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Research

Cadmium and lead assimilation in selected organs of African Catfish (*Clarias gariepinus*) exposed to sub-lethal concentrations of the metal chlorides

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Abstract

Cadmium and lead found naturally in the earth crust and from industrial discharge are capable of contaminating water bodies and aquatic organisms. Contaminated sea foods threaten human health because they pose negative impacts on consumption. The objective of this study was to assess the assimilation pattern of cadmium and lead in selected tissues of the African Catfish (Clarias gariepinus) that is commonly consumed in Nigeria.
Fish samples (36) were randomly distributed into 3 groups of 12 fish each. Group A was exposed to 4.9 mg/L cadmium, B to 24.2 mg/L lead for 4 weeks while group C served as control. Three fish samples were randomly selected from each group weekly. Each fish sample was sacrificed to isolate gills, liver and flesh. All samples were oven-dried, acid digested and analysed for cadmium and lead using atomic absorption spectrophotometer.
Cadmium concentrations in gills, liver and flesh of group A samples were 31.4±10.5 mg/kg, 38.3±11.6 mg/kg and 8.8±5.1 mg/kg respectively. For group B samples, lead concentration in gills, liver and flesh were 123.3±42.9 mg/kg, 82.6±29.1 mg/kg and 44.6±22.5 mg/kg respectively. Cadmium and lead were not detected in the gills, liver and flesh of fish samples in the control group C. This study observed cadmium and lead assimilation in the decreasing order of liver > gills > flesh and gills> liver> flesh respectively. There is a significant increase in cadmium and lead levels of gills and liver compared to flesh ($p<0.05$).
Gills and liver of Clarias gariepinus assimilated more cadmium and lead than the flesh. Thorough washing of fish and eviscerating the gills and liver before consumption will reduce the risk of exposure to cadmium and lead from the Clarias gariepinus.

KEY WORDS: Heavy metals assimilation, bio-concentration, Clarias gariepinus, cadmium and lead

INTRODUCTION

Heavy metals constitute a core group of chemical aquatic pollutants. They are toxic chemicals, persistent in the environment, bioaccumulative and non-biodegradable in food chain (Uysal *et al.*, 2008). Aside the fact that cadmium and lead disrupt and result in contamination of ecosystems, they exert both carcinogenic and non-carcinogenic health impacts on humans.

Heavy metals found naturally or from human activities are capable of being transported into water bodies. 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 (Sarkar *et al*., 2014). Heavy metals in the aquatic environment remain persistent and based on their available concentrations are bioaccumulated into the tissues of aquatic plants and animals.

A water body is referred to as polluted when its quality is changed by manmade contaminants and is no longer fit for man's consumption or unable to support aquatic living communities such as fish. Emerging contaminants are discharged into the water environment through human activities (De Laender *et al*., 2011). Although water pollution is largely accounted to human's activities, major degradation of water quality may also be attributed to natural pollutants from eruption of volcanoes and algae blooms (Prince *et al*., 2008). Industrial effluents, sewage disposal and liquid waste, mining and agricultural waste contribute to the introduction of substances into fresh and ocean water causing pollution of surface and ground water degrading the quality both water and aquatic organisms in the waters (Kanu and Achi, 2011).

Heavy metal pollution is one of the most serious environmental problems (Cheng *et al*., 2012). It has great influences on ecosystem health; water bodies, soil and plants are negatively affected by high concentrations (Duruibe *et al*., 2007; Park *et al*., 2011). Heavy metals are persistent environmental contaminants, they cannot be degraded or destroyed by natural processes, hence, they bioaccumulate in organisms and along food chains. Examples of heavy metals found in water bodies are Lead (Pb), Mercury (Hg), Cadmium (Cd), Chromium (Cr), Arsenic (As), Copper (Cu), Nickel (Ni), Zinc (Zn) etc. Industrial, domestic, agricultural and mineral exploration activities have increased the abundance of heavy metals in water bodies (Sani, 2011).

