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Hoplobatrachus occipitalis AND *Clarias anguillaris* AS SENTINELS FOR ASSESSMENT OF HEAVY METALS IN IRRIGATION WATER KADAWA KANO, NIGERIA

[1]* KUIWA, T. S., [2]M. TUKUR, [3]M. A. AMINU, [1]M. USMAN, [1]A. HAMZA, [1]A. S. MOHAMMED, [3]N. LAWAL, and [1]A. M. BASHIR

1. School of Basics and Remedial Studies, Ahmadu Bello University, Funtua, Katsina.
2. Department of Fisheries and Aquaculture, Faculty of Agric, Ahmadu Bello University, Zaria, Nigeria.
3. Department of Biology, Faculty of Life Science, Ahmadu Bello University, Zaria, Nigeria.

***Corresponding Author: tasiusalehkuiwa@gmail.com, +2347068355044**

ABSTRACT

This study was carried out to evaluate the concentrations of heavy metals (Cd, Cu, Mn, Pb, and Zn) in Crowned bullfrog (Hoplobatrachus occipitalis) and African Catfish (Clarias anguillaris) in irrigation water Kadawa, Kano State, Nigeria. Muscle, liver, lung, and gill were analysed for Cd, Mn, Pb, Cu, and Zn using Atomic Absorption Spectrometer (AAS). It was revealed that Crowned bullfrog has the highest concentrations of Zn, Pb, and Cu with mean values of 35.02 mg/kg, 0.81 mg/kg, and 0.39 mg/kg, the least in African catfish with mean values of 12.85 mg/kg, 0.53 mg/kg and 0.35 mg/kg respectively. The lung of crowned bullfrog has the highest concentrations of Mn (0.68 mg/kg), Pb (1.81 mg/kg) and Zn (77.38 mg/kg) while the highest concentration of Cu (1.02 mg/kg), Mn (7.57 mg/kg), Pb (1.09 mg/kg) and Zn (21.20 mg/kg) were recorded in the liver of African catfish. Concentrations of Mn, Pb, and Zn in Crowned bullfrog, catfishes, and water samples were found to be above the permissible limits set by FEPA (2003) and NESREA (2011). More accumulation of heavy metals was recorded in Crowned bullfrog compared to African catfish, hence frogs may be considered as more sensitive bioindicators of heavy metals compared to African catfish.

Keywords: Crowned Bullfrog, sentinels, Phytoremediation.

INTRODUCTION

Heavy metals are stable metals or metalloids with a density greater than 4.5gcm^{-3} (Karishma, 2014). They are not biodegradable, hence persist in the environment (Abolude *et al*., 2009). Production of goods and services causes a lot of environmental problems by the release of pollutants into the ecosystem which eventually accumulates in the water. Aquatic pollution poses greater concern because all forms of life depend directly or indirectly on water (Faye-ofori *et al.*, 2015). Olgunoˇglu (2014) stressed that heavy metal pollution in the aquatic environment can be harmful to human health. They can concentrate in the food chain and eventually accumulate in the human body (Pigłowski, 2018). Kadawa Irrigation Project is a modern irrigation farming project in Kano State Nigeria where farmers practice different farming activities during both dry and rainy seasons. Many concerned with increasing agricultural productivity and food security are focused on fertilizer and other agrochemicals as a remedy for declining soil fertility and stagnant yields. Agrochemicals such as inorganic fertilizers and pesticides are upon use for improving crop production technology though it is a costly input (Bhandari, 2014). Recently there is global concern on the impact of these modern farming practices on the ecosystem (Ogbodo and Onwa, 2013). Runoff from agricultural fields often contains levels of heavy metals from agrochemicals used. Such runoff gets into different water bodies thereby polluting the aquatic habitat. The water reservoirs of Kadawa irrigation area are used for farming activities and for fishing of crowned bullfrog (*Hoplobatrachus occipitalis*) which is consumed in some parts of Nigeria as a source of protein. Local fishermen also engaged in fishing in the area, the most abundant fish caught are the African catfish (*Clarias anguillaris*) and Tilapia (*Tilapia zilli*).

