Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Medical & Health Sci.
4(1), 2017, 20-29

ISSN 2395 – 6046

Original Research Article

PUBLIC HEALTH EVALUATION OF CADMIUM, LEAD, ARSENIC AND COPPER IN *CLARIAS GARIEPINUS* (CAT FISH) REARED IN TARPAULIN PONDS (NON-NATURAL HABITAT) IN BENIN CITY

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Abstract: Background: Studies have evaluated the level of toxic metals in catfish but no study has been carried out to evaluate the levels of toxic metals in catfish (*Clarias gariepinus*) reared in tarpaulin ponds in Nigeria. Therefore, this study seeks to evaluate the concentration of cadmium, lead, arsenic and copper in catfish (*Clarias gariepinus)* reared in tarpaulin ponds in Benin City and to correlate their concentrations in catfish (*Clarias gariepinus)* obtained from a natural source. **Materials and Methods**: Catfish were obtained from five different farms in Benin City and the controls were gotten from oviariver. The catfish samples were adequately washed and the concentrations of cadmium, lead, arsenic and copper were determined using inductively coupled plasma mass spectrophotometer. **Results**: The concentrations of lead (0.072±0.00and cadmium (0.013±0.00 in the tissues and the concentrations of arsenic (0.011±0.00, in the blood samples of catfish from all five tarpaulin farms were significantly higher (p<0.01) while the cadmium level in the blood samples were lower compared to controls (0.025±0.001mg/g, 0.008±0.00mg/g and with the exception of one tarpaulin pond, Copper concentrations (114.96±1.69, in tissues of catfish (*Clarias gariepinus*) grown in tarpaulin ponds were significantly higher (p<0.01) compared to controls (101.23±0.89). The concentrations of arsenic (0.02±0.00 and 0.015±0.00) in some of the tissue samples were also significantly higher compared to controls (0.014^{b}±0.001). **Conclusion:** Catfish farmed in tarpaulin contain statistically significant (p<0.01) concentrations of cadmium, lead, arsenic and copper and their concentrations are well above the WHO permissible limit.

Key Words: Cat fish, toxic metals, Tarpaulin Ponds

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Received on: February 2017
Accepted after revision: March 2017

Introduction: Catfishes are commonly reared in concrete, earthen and tarpaulin Ponds in Benin-City. Although the use of tarpaulin has not gained much popularity in the state, some catfish farmers prefer the use of tarpaulin because of the different advantages associated with its use including the fact that tarpaulin is collapsible, mobile and easy to maintain. Due to these advantages, several fish farmers are likely to venture into the use of tarpaulin in future. However, research has shown that artificial fish ponds may contain some chemical and physical compositions by virtue of the material makeup of the habitat. Some of the chemicals that may be present in these habitats include; cadmium, lead, arsenic, copper, etc (Umnakwe and Aharanwa, 2014). Some of these metals are required for normal growth and development of the fishes while others have no known biological function. These elements can bioaccumulateto concentrations which can cause toxicity in the fishes (Craford and Avenant, 2010).

In spite of the numerous dietary importance of catfish, research has shown that if the chemical composition of these fishes is significantly elevated it can be toxic to humans who consume them (Adeyemo*et al.*, 2010).

Polyvinyl chloride tarpaulin is the latest material used in rearing Catfish in Benin- city manufactured from polyvinyl Chloride (Bidoki and Wittlinger, 2010). Some of the additives used to modify polyvinyl chloride include Cadmium, Lead, Arsenic, Copper, which are added to the raw polyvinyl chloride in varying concentrations (Evans, 2000). It is therefore possible, that these additives can leach out of polyvinyl chloride products into the environment when subjected to damp or wet condition (Thomas, 1996). If these additives leach out of the polyvinyl chloride product (tarpaulin) during use into the environment (water) they can bioaccumulate in the catfish being farmed. Cadmium, Lead, Arsenic and Copper which are used as additives in polyvinyl chloride formulation and which are therefore, ultimately present in the final manufactured polyvinyl chloride (PVC) products are heavy metals are associated with a number of recognized potential impacts on human health (Sadiki and Williams, 1999). Fish have the ability to bio-accumulate toxic metals over time (Adeniyi and Yusuf, 2007). As have been reported, some of these metals (such as cadmium, lead and arsenic) have no bio-importance in human biochemistry and physiology and consumption even at very low concentrations can be toxic (Nolan 2003.) Concern about the high levels of toxic metals in foods has prompted several statutory bodies such as WHO to establish maximum allowable concentrations for some of the metals in food (WHO 1984). Thus, the world health organization(WHO) as well as the Food and Agriculture Organization (FAO) of the united nations state that monitoring eight elements (mercury, cadmium, lead copper, arsenic, zinc iron and selenium) in fish is obligatory while the monitoring of others though not obligatory may be useful (Staniskiene *et al.*, 2006). This study therefore, was carried out to evaluate the concentration of toxic metals such as Cadmium, Lead, Arsenic and Copper concentration in *Clariasgariepinus* farmed in tarpaulin ponds compared with that of control *Clarias garipinus* from river.