Some heavy metals are beneficial to human health in trace amount. Iron is essential for the synthesis of hemoglobin, cobalt functions as a co-enzyme, copper as co-factor in enzymes while zinc, selenium and chromium are also important components of enzymes (Anderson and Fitzgerald, 2010; Soetan *et al*., 2010). However, a number of heavy metals based on their toxicities have made the list to the world health first ten chemicals of great concern. Arsenic, cadmium, lead and mercury have been declared by the World Health Organization as heavy metals of major public health concern (WHO, 2011). Some are carcinogenic or toxic, affecting, among others, the central nervous system (Hg, Pb, As), the kidneys or liver (Hg, Pb, Cd, Cu) or skin, bones, or teeth (Ni, Cd, Cu, Cr) (Zevenhoven and Kilpinen, 2001; WHO, 2011).

Fish which naturally survives in water bodies is food to man as it's a relatively cheap source of protein. Consumption of fish by man has been found to be of essential nutritional value. Nutritional composition of fish encompasses both macro and trace nutrients beneficial to the human biological system. Water, protein, lipid, mineral and vitamin B2 are major nutritional constituents of fish (Abowei and Tawari, 2011). 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. Heavy metals pollution of the aquatic ecosystem hence threatens the suitability of fish as an important food source for humans.

Man is at risk of either acute effect or chronic effect of heavy metals toxicity from dietary intake of contaminated fish. Cadmium (Cd) and lead (Pb) are heavy metals which can be accumulated by fish and transmitted into man through the ingestion route. Cadmium is released as a by- product in the refining of zinc and occasionally lead; lead is emitted during its mining and smelting activities, from automobile exhausts via the combustion of petroleum fuels treated with tetraethyl lead antiknock and lead paints. Generally, metals are emitted during their mining and processing activities (Lenntech, 2004; Duruibe *et al*., 2007). Increased level of cadmium and lead in various environmental media including water bodies is been observed and attributed to their natural existence in the earth crust and wide industrial production of human consumables (Sinhal *et al*.2010; Mojiri, 2011). Knowledge of the distribution of cadmium and lead in fish organs when assimilated is essential to determine fish parts to be avoided so as to reduce the risk of dietary lead and cadmium from the consumption of fish. Therefore, this study observed the cadmium and lead assimilation into kidney, Liver and flesh of African Catfish (*Clarias gariepinus*).

Materials and methods

Juveniles of *Clarias gariepinus* were purchased from the fish research laboratory of the department of fisheries, Oyo state ministry of Agriculture. The fish samples were raised to mean weight 116.2±8.8 g and standard length 26.5±1.4 cm and acclimatized for four weeks in plastic tanks of 80 litres capacity. The fish were fed with commercial fish pellets (Durhante brand). Adequate feeding was done to the fish during acclimatization while 5% mean body weight of fish feed equivalence was given during the exposure period. Well water in the vicinity of the department was used as the underground water source for maintaining the fish. Physico- chemical analysis of the well water and aquaria water samples was done using standard methods to determine the suitability of the water for breeding fish.

Exposure of fish samples to the test heavy metals

Fish samples (36) were divided into 3 groups of 12 samples each. Each group consists of three aquaria of four fish in each. The first group (A) was exposed to 4.89 mg/l of cadmium while group B was exposed to 24.18 mg/l lead, the third group served as control without cadmium or lead. The test chemicals concentration used are 30% of 24 hour lethal concentration of each heavy metal to the selected fish species predetermined in an experimental study.

Chlorides of cadmium and lead served as the metals source for the experiment. The heavy metal salts were dissolved in well water to make the needed test concentration. The required weight of salt equivalent to the weight of test chemical needed to make the test concentrations was calculated with this formula:

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

Duration of exposure was four weeks; three samples were harvested from each group per week. Harvested fish samples were dissected with stainless dissecting blade to eviscerate the gills and liver. Flesh, gills and liver isolated were collected into smaller plastic sample bottles and labeled appropriately. The samples were oven dried to constant weight at 60°C for 24hrs. Samples when dried were homogenized using a clean stainless mortal and pistil homogenizer in the laboratory before digestion.

