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Determination Of Some Heavy Metals In The Blood, Brain, Flesh And Liver Of Catfish (Clarias Gariepenus) In Gyawana Ecosystem, Adamawa State, Nigeria

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IJIRAS

***Abstract:** Heavy metals occur naturally in the ecosystem, they are metallic elements that are toxic and have high density, specific gravity or atomic weight. This study was conducted in Gyawana ecosystem, Adamawa State, Nigeria to determine the concentration of heavy metals (As, Cd, Co, Cr, Cu, Ni, Pb and Zn) in the blood, brain, flesh and liver of Catfish (Clarias gariepenus). A total of four hundred and eighty (480) male and female catfish were used. The catfish were dissected separately to obtain the organs. The brain, liver and flesh of each catfish sample were dried at 105^{o}C and digested with tri-acid mixture (HNO_3: HCO_4: H_2SO_4). Determination of the heavy metals was done using a Buck Scientific 200A Model, Atomic Absorption Spectrophotometer (AAS). The studied metals (As, Cd, Co, Cr, Cu, Ni, Pb and Zn) were detected at different concentrations in the blood and organs of the studied birds with the exception of Cr which was not detected. Brain of catfish had the highest mean concentration of As (0.036±0.000mg/g) and the lowest was detected in the blood (0.031±0.000mg/g). The highest mean concentration of Cd was detected in the flesh of catfish 0.431±0.005mg/g and the lowest was detected in the brain of catfish which had 0.389±0.004mg/g. The highest mean concentration of Co, Ni and Pb were detected in the blood of catfish; 0.697±0.016mg/g, 0.041±0.000mg/g, 0.932±0.019mg/g in that order. Zn had the highest concentration in the catfish liver 11.734±0.118mg/g. The heavy metal concentrations in the study catfish (Clarias gariepenus) were within the permissible limit, therefore, it is safe to consume the catfish. Regular monitoring of heavy metals in the fish species of Gyawana ecosystem should be carried out to ascertain the level of toxicants.*

***Keywords:** Determination, Heavy Metals, Clarias gariepenus, Gyawana, Ecosystem*

I. INTRODUCTION

Heavy metals are members of loosely defined subset of elements that exhibit metallic properties. It mainly includes the transition metals, some metalloid lanthanides, and actinide. Heavy metals occur naturally in the ecosystem with large variations in their concentration (McDowell *et al.* 2006; Mohsen and Salisu, 2008; Salwa *et al.* 2012).They are metallic elements that are toxic and have high density, specific gravity or atomic weight. Heavy metals occur naturally in the soil from the pedogenetic processes of weathering of parent materials at levels that are regarded as trace (<1000 mg/g) and rarely toxic (Kabata-Pendias and Pendias, 2001; Pierzynski *et al.* 2000). Anthropogenic activities and acceleration of nature’s slowly occurring geochemical cycling of metals by man have caused most soils and environments to accumulate one or more of the heavy metals above defined background values, high enough to cause risks to human health, plants, animals, ecosystems, or other media (D' Amore *et al*. 2005).

Clarias gariepinus is a large, catfish, usually of dark gray or black coloration on the back, fading to a white belly. In Africa this catfish has been reported as being second in size only to the vundu of the Zambesian waters (Froese and Pauly, 2014b). Although FishBase suggests the African catfish surpasses that species in both maximum length and weight (Eslami *et al*., 2011). *C. gariepinus* has an average adult length of 1-1.5m; it reaches a maximum length of 1.7m and can weigh up to 60kg. These fish have slender bodies, flat bony heads, notably flatter than in the genus Silurus and broad terminal mouths with four pairs of barbells. They also have large accessory breathing organs composed of modified gill arches. Also, only pectoral fins have spines (Jansen *et al.* 2013). It is a nocturnal fish like many catfish, it feeds on living, as well as dead, it is able to swallow relatively large prey whole. It has been known to take large waterbirds such as the common moorhen. Catfish can also be able to crawl on dry ground to escape drying pools. It is able to survive in shallow mud for long periods of time, between rainy seasons. The catfish sometimes produce loud croaking sounds, not unlike the voice of the crow (Froese and Pauly, 2014a). *Clarias gariepinus*, belong to the Phylum Chordata, Class: Actinopterygii, Order: Siluriformes, Family: Clariidae, Genus: Clarias, Species: *C. gariepinus* (Anoop *et al.* 2009).

