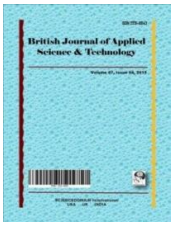

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Lead and Cadmium Levels of African Catfish (*Clarias gariepinus*) and the Effect of Cooking Methods on their Concentrations

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Authors' contributions

This work was carried out in collaboration between both authors. Author OTO designed the study, performed the statistical analysis, wrote the protocol, while author FA wrote the first draft of the manuscript and managed literature searches. Both authors managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Consumption of cadmium (Cd) and lead (Pb) contaminated fish poses carcinogenic and non-carcinogenic health risks to human. Levels of Cd and Pb residues in cooked flesh of *Clarias gariepinus* have not been fully explored inspite of their public health importance. This study therefore, determined the effect of cooking methods on Cd and Pb concentrations in *Clarias gariepinus*.

A laboratory-based experimental study design was adopted. Thirty-six fish were randomly distributed into 3 groups (A, B, C). Group A was exposed to 4.9 mg/L cadmium (30% LC_{50}), B to 24.2 mg/L lead (30% LC_{50}) for 4 weeks, while group C served as control (0% LC_{50}). Flesh samples were cut into 4 pieces; boiled, fried, and roasted on a charcoal grill while the fourth was not cooked. Samples were analyzed for Cd and Pb using atomic absorption spectrophotometer. Data were analyzed using ANOVA at 5% level of significance.

The LC_{50} of Cd and Pb were 16.3±0.5 mg/L and 80.6±0.6 mg/L respectively. In group A, Cadmium

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concentration in boiled, fried and charcoal-grilled flesh samples were 2.2±2.0 mg/kg, 2.8±2.0 mg/kg and 5.7±1.6 mg/kg respectively. Lead concentrations in boiled, fried and charcoal-grilled samples were 25.8±22.0 mg/kg, 30.8±22.3 mg/kg and 38.6±25.5 mg/kg respectively. Mean concentrations of Cd and Pb in the uncooked flesh were 8.8±5.1 mg/kg and 44.6±22.5 mg/kg respectively.
Cadmium and lead were not detected in the control group C. Cadmium and lead concentrations were reduced in all cooked samples (boiled < fried < charcoal-grilled < uncooked samples). Reduction of cadmium and lead concentrations was highest in boiled samples.
Cooking methods reduced heavy metal concentration in African catfish. Effect of cooking methods on concentration of heavy metals in fish is dependent on the specific heavy metal and cooking medium. Boiling of fish before consumption is advocated for the reduction of Cadmium and Lead concentration in *Clarias gariepinus*.

Keywords: Clarias gariepinus; bioconcentration; catfish cooking methods; cadmium and lead bioaccumulation.

1. INTRODUCTION

Toxic chemicals released into the environment, either from point sources such as industrial and municipal discharges, or from non-point sources like agricultural runoff and atmospheric deposition, are capable of contaminating surface waters and their sediments [1]. Heavy metals are group of Stoxic chemicals, persistent in the environment, bio-accumulative and non-biodegradable in food chain [2]. Aside the fact that heavy metals disrupt and result in contamination of ecosystems, they exert both carcinogenic and non-carcinogenic health impacts on humans. Heavy metals in human system do not degrade, this accounts for their chronic toxicities. Heavy metal contaminated air may pollute soil and water resulting in contaminated crops and consumables. Erosion of natural deposit of rock minerals and atmospheric deposition of gaseous emissions from tailpipes of industrial engine allow for the mobility of heavy metals into the aquatic environment. Heavy metals in the aquatic environment remain persistent and based on their available concentrations are bioaccumulated into the tissues of aquatic plants and animals.

Examples of heavy metals released to the environment include cadmium (Cd), Lead (Pb), nickel (Ni), Arsenic (As), Mercury (Hg) and Chromium (Cr) among others which are probable carcinogens in humans. Lead may induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular diseases in adults, while Cadmium accumulates in the human body and may induce kidney dysfunction, skeletal damage and reproductive deficiencies [3].

