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STUDY ON HEAVY METAL CONTENT OF *Oreochromis niloticus, Heteropneustes fossilis* AND *Pangasius sutchi* COLLECTED FROM POND AND OPEN WATER

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ABSTRACT

Present studies were conducted on the study of heavy metal content of three popular freshwater fish, tilapia *Oreochromis niloticus*, shing, *Heteropneustes fossilis* and pangus, *Pangasius sutchi* collected from pond and open water in Mymensingh. All samples were evaluated by studying unwanted heavy metal concentrations. Cadmium (Cd) concentration (µg/g) of pond water *O. niloticus, H. fossilis* and *P. sutchi* was found to be 0.21±0.02, 0.20±0.01 and 0.22±0.02 respectively and open water was 0.28±0.03, 0.25±0.02 and 0.29±0.03, respectively. Copper (Cu) concentration (µg/g) of pond water *O. niloticus, H. fossilis* and *P. sutchi* was 0.25±0.02, 0.29±0.03 and 0.21±0.02 respectively whereas open water had 0.28±0.01, 0.59±0.04 and 0.35±0.01 respectively. Lead (Pb) concentration (µg/g) of pond water *O. niloticus* and *H. fossilis* contained 0.07±0.01 and 0.08±0.01, respectively whereas the open water had 0.15±0.02 and 0.15±0.01, respectively. Pb concentration both in pond and in open water *P. sutchi* was nil. Pb concentration of *O. niloticus* was higher in open water fishes (0.15±0.02 µg/g) than the fishes of pond water (0.07±0.01 µg/g). Heavy metal concentration of *O. niloticus, H. fossilis* and *P. sutchi* was within permissible limits except Cd of open water fishes. The result revealed that open water fishes have higher concentration of heavy metals in their muscle than the fishes of pond water. The results indicate that the open water environment is polluted by various chemical composition as well as various toxic heavy metal pollutants.

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INTRODUCTION

The aquatic environment of Bangladesh is diversified and its bio-diversity is well rich in productivity. Many fish species are surviving in our water body from year after year. Our aquatic resources like; rivers, canals, ponds, haors, baors, lakes, ditches, and flood plains are very much productive for aquatic flora and fauna. But our water body has degraded and its glorious days of fish assets from a few years ago. Now -a-days fish consumption every day is like a nightmare, especially for poor people. Actually there are many reasons which are responsible for this scenery. Few striking causes can be mentioned here like; indiscriminate capture of our indigenous fish species, habitat destruction, water flow change due to making dam, embankment, hydro-power plant and other reasons.

Bangladesh has the third largest aquatic fish biodiversity in Asia with about 800 species in fresh, brackish and marine water (Hussain and Mazid, 2001). Bangladesh is blessed with large water reservoirs containing 260 freshwater species and 475 marine species (DoF, 2014). But the number of so many fish species are in a danger condition due to deteriorate of habitat by both environmental and human activities. The waterbodies are being polluted day by day. Agricultural waste, urban runoff, industrial waste and so many ways water and environment are polluting.

Heavy metal is a general term applied to a metal or metalloid, which has atomic density greater than 4g/cm^3 (at least 5 times or more than water). Toxic heavy metals are persistent environmental contaminants because they can not be degraded or destroyed. To a small extent, they enter the body system through food, air and water. They bio-accumulate over a period of time because they cannot be metabolized (Lenntech, 2004). Heavy metal toxicity can lead to damaged or reduced mental and central nervous functioning, damage of vital internal organs and lowering of energy levels in the body. There are more than 20 heavy metals but this research looked at cadmium, lead and chromium. These metals are highly toxic such that they can cause damaging effects even at low concentrations (Tuberose, 2007). There is great concern of trace metals in foods, which has prompted several bodies such as World Health Organization (WHO) to establish maximum allowable concentrations of these metals in food (WHO, 1984). It is better to know about the degree of heavy metal contamination of different water body. Trace elements can be accumulated by fish, both through the food chain and water (Hadson, 1998). Fish living in the polluted water may accumulated toxic trace metals via their food chains. High levels of arsenic, lead, copper and iron all causes rapid physiological changes in fish (Tarrio et al., 1991). Arsenic is one of the toxic environmental pollutants which has recently attract attention because of its chronic and epidemic effects on human health through widespread water and fish contamination due to the natural release of this toxic elements from aquifer rocks in Bangladesh (Fazal et al., 2001).