Materials and Methods

Study location: This research was carried in Benin City, Edo State, Nigeria. Benin City is the capital of Edo State which is located in south-south Nigeria.

Collection of samples: *Clarias gariepinus* specimens were obtained from five different catfish farms which frequently supply *Clarias gariepinus* (catfish) to major markets in Benin City using questionnaire. The names of the farms are: Sunny fish farm, Funke fish farm, Chucks fish farm, Efex fish farm, Ilo fish farm and the controls were obtained from Ovia river (about 25 kilometres from Benin). In order to prevent haemolysis and achieve easy manipulation, excess water was removed from the fishes with the aid of clean dry towels before they were sacrificed and the samples were obtained under sterile condition. From each fish pond, 20mls of water sample was collected into a clean container and filtered there after using whatman No.1 filter paper into a sterile universal container. Both blood samples and water samples were properly labeled and transported to the laboratory in ice packs for analysis.

Scope of Wet Digestion: A bi-acid digestion procedure was used for the sample preparation of *Clarias gariepinus* tissue and blood.

Sample Preparation: The fish tissues (tail) were carefully oven dried at 105°C to a constant weight. The dried fish samples were homogenized using a clean mortar and pestle. The homogenized samples (1.0g each) were digested in a digestion flask in pentaplicate (n=5) FAO/SIDA manual part 8 (1983).

Ten (10mls) of $HClO_4$ (Perchloric acid) and 10ml of HNO_3 (Nitric acid) was added.

It was swirled gently and heated under the fume cupboard at an increasing temperature for 10minutes. Brown fumes of HNO_3 escaped. A golden yellow liquid was obtained after 3 hours. The liquid was cooled, filtered and transferred into 50ml volumetric flask and made up with HNO_3 solution.

ICP-MS Instrument Set-up: The samples were analyzed following manufacturer's instructions. The instrument was calibrated using the standard blank and various standard dilutions.

Concentrations of the analytes were determined following the individual protocols Cadmium (Link*etal*.,1998),Lead, (Sandstead,1995), Arsenic (Larsen and Sturup, 1994), and Copper (Van Den Berg, 1984).

Statistical analysis: Statistical analysis including descriptive statistics was carried out using the statistical package for social scientists (SPSS) version 16.0. All values were expressed as Mean ± Standard Error of the Mean. Results from all the samples were compared using ANOVA and unpaired T-test. Level of significance was set at (P<0.05)

Ethical approval for the study: Ethical approval was obtained for this study from Edo State Ministry of Health because the research was carried in Benin City because of the public health implication.

Limitations of the study: There are difficulties in locating the tarpaulin ponds in the geographical location, only five different tarpaulin ponds were used for the analysis and obtaining catfish from tarpaulin pond the farmers were afraid of being exposed.

Results: The mean concentration of copper, lead, arsenic and cadmium bio-accumulated in tissues of *Clarias gariepinus* farmed in tarpaulin and control from a river is shown in Table 1. The concentration of lead and cadmium were significantly higher in the tissues of *Clarias gariepinus* farmed in tarpaulin (p<0.01) compared to controls (*Clarias gariepinus* from the river). Copper concentration in tissues of *Clarias gariepinus* farmed in tarpaulin were significantly higher with the exception of the *Clarias gariepinus* obtained from Funke's fish farm which contained lower concentration of copper compared to controls. The concentration of arsenic in the tissues of *Clarias gariepinus* obtained from Sunny's fish farm, Chucks's fish farm and Ilos's fish farm respectively were lower while the concentration in *Clarias gariepinus* from Efex's fish farm and Funke's fish farm were significantly higher compared to controls.