Fishes are organisms which survive mainly in water bodies. Fish is food to human as it remains a relatively cheap source of protein. Food refers to the range of consumables that comprise the essential body nutrients required for life and growth, such as proteins, carbohydrates, fats, vitamins, or minerals. Nutritional composition of fishes encompasses both macro and trace nutrients beneficial to the human biological system. The major nutritional constituents of fish on analysis are water, protein, lipid, mineral and vitamin B2 [4].

However, cadmium and lead pollution of the aquatic ecosystem threatens the suitability of fish as an important food source for humans. Fish being the final chain of aquatic food web is able to bio accumulate heavy metals in aquatic environment. The accumulated metals in fishes are transferable through food chain into humans. Fish safety just as food safety is an important public health issue because there are numerous diseases acquired by humans on the consumption of contaminated fish [5].

Processing of fish into forms suitable for human consumption or to be used as a supplement in animal feed has been a cultural practice as it is unusual for fishes to be consumed raw. Processing methods of fishes are methods applied to the fishes from the time of harvest to consumption period. Processing methods like boiling, frying, roasting and smoking have effect on the texture, nutrient composition and flavor of fishes [6,7]. Processing may be suspected to change the level of heavy metal contamination of a food substance depending on the processing method and the nature of food substance. Determination of heavy metal levels of fish is extremely important for human health [2]. This study therefore aimed at assessing the effect of

boiling, frying and charcoal roasting on cadmium and lead concentrations in African Catfish (*Clarias gariepinus*).

2. MATERIALS AND METHODS

Juveniles of *C. gariepinu*s of mean body weight 14.4±0.7 g and mean standard length 15.3±0.6 cm used for the study were collected from the fish research laboratory of the Department of fisheries, Oyo State Ministry of Agriculture, Ibadan, Nigeria. The fish were acclimatized in the laboratory for four weeks inside plastic tanks of 80 litres capacity before experiment. During the acclimatization, the fishes were daily fed adequately with 5% mean fish body weight equivalence of their regular feed stock i.e Durhante fish pellet. Natural groundwater from well located in the vicinity of the fisheries Department of the Ministry of Agriculture was used as aquarium water to maintain the fish. Water samples were taken in triplicates at different sampling times and analyzed for cadmium and lead with the metals not detected before water sample was accepted adequate for use. Commercial grades of cadmium chloride ($CdCl_2$) and lead chloride ($PbCl_2$) were used as Cd and Pb sources respectively.

2.1 Well Water Sampling Procedure

Composite samples were collected at various depths of the well. A narrow mouth plastic 1 litre sample bottle was used for the collection of the sample. Sample bottle was rinsed thoroughly with the well water before use. Triplicate samples were collected. The aquaria water samples were taken for physicochemical analysis for derivation of pH, alkalinity, dissolved oxygen, hardness and temperature levels.

2.2 Procedure for LC_{50} Determination

Twenty four hours (24 hrs) acute cadmium and lead toxicity experiments were performed for *Clarias gariepinus* after acclimatization at different concentrations. The weight of the fishes was measured by sensitive weighing balance and standard length was obtained using metre rule. Fish samples used for this experiment had mean body weight of 66.6±2.3 g and standard length of 21.1±1.8 cm. Feeding was stopped two days before the fishes were subjected to experiment in order to avoid change in toxicity of metals due to excretory products [8]. The following test concentrations were used:

Cd 0, 5, 10, 20, 40, 80, 160 mg/L
Pb 0, 5, 10, 20, 40, 80, 160 mg/L

Test concentrations were obtained by dissolving the required weight of salt per litre of aquaria water (well water). The required weight of salt equivalent to the weight of test chemical required was calculated using the formula:

$$\frac{\text{Molecular wt of test metal salt (g)}}{\text{Atomic weight of test metal (g)}} \times \text{Weight of test metal (g)} \quad (1)$$

The juvenile fish were randomly selected and divided into six groups of six fish each. The six groups include the control and five test concentrations. Each group occupied an aquarium for each of the two heavy metals (i.e Cd and Pb) for the fish species. The experiment was carried out in triplicates.

Test concentrations were under check and dead fish were removed periodically. Mortality was recorded per dose along the 24 hours of exposure and percentage mortality was calculated. LC_{50} values at 24 hours for each of Cadmium and Lead was calculated by probit graphic method and the unweighted regression method [9].

