Appraisal of heavy metals in the organs of fish species *Cyprinus carpio* and *Heterotis niloticus* in Alaro Stream, Ibadan

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Abstract: Studies were carried out on the evaluation of heavy metals in the organs of the fish species *Cyprinus carpio* and *Heterotis niloticus* in Alaro Stream located in Ibadan, Nigeria. The study was to assess the extent of heavy metals which have the chemical and ecological potential to be become elevated with the tendency to bioaccumulate in fish which are higher consumers in the food chain of aquatic ecosystems. A total of 64 fish representing two fish species of *H. niloticus* (32) and *C. carpio* (32) were collected from the sampling sites. Fish were dissected to remove the gills, gut, liver, fins, bones and muscle (flesh) and dried separately in an oven at 105^0C for 6hours for pulverization. The pulverized samples were acid digested for analyses with Inductively Coupled Plasma Spectrometer (ICP-MS).The results of mean Cu, Zn, Mo, V and Co in the fish organs in this study supports earlier findings that indicated elevated levels in polluted aquatic ecosystems. For instance, all the organs of *H. niloticus* had higher mean values of Ni that were above World Health Organization's (WHO) limit guideline limit in food while in *C. carpio*, only the intestine, gut , bones and fins were higher than the permissible level . The mean V concentration in the two fish species was above the WHO permissible limit guideline of 0.02ppm in the order in the order: Liver<Gills<Fins<Intestine<Gut<Muscle *C. carpio* and Gills<Bone<Fins<Intestine<Muscle<Gut (*H. niloticus*). The study shows that these fish caught in Alaro Stream is not safe for human consumption.
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Introduction

In the aquatic habitat, fish are a very important indicator of heavy metal pollution since heavy metals are transferred to the fish through food chain that could ultimately affect the health of people consuming the fish (Gernhofer *et al.*, 2001). Heavy metals have the chemical and ecological potential to be persistent in the environment which elevates their toxicity and tendency to bioaccumulate in aquatic ecosystems (Miller *et al.,* 2002; Jabeen *et al.*, 2012). Thus, heavy metal ecotoxicity in the aquatic ecosystems has become a major global health concern over the years (Mendil *et al.* 2010). Bioaccumulation of toxic heavy metals in the tissues of aquatic biota to elevated hazardous levels has becomes a problem of increasing public health concern to both animals and humans (Raju *et al*. 2013). This is due to the presence of excessive heavy metal concentration in surface water that could lead to health hazards in man, either through drinking of water and unknowing consumption of contaminated fish (Mathias and Cummings, 1973; Kargin, 1998; Ashraf, 2001). This public health concern posed by heavy metals in the environment creates an immense threat to the existence of organisms thriving in an ecosystem and the ecological integrity of the habitat as these pollutants may enter the food chains, persist in the environment, bioaccumulate and biomagnify and increase the exposure to environmental health risks (Su *et al.,* 2009). However, what becomes bioavailable in the aquatic ecosystem depends on the heavy metal speciation in bottom sediments and entire water body that affects uptake in the food chain (Bochenek *et al.,* 2008). Fish being the higher consumer in the aquatic food chain are able to accumulate large quantities of heavy metals in their organs (Chezhian *et al.* 2010). The heavy metals once absorbed are transported by the blood stream to either a storage organ (bones, liver), or to other organs such as the kidney, gills, fins and intestine (Dural *et al.* 2007; Kousar and Javed, 2014) . The objectives of this study are to assess heavy metals Nickel (Ni), Copper (Cu), Zinc (Zn), Cobalt (Co), Vanadium (V) and Molybdenum (Mo) in organs of the fish, *Heterotis niloticus* and *Cyprinus carpio* in Alaro Stream and to compare it with World Health Organization's (WHO) limit guidelines.

Experimental Methods

Study Area

Alaro stream flows through Oluyole Industrial Estate in a west to south east direction from its source at Agaloke near Apata in Ibadan. It joins River Ona as its main tributary at the south east of a meat processing factory. The stream receives effluents from industries, agricultural activities and domestic wastes. These effluents sources are discharged into Alaro stream directly or indirectly as run-off, leachate and deposition.