Water sampling procedure

For the underground water source, composite samples were collected at various depths of the well into a clean narrow mouth plastic 1litre sample bottle. Sample bottle was rinsed thoroughly with the well water before use. Samples were collected in triplicates.

The aquaria water samples were collected weekly. Well water was analyzed for baseline cadmium and lead as well as pH, alkalinity, dissolved oxygen, hardness and temperature levels.

Acid digestion of samples

Water sample (10 mls) was measured into a pyrex digestion flask. Acid mixture of 5 mls (2:1) analytical grades of nitric acid (HNO_3) and hydrochloric acid (HCl) 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 mls. Digest was made up to 25 mls with deionized water in a volumetric flask. Blank digests were also prepared using the same procedure.

Homogenized sample (1 g) of fish flesh was weighed in a pyrex digestion flask. Analytical grade Nitric acid (HNO_3) with perchloric acid 5 mls (4:1) 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 filterate was made up to 25 mls with deionized water in a volumetric flask.

Results

Physico-chemical parameters of water samples

The samples collected from the well and aquaria were analyzed for their physico-chemical properties. Mean values of the parameters investigated for the well and aquaria follows respectively; Temperature (25.2±0.8 ^oC) : (24.8±0.6 ^oC) , pH (6.7±0.3) : (6.8±0.3), Dissolved Oxygen (7.8±0.8mg/l) : (7.1±0.6mg/l) , Hardness (54.2±2.1 mg $CaCO_3$/l) : (45.3±9.9 mg $CaCO_3$/l) and Total alkalinity (119.5±3.9 mg $CaCO_3$/l) : (128.9±19.4 mg $CaCO_3$/l). Cadmium and lead were not detected in the samples of well (source) water used for the experiment. Results were compared with the Food and Agriculture Organization (FAO) guidelines for fish pond water quality as shown in Table 1..

Table 1: Physico-chemical parameters of water samples

Parameter	Range	Mean ± SD*	Mean ± SD**	FAO Guideline
Temperature (ºC)	23-26	25.2±0.8	24.8±0.6	25 – 30
pH	6.1 – 7.9	6.7±0.3	6.8±0.3	6.5 – 8.5
Dissolved Oxygen (mg/l)	5.3 – 8.9	7.8±0.8	7.1±0.6	>3
Hardness (mg $CaCO_3$/l)	32.4 – 64.6	54.2±2.1	45.3±9.9	>25*
Total Alkalinity (mg$CaCO_3$/l)	100 – 162	119.5±3.9	128.9±19.4	>25*
Cadmium (mg/l)	ND			
Lead (mg/l)	ND			

Food and Agriculture Organization (FAO, 2013a).
*Food and Agriculture Organization (FAO, 2013b).
ND - Not Detected in water source (well) samples

Mean ± SD* - results for water source (well) samples
Mean ± SD** - results for aquaria water samples

Heavy metal assimilation in tissues of *Clarias gariepinus*

The cadmium and lead assimilation in the selected tissues of *Clarias gariepinus* shows various concentrations as indicated in Figure 1.

i. Cadmium assimilation in tissues of *Clarias gariepinus*

The mean concentrations of cadmium in mg/kg dry weight of flesh, gills and liver of the test fish were 8.8±5.1 mg/kg, 31.4±10.5 mg/kg and 38.3±11.6 mg/kg respectively. Concentrations detected in the gills and liver are significantly higher than the flesh ($p<0.05$). Results are summarized in Table 2 below.

ii. Lead assimilation in tissues of *Clarias gariepinus*

The mean concentrations of lead in mg/kg dry weight of flesh, gills and liver of the test fish were 44.6±22.5mg/kg, 123.3±42.9mg/kg and 82.6±29.1mg/kg respectively. Concentration detected in the gills was significantly higher than that of the liver which was also higher than the flesh ($p<0.05$) as shown in Table 3 below.