2.3 Exposure of Fish Samples to 30% LC_{50} of Test Heavy Metals

A set of fish samples of mean weight 116.2±8.8 g and standard length 26.5±1.4 cm were exposed to 30% LC_{50} obtained for cadmium and lead. Three groups of 12 fish samples each were set. Group A was exposed to 30% LC_{50} of cadmium, B to 30% LC_{50} of lead, and C was the control group (0% LC_{50} of cadmium and lead). Each group was made up of three aquaria with 4 fish sample in test concentration. Three fish samples were harvested per week from each group i.e 1 per aquaria. The aquaria water sample was also collected weekly for analysis of some physicochemical parameters. Harvested fish samples were dissected with stainless dissecting blade to eviscerate the gills and liver. The flesh samples were isolated into plastic sample bottles and labeled appropriately. Fish samples were stored in the refrigerator of the Department of fisheries.

2.4 Fish Sample Preparation and Processing

The fish samples were removed from refrigerator and taken for processing within two hours. Fish samples were thoroughly washed with tap water to remove ice and blood from flesh. Samples were beheaded and divided into four pieces. A piece of the flesh was left raw. For the other three pieces, a piece was boiled on the electric stove at 180°C for 5 minutes, the other was fried with refined vegetable oil in a pan at 180°C of the electric stove for 5 minutes and the last piece was grilled on a charcoal grill until the two side of fish sample was completely grilled for 10 minutes. The charcoal grill was constructed by placing a stainless wire mesh on a local household charcoal burner. Fish samples were placed on the mesh for grilling after the ignition of charcoal.

All samples were oven dried to constant weight at 60ºC for 24 hours. Dried samples were homogenized in a clean stainless mortar and pistil homogenizer in the laboratory before digestion.

2.5 Acid Digestion

Homogenized sample (1 g) of fish flesh was weighed in a pyrex digestion flask. Analytical grade Nitric acid (HNO_3) with perchloric acid in ratio 4:1 (5 ml) was added into the flask content. The flask was placed on an electric heater in a fume cupboard until complete dissolution and almost dryness. Digest was filtered using Whattman No.42 filter paper and filtrate was made up to 25 ml with de-ionized water in a volumetric flask.

Water sample (10 ml) was measured into a pyrex digestion flask. Acid containing analytical grades of nitric acid (HNO_3) and hydrochloric acid (HCl) in ratio 2:1 (5 ml) was added to the sample. The flask was placed on an electric heater and heated slowly in a fume cupboard until reduction of volume to about 2 ml. Digest was made up to 25 ml with deionized water in a volumetric flask.

Blank digests were also prepared using the same procedure as above.

3. RESULTS

3.1 Physico-chemical Characteristics of Well Water Samples

The samples collected from the well were analyzed for their physico-chemical properties. The mean values of the parameters investigated are as follows; temperature (25.2±0.8ºC), pH (6.7±0.3), dissolved oxygen (7.8±0.8 mg/l), hardness (54.2±2.1 mg $CaCO_3$/l) and total alkalinity (119.5±3.9 mg $CaCO_3$/l). Cadmium and lead were not detected in the samples. Results were compared with the Food and Agriculture Organization (FAO) guidelines for fish pond water quality as shown in Table 1.

3.2 Fifty Percent Lethal Concentration (LC_{50}) at 24 hours

The 24 hours fifty percent lethal concentration (LC_{50}) for both cadmium and lead were calculated by the regression method. Number of deaths of test fish observed at each concentration after 24 hours of exposure for each of the three replicates is shown in the probit Tables 2 and 3. Twenty four hours 50% lethal concentration (LC_{50}) of cadmium and lead for *Clarias gariepinus* and the physico-chemical characteristics of the fish aquarium water samples are shown in Tables 4 and 5.