A substantial toxicological data base for cadmium and fresh water biota demonstrates that ambient cadmium water concentrations exceeding 10 ppb are associated with high mortality, reduced growth, inhibited reproduction and other adverse effects. In case of Cd accumulates is higher in liver, kidneys and other organs but low in fish. Cadmium encourages kidney disease, high blood pressure to man and also may lead to kidney and lung damage. Resistance to cadmium is higher in marine then in freshwater organisms; survival usually is higher at the lower temperatures and higher salinities for any given level of cadmium in the medium. Cadmium is a known teratogen and carcinogen, a probable mutagen and has been implicated as the cause of severe deleterious effects on the fish and wildlife. The concentrations of 0.8 to 9.9 µg/l in water is lethal to several species of aquatic insects, crustaceans, and teleosts and concentrations of 0.7 to 570 µg/L is associated with sub-lethal effects such as decreased growth. The main objective of present study was to assess the content of heavy metals of the stated samples of tilapia (*Oreochromis niloticus),* shingh (*Heteropneustes fossilis)* and pangus *(Pangusius sutchi*) that collected from different pond and open waterbodies of Mymensingh region, Bangladesh.

MATERIALS AND METHODS

Organoleptic assessment

Physical characteristics such as color, odour, taste, flavor and texture of Tilapia (*Oreochromis niloticus),* Shing (*Heteropneustis fossilis)* and Pangus (*Pangasius sutchi)* were observed by organoleptic method (Howgate *et al*., 1992). The organoleptic assessment is a simple and widely used method in selecting quality of fish in the industry. This chapter represents the results on post-harvest quality loss and studies on the physical or organoleptic in fish and their relationship with the freshness of koi and tilapia. Sensory methods are the most accurate and most widely used for organoleptic evaluation. A large number of schemes have been proposed for sensory evaluation of various types of fish. The evaluation method used in the study was based on the one currently in use in various institutes and industries of the world. The following set of guidelines has been prepared to get maximum value from them by being able to compare the results. The guidelines and methods given here using score on the organoleptic characteristics of fish as described by EC freshness grade for fishery products which is shown in (table 1 and 2).

Table 1. Grading of fresh fish.

Grade	Points	Degree of freshness
A	<2	Excellent/Acceptable
B	2 to <5	Good/Acceptable
C	5	Rejected

Table 2. Determination of standard defect points for organoleptic test of samlpe fish as quality assured.

Characteristics of whole fish	Defect characteristics	Defect points	Grade
1. Odour of neck when broken	Natural odour	2	Acceptable
	Natural odour	1	Excellent
2. Odour of gills	Faint sour odour	2	Acceptable
	Slight moderate sour odour	3	Acceptable
	Slight pinkish red	1	Excellent
3. Color of gills	Pinkish red or brownish red., some mucus may be present	2	Acceptable
	Brownish or grey	3	Acceptable
4. General appearance	Full bloom, bright, shining, iridescent	1	Excellent
	Slight dullness and loss of bloom	2	Acceptable
	Bulging with protruding lens, transparent eye cap	1	Excellent
5. Eyes	Slight clouding of lens and sunken	2	Acceptable
	Dull, sunken, cloudy	5	Reject
	Usually clear, transparent and uniformly spread but occasionally may be slightly opaque or milky	1	Acceptable
6. Slime	Becoming turbid opaque and milky, amount of slime present in skin	1	Acceptable
	Thick, sticky, yellowish greenish in colour	5	Reject
	Firm and elastic	1	Acceptable
7. Consistency of flesh	Moderately soft and some loss of elasticity	2	Acceptable
	Some softening	3	Acceptable

Calculation of organoleptic assessment

$$\text{Average grade points} = \frac{\text{Total Grade Point}}{\text{No. of Characteristics}}$$