Frogs are ancient animals that have been around for about 200 million years (David and Carola, 2009). They can absorb toxic substances through their highly permeable skin. Frogs and toads are commonly used as suitable biological indicators of metal pollution (Burger and Snodgrass, 1998). The amphibious lifestyle of crowned bullfrogs making them be an excellent sentinel animal (Mitchell, 2000; IUCN SSC Amphibian Specialist Group, 2014). They are described as a good experimental model for being easy to handle, economical to use, and highly prolific (Abosede *et al*., 2016).

Fish among other animals are used to determine the health condition of the aquatic ecosystem. Fish and bivalve Molluscs are used in bioaccumulation tests because they are higher trophic level organisms and are usually eaten by man (Olaifa *et al*., 2004). Among aquatic species, fishes are the inhabitants that cannot escape the harmful effects of heavy metal pollution (Djibrine *et al*., 2018). The chemical composition of aquatic organisms such as fish is related to the organic and chemical pollutants in their environment (Abolude *et al.*, 2007). Fish biota has been recognized as suitable bioindicators in the assessment of heavy metal contamination in aquatic systems, largely due to the ease of accumulation through uptake of metals in water and diet (Idowu and Oloye, 2017). Trace metals in the hydrosphere come from natural and anthropogenic sources. The presence of heavy metals in the water body is becoming a threat to aquatic animals thereby making them unfit for human consumption (Abolude *et al.*, 2013). Imasuen and Egai (2013) reported significantly high concentrations of some heavy metals in rivers and pond waters in Bayelsa State. All the heavy metals analyzed by Obaroh *et al*., (2015) except zinc were above the WHO acceptable limit.

The study was conducted to evaluate the sensitivity of crowned bullfrog and African catfish in assessing heavy metals (Cd, Cu, Mn, Pb, and Zn) toxicity in selected water reservoirs in Kadawa irrigation project Kano, Nigeria.

MATERIALS AND METHODS

Study Area

Kadawa irrigation project is part of the Kano River Project that lies on both sides of Kano-Zaria and Kano-Rano roads. The project is situated in Garun Malam Local Government Area in the southern part of Kano State. Kadawa is located between latitude (11° 35′N and 11° 50′N) and longitude (08°25′E and 08°35′E) (Adamu *et al*., 2014). The area has a tropical wet and dry climate with an average temperature of about 25°C ± 7°C (Olofin and Tanko, 2002).

Sampling and Sample Preparation

Crowned bullfrog (*Hoplobatrahus occipitalis),* African catfishes (*Clarias anguillaris*), and water were collected biweekly for six months (July to December 2018), 36 frogs and catfishes each was collected from the three reservoirs: Dorawar Sallau, Kadawa, and Gafan.

Samples Collection and preparation

The frog samples were caught using a hook and line fishing tool with a piece of meat tied onto the line as bait to attract the frogs without using a hook. The frog holds onto the bait as it is swallowed and pulled out of the water uninjured (Kuiwa *et al*., 2019), then taken to the Zoological Museum Department of Zoology, Ahmadu Bello University, Zaria for identification. The Catfish samples were caught using hook and line fishing tools. Identification of the fish was done at the Department of Zoology, A.B.U. Zaria laboratory.

Crowned bullfrog and African catfish samples were washed using pipe-borne water and rinsed with distilled water. Dissection of the frogs and African Catfish was carried out, livers, lungs/gill, and muscle were collected. The samples were dissected; gills, livers, and muscles were collected. The organs/parts collected were oven-dried at 120°C for 8 hours to a constant weight (Tyokumbur, 2016). Dried samples were crushed to powder using porcelain mortar and pestle after which they were weighed using Mettler MP600 model electric balance, stored in Ziploc bags, and labeled before acid digestion.

