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Determination of Heavy Metals in *Hoplobatrachus occipitalis* (Crowned Bullfrogs) and Water from Some Reservoirs in Kadawa Irrigation Project Kano, Nigeria

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ABSTRACT: This study was carried out to evaluate the concentrations of heavy metals (Cd, Cu, Mn, Pb, and Zn) in water and crowned bullfrog (*Hoplobatrachus occipitalis)* organs/parts (muscle, liver, leg, lung and trunk) from Kadawa irrigation project, Kano State, Nigeria. Atomic Absorption Spectroscopy was carried out using Atomic Absorption Spectrometer (AAS) Buck Scientific VGP-210 model (2008). The mean concentration of heavy metals in water samples were 0.11 mg/L, 0.18 mg/L, 0.26 mg/L, and 3.65 mg/L for Cu, Mn, Pb and Zn respectively. The sequence of metal accumulation in all the organs was Zn > Pb > Mn > Cu. The highest concentration of Zn (77.38 mg/kg), Pb (1.81 mg/kg) and Mn (0.68 mg/kg) were found in the lung while Cu (0.07 mg/kg) was deposited more in the liver. Cadmium was not detected in all the samples analysed. Zinc and lead were the most accumulated metals in all the organs/parts with the range of 77.38 mg/kg - 18.10 mg/kg and 1.81 mg/kg - 0.13 mg/kg respectively. The highest accumulation of metals was found in the lung and liver. The organ/parts accumulation pattern was: lung > liver > trunk > muscle > leg for Zn, Pb and Mn, while liver > lung > trunk > muscle > leg was for Cu. Lung and liver have the highest bioaccumulation of heavy metals while the leg and muscle bioaccumulated the least heavy metals. Hence the water, liver and lung of *H. occipitallis* are unsafe for consumption, and therefore posed a threat to public health. Farmers should be trained on proper usage of agrochemical.

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Most environmental problems are as a result of production of goods and services which leads to the release of pollutants that eventually accumulates in water. Because all other life forms depend directly or indirectly on water, aquatic pollution is often regarded as one of greater concern (Faye-ofori *et al*., 2015). Most of our water resources are gradually becoming polluted due to the addition of foreign materials from the surroundings. Lower aquatic organisms absorb and transfer them through the food chain to higher trophic levels, including fish (Ijeoma *et al.,* 2015). Kadawa Irrigation Project is one of the modern irrigation farming area in Nigeria, especially Kano state. Farmers in the area engaged in different farming activities to increase productivity. Efforts toward increasing agricultural productivity and food security are focused on fertilizer and other agrochemicals as a remedy for declining soil quality and stagnant yields. Agrochemicals (pesticides and fertilizers) are considered as a vehicle for improved crop production technology though it is a costly input. Balance use, optimum doses, correct method and right time of application of agrochemicals ensures increased crop production (Bhandari, 2014). There is currently world-wide concern regarding the impact of these modern farming practices on soil and water quality (Ogbodo and Onwa, 2013). Agricultural runoff often contains developed levels of heavy metals from agrochemicals applied to the fields. These chemicals are carried with rainfall runoff into rivers, streams and reservoirs, polluting water bodies and modifying aquatic habitats (Ogbodo and Onwa, 2013). The water reservoirs of Kadawa irrigation area are used for farming activities and as a source of crowned bullfrogs (*Hoplobatrachus occipitalis*) which are transported to other part of Nigeria and consumed as source of protein. This study focused on the assessment of heavy metals in the water and crowned bullfrogs in Kadawa Irrigation Area. Different organs and parts of the frogs were analysed and compared for heavy metals concentration. Frogs are amphibians in the order Anura (from Greek word an – without and oura – tail), formerly referred to as Salientia (from Latin word salere -"to jump"). The Order Anura contains 4,810 species in 33 families, of which the Leptodactylidae (1100 spp.), Hylidae (800 spp.) and Ranidae (750 spp.) are the richest in species. About 88% of amphibian species are frogs (Oduntan *et al.,* 2012). Frogs are ancient animals that have been

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around for about 200 million years (David and Carola, 2009). Frogs highly permeable skin means they can rapidly absorb toxic substances. The use of frogs and toads, as biological indicators of metal pollution is becoming more common (Burger and Snodgrass, 1998).