Table1: Level of Cu, Pb, Cd and As Bio-accumulated in Tissues of *Clarias garipinus* Reared in Tarpaulin Ponds

	SUNNY FARM(1) FISH TISSUE	FUNKE FARM(2) FISH TISSUE	CHUCKS FARM (3) FISH TISSUE	EFEX FARM(4) FISH TISSUE	ILO FARM(5) FISH TISSUE	RIVER (6) FISH TISSUE	p- Value
Cu (mg/g)	$114.96^{b}\pm1.69$	$98.56^{d}\pm2.69$	135.s49$^{a}\pm0.96$	$105.59^{c}\pm0.99$	$113.14^{b}\pm0.83$	$101.23^{d}\pm0.89$	**P<0.01
Pb (mg/g))	$0.041^{c}\pm0.001$	$0.072^{a}\pm0.004$	$0.037^{c}\pm0.001$	$0.058^{b}\pm0.001$	$0.041^{c}\pm0.001$	$0.025^{d}\pm0.001$	**P<0.01
Cd (mg/g)	$0.013^{c}\pm0$	$0.023^{a}\pm0.001$	$0.012^{c}\pm0.00$	$0.019^{b}\pm0$	$0.013^{c}\pm0$	$0.008^{d}\pm0$	**P<0.01
As (mg/g)	$0.01^{c}\pm0$	$0.02^{a}\pm0.001$	$0.01^{c}\pm0.00$	$0.015^{b}\pm0$	$0.01^{c}\pm0$	$0.014^{b}\pm0.001$	**P<0.01

*Note: **P<0.01- highly significant*

Note: subscripts a, b, c and d represent concentration levels from highest to lowest

Table **2** shows the mean concentration of copper, lead, arsenic and cadmium bio-accumulated in blood samples of *Clarias gariepinus* farmed in tarpaulin and control from ovia river. The concentrations of arsenic were significantly higher while the concentrations of cadmium were significantly lower in the blood samples compared to controls (*Clarias gariepinus* from the river). On the other hand, Copper concentrations in blood samples of *Clarias gariepinus* obtained from Chucks, Sunny, Efex and Ilo were significantly higher while those obtained from Funke's farm had lower concentrations of copper compared to controls. The blood samples of *Clarias gariepinus* obtained from sunny, chucks, Efex and Ilo's tarpaulin ponds contained higher concentrations of lead while those obtained from Funkes's tarpaulin pond had reduced concentrations of lead compared to controls

Table2: Level of Cu, Pb Cd and As Bio-accumulated in Blood of *Clarias gariepinus* Reared in Tarpaulin Ponds

	SUNNY(1) FISH BLOOD	FUNKE(2) FISH BLOOD	CHUCKS(3) FISH BLOOD	EFEX(4) FISH BLOOD	ILO(5) FISH BLOOD	RIVER(6) FISH BLOOD	p- Value
Cu (mg/g)	112.2^{d}±0.95	98.50^{f}±0.79	140.31^{b}±1.31	125.32^{c}±1.58	146.92^{a}±1.43	105.21^{e}±0.93	**P<0.01
Pb (mg/g)	0.04^{c}±0	0.030^{d}±0	0.043^{a}±0	0.039^{b}±0	0.045^{a}±0.00	0.038^{b}±0.001	**P<0.01
Cd (mg/g)	0.007^{d}±0	0.006^{e}±0	0.009^{b}±0	0.008^{c}±0	0.009^{b}±0	0.013^{a}±0.001	**P<0.01
As (mg/g)	0.011^{d}±0	0.01^{e}±0	0.014^{b}±0	0.013^{c}±0	0.015^{a}±0	0.006^{f}±0	**P<0.01

*Note: **P<0.01- highly significant*

Note: subscripts a, b, c and d represent concentration levels from highest to lowest

Table **3** shows the concentration of copper, lead, arsenic and cadmium bio-accumulated in water samples obtained from tarpaulin ponds and water sampled from a river .The concentrations of copper in water samples obtained from sunny, Chucks and Ilo's ponds were higher while the water samples from Funke and Efex's ponds had lower copper concentration compared to control. The concentration of lead in water sample obtained from Funke and Efexponds were significantly higher compared to the controls while the copper concentrations in water samples obtained from Ilo, Chucks and Sunny ponds correspond with that of controls. Cadmium concentrations in water samples obtained from Ilo and Efex ponds were significantly higher while the concentration of cadmium in the water samples obtained from Sunny, Chucks and Ilo's ponds respectively were significantly higher compared controls. Arsenic concentrations of water samples obtained from all the ponds were significantly lower compared to controls.