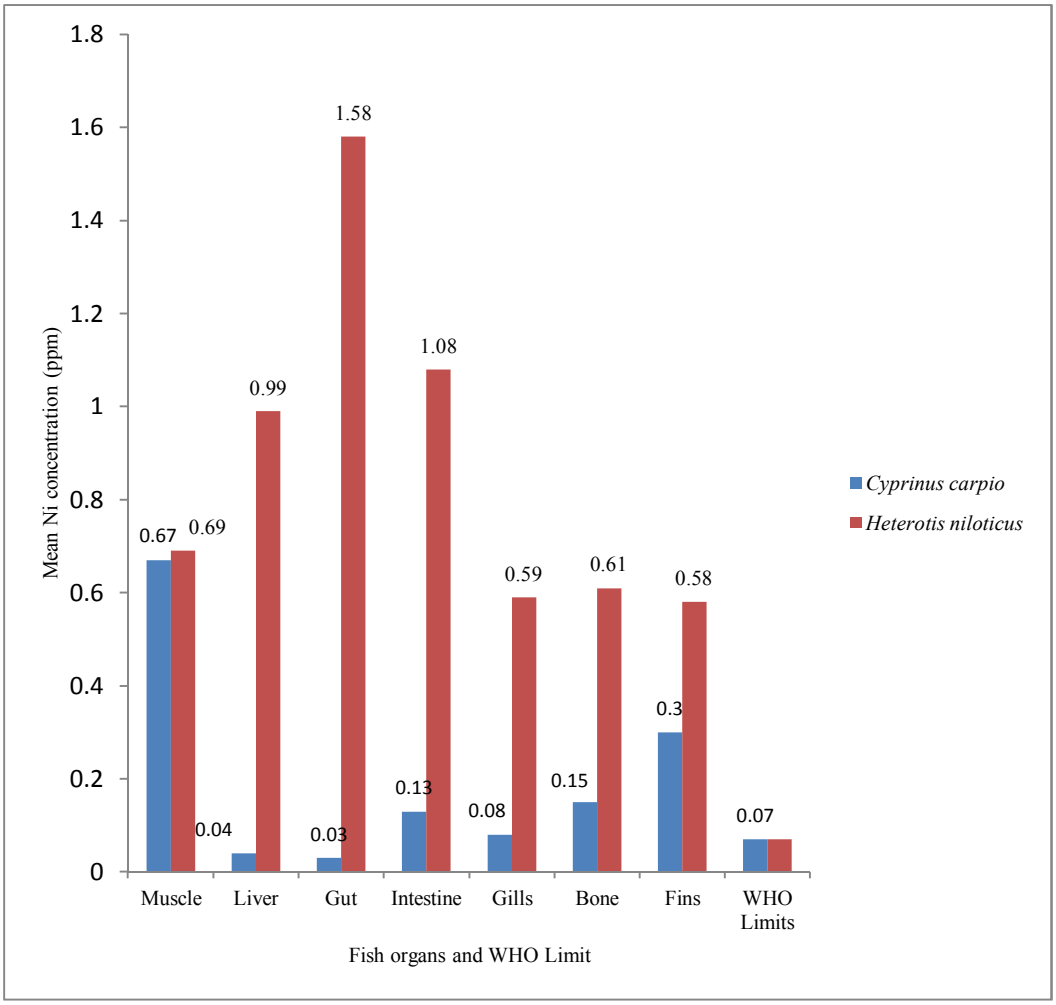

Figure 1: Mean Ni concentration (ppm) in the fish organs.

The Oluyole industrial estate is located between latitude 7º21'N -7 º 22'N and longitude 3 º 50'-3 º 52'E.

Sampling Sites

Sampling sites were numbered 1 to 5, with Site 1 located before Oke Alaro Bridge I just before the stream flows through the industrial estate. Sampling Site 2 was located downstream of Oke Alaro bridge II at a distance of about 500 metres from Site 1. This site receives run-off water from Alaro dwellings and some areas of Oluyole residential area. A shopping arcade also discharges effluents into a gutter that drains into the stream.