Therefore due to the property to accumulate persistent pollutants, fish are an excellent bioindicators reflecting the relative health of aquatic ecosystems. Tissues concentrations of chemicals are a function of uptake, storage and excretion and therefore are excellent indicators of environmental load of a specific toxicant but usually do not directly reflect the physiological and ecological consequences (NIST, 2004; Gintare and Gintaras, 2017). Meanwhile, heavy metals can affect fish at all levels of biological organization; biochemical, physiological, cellular behavioral, increase mortality and reduce reproduction, individual growth and susceptibility to multiple types of disease (Froese and Pauly, 2014b; Fabio *et al.* 2016).

Gyawana village in Lamurde Local Government Area of Adamawa State, Nigeria, receives a wide variety of waste from almost every significant human activity. These include mostly the dumping of domestic wastes, sewage, agricultural wastes and industrial smokes and effluent. Such contaminations might accumulate in various organs of organisms and may affect humans and other species that prey on such organisms as food. The presence of heavy metals in aquatic animals like fish is becoming a threat, thereby making them unfit for human consumption. For this reason, the investigation of heavy metals in fish is essential since even slight changes in their concentration above the acceptable levels, whether due to natural or anthropogenic factors, can result in serious environmental and subsequent health problems. This research work was carried out mainly to determine the concentrations of heavy metals in the blood, brain, flesh and liver of catfish (*Clarias gariepenus)* in Gyawana Ecosystem, Adamawa State, Nigeria. The determination of heavy metals in Gyawana aquatic organisms is relatively less studied; hence this study is a step in that direction. This will create awareness to the people of Gyawana about the levels of these heavy metals in their community.

STUDY AREA

The study was carried out in Gyawana ecosystem, Lamurde Local Government Area, Adamawa State of Nigeria. Gyawana is located at latitude 9˚.35' N and longitude 11˚.55' E and is 135 meters above Sea level. Lamurde Local Government Area lies between longitude 9˚.36' 03.92"N and latitude 11˚.47' 36.25"E at an elevation of 137 meters above sea level and has a population of 77,522 people (Adebayo and Tukur, 2004). Adamawa State is located in the North Eastern part of Nigeria, and lies between latitudes 7˚ and 11˚ N and between longitudes 11˚ and 14˚ E. It is on an altitude of 185 meters above Sea level and covers a land area of about 39,741km^2. The study was conducted for a period of twelve (12) months (December 2015 to November, 2016). fish (*Clarias gariepinus*) were collected once every first week of a month from various locations (Gokumbo, Italiah, Nguro Bemun, Canal Rivers and sugar cane farms of the Savanna Sugar Compay); in Gyawana ecosystem, Lamurde local Government Area, Adamawa State, Nigeria.

SAMPLING AND PREPARATION

A total of four hundred and eighty (480) freshly caught male and female C*larias gariepinus* were bought from fishermen at Italiah stream, Canal Rivers, and Gokumbo stream, (that is forty 40 fish each month). Random composite sampling techniques were used for both sexes and the samples were identified taxonomically. The fish were transported to the Department of Fisheries Laboratory of Adamawa State University, Mubi, Adamawa State, Nigeria, for identification using standard reference sources (www. Fishbase.org) and biometric measurements. Dissection and digestion were done in the Department of Animal Production Laboratory, Adamawa State University, Mubi, Adamawa State, Nigeria as in Olatunde (1983). Total length was measured from the top most part of the mouth to the tip of the caudal fine and standard length were measured from the top most part of the mouth to the tip of hypural bone, using a meter rule. Fresh weights were measured using an electronic weighing balance after removing water and other substances (Froese and Pauly, 2014a). Male catfish are smaller and narrower, has large and wider head; longer dorsal fins and male genital opening are raised and shaped like nipple, while female catfish are wider and rounded, smaller head, shorter dorsal fin and they have rounded opening at their genital area (Anoop *et al.* 2009).