The Crowned bullfrog (*Hoplobatrahus occipitals)* (Günther, 1858), is an important species of Anurans that is considered a delicacy as local people catch them for food due to their taste and fleshy legs. *H. occipitalis* is a species of frog in the Dicroglossidae family. It is found in the Sub-Saharan Africa (Abosede *et al*., 2016). It naturally inhabits both aquatic and terrestrial areas therefore making it an excellent sentinel animal (Mitchell, 2000; IUCN SSC Amphibian Specialist Group, 2014). They are also highly prolific, easy to handle and comparatively economical to use for field evaluation and as an experimental model (Abosede *et al*., 2016). Frogs have been eaten in Europe for centuaries, but after the Second World War demand escalated. An increase in the size of human population tends to increase the existing pressure on wildlife resources population (Akinyemi and Efenakpo, 2015). Frog legs are consumed in many part of the world. The leading importer for frog legs worldwide is EU, with France 23%, Belgium 53%, Italy 6% and Netherland 17% being the major destination (Sandra *et al*., 2011). Tao *et al.* (2012) found that Zn and Cu are the most likely to be accumulated in all aquatic organisms of different tropic levels. Taiwo *et al*. (2014) analysed *H. occipitalis* and reported that there was widespread heavy metal pollution in Lagos State emanating from diverse sources and bioaccumulative potentials of measured heavy metals in *H. occipitalis* in Lagos metropolis raises salient pollution management questions. Nasir *et al*., (2017) determined Zn concentrations in different tissues (liver and kidney) of *R. tigrina* from different habitats (canal water, fish pond and sewage water) and reported that liver tissues of *R. tigrina* taken from all the three habitats contain higher levels of Zn than kidney samples. Therefore, the objective of this paper is to evaluate the concentrations of selected heavy metals such as Cd, Cu, Mn, Pb and Zn in Water samples and organs/body parts (muscle, liver, leg and trunk) of Crowned bullfrog (*Hoplobatrahus occipitals)* from Kadawa irrigation project, Kano State, Nigeria.

MATERIALS AND METHODS

Study Area: Kadawa irrigation project is part of the Kano River Project lies on both sides of Kano-Zaria and Kano-Rano roads, positioned in Garun Malam Local Government area in southern part of Kano State. Kadawa is enclosed between latitude (11° 35′N and 11° 50′) and longitude (08°25′E and 08°35′E) (Adamu, *et al*., 2014). The area has been identified as an area where mechanized and intensive irrigation activities are taking place. The climate of Kano State (Kadawa inclusive) is the tropical wet and dry type. The temperature is averagely warm to hot throughout the year at about 25°C ± 7°C (Olofin and Tanko, 2002).

Sampling and Sample Preparation: Samples (frogs, catfishes and water) were collected weekly for three months (October, November, and December 2018). A total of 36 frogs (14 males and 22 females) were collected from the three locations: A (Dorawar sallau), B (Kadawa) and C (Gafan) reservoirs. The samples collected were prepared for heavy metal analysis.

Samples collection: The frog samples were caught using rod and line fishing tools, bait (piece of meat) was tightened onto the line to attract the frogs without using hook, when the frog swallow the bait it is then pulled out of the water without any injury. The frog samples caught were taken to the Zoological Museum Department of Zoology, Ahmadu Bello University, Zaria for identification. The sexes of the frogs were recorded. Guide lines for human use of animals for scientific research as contained in ABU Committee on Animal Use and Care (ABUCAUC) was strictly followed.

Samples preparation: Frog samples were washed and dissected respectively; while livers, lungs, muscle, trunks and legs were collected and prepared for analysis. The organs and tissues collected were oven-dried for three days at 105°C. Dried samples were pulverized to powder in a porcelain mortar using a pestle after which they were weighed using Mettler MP600 model electric balance, stored in Ziploc bags and labeled prior to acid digestion.

Samples Digestion: Digestion was done in accordance with Millam, *et al.* (2015) with little modification. Powdered organ digestion was carried out by adding 1g of each pulverized organ into 1000cm^3 flasks, 10cm^{3}of distilled de-ionised water was added to the samples, followed by 10cm^3 of concentrated HNO_3. The mixture was boiled at 100°C for 60 minutes then allowed to cool, after which 5cm^3 of H_2SO_4 was added to the cooled mixture and heated again at 140°C for 20 minutes until a dense white fume of H_2SO_4 was observed. The solution was allowed to cool and transferred into 100cm^3 volumetric flask and diluted using distilled de-ionised water to a final volume of 100cm^3 and then stored in a plastic bottle for Atomic Absorption Spectrometry (A.A.S.) analysis.