Sample collecton and processing

The study was conducted during July 2013 to December 2014 and the freshwater fish species of tilapia (*O. niloticus)*, shing (*H. fossilis)* and pangus *(P. sutchi)* were collected from Mymensingh Messua Bazar, Mymensingh Town, Bangladesh. The collected fishes were brought to the laboratory of Fisheries Technology Department, Bangladesh Agricultural University, Mymensingh with ice in insulated box. The sample collection procedure was strickly followed by organoleptic test. During sample collection organoleptic characters and overall quality of *O. niloticus, H. fossilis* and *P. sutchi* were excellent. Sensory characteristics were fresh, bright, soft and fresh odour. The average weight of the experimental species was tilapia 350g/species and 170g/species, shing 115g/species and 135g/species and pangus 1.25kg and 4.50kg, pond and open water respectively. Two representative samples of each fish were packed and kept in -20°C for further study.
The frequency of monitoring was once a week up to 12 weeks. Frozen fishes were thawed and soaked with wet tissue paper. Only fish muscle was collected for the further examination. Then the muscle was chopped and finally ground with a blender for homogenous mixture.

Sample analysis

The samples were digested in nitric acid and concentration of heavy metals was determined using atomic absorption spectrophotometer method (AOAC method 1990). All chemicals and reagents used in this procedure were of analytical grade (AR). Buck Scientific Atomic Absorption Spectrophotometer Model 200A was used in this research.

Collected tissues were weighed by electronic balance and 5 ml of diacid mixture (5 ml conc. HNO_3: 3 ml 60% $HClO_4$) were added to each sample. The content mixed for overnight. Samples were then digested, initially at 80ºc temperature and later on 150ºc for 2 hours. The completion of digestion was indicated by almost colorless material. The brown fumes also cease to exist at completion of digestion. The samples were separately filtered by using an ash less filter paper and volume made up to 25 ml with 0.5% HNO_3 which prepared for the determination of arsenic, cadmium and chromium (Eboh *et al*., 2006). The samples were subjected to analysis by Atomic Absorption Spectrophotometer (HG-AAS, PG-990, PG Instrument Ltd. UK) at Chemical Analyst Md. Tarikul Alam, Bangladesh Institute of Nuclear Agriculture (BINA), Bangladesh Agricultural University, Mymensingh, following the method of Clesceri *et al*. (1989). The concentration of Cd, Cu and Pb in fish samples were calculated by the following formula:

$$\text{Metal concentration} = \frac{\text{ppm conc.Observed} \times \text{final volume of sample in ml}}{\text{Weight of tissues taken in gm}}$$

RESULTS AND DISCUSSION

Organoleptic quality of Tilapia*(Oreochromis niloticus)*, Shing (*Heteropneustis fossilis)* and Pangus *(Pangasius sutchi*)

During sample collection organoleptic characters and overall quality of *O. niloticus, H. fossilis* and *P. sutchi* were excellent. Sensory characteristics were fresh, bright, soft and fresh odour. The results of the organoleptic quality assessment during sample collection are presented in Table 3.

Table 3. Organoleptic characteristics of *Oreochromis niloticus, Heteropneustis fossilis* and *Pangasius sutchi*

Fish species	Source of fish	Organoleptic quality (Physical characteristics)	Defect point	Overall quality
O. niloticus	Pond water	Fresh, bright appearance, soft and firm texture with characteristics fresh odor.	1.54	Excellent
	Open water	Fresh, bright appearance, soft and firm texture with characteristics fresh odor	1.63	Excellent
H. fossilis	Pond water	Fresh, bright appearance, soft and firm texture with characteristics fresh odor.	1.00	Excellent
	Open water	Fresh, bright appearance, soft and firm texture with characteristics fresh odor	1.00	Excellent
P. sutchi	Pond water	Fresh, bright appearance, soft and firm texture with characteristics fresh odor.	1.53	Excellent
	Open water	Fresh, bright appearance, soft and firm texture with characteristics fresh odor.	1.20	Excellent

It is essential to use sensory assessment wherever standards of quality need to be established, controlled or assured. Since the prosperity of most fish businesses depends on maintaining the quality of their products at a consistently high level, the importance of sensory assessment is obvious (FAO, 1989).