Table3: Level of Cu, Pb, Cd and As bio-accumulated in Water Sample from Tarpaulin Ponds and Controls

Parameters	SUNNY(1) WATER	FUNKE(2) WATER	CHUCKS(3) WATER	EFEX(4) WATER	ILO(5) WATER	RIVER(6) WATER
Cu (mg/g)	116.652	95.858	134.524	104.599	113.976	106.378
Pb (mg/g)	0.042	0.076	0.035	0.059	0.040	0.045
Cd (mg/g)	0.014	0.025	0.011	0.019	0.013	0.016
As (mg/g)	0.011	0.019	0.009	0.015	0.010	0.023

Table 4: Test of relationship between the heavy metals of Clarias gariepinus tissue, Blood and water of sampled ponds

	mg/g Cu.fish .tissue	*mg/g Pb.fish .tissue*	*mg/g Cd.fish .tissue*	*mg/gAs. fish .tissue*	*mg/dl Cu.fish .blood*	*mg/dlP b.fish ..blood*	*mg/dl Cd.fish .blood*	*mg/dl As.fish. blood*	*mg/L Cu.fish .water*	*mg/L Pb.fish ..water*	*mg/L Cd.fish water*	*mg/L As.fish water*
mg/g Cu.fish .tissue	1											
mg/g Pb.fish .tissue	-0.404	1										
mg/g Cd.fish .tissue	-0.389	**1**	1									
mg/g As.fish .tissue	**-0.815**	**0.691**	**0.677**	1								
mg/dl Cu.fish .blood	**0.685**	-0.298	-0.284	**-0.739**	1							
mg/dlPb.fish ..blood	0.6	-0.541	-0.531	**-0.728**	**0.93**	1						
mg/dl Cd.fish .blood	-0.079	**-0.785**	**-0.796**	-0.162	0.036	0.386	1					
mg/dl.Asblood	**0.639**	0.147	0.165	-0.557	**0.856**	0.627	-0.477	1				
mg/LCufish(H_2O)	-0.34	0.538	0.538	0.538	-0.271	-0.271	-0.271	-0.271	1			
mg/LPb.fish(H_2O)	-0.155	-0.165	-0.165	-0.165	-0.197	-0.197	-0.197	-0.197	**-0.839**	1		
mg/L Cd.fish(H_2O)	-0.092	-0.204	-0.204	-0.204	-0.137	-0.137	-0.137	-0.137	**-0.869**	**0.994**	1	
mg/L As.fish(H_2O)	0.388	-0.4	-0.4	-0.4	0.338	0.338	0.338	0.338	**-0.741**	0.563	**0.649**	1

N=12

CRITICAL r(0.05,df=10(α2))=0.632,

CRITICAL r(0.01,df=10(α2))=0.765

bolded values are significant, P<0.05

while bolded values with underline are highly significant ;P<0.01

Table 4 shows the test of relationship between the heavy metals in tissue and blood samples of *Clarias gariepinus* as well as water sample from each pond. The result on the relationship between lead and copper in the tissue of *Clarias gariepinus* shows a negative correlation. The relationship between arsenic, lead, cadmium and copper in the tissue of *Clarias gariepinus* is highly significant ($P<0.01$), but the correlation between arsenic and copper is negative. The result on the relationship between copper in the blood samples and copper in the tissue samples of *Clarias gariepinus* shows a significant correlation ($P<0.05$) while there is a negative significant correlation between copper in the blood sample and arsenic in the tissue samples. The result on the relationship between lead in the blood, arsenic in the tissue and copper in the blood of *Clarias gariepinus* shows a significant correlation ($P<0.05$) with the correlation between lead in blood and arsenic in tissue of *Clarias gariepinus* being negative. The result on the relationship between cadmium in the tissues and cadmium in the blood samples shows a negative but highly significant correlation ($P<0.01$). The result on the relationship between arsenic in blood, copper in tissue and copper in the blood of *Clarias gariepinus* shows a highly significant correlation ($P<0.01$). There is a highly significant($P<0.01$) but negative correlation between lead and copper in the water sample. The relationship between cadmium, copper and lead in the water sample is highly significant ($P<0.01$) but the relationship between the cadmium and copper in the water sample is negative. The result on the relationship between arsenic, copper and lead in the water sample is highly significant ($P<0.01$) but the relationship between arsenic and copper in the water sample is negative.