Table 1. Physico-chemical parameters of well water samples

Parameter	Range	Mean ± SD	FAO guideline
Temperature (ºC)	24-26	25.2±0.8	25 – 30
pH	6.2 – 7.9	6.7±0.3	6.5 – 8.5
Dissolved Oxygen (mg/l)	6.9 – 8.9	7.8±0.8	>3
Hardness (mg $CaCO_3$/l)	50.3 – 55.7	54.2±2.1	>25*
Total alkalinity (mg $CaCO_3$/l)	115 – 125	119.5±3.9	>25*
Cadmium (mg/l)	ND**		
Lead (mg/l)	ND**		

*Food and agriculture organization [11a], *Food and agriculture organization [11b], ** not detected*

Table 2. Probit for 24 hours exposure to cadmium

Concentration (mg/l)	Log_{10} concentration	Total no of test fish	Replicate 1			Replicate 2			Replicate 3		
			No of death	% mortality	Probit	No of death	% mortality	Probit	No of death	% mortality	Probit
0	-	6	-	-	-	-	-	-	-	-	-
5	0.699	6	1	16.67	4.05	-	-	-	1	16.67	4.05
10	1.000	6	2	33.33	4.56	2	33.33	4.56	2	33.33	4.56
20	1.301	6	3	50.00	5.00	3	50.00	5.00	3	50.00	5.00
40	1.602	6	5	83.33	5.95	5	83.33	5.95	5	83.33	5.95
80	1.903	6	6	100	-	6	100	-	6	100	-
160	2.204	6	6	100	-	6	100	-	6	100	-

Table 3. Probit for 24 hours exposure to lead

Concentration (mg/l)	Log_{10} concentration	Total no of test fish	Replicate 1			Replicate 2			Replicate 3		
			No of death	% mortality	Probit	No of death	% mortality	Probit	No of death	% mortality	Probit
0	-	6	-	-	-	-	-	-	-	-	-
5	0.699	6	-	-	-	-	-	-	-	-	-
10	1.000	6	-	-	-	1	16.67	4.05	-	-	-
20	1.301	6	-	-	-	1	16.67	4.05	1	16.67	4.05
40	1.602	6	2	33.33	4.56	2	33.33	4.56	2	33.33	4.56
80	1.903	6	3	50.00	5.00	3	50.00	5.00	3	50.00	5.00
160	2.204	6	4	66.67	5.44	4	66.67	5.44	4	66.67	5.44

Table 4. Twenty four hours 50% lethal concentration (LC_{50}) of cadmium (Cd) and lead (Pb) for *Clarias gariepinus*

Heavy metal	Replicate 1 LC_{50}(mg/l)	Replicate 2 LC_{50}(mg/l)	Replicate 3 LC_{50}(mg/l)	Mean LC_{50}(mg/l) ±SD
Cadmium	16.01^{a}	16.88^{a}	16.01^{a}	16.30 ± 0.5^{a}
Lead	79.98^{b}	81.03^{b}	80.83^{b}	80.61 ± 0.56^{b}

Values with different superscript are significantly different at ($p<0.05$)

Table 5. Physico-chemical characteristics of the fish aquarium water samples

Parameter	Range	Mean ± SD	FAO guideline
Temperature (ºC)	23-26	24.8±0.6	25 – 30
pH	6.1 – 7.2	6.8±0.3	6.5 – 8.5
Dissolved Oxygen (mg/l)	5.3 – 8.4	7.1±0.6	>3
Hardness (mg $CaCO_3$/l)	32.4 – 64.7	45.3±9.9	>25*
Total alkalinity (mg $CaCO_3$/l)	100 – 162	128.9±19.4	>25*

*Food and agriculture organization [11a], *Food and agriculture organization [11b]*

3.3 Effect of Cooking Methods on Cadmium Concentration in *Clarias gariepinus*

Mean concentrations in mg/kg dry weight (wt) of cadmium in boiled, fried and charcoal-grilled flesh samples of the test fish were 2.2±2.0 mg/kg, 2.8±2.0 mg/kg and 5.7±1.6 mg/kg respectively. Concentrations of the boiled and fried samples were lower than the charcoal grilled ($p<0.05$). Cadmium concentrations of all cooked flesh samples were significantly lower than the level detected in uncooked flesh (8.8±5.1 mg/kg).