Heavy metal concentration of *Oreochromis niloticus, Heteropneustis fossilis* and *Pangasius sutchi*

Arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), silver (Ag), zinc (Zn) and the platinum group elements constitute heavy metals. The metals get into the tissues of aquatic animals mainly via their food mostly the fishes are in polluted water. A great number of anomalies and abnormalities in the fishes are reported due to the concentrations of metals exceed the environmental standards (Shesterin, 2001).

Fish living in the polluted water may accumulate higher amount of toxic heavy metals through their food chain (Hadson, 1998). Various factors such as season, physical and chemical properties of water can play a significant role in metal accumulation in different fish tissues (Kargin et al., 1996). Stress due to heavy metals present in the waste water pond, does create hematological disturbances, erythrocyte destruction (hemolysis), and leukocytosis in fish population, affecting the immune system and making the fish vulnerable to diseases (Javed and Osmani, 2013). There is high accumulation of some heavy metals in fishes in the river (Moody et al., 2013). In the present study, Cd, Cu and Pd were determined in the muscle of *O. niloticus H. fossilis* and *P. sutchi*

Table 4. Cadmium (Cd), Copper (Cu) and Lead (Pb) concentration of *Oreochromis niloticus, Heteropneustis fossilis* and *Pangasius sutchi*

Heavy metal, conc. (µg/g)	***O. niloticus***		***H. fossilis***		***P. sutchi***		**Permissible limit** (FAO/WHO, 2011)
	Pond water	**Open water**	**Pond water**	**Open water**	**Pond water**	**Open water**	
Cd	0.21±0.02	0.28±0.03	0.20±0.01	0.25±0.02	0.22±0.02	0.29±0.03	0.20 µg/g
Cu	0.25±0.02	0.28±0.01	0.29±0.03	0.59±0.04	0.21±0.02	0.35±0.01	10 µg/g
Pb	0.07±0.01	0.15±0.02	0.08±0.01	0.15±0.01	ND	ND	2 µg/g

Cadmium (Cd) concentration

Open water *P. sutchi* contains higher Cd in the muscle than pond water *O. niloticus* and *H. fossilis*. The average Cd concentration of *P. sutchi* collected from pond water and open water was 0.22±0.02µg/g and 0.29±0.03µg/g respectively. Pond water *O. niloticus* and *H. fossilis* contains lower concentration of Cd in the body than open water *O. niloticus* and *H. fossilis.* Average Cd concentration of *O. niloticus* collected from pond water and river water was 0.21±0.02µg/g and 0.28±0.03µg/g respectively.Average Cd concentration of *H. fossilis* collected from pond water and river water was 0.20±0.01µg/g and 0.25±0.02µg/g respectively. The results are compared in Table 4 and Figure 1.

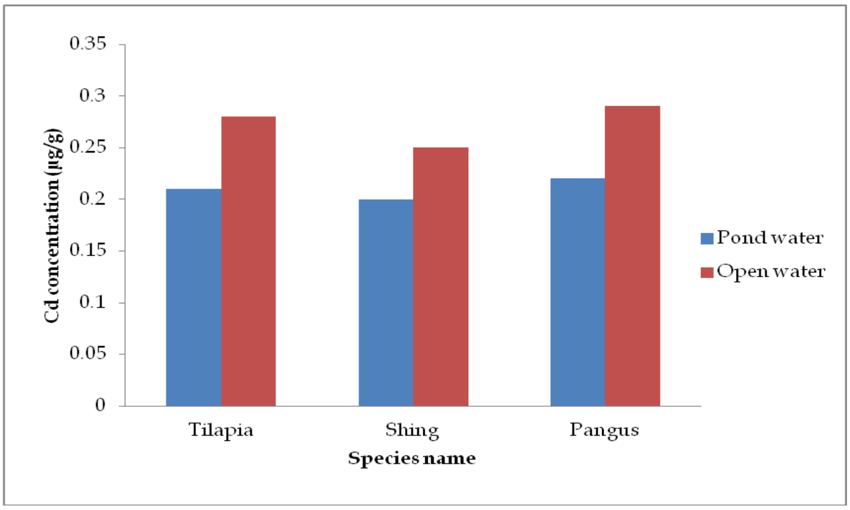

Figure 1. Cadmium (Cd) concentration of *O. niloticus, H. fossilis* and *P. sutchi*

Bioaccumulation of some heavy metals (Lead, chromium and cadmium) in some freshwater fishes (*T. zilli*, *L. coubie* and *S. membranaceous*) are Cr > Pb> Cd. There is high accumulation of some heavy metals in fishes in river (Moody et al.*,* 2013). The concentration of Cd in fish muscle tissue range was 0.01-1.10, µg/g dry weight (Kumar et al.*,* 2012). In the present study Cd accumulation is lower than Cu accumulation and does not exceed the recommended limits. Pond water fishes have lower concentration of Cd than open water species. Cadmium levels usually increase with age and pollution (WHO, 1992).