DETERMINATION OF HEAVY METALS

The fish samples (C*larias gariepinus*) were washed with tap water before dissecting with dissecting instruments to remove the brain, liver and flesh. Blood was collected on Whatman No.1filter paper immediately after dissection and adequately labelled in the laboratory. The brain, liver and flesh of each fish sample was transferred into individual sterile sample bottles, labeled and kept for digestion and analysis to determine the heavy metals. Each fish organ was dried at

IJIRAS

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105°C until a constant weight was obtained and ground separately by sex, using porcelain mortar and pestle. The ground fish samples were transferred to a porcelain basin and put into a muffle furnace and the temperature was increased gradually until 550°C was reached. The samples were digested with tri-acid mixture (HNO_3: HCO_4: H_2SO_4) in the ratio of 10:4:1, respectively at a rate of 5ml per 5.0g of sample and were placed on a hot plate at 100°C temperature. Digestion was allowed to continue until the liquor became clear. All the digested liquor was filtered through Whatman 541 filter paper and diluted with 25mls of distilled water. Determination of the heavy metals was done directly on each final solution using a Buck Scientific 200A Model, Atomic Absorption Spectrophotometer (AAS). Values obtained were expressed in milligram per gram (mg/g) (APHA, 1995; AOAC, 2000; APHA, 2005).

DATA ANALYSIS

Data obtained were analyzed by one way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) for means separation.

II. RESULT

The result of this research work shows the mean concentrations of all the studied heavy metals in blood, brain, liver and flesh of catfish samples. Chromium was not detected in the blood and organs of the catfish samples. Arsenic had the highest mean concentration (0.036±0.000mg/g) in the brain, followed by flesh and liver (0.032±0.000mg/g). The least mean concentration was found in the blood with (0.031±0.000mg/g) and the differences of the mean concentrations were not significant at (P>0.05). For Cadmium, the result shows that flesh had the highest mean concentration of 0.431±0.005mg/g, followed by brain with mean concentration of 0.401±0.004mg/g, then blood had 0.400±0.005mg/g. The least mean concentration was found in the liver with a value of 0.389±0.004mg/g, but there was no significant difference (P > 0.05). The result for Cobalt, shows that the blood of the fish sample had the highest mean concentration (0.697±0.016 mg/g), followed by brain (0.553±0.013mg/g) and flesh had 0.441±0.011mg/g. Catfish liver had the least mean concentration of 0.235±0.003mg/g, and the differences were significant (P < 0.05).

Copper had the highest mean concentration of 18.738±0.074mg/g in the liver of catfish, followed by flesh with mean concentration of 18.216±0.071mg/g and blood with 17.947±0.082mg/g. The least mean concentration of Cu was found in the fish brain with a value of 13.462±0.082mg/g and the differences were significant (p < 0.05). The highest concentration of Nickel was observed in the blood, flesh and liver of the fish samples with a mean value of 0.041±0.000mg/g, while the brain had the least mean concentration of 0.028±0.000mg/g. The differences were also significant (p < 0.05). Lead concentration was highest in the blood of catfish 0.932±0.019 mg/g, followed by brain with mean concentration of 0.834±0.017mg/g and flesh with 0.468±0.011mg/g. The least mean concentration of 0.286±0.004mg/g was found in the liver; and the differences were significant (P<0.05). Zn was highest in the liver (11.734±0.118mg/g) followed by blood, with 10.725±0.121mg/g and catfish flesh with 9.514±0.093mg/g. The least mean concentration of 8.766±0.105mg/g was found in the brain; and the differences were significant (P< 0.05).

Organs	Concentrations mg/g) of heavy Metals							
	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
Blood	0.031±0.000^a	0.400±0.005^a	0.697±0.016^a	ND	17.947±0.082^a	0.041±0.000^a	0.932±0.019^a	10.725±0.121^{ab}
Brain	0.036±0.000^a	0.400±0.004^a	0.553±0.013^b	ND	13.462±0.082^b	0.028±0.000^b	0.834±0.017^c	8.766±0.105^b
Flesh	0.032±0.000^a	0.431±0.005^a	0.441±0.011^{ab}	ND	18.216±0.071^a	0.041±0.000^a	0.468±0.011^{bc}	9.514±0.093^{ab}
Liver	0.032±0.000^a	0.389±0.004^a	0.235±0.003^a	ND	18.738±0.074^a	0.041±0.000^a	0.286±0.004^{ab}	11.734±0.118^a
	Ns	ns	*		*	*	*	*