3.4 Effect of Cooking Methods on Lead Concentration in *Clarias gariepinus*

Mean concentrations in mg/kg dry weight (wt) of lead in boiled, fried and charcoal-grilled flesh samples of the test fish were 25.8±22.0 mg/kg, 30.8±22.3 mg/kg and 38.6±25.5 mg/kg respectively. Although the concentration of lead in cooked samples varied in the order boiled< fried<charcoal-grilled, however the difference in concentration was not significant ($p>0.05$). Lead concentrations of all cooked flesh samples were not significantly lower than the level detected in uncooked flesh (44.6±22.5 mg/kg). Details of the concentrations of Cd and Pb in the cooked flesh are shown in Table 6. Fig. 1 shows the concentrations of Cd and Pb in the cooked flesh relating to the various cooking methods used in the study.

4. DISCUSSION

4.1 Physico-chemical Analysis of Water

Cadmium and lead were not detected in the well water sample which served as the source of water for culturing fish for this study. In view of this, the concentration of each heavy metals introduced into culture water for fish exposure was regarded as the concentration of heavy metal available in the fish external environment as there are no expected cadmium and lead contamination from the water source. Survival of aquatic lives like fishes depends solely on the physical, chemical and biological characteristics of the water they reside [10]. Water of good quality characterized by adequate oxygen, optimum pH and temperature, proper dissolved solids to allow transparency is critical for fish culture. Maintenance of all physico-chemical factors is essential for optimum yield in a fish pond.

The water source for maintaining the fish had a temperature of 25.2±0.8ºC and temperature of the ponds for the four weeks of exposure was 24.8±0.6ºC as shown in Table 5. These values are not significantly different ($p>0.05$) from the FAO (2013a) suitable water temperature for fish culture (25ºC-30ºC). Temperature controls the rate of biochemical reactions and determines the amount of dissolved gases. An optimum temperature range of 25ºC-27ºC is regarded

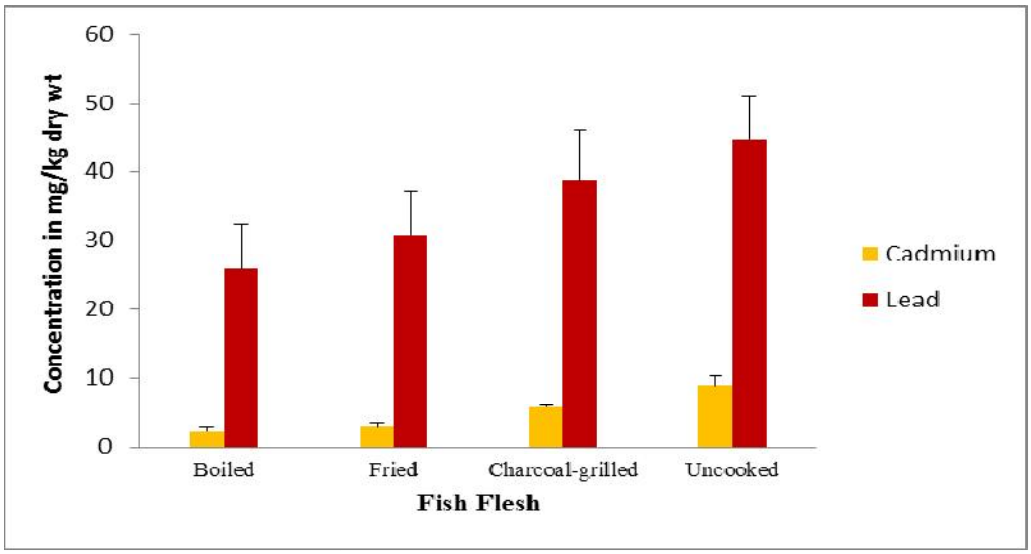

Fig. 1. Mean Cd and Pb concentrations in *Clarias gariepinus* flesh
***(Note:** There is a significant difference in comparison with the uncooked group)*

Table 6. Mean concentrations of Cd and Pb in cooked *Clarias gariepinus* flesh

Sample	Cd (mg/kg dry wt)	Decrease in Cd level relative to uncooked flesh (%)	Pb (mg/kg dry wt)	Decrease in Pb level relative to uncooked flesh (%)
Uncooked	8.8±5.1[a]	-	44.6±22.5	-
Boiled	2.2±2.0[b]	75	25.8±22.0[a]	37
Fried	2.8±2.0[b]	68	30.8±22.3[a]	26
Charcoal-grilled	5.7±1.6[c]	35	38.6±25.5[a]	18