Water sample collection and preparation: Water samples were randomly collected in sampling bottles;

pre-conditioned with 5% nitric acid and later rinsed thoroughly with distilled de-ionised water. At each sampling site, the plastic sampling bottles were rinsed at least three times water at the sampling stations before collecting the samples. The sampling bottles were immersed about 10 cm below the water surface. About 0.5 L of water sample was collected at each sampling site. Samples were acidified with 10% HNO_3, placed in an ice bath and brought to the laboratory. The samples were filtered through a 0.4 µm micropore membrane Whatman filter and kept at 4°C until analysis (Ozturk *et al*., 2009).

Heavy Metals analysis: Digested samples were analysed for Cadmium (Cd), Copper (Cu), Lead (Pb), Manganese (Mn) and Zinc (Zn) using Atomic Absorption Spectrometer (AAS) Buck Scientific VGP-210 model (2008). Air acetylene was used as lean fuel, appropriate hollow cathode lamp for each element determined was employed. Blank and standard solutions were aspirated and the absorbance readings in nanometers were recorded. Three separate absorbance readings were taken for each sample aspirated and their average values computed. The concentration of heavy metals was calculated using the formula (Olaifa *et al.,* 2004).

***Kindly crosscheck this equation and make the necessary corrections
Actual concentration of metal in Sample = PPMR X Dilution factor
Where; PPMR = AAS reading of Digest.

$$\text{Dilution factor} = \frac{(volume\ of\ digest\ used)}{weight\ of\ sample\ digested}$$

The Bioaccumulation factor (BF) of heavy metals was calculated using the formula (Djibrine *et al.,* 2018)

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

Statistical Analyses: Mean concentration of heavy metals in the samples was obtained at 5% level of significance using Analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was used in separating the means where significant difference was observed. All analyses were performed using SPSS V21.0.

RESULTS AND DISCUSSION

The analysis of heavy metals concentration was carried out to assess the levels of some heavy metals in organs/parts of *Hoplobatrachus occipitalis* and water in Kadawa Irrigation Project, Kano. A total of thirty six (36) *H. occipitalis* and water sample each were collected from three different locations in Kadawa Irrigation Project within the period of August to December 2018. The samples collected were analysed for Cd, Cu, Mn, Pb and Zn. In all the samples analysed Cd was found to be below the detection limit by Atomic Absorption Spectrometer (AAS) Buck Scientific VGP-210 model (2008). The physico-chemical parameters in water analysed were: Temperature (27.0-25.4 ^{0}C), pH (8.0-7.5), Dissolved Oxygen (3.5-3.1 mg/l), Total Dissolved Solids (50.1-40.2 mg/l) and Electric Conductivity (111.8-84.4 µS/cm).

Table 1: Heavy Metals in Organs/Parts of *Hoplobatrachus occipitalis* (Crowned bullfrog) and Different Permissible Limits

Organs	Cd (mg/kg)	Cu(mg/kg)	Mn(mg/kg)	Pb(mg/kg)	Zn(mg/kg)
Muscle	ND	0.07 ± 0.01^c	0.06 ± 0.04^b	0.20 ± 0.04^b	$18.10 \pm 2.28b^c$
Liver	ND	1.21 ± 0.20^a	0.61 ± 0.07^a	1.78 ± 0.22^a	42.99 ± 5.18^b
Leg	ND	0.09 ± 0.02^c	0.06 ± 0.02^b	0.17 ± 0.03^b	16.43 ± 1.18^c
Lung	ND	0.48 ± 0.08^b	0.68 ± 0.10^a	1.81 ± 0.30^a	77.38 ± 14.14^a
Trunk	ND	0.10 ± 0.03^c	0.12 ± 0.02^b	0.13 ± 0.02^b	$23.06 \pm 1.50b^c$
p-value	-	0.000	0.000	0.000	0.000
FAO (1983)#	0.05	30	1.0	0.5	30
FAO/WHO(1989)#	0.5	30	0.5	0.5	40
FEPA(2003)#	-	0.5-1.5	0.05	-	5-10
USFDA(2001)#	-	1.3	0.05	-	5

Note*: Values with different superscript differ significantly at $p \leq 0.05$. Average number of frogs = 36.; Key: ND= Not detected by Atomic Absorption Spectrometer (AAS) Buck Scientific VGP-210 model, # = Maximum permissible limit in fish and fishery products.*