Discussion: The findings show significant increase in the concentration of cadmium in the tissues of *Clarias gariepinus* grown in tarpaulin ponds compared to controls ($p<0.05$). This maybe as a result of thee leaching of additives (Evans,2000) used in the formulation of polyvinyl chloride(PVC) which in turn is used for the manufacture of tarpaulin (Bidoki and Wittlinger,2010). Highly significant correlation was also observed to exist between the concentration of cadmium and the concentration of lead in the tissues of *Clarias gariepinus* reared in tarpaulin ponds ($P<0.01$). According to Van *et al* (2008), cadmium, lead and other metals are used as pigments and stabilizers in polyvinyl chloride and polyvinyl chloride is the chief constituent of tarpaulin. Therefore, the increased levels of cadmium and lead observed in this study could be attributed to their leakage into the fishpond (Thomas, 1996) and their increased accumulation in the tissues of the catfish since fishes are notorious for the ability to accumulate metals (Adeniyi and Yusuf, 2007). The increased concentrations of lead in the tissues of *Clarias gariepinus* reared in tarpaulin may result in toxicity such as reduced spermatogenesis and development of sperm (Sokol *et al*., 2002). Literature supports the view that cadmium which often accumulate in human body through food negatively affects several organs: liver, kidney, lungs, bones, placenta, brain and the central nervous system (Chouba *et al*., 2007). Possible effects of cadmium on consumers include reproductive damage, immunological and haematological and developmental toxicity. These disorders may result because cadmium even at low concentration is harmful to living organisms (Tsui and Wang, 2004). However, the concentration of cadmium in the blood samples of *Clarias gariepinus* was significantly lower compared to control ($p<0.05$), showing that this metal bio-accumulates more in the tissues than in the blood of *Clarias gariepinus*. There is also a significant correlation between the concentration of cadmium and lead in the water sampled from each tarpaulin pond (($P<0.05$), where increase in the concentration of cadmium in the water samples corresponds to increase in the concentration of lead

The mean concentration of cadmium in the tissues of *Clarias gariepinus* obtained from the five different tarpaulin ponds increased significantly above the WHO standard for maximum permissible limit of cadmium in tissues of catfish(WHO=0.0002mg/g) (WHO and FAD, 2011).Acute ingestion may result in symptoms such as abdominal pain, burning sensation, nausea, vomiting, salivation, muscle cramps, vertigo, shock, loss of consciousness and convulsions usually appear within 15 to 30 minutes. Other damages in human include; gastrointestinal tract erosion, pulmonary, skeletal demineralization by increasing calcium excretion leading to bone fragility and risk of fracture, and coma, depending on the route of poisoning (Basalt and Cravey,1995).

Significant increase in the concentration of lead was observed in the tissues of *clarias gariepinus* reared in tarpaulin ponds ($p<0.05$). But the concentrations of lead in the blood samples of *Clarias gariepinus* obtained from sunny, chucks, Efex and Ilo's tarpaulin ponds were higher showing a significant correlation between the concentration of this metal in tissue and blood of *Clarias gariepinus* grown in tarpaulin ponds ($p<0.05$). The mean concentrations of lead in the tissues of *Clarias gariepinus* reared in tarpaulin ponds were above the WHO's maximum permissible limit of(0.0004mg/g). Lead may cause several disorders including disruption of both pituitary glands and hypothalamus functions in both experimental animals and humans which may result in hormonal imbalance (Sokol *et al*., 2002). Lead is also considered by the IARC as a probable human carcinogen (IARC, 1987)