Superscripts [a], [b] and [c] are only related to each metal group, [a b c] Different superscripts on the same column denote significant mean difference at p< 0.05

adequate for *Clarias gariepinus* adults [11] and is similar to what was obtained in this study. Very high temperature encourages depletion of dissolved oxygen through increased biochemical activities in water bodies while low water temperature has been implicated in the buildup of toxic gases like hydrogen sulphide and methane which poses a negative effect on fish health. The fish matrix takes up the temperature of their residence water body. A temperature range of 28ºC-30ºC is suggested to be necessary for the optimum growth of *Clarias gariepinus* [12]. A study on pond temperature monitored for a 24 weeks Catfish culture showed average of 30.2ºC, which is higher than what was obtained in this study [13]. Analysis of the temperature of well water used for the culture of *Clarias gariepinus* by some researchers was also found to be 23.40±1.21ºC [14]. This result is also similar to the one obtained in this study.

The pHs of source water and test aquarium water samples were 6.7±0.3 and 6.8±0.3. This result is similar to those documented by earlier mentioned study on well water [14]. Fish production is greatly influenced by excessively high or low pH. Excessively high and low pH could lead to high fish mortality. Although fish species characteristic, fish size and environmental conditions of the pond determine the optimum pH for cultured fish, a culture water pH within 6.5–8.5 is considered adequate for pond fish production [11a]. The result of pH obtained falls within this range. Some works done recently, however, obtained pH values of within 7.4 to 7.9 higher than those obtained in this study [15].

Dissolved Oxygen for the source water and aquaria were 7.8±0.8 mg/l and 7.1±0.6 mg/l respectively. Similar level of dissolved oxygen (7.4 mg/l) was used in a study to maintain *Clarias gariepinus* fingerlings [16] while in another study, the same fish species were maintained with water of lower dissolved oxygen level (5.7±0.42 mg/l) [14]. Dissolved oxygen is regarded as the most important water quality factor in aquaculture systems. Fish require dissolved oxygen in water for respiration and other metabolic activities necessary for survival. The oxygen requirement of fish in water depends on the rate of metabolism of fish and physical chemical factors like water temperature, pH and carbon dioxide level [11a]. Dissolved oxygen level of >20 mg/l causes physiological dysfunction; hence toxic to fish, while at lower level than 3 mg/l, fish exhibit reduced fecundity with low egg and sperm viability [12]. Low dissolved oxygen or excessive oxygen depletion causes poor feeding of fish, starvation, reduced growth and ultimately death [17]. The optimal dissolved oxygen concentration for growth of eggs and juveniles of African catfish is 9 mg/l, while adults would survive in water of at least 3 mg/l dissolved oxygen [11a].

Alkalinity of culture water is an important criterion for determining the effect and concentration of essential water quality constituents and hence necessary for the suitability of a water source for fish culture. For fish culture, the pH of test water is closely related to and interpreted for its alkalinity. The South African water quality guideline for aquaculture stipulates a water alkalinity of below 20 mg $CaCO_3$/ l and greater than 170 mg $CaCO_3$/l to be unsuitable for fish culture. A total alkalinity value of at least 20 mg $CaCO_3$/l is essential for catfish production [18]. An alkalinity of between 100 - 150 mg $CaCO_3$ / l is suitable for exerting less energy on osmo-regulation which translates into better growth for a fresh water fish like *Clarias spp* [12]. For this study, the total alkalinity of culture water from source (119±3.9 mg $CaCO_3$ / l) and aquaria (128.9±19.4 mg $CaCO_3$ / l) are similar to values stipulated by the guideline.

Water hardness was 45.3±9.9 mg $CaCO_3$ / l (See Table 5) for the aquaria and 54.2±2.1 mg $CaCO_3$ / l for the source water. A study conducted in

2007, recommended a range of 30 - 180 mg $CaCO_3$ / l as desirable for fish culture [19]. Hardness of <20 mg $CaCO_3$ / l causes fish stress while at above 300 mg $CaCO_3$ / l, an increase in pH occurs leading to unavailability of nutrient [20]. Total hardness ranging from 200-230 mg $CaCO_3$ / l was utilized in a recent study; a range higher than what was obtained in this study for the maintenance of a similar freshwater air breathing fish species (*Channa punctatus*) [15]. An experimental culture of *Clarias gariepinus* fingerlings was achieved with water of hardness measurement of 78.56±3.89 mg $CaCO_3$ / l, relatively similar to the measurement obtained for the present investigation [16].