The Cd contents in *C. carpio* varied closely. Detected values for individual tissues ranged in muscle 0.02-0.13, hepatopancreas 0.05-0.14, kidney 0.07-0.24, gonads 0.02-0.13, skin 0.07-0.76, gill 0.26-0.38, fin 0.52-0.84 mg.kg-1 (Toth et al.*,* 2012). Anim et al.*,* 2011 investigated the accumulation profile of Cd in five fish species namely; *H. niloticus*, *C. obscura*, *H. odoe*, *T. zilli* and *C. gariepinus*. The trend of heavy metals concentration was represented as: Cr > Zn > Cu > Fe > Mn > Cd > Pb for *T. zilli*, while that of *C. gariepinus* was Cr > Zn > Fe > Cu > Mn > Cd > Pb (Eneji et al.*,* 2011). The concentration of heavy metals was in order of, Fe > Zn > Cu > Mn > Ni > As > Hg > Cd (Kumar et al.*,* 2011). In the present study, the trend of heavy metals concentration can be represented as: Cu> Cd> Pb in pond water and open water *O. niloticus,* Cu> Cd> Pb in pond water and open water *H. fossilis* and Cu> Cd> Pb in pond water and open water *P. sutchi*.

The concentration of Cd was in range of 0.01-2.10 µg g-1 dry wt. The average Cd concentration was 0.47±0.07 µg g-1 dry wt. The concentration of heavy metals is species specific and metal specific significantly varied (Mukherjee and Kumar, 2011). The result of the present study was between the range of Mukherjee and Kumar, 2011. The acute toxicity of Cd in freshwater teleost, *C. punctatus* (Bloch) studied by Tiwari et al.*,* 2011.

The concentration of Cd varied between 0.13– ND (not detected) mg/kg (Islam et al.*,* 2010). The average status of Cd in *Myllus spp.*, *M. merlucius*, *D. labrax* and *S. aurata* in small sized fish (mean weight of 158 g) was 0.20 mg/kg wet weight whereas in medium sized fish (mean weight of 245 g) leves was 0.40 mg/kg wet weight (Ozuni et al.*,* 2010). These support the results of the present study. The results of the present study are more or less similar. In the present study Cd concentration is lower in *O. niloticus*, *H. fossilis* and *P. sutchi* than Cu accumulation.

Copper (Cu) concentration

Open water *O. niloticus* contains higher Cu in the muscle than pond water *O. niloticus*. The average Cu concentration of *O. niloticus* collected from pond water and open water was 0.25±0.02µg/g and 0.28±0.01µg/g respectively. Pond water *H. fossilis* contains lower concentration of Cu in the body than open water *H. fossilis*. Average Cu concentration of *H. fossilis* collected from pond water and open water was 0.29±0.03µg/g and 0.59±0.04µg/g respectively. Pond water *P. sutchi* contains lower concentration of Cu in the body than open water *P. sutchi.* Average Cu concentration of *P. sutchi* collected from pond water and open water was 0.21±0.02µg/g and 0.35±0.01µg/g respectively. The results are compared in the Table 4 and Figure 2.

The Cu accumulation is higher than Cd concentration in the fish living in polluted water in their tissues (Jezierska and Witeska, 2006) which is similar to the present study. The order of heavy metal concentration in *M. cephalus* was Zn>Pb>Mn>Cu>Cr>Hg and average concentrations in liver and muscle (mg/kg) was 32.4, 10.8, 8.9, 6.4, 2.3 and 2.2 respectively (Krishna et al., 2014).