NB: Mean with the same superscripts in each column are not significantly different at p > 0.05, using ANOVA and DMRT for mean separation.
Key: ns = Not significant
** = significant*
ND = Not detected

Table 1: Mean Concentrations of Heavy Metals in the Blood, Brain, Flesh and Liver of Fish

III. DISCUSSION

The results of this study shows a significant variability in the accumulation of heavy metals in the different organs of catfish (*C. gariepinus*), which is principally dependent on the bioavailable metal concentration in the biotic components of their habitats as stated by Peakall and Burger, (2003) and Marchovecchio, (2014). The findings of this study indicate that the highest mean concentration of arsenic was in the catfish brain followed by flesh and liver, the least mean concentration in the blood. However, even the highest mean concentration of arsenic in this study did not exceed the permissible level for some of the toxic heavy metals in fish. FAO (1983) gave the minimum allowable level as 0.05mg/g body weight. The results of this study is at variance with the findings of Stancheva *et al.* (2014) who reported that the concentration of As in most organs of consumed fish species from Bulgarian Black Sea was 0.38mg/g. This disparity may be due to geographical location, anthropogenic activities and higher availability of chemicals in their habitat. The Joint Expert Committee on Food Additives (JECFA), (FAO/WHO 2005) established a Provisional Tolerable Weekly Intake (PTWI) for inorganic arsenic as 0.015mg/g body weight/ week and 0.05mg/g body weight/week for organic arsenic intakes. The maximum arsenic level permitted for fishes is 1.0mg/g according to Australian standards. The finding in this study was not above the tolerable intake. The trend of accumulation of arsenic in this study was blood > liver > flesh > brain. Exposure to arsenic is mainly via intake of food and drinking water, food being the most important source in most populations. Long term exposure to arsenic in drinking water

IJIRAS

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and food/feed is mainly related to increased risks of skin cancer, but also some other cancer as well as other skin lesions such as hyperkeratosis and pigmentation (Mandal, 2017). It has been established that inorganic arsenic is extremely toxic both in acute and chronic intakes. Initially it enters into the human body through ingestion, inhalation, or skin absorption. After entering into the body it is distributed to a large number of organs including the lungs, liver, kidney and skin (Khan *et al.* 2010)

In this study flesh had the highest mean concentration of cadmium followed by the brain, blood and the liver. However cadmium levels in this study was within the permitted level for fish, which is 0.10 mg/g (Turkish Food Codex Anonymous, 2008). But the result for cadmium in all the organs of *C. gariepinus* in this study was above the recommended PTWI for cadmium which is 0.07mg/g of body weight (FAO/WHO, 2005; Stancheva *et al.* 2014). The findings of cadmium concentration in this study did not also agree with the results of Stancheva *et al.* (2014) who reported a value below 0.01mg/g for the muscle of *C. gariepinus*. This may be because Gyawana is full of human activities and the industrial wastes from the Sugar Company and the fertilizers used by farmers in both dry and wet season farming. The blood of fish had the highest mean concentration of cobalt (0.697±0.016 mg/g) in this study, followed by the brain (0.553±0.013mg/g), flesh (0.441±0.011mg/g) and liver (0.235±0.003mg/g). The trend of accumulation of cobalt in this study was blood > brain > flesh > liver; this revealed that the means concentration of cobalt was higher in blood compared to the other organs studied in fish. Cobalt can accumulate to toxic levels in the liver, kidney, pancreas and heart, as well as the skeleton and skeletal muscles. Cobalt has been found to produce tumors in rats and rabbits and is likely a human carcinogen as well (Edward, 2015).