4.2 Twenty Four (24) hours Bioassay

Bioassays are important for the estimation of toxicant concentration capable of posing severe irreversible negative effects to experimental organisms [21,22]. Aquatic bioassay serves as tool for pollution control as it helps to determine the measure of a toxicant which may be allowed into a water body without inducing adverse effects on the resident living organisms [16]. The concentration of a toxicant found lethal to 50% of test organisms exposed to it in a toxicity test is referred to as the LC_{50} of the toxicant and may be determined for a 24 hour exposure time [23].

The 24 hour LC_{50} determined shows that there was no significant difference in the values obtained for the three replicates for both Cd and Pb. Although no death was recorded in the control groups for both heavy metals, the percentage mortality of test organism increased with increase in test concentration of both cadmium and lead. The increase in mortality with increase toxicant concentration may be due to the increased toxicant solubility and increased species susceptibility which accompanies high toxicant concentration in aquatic medium [24,25]. Lethal concentration (LC_{50}) obtained for cadmium in the three replicates were significantly lower than those obtained for lead ($p<0.05$). This result suggests that cadmium is more toxic to *Clarias gariepinus* than lead. Higher LC_{50} connotes lesser toxicity since higher concentration is required to achieve a 50% mortality of test organisms [26].

The test organism could tolerate more lead contamination than cadmium in terms of dose – response relationship. This may be due to the fact that cadmium exerts a more fatal effect on the test organism than lead. It has been proven that cadmium elicits a necrotic damage on the gill epithelium of *Clarias gariepinus* leading to desruption of gaseous exchange and salt water balancing of the fish [27,28]. A negative effect on the gills which serves as a respiratory organ of the fish may cause asphyxiation which would lead to death of organism more instantly than other toxic influence from heavy metals. Cadmium elicits respiratory distress and increased operculum beats per minutes in fish [29]. The fusion of the lamella leading to reduction in available surface area for gaseous exchange was observed in cadmium toxicity on fresh water catfish *Heteropneustes fossilis* [29]. The higher sensitivity of the test fish to cadmium than lead may be due to the fact that the gills stands as the primary organ to suffer immediate toxic damage of cadmium toxicity.

Similarly, there was a report from a study revealing that a 96 hour LC_{50} for lead (300 ppm) was greater than cadmium (6.5 ppm) for test fish Tinca (*Tinca tinca* L., 1758) [25]. It was also established of a higher 24 hour LC_{50} for lead than cadmium in the common Carp (*Cyprinus carpio*) and Sutchi catfish (*Pangasius hypophthalmus*) [30]. The 24 hour LC_{50} values obtained for cadmium and lead in Sutchi catfish was however higher than those obtained in this study [30]. A similar 72 hour LC_{50} of 83.3±0.9 mg/l for lead chloride on common carp which is similar to values obtained for this study has been documented [31]. It was also discovered that lead chloride was less toxic to common carp than zinc chloride and mercury chloride.

4.3 Effect of Cooking Methods on Concentrations of Cadmium and Lead in Flesh of *Clarias gariepinus*

Fish is an important aquatic vertebrate whose flesh is consumed by human as a valuable source of high quality protein, n-3 polyunsaturated fatty acids, vitamins and minerals beneficial to health [32]. Fish flesh is usually not consumed raw and hence further processed with several cooking methods before consumption by man [33]. Result of this study show that cooking methods affect the concentrations of heavy metal in fish flesh. Though cadmium and lead were not detected in all cooked and uncooked samples of control fish samples, cadmium and lead levels of test fish varied in the order uncooked samples > charcoal- grilled > fried > boiled samples. Variation suggests that cooking methods achieved a reduction in heavy metal content of

fish. This observation may be attributed to the ability of fish flesh to act as a permeable membrane most especially under the influence of heat. This permeability may result in appreciable movement of fish water and oil with heavy metals away from flesh layer into the surrounding cooking medium [34].

