Pb in the environment studied are printing press, batteries storage and repair, pipes and various alloys, cable sheathings. Emission from automobile exhaust pipes is also a major source of high Pb concentration in the environment.

Table 3: Bioaccumulation of Pb in Selected tissues of *Clarias gariepinus* (µg/g)

Sampling Stations	Gill	Liver	Skin
1	0.00243	0.01044	0.00335
2	0.00298	0.01054	0.00374
3	0.00298	0.01059	0.00398
4	0.00353	0.01134	0.00398
5	0.00396	0.01132	0.00398

The accumulation factor (AF) is the ratio of the accumulated concentration of a given pollutant in any organ to its dissolved concentration in water. It gives an indication about the accumulation efficiency for any particular pollutant in any fish organ. Table (4) shows the AF values of Pb in gills, livers and skin *Clarias gariepinus*. In general the pattern of Pb was in the order of liver > skin > gill tissues of *Clarias gariepinus*. This is constant for all the five sampling stations and it follows the general trend for Pb bioaccumulation in the concerned tissues.. For all the analyzed metals, accumulation of metals in muscle might show time integrated storage while liver and gill show the present condition or episodic inputs of the contaminants in fish as well as in the lake[17]. Generally, trace elements accumulations are organ specific[18]. From the results obtained, it can be noted that there would be two factors affecting the element distribution in fish tissues: the one is physiological role of each element, and the other is the preference of an element to bind to or replace some elements in the tissue. This information will greatly increase the sensitivity of bio-monitoring of lake water quality using fish organs. AF values below 25 levels indicates that each fish species excrete trace metal after ingesting or do not consume in excess from water[18]. However, to get more comprehensive generalization, analysis of other organs and tissues, such as kidney, spleen, scales, intestine, heart, and bile requires consideration.

Table 4: Accumulation Factor of Pb in gills, liver and skin of *Clarias gariepinus*

Sampling Stations	Gill	Liver	Skin
1	0.0116	0.0497	0.0160
2	0.0115	0.0405	0.0144
3	0.0110	0.0392	0.0147
4	0.0065	0.0210	0.0074
5	0.0116	0.0333	0.0117

Conclusion

It has been indicated that the level of contaminants in fish depends on habitats [19,20] duration of exposure of fish to contaminants, feeding habit and metabolism, [21] age, size and length of the fish[22] concentrations of contaminants in water column and the water chemistry. The bioaccumulation and AF values of Pb in the considered tissues of *Clarias gariepinus* therefore need to be closely monitored, since it has earlier been confirmed that Pb is a cumulative poison which is very toxic even at low concentrations.

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