Sampling site 3 receives effluents discharged from some beverage, crown cork and confectionery factories.

Sampling Site 4 receives effluents from a meat processing factory. Sampling site 5 is located just before the confluence between River Ona and Alaro Stream.

Sample Collection and Processing for Analyses

A total of 64 fish representing *H. niloticus* (32) and *C. carpio* (32) were collected from the sampling sites using cast nets of mesh size between 30-50mm.The nets were cast and retrieved with a drawing string to check for caught fish. In addition, gill nets with mesh size of 30-50mm were tied to stakes with a lead weight resting on the benthic surface vertically with the aid of floats and left overnight, pending retrieval the following morning.

Fish were dissected to remove the gills, gut, liver, fins, bones and muscle .These organs were dried separately in an oven at 105^{0}C for 6hours .Each organ was pulverized into powdered form with using a porcelain mortar and pestle .The pulverized samples were kept in Ziploc bags pending acid digestion.

Pulverized samples were digested by adding 2mL trace metal grade HNO_3 to 0.5g of each sample in Teflon tubes and heated at 105 ^{0}C for 1hour in a heat block after which the clear solution was then

allowed to cool down, followed by addition of 1ml H_2O_2. After the simmering reaction, the digestate was boiled and left overnight. The digestate was thereafter diluted to the 10ml mark using MilliQ water and transferred into test tubes rinsed with deionized water for analyses using Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

The Standard Reference Material (SRM) used to evaluate the level of detection and recovery of heavy metals by the ICP-MS was bovine liver procured from the National Institute of Standards and Technology (NIST-1577).

Results

The results of the mean heavy metal concentration in the organs of the fish in the study is shown in Figures 1-6 below.

The highest mean Ni was recorded in *H. niloticus* (1.58ppm, gut) while the lowest was 0.03ppm in *C. carpio* also in the gut. The least mean value for *H. niloticus* was 0.58ppm in the fins while the highest for *C. carpio* was 0.67ppm in the muscle (flesh). All the mean Ni concentration in *H. niloticus* organs were above the World Health Organization (WHO) permissible limit guideline of 0.07ppm [14]. In *C. carpio*, with the exception of the liver (0.04ppm) and gut (0.03ppm) all the other organs were above the WHO permissible limit guideline. Comparatively, all the organs of *H. niloticus* had higher mean values of Ni that were above WHO limit while in *C. carpio*, only the intestine, gut, bones and fins were higher than the permissible level.

With the exception of the liver (77.79ppm) of *H. niloticus*, all the other organs of the two fish were below the WHO permissible guideline of 30ppm.