Table 2: Mean concentrations of Cd assimilated in flesh, gills and liver of *Clarias gariepinus* in mg/kg dry weight (wt)

Fish Organ	Cd (mg/kg dry wt)	Increase in Cd level relative to flesh (%)
Gills	31.4±10.5 [a]	256.8
Liver	38.3±11.6[a]	334.1
Flesh	8.8±5.1[b]	-

[a b] Different superscripts on the same column denote significant mean difference at $p< 0.05$

Table 3: **Mean concentrations of Pb assimilated in flesh, gills and liver of *Clarias gariepinus* in mg/kg dry weight (wt)**

Fish Organ	Pb (mg/kg dry wt)	Increase in Pb level relative to flesh (%)
Gills	123.3±42.9 [a]	176
Liver	82.6±29.1[b]	85
Flesh	44.6±22.5[c]	-

[a b c] Different superscripts on the same column denote significant mean difference at $p < 0.05$

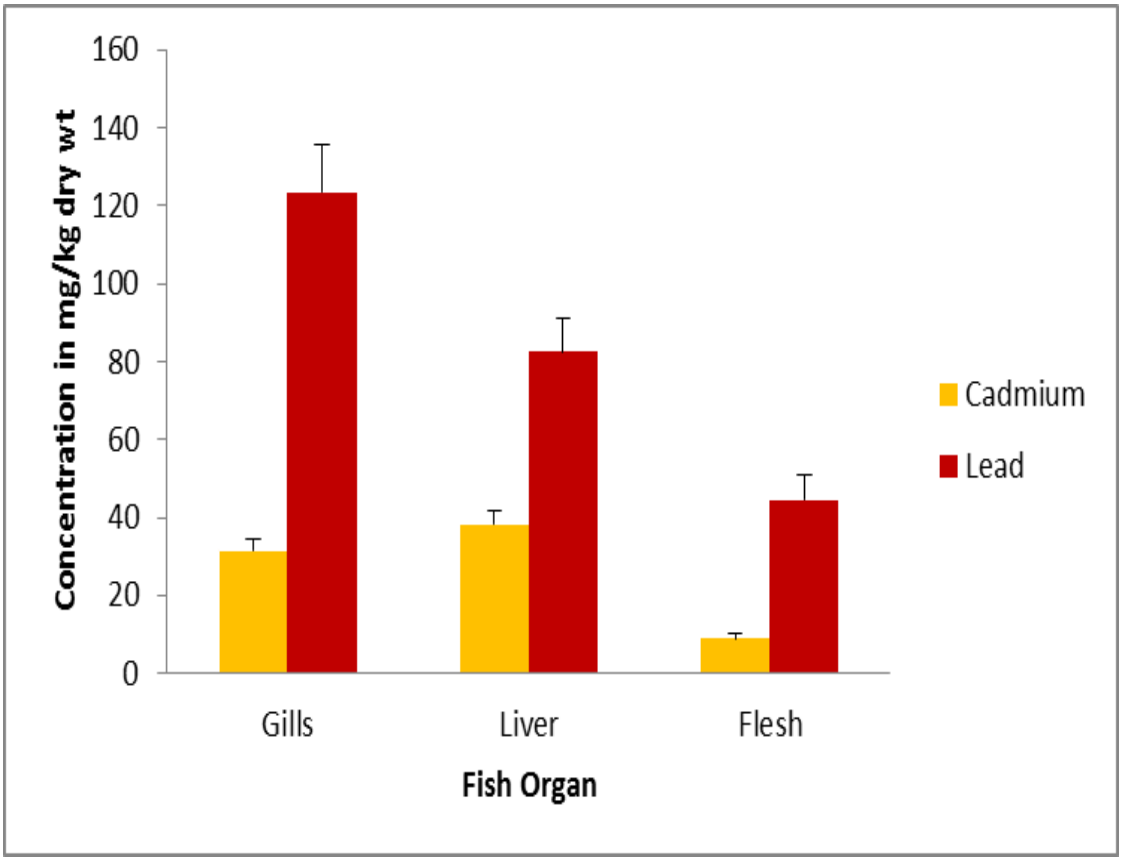

Figure 1: **Mean Cd and Pb concentrations in *Clarias gariepinus* organ**

Discussion

Physico-chemical analysis of water

The physical, chemical and biological characteristics of a water body determine the survival of its aquatic residents (Bhatnagar and Devi, 2013). Adequate dissolved oxygen, optimum pH and temperature, proper dissolved solids of water among others are essential for fish culture.