Water sample collection and preparation

The water samples were randomly collected in sampling bottles pre-conditioned with 5% nitric acid and later rinsed thoroughly with distilled water. At each sampling site, the sampling bottles were rinsed three times before collecting the sample. The sampling bottles were deep into the water to about 10 cm depth. About a half-liter of water was collected at each sampling location. Samples were acidified with 10% HNO_3, taken to the hydrobiology laboratory Ahmadu Bello University, Zaria in an ice bath. Filtration of the samples was done using Whatman filter with 0.4 µm micropore membrane, kept at 4°C until analysis (Ozturk *et al*., 2009).

Samples Digestion

Digestion was done following Milam, *et al.* (2015) with little modification. Pulverized organ digestion was carried out by adding 1g of each pulverized organ into 1000cm^3 flasks, 10cm^{3}of distilled water was added to the samples, followed by 10cm^3 of concentrated HNO_3. The mixture was boiled at a temperature of about 100°C for one hour then cold, 5cm^3 of H_2SO_4 was added to the cold mixture and heated again for 20 minutes at 140°C until a dense white fume of H_2SO_4 was observed. The solution was allowed to cool, then transferred into a 100cm volumetric flask and diluted using distilled water to a final volume of 100cm^3 and stored in a polyethylene sample bottle for heavy metal analyses.

Heavy Metals Analysis

Atomic Absorption Spectrometer (AAS) Buck Scientific VGP-210 model (2008) was used for the analysis of digested samples for Cadmium (Cd), Copper (Cu), Lead (Pb), Manganese (Mn), and Zinc (Zn).
The concentration of heavy metals was calculated using the formula as described by Djibrine *et al.*, (2018).

$$C = \frac{C1 \times V}{P}$$

Where
C: Concentration of heavy metal in the sample (mg/kg)
C1: AAS reading (mg/l)
V: Final volume of the mineralization solution in (ml)
P: Mass of the sample in (g)

The Bioaccumulation of heavy metals was calculated using the formula as described by Djibrine *et al.*, (2018)

$$BF = \frac{\text{Concentration of Heavy Metal in Animal Tissue}}{\text{Concentration of Heavy Metal in Abiotic Medium}}$$

Statistical Analyses:

The mean concentration of heavy metals in the samples was obtained using one-way and two-way Analysis of variance (ANOVA) at a 5% level of significance. Duncan's Multiple Range Test (DMRT) was used in separating the means where a significant difference was observed. All the analyses were performed using SPSS V.21.0.

RESULTS

The result (Table 1) revealed that all the metals excluding Mn accumulated more in the crowned bullfrog samples with the pattern in increasing order of frog > fish > water while that of Mn was: fish > frog > water. In general, all the heavy metals analysed were found to be more in crowned bullfrog and fish compared to the water sample (Table 1). Copper was the least concentrated heavy metal in all the samples with the highest value of 0.39 mg/kg in the crowned bullfrog and the least value of 0.11 mg/kg in the water (Table 1). Manganese concentration in the fish was reported with the highest value of 2.45 mg/kg which was far above the 0.3 mg/kg and 0.18ppm recorded in the crowned bullfrog and water respectively (Table 1). Lead was recorded with the highest value of 0.81 mg/kg in the crowned bullfrog and the least value of 0.26 ppm in the fish. Zinc was recorded to be the most accumulated heavy metals in all the samples analysed. The highest concentration of Zn was recorded in the crowned bullfrog with the mean value of 35.02 mg/kg and the least was obtained in the water with a mean value of 3.65 mg/kg (Table 1).