There was significant increase in the concentrations of arsenic in the tissue samples of *Clarias gariepinus* obtained from Efex's and Funke's tarpaulin ponds while *Clarias gariepinus* obtained from other tarpaulin ponds did not contain significant concentrations of arsenic compared to control ($p<0.05$) . Arsenic concentrations contained the blood samples of *Clarias gariepinus* were significantly increased compared to controls. The concentrations of arsenic in water samples obtained from each pond was significantly reduced compared to control. However, the concentrations of arsenic in the tissues of *Clarias gariepinus* obtained from each tarpaulin pond were above the maximum limit set by the WHO(0.0001mg/g). According to past research, As (III) can cause inactivation of over 200 enzymes by binding to thiol or sulfhydryl groups on proteins in the body. This is the Likely mechanism responsible for arsenic's widespread effects on different organ systems. Arsenic has been shown to cause impairment of cellular respiration by the inhibiting of various mitochondrial enzymes uncoupling of oxidative phosphorylation. Arsenic compounds have also been shown to induce gene amplification, arrest cells in mitosis, inhibit DNA repair, and induce expression of the *c-fos*gene and the oxidative stress protein heme- oxygenase in mammalian cells (Saleha *et al.*, 2001)

The findings of this study shows a significant elevation of copper concentrations in the tissues and even greater elevation of copper concentrations in the blood samples of *Clarias gariepinus* grown in tarpaulin ponds with the exception of *Clarias gariepinus* obtained from Funke's tarpaulin pond which had decreased concentration of copper in both tissue and blood samples compared to controls($p<0.05$). The greater concentrations of copper in the blood samples of *Clarias gariepinus* may have resulted from the fact that copper is an essential nutrient that is incorporated into a number of metallo-enzymes involved in hemoglobin formation (Stern, 2010). The concentrations of copper in the water samples were equally increased except for the water sampled from Efex's and Funke's tarpaulin ponds compared to controls. The mean concentrations of copper (114.96mg/g, 98.56mg/g,135.49mg/g, 105.59mg/g, 113.14mg/g, and 101.23mg/g respectively)) in all five tarpaulin ponds were above the maximum permissible limits set by WHO(0.0133mg/g). Copper is a trace element present in all tissues and is required for cellular respiration, peptide amidation, neurotransmitter biosynthesis, etc. Copper deficiency causes Menkes disease and anaemia (Kaler *et al*., 2008). According to Cox and Moore, 2002, deficiency of copper in the body can also result in Wilson disease. An increased concentration of copper in cerebrospinal fluid with normal plasma copper concentration has been noted in some patients with Alzheimer disease and this may contribute to the pathogenesis of the disease (Roos *et al*., 2006). Elevated copper concentration is also associated with human Huntington disease (Fox *et al*., 2007) linked to cellular damage leading to Wilson disease in human (Tchnuowou *et al*.,2008). Fatal dose of copper causes heartburn, vomiting, diarrhoea, metallic taste in the mouth, gastrointestinal haemorrhage, haemolysis and jaundice (Guany*et al*., 2006). Other research has shown that cellular toxicity of copper affects erythrocytes, hepatocytes and myocytes and that copper inhibits the sulfhydryl moieties of glucose-6-phosphate dehydrogenase and glutathione, reducing their free radical scavenging activities. Also, intracellular haemolysis is caused by the inhibition of glucose-6-phoosphate dehydrogenase, which leads to the oxidation of the sulfhydryl groups of haemoglobin.

Although no study has been carried out on the concentration of lead, cadmium, arsenic and copper in *Clarias gariepinus* reared in tarpaulin, this study shows that *Clarias gariepinus* grown in tarpaulin pond contain statistically significant concentrations of these metals compared to control. The concentration of some of the metals in water sampled from some of the tarpaulin ponds and samples of *Clarias gariepinus* tissues were decreased, this may be due to frequent change of water and proper maintenance of the ponds by the fish farmers .The concentration of some of the metals in water were reduced but increased in the tissues of *Clarias gariepinus*. This finding agrees with reports from previous research that fishes are notorious for their ability to accumulate heavy metals in their muscles (Adeniyi and Yusuf, 2007) and concentrations of trace metals in fish tissues were always higher than that of water (Chale, 2002)

Conclusion:

Clarias gariepinus reared in tarpaulin ponds contained cadmium, lead, arsenic and copper and that the concentrations of these metals in the tissues of the *Clarias gariepinus* was high enough to cause toxicity in man compared to the maximum permissible limits set by the World health Organization/Food and Agriculture Organization.

Because of the limited data used in the evaluation of cadmium, lead, arsenic and copper concentrations in *Clarias gariepinus* reared in tarpaulin in this geographical location, further research is required to evaluate the concentration of these metals in *Clarias gariepinus* reared in tarpaulin using a larger sample size.

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