Concentration of each heavy metal i.e Cd and Pb introduced into aquaria water was taken as the available concentration to which test fish samples were exposed since cadmium and lead were not detected in samples of the water source used for culturing (well).

The water source had a temperature of 25.2±0.8ºC and temperature of the ponds for the four weeks of exposure was 24.8±0.6ºC. These values are not significantly different ($p>0.05$) from (25ºC -30ºC) which is the range stipulated by FAO (2013a) as suitable for fish culture. Optimum temperature range of 25 ºC -27 ºC is regarded adequate for *Clarias gariepinus* adults (FAO, 2013a) and is similar to the findings of this study. The rate of biochemical reactions and level of dissolved gases of pond water is controlled by the temperature of the pond water. Low water temperature encourages the buildup of toxic gases like hydrogen sulphide and methane which poses a negative effect on fish health. Kusemiju *et al*., 2012 similarly analysed the temperature of well water used for the culture of *Clarias gariepinus* to be 23.40±1.21ºC. Okonji and Ewutanure, 2011 monitored the pond temperature for a 24weeks Catfish culture to be 30.2 ºC which is higher than what obtains in this particular study.

The pHs of source water and test aquarium water samples were 6.7±0.3 and 6.8±0.3 respectively. This is similar to the value documented by Kusemiju *et al.*, 2012 (6.9±0.64). Fish production is greatly affected by excessively high or low pH. Extremely high and low pH could lead to high fish mortality. A culture water pH within 6.5 – 8.5 is considered adequate for pond fish production (FAO, 2013a). The result of pH obtained falls within this range.

For the water source and aquaria, dissolved Oxygen (DO) was 7.8±0.8mg/l and 7.1±0.6mg/l respectively. Olaifa *et al*., 2003 used water with similar level of dissolved oxygen (7.4mg/l) in maintaining *Clarias gariepinus* fingerlings and Kusemiju et al., 2012 cultured the same species with water of lower dissolved oxygen (5.7±0.42mg/l). DO is considered the most important water quality criterion in aquaculture systems. It is vital for respiration and other metabolic activities necessary for fish survival. Dissolved oxygen level of >20 mg/l causes physiological dysfunction; hence toxic to fish while at lower level than 3mg/l, fish exhibit reduced fecundity with low egg and sperm viability(Department of Water Affairs and Forestry, 1996). Poor feeding, starvation, reduced growth and death are possible consequences of low dissolved oxygen on fish (Bhatnagar and Garg, 2000). Adult African catfish survives in water of at least 3mg/l dissolved oxygen and 9mg/l is optimal for growth of eggs and juveniles (FAO, 2013a).

Alkalinity of culture water is an important criterion pertinent 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. Total alkalinity value of at least 20 mg $CaCO_3$/l is necessary for catfish production (Swann, 1997). An alkalinity of between 100 - 150 mg $CaCO_3$ / l is suitable for exerting less energy on osmoregulation which translates into better growth for a fresh water fish like *Clarias spp* (Department of Water Affairs and Forestry,1996). Total alkalinity of culture water from source (119±3.9 mg $CaCO_3$ / l) and aquaria (128.9±19.4 mg $CaCO_3$ / l) obtained for this study are similar to values stipulated by the guideline.

Water hardness was 45.3±9.9 mg $CaCO_3$ / l for the aquaria and 54.2±2.1 mg $CaCO_3$ / l for the source water. Hardness of 30 - 180 mg $CaCO_3$ / l is desirable for fish culture (Santhosh and Singh, 2007). Hardness of <20 mg $CaCO_3$ / l leads to fish stress while at >300 mg $CaCO_3$ / l, an increase in pH occurs leading to unavailability of nutrient (Bhatnagar *et al*., 2004). Nwani *et al*., 2010 utilized water of total hardness ranging from 200 - 230 mg $CaCO_3$ / l higher than what obtains in this study for the maintenance of a similar freshwater air breathing fish species (*Channa punctatus*). Olaifa *et al*., 2003 maintained fingerlings of *Clarias gariepinus* with water of hardness (78.56±3.89 mg $CaCO_3$ / l) relatively similar to what obtains in this investigation.