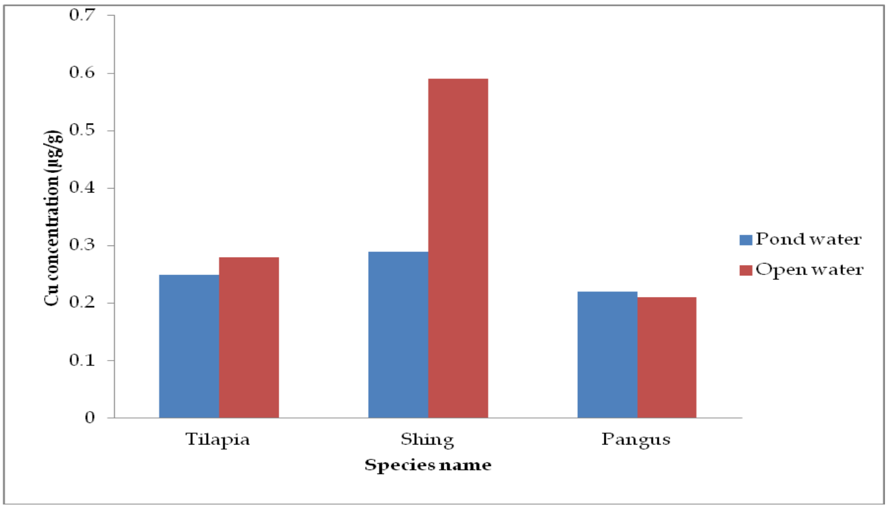

Figure 2. Copper (Cu) concentrations of *O. niloticus, H. fossilis and P. sutchi*

The order of accumulation of heavy metals in *H. fossilis* was liver > kidney > gills > integument > muscle. Accumulation of Fe (140.2 to 1533.08 mg kg-1.dw) was highest in all the organs. The average accumulation of Cu in the muscle and integument was 13.65 mg kg-1 dry weight. Cu concentration was highest (236.66 mg kg-1) in kidney and least (13.25 mg kg-1) in muscle and the sequence of their presence in organs/tissues were kidney > gills > liver > integument > muscle (Javed and Osmani, 2014). This is higher than Cu concentration of *A. mola* and *C. punctatus* found in the present study. In a heavily polluted river of Bangladesh, Buriganga River, highest Cu was found in *C. punctatus* (5.27 mg/kg) and lowest in *G. chapra* (4.25 mg/kg) in pre-monsoon fish samples (Ahmed et al., 2010).

The Cu levels in liver, gills and muscles of tilapia fish were 491.30, 3.70 and 1.82 µg/g dry weight (dw), respectively and significant changes occurred in Cu levels in tilapia fish organs (Taweel et al., 2013). The results are higher than the present study. The Cu concentration in *M. armatus* was 0.86 mg kg-1 (Javed and Osmani, 2012). This result is also higher than the results of the present study.

The concentrations of Cu in fish muscle collected from North East coast of India were 0.5-28.2 µg/g dry weight (Kumar et al., 2012). Concentrations of Fe, Mn, Cu, Zn, Ca, Cd, and Pb were measured in fish samples by (Anim et al., 2011). The Cu concentration is higher than Cd concentration also found (Kumar et al., 2011). In the present study Cu concentration is higher in *O. niloticus*, *H. fossilis* and *P. sutchi* than Cd concentration.

Lead (Pb) concentration

The average Pb concentration of *A. mola* collected from pond water and open water was 0.14±0.03 µg/g and 0.23±0.05 µg/g, respectively. Average As concentration of *C. punctatus* collected from pond water and open water was Nil. Pond water *A. mola* contains lower As than open water *A. mola*. Comparison of As concentration of *A. mola* is shown in Table 4 and Figure 3.

Lead is a devastating environmental pollutant that causes severe ground water pollution in Bangladesh. Fish is a major source of arsenic exposure. Organic arsenic compounds (such as arsenobetaine) are primarily found in fish by Jarup (2003). Concentration of As is lower in freshwater fish species and below the regulatory maximum level (Olmedo et al., 2013). The concentrations of As in fish muscle tissue collected from North East coast of India and the concentrations range were 0.02-2.37 µg/g dry weight (Kumar et al., 2012).