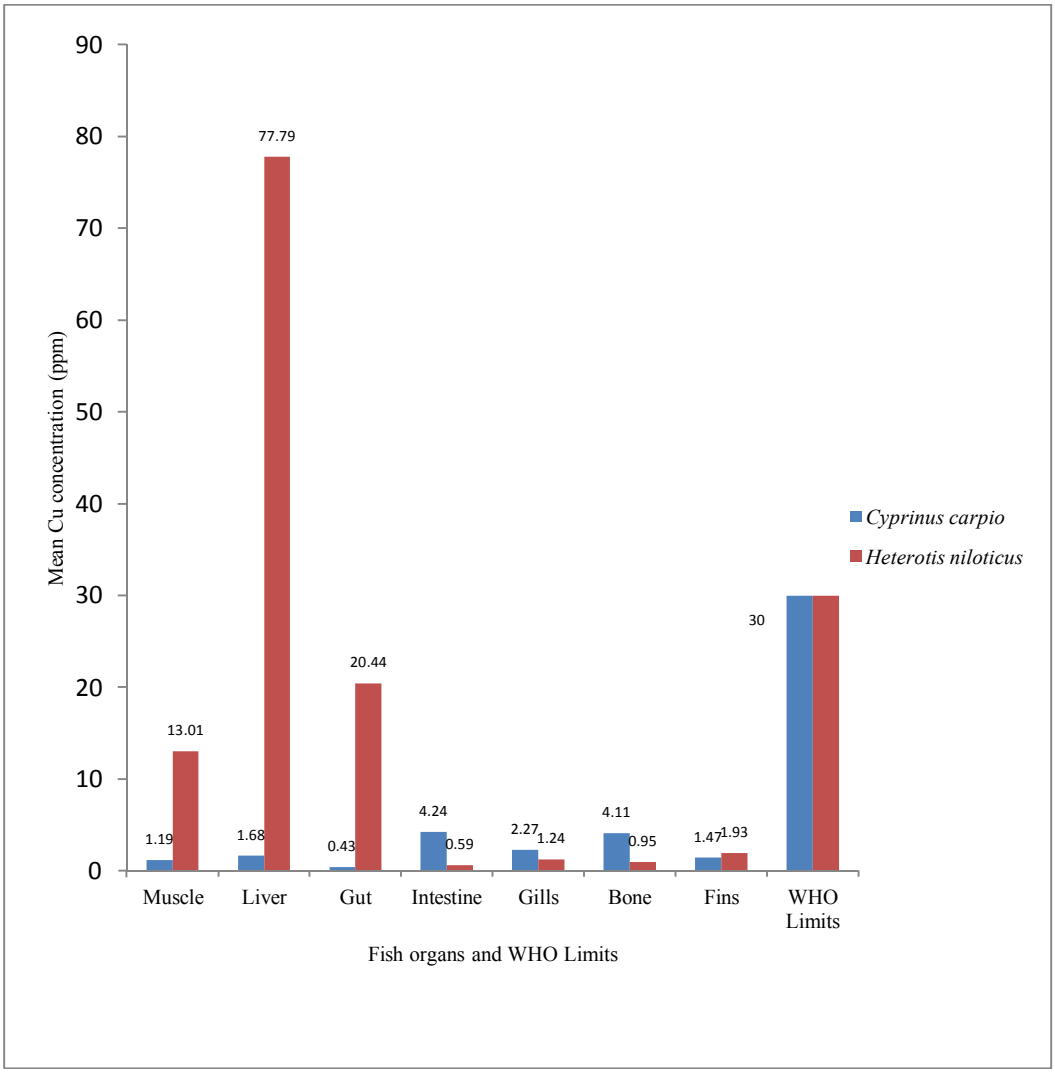

Figure 2: Mean Cu concentration (ppm) in the fish organs.

The mean Cu levels in *C.carpio* were in the order: Gut<Muscle<Liver<Fins<Gills<Bone<Intestine (4.24ppm), while in *H. niloticus*, it was in order: Intestine<Bone<Gills<Fins<Muscle<Gut<Liver as shown in fig.2.