Cadmium assimilation pattern in *Clarias gariepinus*

Tissues of a body system accumulate heavy metals from the same medium at different levels (Al-Kahtani, 2009; Kusemiju *et al*., 2012). The tissues showed a significant variation in their pattern of assimilating both cadmium and lead. The difference in physiology and location of tissue may be initiating factors for the preference of metal assimilation of a specific tissue

over another (Mohanambal and Puvaneswari, 2013). Cadmium and lead were not detected in the tissues of control fish samples. For the test group, Cadmium residues in dry weight of gills, liver and flesh of fish samples were 31.4±10.5mg/kg, 38.3±11.6mg/kg and 8.8±5.1mg/kg. This study observed cadmium assimilation in the decreasing order of liver > gills > flesh. There is a significant increase in cadmium levels of gills and liver compared to flesh ($p<0.05$).

In fish the cadmium binding protein methalothioneins have been found to play a very important role in cadmium accumulation and are localised mostly in the liver, kidney and gills (Campenhout *et a*l., 2004). This may account for the high cadmium level discovered in the liver and gills relative to the fish flesh. Staniskiene *et al*., (2006) similarly discovered higher levels of cadmium in liver than the flesh of various fresh water fish in Lithuania.

Lead assimilation pattern in *Clarias gariepinus*

Lead residues in dry weight of gills, liver and flesh of fish samples were 123.3±42.9mg/kg, 82.6±29.1mg/kg and 44.6±22.5mg/kg. There is a significant difference between lead levels in gill, liver and flesh of fish sample ($p<0.05$). Lead assimilation was in the decreasing order of gills> liver> flesh. This observation is consistent with the findings of Kusemiju *et al*., (2012); Eneji *et al*., (2011) and Ganbi, (2010). Lead forms a complex with mucous around the gill tissues which are not easily dissociated with sample preparation during analysis, this may account for the high level of lead determined in the gills of fish species. The important role of the gills for respiration and its large contact surface area to the culture medium may also be responsible for high level of lead detected in the gills relative to the liver and flesh (Kusemiju *et al*., 2012).

Gills and liver of *Clarias gariepinus* assimilated more cadmium and lead than flesh. This result is similar to that of Ganbi, (2010), Yehia and Sebaee, (2012) but conflicts the report of Sani, (2012). The abundance of metal binding proteins in the gills and liver than the flesh coupled with the fact that the liver stands as a major site of detoxification of xenobiotics may be reasons for this observation. Level of lead in the gills was greater than the liver as opposed to this comparism for cadmium; this may be due to the high concentration of cadmium binding metallothioneins in the liver than the gills (Lange *et al*., 2002, Campenhout *et a*l., 2004).

Conclusion

The tissues of *Clarias gariepinus* showed a variation in the extent of heavy metal assimilation. Gills and liver of *Clarias gariepinus* assimilated more cadmium and lead than the flesh. Consumption of the fish without these tissues or their residues would be a proactive measure of reducing the burden of cadmium and lead from diets. Thorough washing of fish and removing the gills and liver before consumption will reduce the risk of exposure to cadmium and lead from the *Clarias gariepinu*, since this fish is highly consumed in Nigeria as protein source. Knowledge of these assimilation patterns and adopting proactive measures in ameliorating the burden of cadmium and lead from the commonly consumed fish in Nigeria, will provide improved and safe *Clarias gariepinu* for consumption.

Recommendations

Thorough washing of fish and evisceration household techniques which allows removal of internal organs of the fish before consumption is advocated in order to reduce risk of dietary cadmium and lead. The internal organs removed should be disposed in a safe manner that it will not pose risks to man and the environment.

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