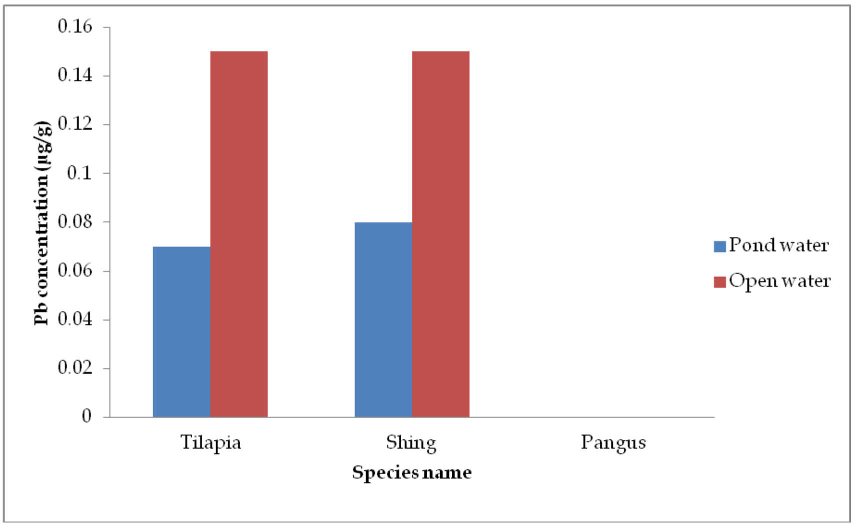

Figure 3. Lead (Pb) concentration of *O. niloticus, H. fossilis* and *P. sutchi*

WHO (µg/g) suggests the maximum limits Mn, Cu, Zn, Pb and Cd for fish are 1µg/g, 30µg/g, 100µg/g, 2µg/g, 1µg/g and FAO suggests the maximum limits Cu, Zn, Cd for fish are 10µg/g, 100µg/g and 0.2µg/g (Adedeji et al., 2011).

Considering human health concern it can be finally recommended that the mean concentrations of arsenic, cadmium and chromium accumulated in fish tissues was less than that for human acceptable range compare with drinking water. The recommended value arsenic, cadmium and chromium are 0.050 ppm, 0.1 ppm and 0.1 ppm respectively (WHO, 2001). Hadson, P.V et al., 1998, reported that the trace elements can be accumulated by fish, both through the food chain and water. Fish living in the polluted water may accumulated toxic trace metals via their food chains. Tarrio, et al., 1991, observed that the high levels of arsenic, lead, copper and iron have been to cause rapid physiological changes in fish. Fazal, et al., 2001, reported that the arsenic is one of the toxic environmental pollutants which has recently attract attention because of its chronic and epidemic effects on human health trough widespread water and crop contamination due to the natural release of this toxic elements from aquifer rocks in Bangladesh..

Fish living in polluted waters tend to accumulate heavy metals in their tissues and accumulation depends on metal concentration, time of exposure, way of metal uptake, environmental conditions (water temperature, pH, hardness, salinity), and intrinsic factors (fish age, feeding habits). Various metals show different affinity to fish tissues. Most of the metals accumulate mainly in liver, kidney and gills. Metal distribution in various organs is time-related. Accumulation of metals in various organs of fish may cause structural lesions and functional disturbances (Jezierska and Witeska, 2006).

CONCLUSIONS

Heavy metal concentrations of pond and open water harvested *O. niloticus, H. fossilis* and *P. sutchi* were within the acceptable level for human consumption. Open water fishes accumulate more heavy metals in the muscle than pond water fishes. Because, it may be due to aquatic pollution by industrial effluent or municipal waste discharge in the open water bodies. WHO suggests the maximum limits of Mn, Cu, Zn, Pb and Cd for fish are 1µg/g, 30µg/g, 100µg/g, 2µg/g, 1µg/g and FAO suggests the maximum limits Cu, Zn, Cd for fish are 10µg/g, 100µg/g and 0.2µg/g (Adedeji et al., 2011). Considering human health concern it can be finally recommended that the mean concentrations of arsenic, cadmium and chromium accumulated in fish tissues was less than that for human acceptable range compare with drinking water. The recommended value arsenic, cadmium and chromium are 0.050 ppm, 0.1 ppm and 0.1 ppm respectively (WHO, 2001).

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