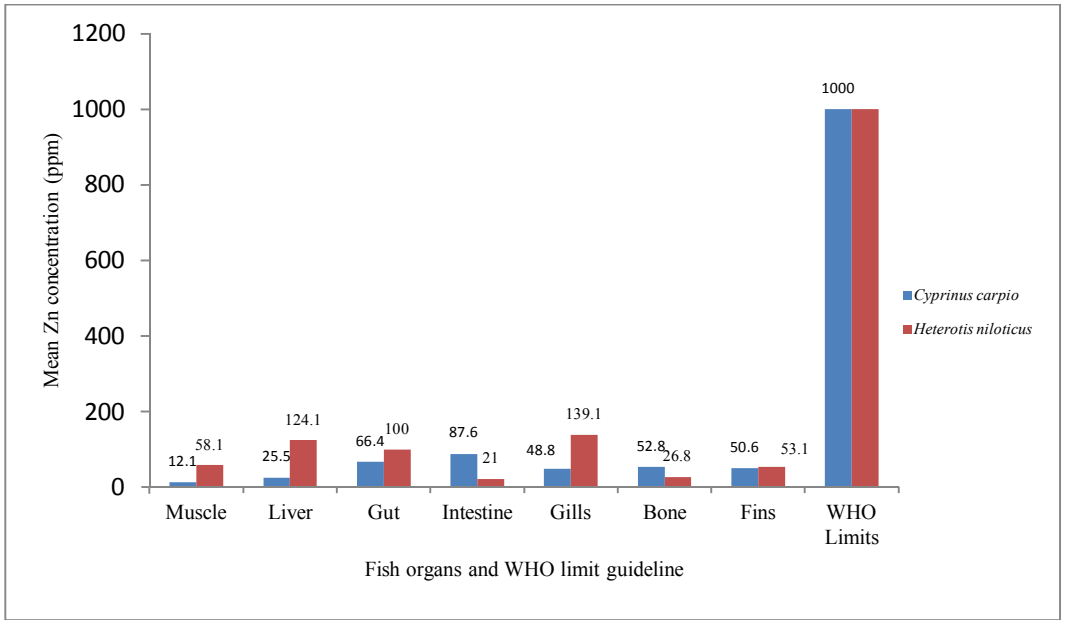

Figure 3: Mean Zn concentration (ppm) in the fish organs.

All the mean Zn concentration in the organs of the two fish was below the WHO permissible guideline of 1000ppm. The highest mean level was in the gills (139.1ppm) of *H. niloticus* while the least was in the muscle (12.1ppm) of *C. carpio*. The mean Zn concentration in the organs of *C. carpio* was in the order: Muscle<Liver<Bone<Gills<Fins<Gut while in *H. niloticus* it was: Intestine<Bone<Fins<Muscle<Gut <Liver.

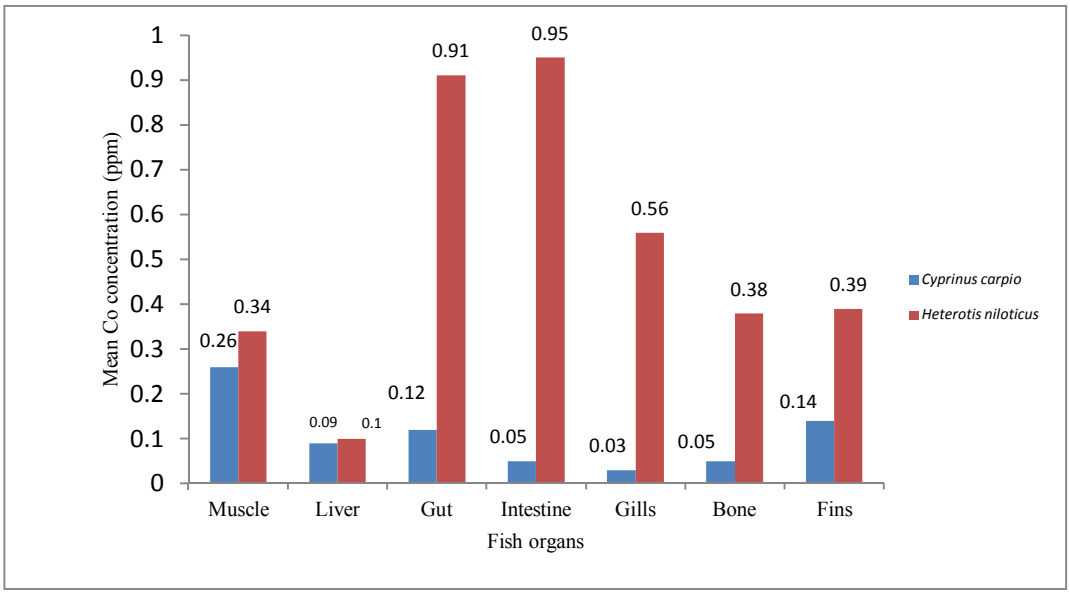

Figure 4: Mean Co concentration (ppm) in the fish organs.

The highest mean Co concentration in the organs of C. carpio was in the muscle (0.26ppm) while the least was in the gills (0.03ppm) with the order: Gills<Intestine and Bone<Liver<Gut<Muscle. The highest mean value for H. niloticus organs was 0.95ppm (Intestine) while the least was 0.1ppm (Liver) in the order: Liver<Muscle<Bone<Fins<Gut<Intestine. There is however, no listed limit for Co.