Cadmium concentration of samples varied in the order uncooked samples > charcoal-grilled > fried > boiled samples. Concentration of cadmium detected in uncooked flesh, charcoal-grilled, fried and boiled samples were 8.8±5.1 mg/kg, 5.7±1.6 mg/kg, 2.8±2.0 mg/kg and 2.2±2.0 mg/kg (Table 6, Fig 1). The difference in the concentrations of cadmium for all cooked samples were significantly reduced compared to that of uncooked samples ($p<0.05$). There was a similar documentation of a 100% and 20.7% loss in cadmium due to frying and broiling respectively compared to uncooked flesh of Hammour fish [35]. Cadmium level detected in the boiled samples was not significantly different from fried samples. Cadmium level of samples cooked through the moisture medium process (i.e boiling and frying) were significantly lower than those cooked with the non- moisture dry process (charcoal grilling) and uncooked samples. This may be as a result of available cooking medium of the wet cooking method which will facilitate the softening of fish flesh and enhance permeability. In contrast to our findings, there was an observation of a significant increase in cadmium levels of thermally processed squid compared to raw samples [36].

Lead levels of samples also varied in the order; uncooked samples > charcoal-grilled > fried > boiled samples. Concentration of lead detected in uncooked flesh, charcoal-grilled, fried and boiled samples were 44.6±22.5 mg/kg, 38.6±25.5 mg/kg, 30.8±22.3 mg/kg and 25.8±22.0 mg/kg (Table 6, Fig. 1). The variations were however not found to be statistically significant ($p>0.05$). Lead levels of cooked samples were significantly different from the uncooked sample. Lead possesses a peculiar chemical stability obvious in its very high melting and boiling point which may render its movement away from fish flesh to surrounding cooking medium difficult. Similar findings reported [36-38] on the effect of thermal processing on cadmium and lead levels of squid is also consistent with this observation.

The difference in the results of cadmium and lead exposed fish flesh may be attributed to variation in chemical and physical properties of both heavy metals. Lead exhibits a higher boiling (1740ºC) and melting point (327.5ºC) than cadmium (Boiling point: 765°C; Melting point: 321°C) which could account for the greater stability of lead relative to cadmium. The influence of heat processing method, reported in some studies on the concentration of cadmium and lead in squid are similar to the observation made in this study [36]. However, a specific report not consistent with the findings of this study discovered a significant increase in cadmium and lead levels of boiled mushroom compared to raw and a significant reduction in cadmium levels of boiled mushroom compared to fried [39]. It therefore suggests that the influence of heat processing on cadmium and lead residues in a food may depend on the type of the food.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Lethal concentration (LC_{50}) obtained for cadmium were significantly lower than those obtained for lead. This was evident in the higher concentration of lead obtained to cause mortality of 50% of test organisms than the concentration of cadmium which elicited the same effect. The fish could accumulate a high concentration of lead and still remain active and alive, when compared with their exposure to cadmium. In view of this, it might be concluded that the consumption of *Clarias gariepinus* is capable of presenting a higher risk of lead contamination than cadmium.

Boiling, frying and charcoal-grilling of *Clarias gariepinus* resulted in a significant reduction in cadmium level, while no significant reduction was recorded for lead. This suggests that the ameliorating effect of cooking methods on heavy metals in *Clarias gariepinus* could be influenced by the specific heavy metal residue. Boiling produced the highest reduction of cadmium followed by frying and charcoal-grilling. The effect of cooking methods on heavy metals concentrations in *Clarias gariepinus* therefore, depends on the specific heavy metal and cooking medium.

5.2 Recommendations

In view of the high tolerance of *Clarias gariepinus* for lead, fish culturist should not rely on physical

examination and mortality rate as a means of detecting heavy metal pollution of fish ponds. Periodic monitoring of culture water should be done using standard laboratory analytical methods and techniques to allow prompt detection of heavy metal pollution level of fish ponds.

Boiling and frying should be utilized as cooking methods for fish than charcoal-grilling method. Boiling is particularly advocated for cooking *Clarias gariepinus* because it presented the greater reduction of cadmium when compared with the other methods. Integrated cooking method involving combination of two or more of the afore-mentioned methods, for instance, boiling and frying of *Clarias gariepinus* could be adopted for improved safe consumption.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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