Table 1 shows significant difference in heavy metals concentration between different organs/parts. Zinc was reported to be accumulated more in all the organs of *H. occipitalis*. High accumulation of Zn in all the organs/parts of frogs may be due to the specific adaptive mechanism of the frogs to absorb Zn from the habitat for onward transfer to kidney for metabolism. It may also be from agro-chemicals (fungicides) used in the farms as reported by Qureshi *et al*., (2015). This is in line with the finding of Taiwo *et al*., (2014) who recorded high concentration of Zn in tissue of *H. occipitalis.* Zinc concentration differs significantly between the organs studied (p-value = 0.00). The concentration of Zn in all the parts/organs of frog was found to be above the 5-10 mg/kg permissible limit of FEPA (2003) and also above the FAO/WHO (1989) and FAO (1983) permissible limits of 40 mg/kg and 30 mg/kg in lungs and livers respectively. There was significant difference observed in the concentration of Pb among different organs/parts of frogs (p-value = 0.00). Concentration of Pb in the lungs and livers was above the FAO/WHO (1989) permissible limit of 0.5 mg/kg but below the limit in muscle, trunk and leg. This is in line with the report of Amuzie and Daka (2018) who recorded higher concentration of Pb in liver of *H. occipitallis* in Niger Delta. This may be connected to the use of inorganic fertilizer as stated by Raymond and Felix (2011) that compounds used to produce fertilizers contain trace amounts of heavy metals such as Cd and Pb as impurities. All these can get into water as runoff or get into the frog through feeding on herbivorous insects. Manganese concentration in the organs/parts was above the 0.05 mg/kg permissible limit of FEPA (2003) but below FAO (1983) permissible limit of 1.00 mg/kg. Concentration of Mn in organs/parts of frog differed significantly (p-value = 0.00). Manganese distribution pattern in organs was: lung > liver > trunk > leg, with the lung having the highest (0.68 mg/kg) and least in the muscle and leg (0.06) each. Copper was recorded more in liver with mean value of 1.21 mg/kg and less in muscle with a value of 0.07 mg/kg. Qureshi *et al.,* (2015) also reported high level of Cu in *Rana tigrina* in Pakistan. Organ/parts accumulation pattern of Cu was: liver > lung > trunk > leg > muscle which is also in concordance with the findings of Amuzie and Daka (2018) who reported the similar pattern of Cu accumulation (liver > lung > gut > muscle). Although there was high accumulation of Cu in the liver and lung the level was below the FAO/WHO (1989) permissible limit of 30.0 mg/kg and 0.5-1.5 mg/kg of FEPA (2003). All the heavy metals studied accumulated more in the lung and liver.

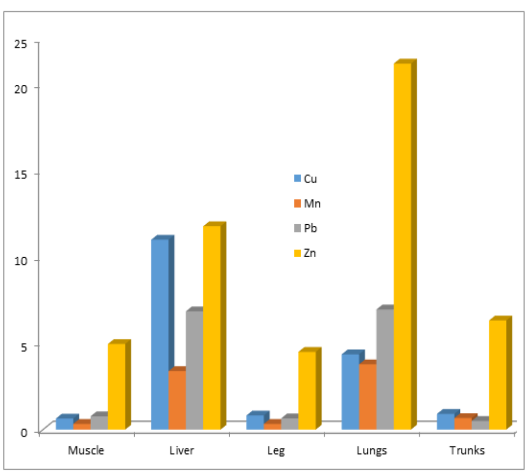

Fig 1: Bioaccumulation of heavy metals in *Hoplobatrachus occipitalis* (Crowned bullfrog) organs/parts.

This agreed with the finding of Bawuro (2018) who recorded more heavy metals in the lung of fish and Qureshi *et al*., (2015) who recorded more metals in the liver of frogs. Lung accumulated more heavy metals likely because of its prominent function in gaseous exchange (Singh *et al*., 2011). High heavy metals concentration in the liver may be attributed to the fact that liver act as an organ mostly associated with detoxification of toxic chemicals that entered the body (Soufy *et al.,* 2007). Shaapera *et al.,* (2013) also reported high concentration of Cu and Cd in the liver. Bioaccumulation of heavy metals in organs/parts of *H. occipitalis* as shown in figure 1 indicate that Lung was found to have highest bioaccumulation of Zn, Pb and Mn with mean values of 21.1ppm, 6.96 mg/kg and 3.78 mg/kg respectively. This could be due to the respiratory function of the lung which lead to accumulation of the heavy metals from the air (Singh et al., 2011). Tyokumbur and Okorie (2011) also reported higher bioaccumulation of Zn (29.84 mg/kg) in *Rana esculentus*. Lawal-Are *et al*., (2017) in their work reported higher Zn concentration in Lagoon crab and Shrimp. Liver has more bioaccumulation of Cu with mean value of 11.00 mg/kg. Higher bioaccumulation of Cu in the liver may be due to its high chemical metabolism (Bawuro, 2018). Bioaccumulation pattern in organs was in the order of lung > liver > trunk > muscle > leg for Zn, Pb and Mn, while liver > lung > trunk > leg > muscle pattern was for Cu. In general lung and liver have higher bioaccumulation of heavy metals.