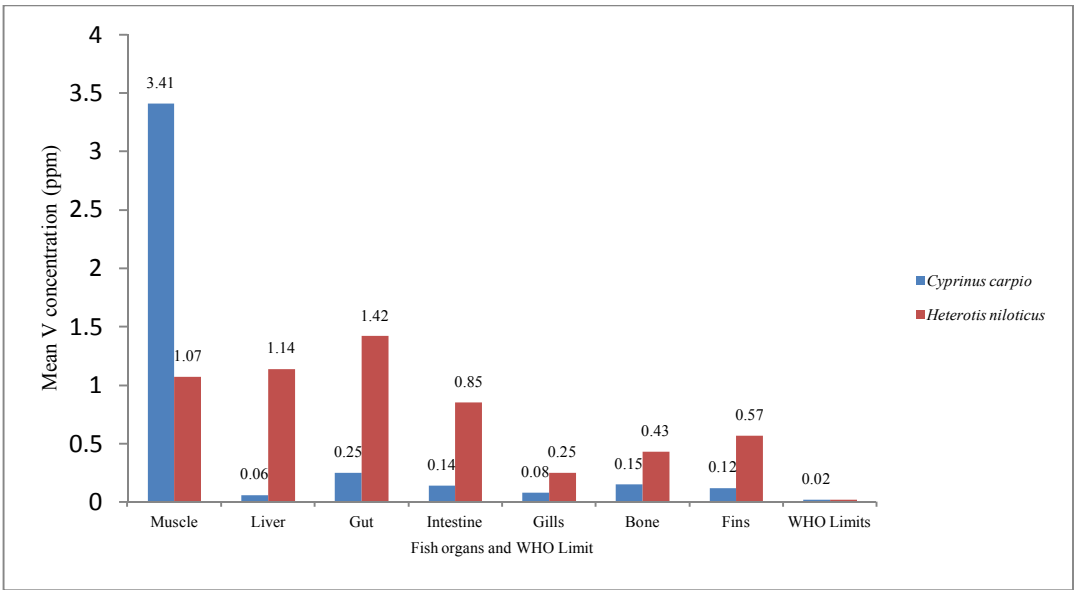

Figure 5: Mean V concentration (ppm) in the fish organs.

All the mean V concentration in the two fish was above the WHO permissible limit guideline of 0.02ppm.

The highest mean V concentration in *C. carpio* was 3.41ppm (Muscle) while the lowest was 0.06ppm (Liver) in the order: Liver<Gills<Fins<Intestine<Gut <Muscle. The highest mean V value in *H. niloticus* was 1.42ppm (Gut) while the lowest was 0.25ppm (Gills) in the order: Gills<Bone<Fins<Intestine <Muscle<Gut. All the mean V concentration in the organs of *H. niloticus* was all above that of *C. carpio*.