Table 2 showed the concentration of heavy metals in different organs/parts of crowned bullfrog and African catfish. Results recorded for the crowned bullfrog revealed that the lung and liver were recorded to have the highest concentration of all the heavy metals studied the least was recorded in the muscle (Table 2). The highest concentration of Cu was recorded in the liver with a mean value of 1.21 mg/kg and the least was in the muscles with a mean value of 0.07 mg/kg (Table 2). The accumulation pattern of Cu in organs/parts of frog was; liver > lung > muscle. Manganese was recorded more in the lung with a mean value of 0.68 mg/kg and least in the muscle with a mean value of 0.05 mg/kg. Manganese accumulation pattern in organs/parts were; lung > liver > muscle. Lead accumulated more in the lung and the least was in the muscle. The pattern of Pb accumulation in organs/parts was; lung > liver > muscle. Zinc was found to be the most accumulated heavy metal in all the organs/parts of the Crowned bullfrog with the highest mean values of 77.38 mg/kg in the lung and the least of 18.10 mg/kg in the muscle (Table 2). In the case of the African catfish, the liver was reported to have the highest concentration of all the heavy metals recorded and the least value recorded in the muscle. The accumulation pattern of heavy metals in organs/parts of catfish was in decreasing order of liver > gill > muscle (Table 2). Copper was the least accumulated heavy metal in organs/parts of the African catfish with the highest value of 1.02 mg/kg in the liver and the least of 0.02 mg/kg in muscle (Table 2). Manganese was the second most accumulated heavy metal in the African catfish after Zn, with the highest concentration of 7.57ppm in the liver and the least of 0.53 mg/kg in the muscle. Lead has the highest accumulation of 1.09 mg/kg in the liver and the least of 0.43 mg/kg in the muscle of the catfish. Zinc was the most accumulated heavy metal in the organs/parts of the catfish with the highest concentration of 21.20 mg/kg in the liver and the least concentration of 7.42 mg/kg in muscle (Table 2). Figure 1 indicates the bioaccumulation of heavy metals in crowned bullfrog and catfish. The bioaccumulation pattern of heavy metals in frogs was: Zn > Cu > Pb > Mn and that of catfish was: Mn > Zn > Cu >Pb. Crowned bullfrog was reported to have the highest bioaccumulation of Zn, Pb, and Cu while African catfish have the highest bioaccumulation of Mn.

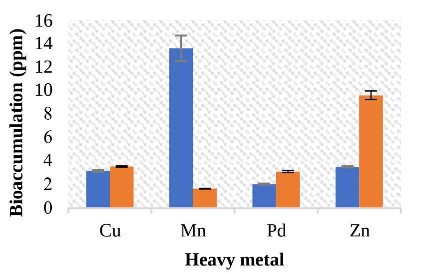

Fig. 1 Bioaccumulation of metals in *Hoplobatrachus occipitalis* and *Clarias anguillaris*

DISCUSSION

Higher values of heavy metals were recorded in the crowned bullfrog parts/organs compared to the African catfish parts/organs. This may be due to the difference in feeding habits, life cycle, behavior, and lifestyle, which may lead to the deposition of more heavy metals in the frog. This was similar to the result of Somsak *et al.* (2016) who reported higher concentrations of Cu and Cd in frogs compared to fish. Frogs being carnivores may ingest contaminated sediments and soil particles during prey capture (Somsak *et al.*, 2016). Their biphasic lifestyle and permeable skin may also make them more susceptible to accumulate more heavy metals than fishes. Qureshi *et al*. (2015) stated that amphibian species are more sensitive bioindicators of aquatic contaminants than any other aquatic vertebrates.