Copper is an essential part of several enzymes and it is necessary for the synthesis of haemoglobin. Copper toxicity in fish is taken up directly from the water via gills, and accumulation of Cu in various organs and tissues depends upon the way of exposure such as through diet or their elevated level in the surrounding environment (Alam *et al.* 2002). Morphological and behavioral abnormalities such as alteration in sensory reception, reduced responses to normal olfactory function (feeding, mating, selection or homing), reduction in swimming performance, gills purge, ventilation, coughs, learning impairment, loss of equilibrium that will lapse into paralysis, loss of reproductive efficiency and irregular metamorphosis are symptoms of toxic exposure of Cu (Mansour and Sidky, 2002). The trend of mean concentration of Copper in this study is liver (18.738±0.074mg/g) > flesh (18.216±0.071mg/g) > blood (17.947±0.082mg/g) > brain (13.462±0.082mg/g). However, the highest Copper means concentrations in this study did not exceed the allowable limit for some of the toxic heavy metals in fish. FAO (1983) gave a maximum allowable limit of 30mg/g, but Cogun and Kargin (2004), reported that low pH promotes the accumulation of Copper in fish.

Blood, flesh and liver of catfish had the same mean concentrations of nickel (0.041±0.002mg/g), and brain had the least mean concentration of (0.027±.001mg/g). The result of this study is lower than the maximum recommended range of 0.5-0.6mg/g (FAO, 2003; WHO, 2005). This study agrees with the study of Orosaye *et al.* (2010), who reported low (0.25 - 0.30mg/g) concentration of Nickel in the body of fish in Ikpoda River Dam, Benin City, Nigeria. However, a higher level has been reported by Idodo Umeh (2002); Mohammed *et al*. (2012); Ayeloja *et al*. (2014); and Lalel *et al.* (2016), which was above the permissible limit. This may be due to high level of biochemical substances in that ecosystem. The major source of Ni for humans is food and uptake from natural sources, as well as food processing (Nas-NRC 1975; Akan *et al*. 2012). Increased incidence of cancer of the lungs and nasal cavity caused by high intake of Ni in animals has also reported (Anonymous, 2013).

A significant variation was observed in the mean concentration of lead in the organs of fish. The highest mean concentration was observed in the blood, followed by brain, flesh and the liver. These results are lower compared to the findings of Doherty *et al.* (2010), who reported a higher value of 55.12mg/l concentration level of Lead from Lagos Lagoon in some fish species. However, it agrees with Farombi *et al.* (2007), who reported a low concentration (0.3002±0.0167µg/L) of lead in *C. gariepinus* from Ogun River Nigeria. The values of lead obtained in the blood of this study in *C. gariepinus*, have exceeded the maximum recommended limit of 0.6mg/g of body weight by FAO /WHO, (2003). Lead is classified as one of the most toxic heavy metals. The biological effects of sublethal concentrations of lead in fish include delayed embryonic development, suppressed reproduction and inhalation of growth, increased mucous formation, neurological problems, enzyme inhibition and kidney dysfunction.

The mean concentration of zinc observed in the current study was within the permissible limit of 10 -20.00mg/g for fish food as reported by FAO, (1983); WHO (2006); FAO/WHO (2005). This study has shown a mean concentration of 11.734±0.118mg/g in the liver, which agrees with Taweel *et al. (*2013) and Fabio *et al.* (2016), who found variations in heavy metal concentrations between some fish organs they studied. The trend of accumulation of Zinc in this study was slightly different from the results obtained by Akan *et al.* (2009), who reported a trend of bioaccumulation of heavy metals in six fresh water fishes from Lake Chad as liver > gills > muscles. This may be due to the differences of the organs and species of fish used and also ecological factors. Zinc is an essential trace metal for both retarded growth, loss of taste and hypogonadism, leading to decreased fertility (Sivapermal *et al.* 2007). Zinc toxicity is rare, but at concentrations in water of up to 40 mg/g, may induce toxicity, characterized by symptoms of irritability, muscular stiffness and pain, loss of appetite, and nausea (Nas-NRC 1975; Akan *et al.* 2014).

In conclusion, the results obtained in this research work shows that the concentrations of arsenic, cadmium, cobalt, copper, nickel, lead and zinc were within the permissible limit in the blood and organs of fish studied. The heavy metal concentrations in the study catfish (*Clarias gariepenus*) were within the permissible limit, therefore, it is safe to consume the catfish. Regular monitoring of heavy metals in the fish species of Gyawana ecosystem should be carried out to ascertain the level of toxicants.

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