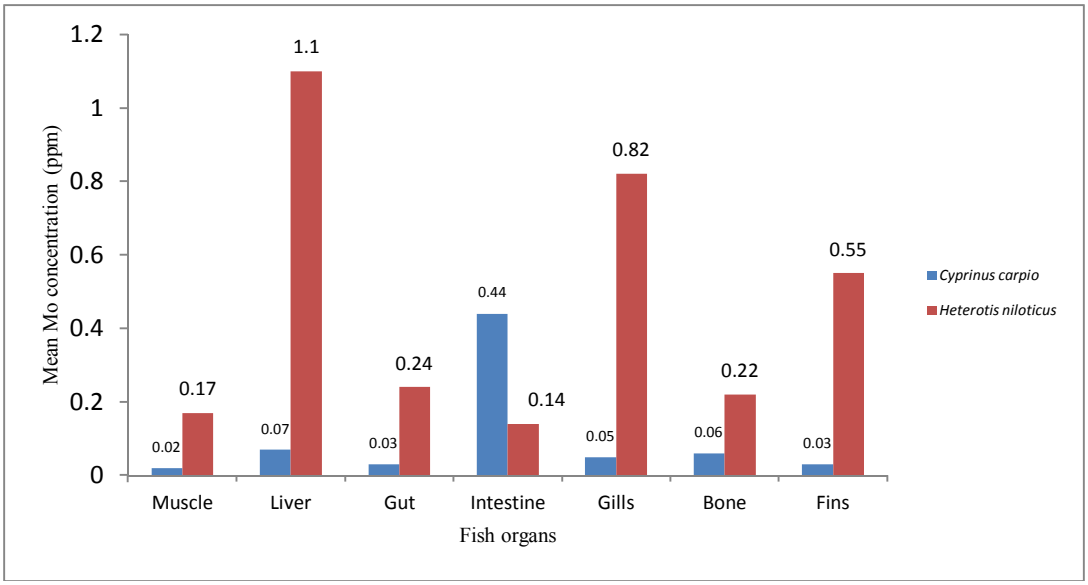

Figure 6: Mean Mo concentration (ppm) in the fish organs.

The highest mean Mo concentration in the organs of *C. carpio* was 0.44ppm (Intestine) while the lowest was in the muscle (0.02ppm) in the order: Muscle<Gut and Fins<Gills<Bone<Liver<Intestine. The lowest mean Mo in *H. niloticus* was 0.14ppm (Intestine) while the highest mean value was 1.1ppm (Liver) in the order: Intestine<Muscle<Bone<Gut <Fins<Gills<Liver. However, there is no WHO permissible limit for Mo because in 2011 the 70 µg/L guideline was withdrawn with the assertion that molybdenum occurs in drinking-water at concentrations well below those of health concern and therefore, it is not considered necessary to set a formal guideline value (WHO, 2011).

Discussion

Heavy metals are toxic aquatic pollutants whose uptake and accumulation in the aquatic biota beyond optimally safe limits could cause direct impacts on the aquatic food chain and eventually to man as a higher consumer (Rauf, 2009). This is so because heavy metals are inorganic contaminants that are widespread in the aquatic environment (Bochenek *et al.,* 2008). The relatively high Ni concentration in the organs of *C. carpio* and *H. niloticus* which exceeded WHO permissible guideline limit can be attributed to the level of pollution of Alaro Stream and the high retention capacity in the fish (Nagabhushanam *et al.* 1999; Heath, 1987). This corroborates similar findings by Jabeen *et al*. (2012), in which uptake and accumulation of nickel in the liver fish species were significantly higher, followed by that in kidney and gills

with statistically significant differences. The results of this study also compare favourably with other findings in which among exposed freshwater fish species, *Cirrhina mrigala* exhibited significantly higher ability to amass Ni (146.8±149.1 µg g^{-1} or ppm). The findings for Cu, Zn, Mo, V and Co in the fish organs in this study supports earlier findings by Jabeen *et al* (2012) where freshwater fish exhibited significant tendencies to bioaccumulate zinc in their liver with the concentrations of 84.77±26.23 and 124.79±11.91 µgg^{-1} respectively, followed by the accumulation of this metal in the gills with statistically significant differences (Chezhian, 2010).Similarly, Raju and others found that the concentration of all the analyzed metals (Fe, Pb, Zn, Ni, Mn, Cu, Cr and Cd) in freshwater fish organs along the River Cauvery in India were observed to be high at significant level in downstream stations which was also attributed to the dumping of agricultural and industrial wastes (Raju *et al.* (2013). The liver of both fishes also contained significantly higher heavy metals due to its affinity as a storage organ and formation of metallothionein protein with the metals (Nagabhushanam *et al.* 1999). Metallothioneins are proteins that bind to heavy metals such as zinc, copper, mercury, cadmium and silver thereby maintaining a balance between copper and zinc concentrations in order to protect the organism from the toxic effects of cadmium and mercury, which are its inhibitors (Ikem *et al.,*2003; Bochenek *et al.,* 2008). The differences between the heavy metal concentrations in the two fish species could be due to different feeding habit, diet and habitat preferences (Karadede-Akin and Unlu, 2007; Rauf, 2009). Heavy metal distribution in the two fish species depends on the metal properties and physicochemical factors while the effects of the toxicants depend on their concentration, dose, duration of exposure, and route of exposure (Bochenek *et al.,* 2008; Ladipo *et al.,* 2012).

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