The values of Cu recorded in catfish and crowned bullfrog differed significantly from the values obtained in the water at $p \leq 0.05$. The highest concentration of Cu was recorded in the crowned bullfrog compared to that of African catfish and the least in the water sample. This was similar to the finding of Putshaka *et al*. (2015) who recorded more Cu concentration in bullfrog (12.96 mg/kg) and less concentration (9.63mg/kg) in fish and the least in water (3.11mg/l) from River Challawa, Kano State. The concentration of Cu in the frog and fish was below the permissible limit of 30 ppm set by NESREA (2011), while the level in water was above the 0.01 ppm permissible limit set by NESREA (2011). The concentration of Manganese in African catfish differed significantly from that obtain in the crowned bullfrog and water samples at $p \leq 0.05$ but no significant difference was observed in the values in crowned bullfrog and water samples. A higher value of Mn recorded in fish may probably be because the concentration of Mn that passes into the fish body was above the regulation Wangboje (2015). It may also be because Mn is a metal with low toxicity but has a considerable biological significance and seems to accumulate in fish (Kumar *et al.,* 2011). The concentration of Mn recorded in the catfish was far above the recommended limit of 0.05ppm set by FEPA (2003) and that of crowned bullfrog and water was slightly above the permissible limits of 0.05ppm set by FEPA (2003). Lead concentration recorded in crowned bullfrog did not differ significantly at $p \leq 0.05$ from the value obtained in the African catfish but differed significantly from the value recorded in water. The concentrations of Pb recorded in the African catfish, crowned bullfrog, and water were found to be far above the recommended limit of 0.2ppm and 0.1ppm set by NESREA for fish and water respectively (2011). This was in line with the result obtained by Lawrence *et al.,* (2019) in which Pb was recorded above the permissible limit in the water shrimp and fish compared to the water sample from Benin River, Nigeria. Zinc as essential metal was reported to be the most accumulated heavy metal and its concentration differed significantly in the African catfish, crowned bullfrog, and water at $p \leq 0.05$. A significantly higher value of Zn obtained in crowned bullfrog compared to African catfish may be due to the specific adaptive mechanism of the frog to absorb Zn from the habitat for onward transfer to the kidney for metabolism (Taiwo *et al*., 2014). The result was similar to that of Djibrine *et al*. (2018) who recorded more Zn value (36.48-50.24 mg/kg) in tilapia compared to (0.01-0.15mg/l) value recorded in the water from Fitri Lake. The value of Zn recorded in the crowned bullfrog, African catfish, and water was above the permissible limit of 5-10 ppm set by FEPA (2003) for fish and 0.2 ppm permissible limit set by NESREA (2011) for water. This may be linked to the fact that Zn is found in natural waters in larger quantity compared to Cu, Pb, Cd, and Hg (Pajtim *et al*., 2018). Falfyshynka *et al*. (2017) reported that exposure to Zn pollution may cause physiological disturbance in frogs. Consumption of fish and frogs from the study site at present has potential health risk due to Zn contamination, which could lead to diarrhea, blood urine, liver, and kidney failure, and anemia (Pajtim *et al.*, 2018).

All the heavy metals recorded in the study accumulated more in the lung and liver of *H. occipitalis* compared to other organs probably because the lungs are actively involved in gaseous exchange while the liver is the largest organ in the body concerned with detoxification of toxic materials in the body including heavy metals. This is in agreement with the findings of Qureshi *et al*. (2015) who recorded more heavy metals in the liver of *Rana tigrina* compared to the muscle in Pakistan and Shaapera *et al*., (2013) who reported a high concentration of heavy metals (43.6%) in the liver of *Rana esculenta* from River Guma, Benue State. In the case of catfish, the concentrations of all the heavy metals studied were recorded more in the liver of *Clarias anguillaris* compared to other organs probably because the liver is the largestorgan in the body actively concerned with detoxification of toxic materials (Soufy *et al.*, 2007), and storage of essential elements such as Feand Cu. Similarly, Bawuro (2018) also recorded more heavy metals in the liver of fish in LakeGeriyo, Adamawa State. Idowu and Oloye, (2017) also recorded more heavy metals in the liver of *Clarias gariepinus* from Ilushi River, Edo state. Theleast concentration of heavy metals recorded in the muscle compared to other organs was in support ofthe finding of Abolude *et al*. (2007a) who recordedless heavy metal in the flesh of *Lates niloticus*compared to gill and bone. The highest bioaccumulation of Zn and Cu recorded in crownedbullfrog was in line with the finding of Tyokumbu and Okorie (2011) who reported higher bioaccumulation of Zn (29.84mg/kg) in *Rana esculentus.* Manganese was the most bioaccumulated heavy metal in catfish while lead was the least bioaccumulated heavy metal.