Heavy metal accumulation in frogs may adversely affect the function of different systems and behavior patterns of the amphibians (Brunt *et al*., 2012; Singha *et al.* 2014) Result in Table 2 shows that higher concentration of all the heavy metals were recorded in water in Location A, followed by location B, and the least were found in Location C. The pattern of heavy metals accumulation in water across locations was: A > B > C. This may be attributed to the fact that Water in location A was closer to the express way just 50 m away while location B was 200 m from express way and location C was 1000 m from express way. This shows that location A may be more polluted due to vehicular exhaust. Chiroma *et al.,* (2014) also reported more heavy metal in irrigation water from Kano city that has more traffic congestion than irrigation water from Yola with less traffic congestion. Taiwo *et al*., (2019) also stated that heavy metals variation in water may be due to level of pollution, oxygen concentration and transparency. Although all the metals analysed were more in location A compared to B and C but statistically only Zn and Pb concentrations were significantly different between the locations (P-value = 0.012 and 0.000) respectively. .

Table 2: Heavy Metal Concentration in Water in different Locations and Different Permissible Limits

	Heavy metals				
Locations	**Cd (mg/L)**	**Cu (mg/L)**	**Mn(mg/L)**	**Pb(mg/L)**	**Zn (mg/L)**
Loc. A	ND	0.13±0.05[a]	0.22±0.02[a]	0.33±0.02[a]	4.35±0.20[a]
Loc. B	ND	0.11±0.01[a]	0.19±0.03[ab]	0.26±0.02[b]	3.78±0.46[ab]
Loc. C	ND	0.09±0.01[a]	0.15±0.02[b]	0.17±0.00[c]	2.80±0.33[b]
p-value	-	0.699	0.058	0.000*	0.012*
FEPA (1991)#	-	1.0	0.1	-	0.5
WHO (2006)#	0.003	0.5	0.1	0.01	0.3
SON (2002)#	0.003	1.0	0.05	0.01	3.0

Note: Values with different superscript differ significantly at $p \leq 0.05$. Keys: ND= Not detected by Atomic Absorption Spectrometer (AAS) Buck Scientific VGP-210 model. * = $P < 0.05$, # = Maximum permissible limit in water, Loc. A= 50m from Expressway, Loc. B = 200m from Expressway, Loc. C = 1,000m from Expressway

There was no significant difference in the concentrations of Mn and Cu in all the locations (P-value =0.058 and 0.699 respectively). Zn concentrations in all the locations were found to be above the FEPA (1991) and WHO (2006) permissible limits of 0.5ppm and 0.3ppm and below the SON (2002) permissible limit of 3.0ppm in locations A and B. This was similar to the finding of Uzairu *et al.,* (2014) who observed highest value of Zn in water during hot season. Lead (Pb) concentration in all the locations ranged from 0.33 mg/L to 0.17 mg/L, which was above the permissible limits of 0.01 mg/L recommended by SON (2002) and WHO (2006). High lead concentration in the water may be from the fertilizer, pesticides and vehicular exhaust (Raymond and Felix, 2011). Concentration of Cu in all the locations was below the permissible limits of 1.0 mg/L of SON (2002) and FEPA (1991) and 0.5 mg/L set by WHO(2006). This is in line with the findings of Uzairu *et al.,* (2014) who reported that Cu was generally within the recommended limits.

Conclusion: There was Zn, Pb and Mn pollution in both the frog and water from diverse sources in the area under study. Zn and Pb was the most accumulated heavy metals in the frog organs/parts and water and were above the various standard permissible limis. Lung and liver have the highest bioaccumulation of heavy metals while the leg and muscle have the least. Hence the water, liver and lung of *H. occipitallis* are unsafe for consumption, and therefore posed a threat to public health. Consumers should go for the leg and muscle, internal organs should be avoided.

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