CONCLUSION

There was Zn and Pb contamination in the crowned bullfrogs, African catfish, and water samples analysed, while Mn contamination was recorded in African catfish and water. The highest accumulation of heavy metals was recorded in crowned bullfrog compared to African catfish, hence the crowned bullfrog may be considered as more sensitive bioindicators of aquatic pollutants compared to African catfish. Zinc was the most accumulated heavy metals in crowned bullfrog while African catfish accumulated more Mn. Bioaccumulation of Zn, Pb, and Cu was more in crowned bullfrog while Mn bioaccumulation was recorded more in the catfish. The water, crowned bullfrog and catfish are unsafe for human consumption, and therefore posed a threat to public health.

RECOMMENDATION

Farmers should be encouraged on organic farming and minimize the usage of agrochemicals. Phytoremediation of heavy metals should be employed by the farmers to reduce the heavy metal contamination in the area.

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Table 1: Heavy metals concentrations in *Hoplobatrachus occipitalis* (Crown bullfrog), *Clarias anguillaris* (African Catfish), and water

Sample	Cd	Cu (ppm)	Mn (ppm)	Pb(ppm)	Zn (ppm)
Fish	ND	0.35 ± 0.07^{ab}	2.45 ± 1.09^{a}	0.53 ± 0.05^{ab}	12.85 ± 1.01^{b}
Frog	ND	0.39 ± 0.05^{a}	0.30 ± 0.03^{b}	0.81 ± 0.09^{a}	35.02 ± 3.37^{a}
Water	ND	0.11 ± 0.02^{c}	0.18 ± 0.01^{b}	0.26 ± 0.02^{b}	3.65 ± 0.22^{c}
p-value	-	0.052	0.000*	0.012*	0.000*
FEPA (2003)#	-	0.5-1.5	0.05	-	5-10
NESREA (2011)#	0.5	30	-	0.2	
NESREA (2011)##	0.003	1.0	0.05	0.01	3.0

Note: Values with different superscript differ significantly at $p\leq0.05$.The average number of fish = 36.Key: ND= Not detected because it was below the levels that can be detected by Atomic Absorption Spectrometer (AAS) Buck Scientific VGP-210 model. * = $P < 0.05$, # = Maximum permissible limit in fish and fishery products, ## = Maximum permissible in water.

Table 2: Heavy Metals Accumulation in Organs/Parts of Crowned bullfrog and African Catfish

	Hoplobatrachus occipitalis			*Clariasanguillaris*			
HM	Muscle	Lung	Liver	Muscle	Gill	Liver	NESREA (2011)
Cd	ND	ND	ND	ND	ND	ND	0.5 #
Cu	0.07±0.01	0.48±0.08	1.21±0.20	0.02±0.01	0.09±0.03	1.02±0.24	30 #
Mn	0.06±0.04	0.68±0.10	0.61±0.07	0.53±0.11	3.50±1.14	7.57±2.18	-
Pb	0.20±0.04	1.81±0.30	1.78±0.22	0.43±0.06	0.88±0.17	1.09±0.15	0.2 #
Zn	18.10±2.28	77.38±14.14	42.99±5.18	7.42±0.98	11.65±1.27	21.20±3.18	-

Key: HM= Heavy metals, n = 36, ND= Not detected because it was below the levels that can be detected by Atomic Absorption Spectrometer (AAS) Buck Scientific VGP-210 model, # = Maximum permissible limit in fish and fishery products.