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Bioaccumulation of zinc in *Rana tigrina* in different aquatic habitats

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Some creatures are very sensitive to water borne pollutants and can be used as useful bio-indicators of water pollutions. The present study used these bio-indicators to determine the status regarding health of freshwater habitats. The study was carried out to quantify Zn in kidney and liver of *Rana tigrina* from different aquatic habitats (canal, fish pond and sewage water) by atomic absorption spectrometry (AAS). Liver and kidney tissues of *R. tigrina* from sewage habitat showed elevated levels of Zn compared to those collected from canal and fish pond water habitats. Hence, it may be concluded that *R. tigrina* is one of the good bio-indicator species for heavy metals. It can be used to assess the heavy metal load and pollution of aquatic ecosystems.

Key words: Zinc, *Rana tigrina*, atomic absorption spectrometry (AAS), aquatic habitat.

INTRODUCTION

Environmental scientists widely use the term "heavy metals”. Heavy metals have specific gravities range of 4.5 to 22.5. The term heavy metal is used for elements which are toxic. At the same time, many authors (Phipps, 1981; Duffus, 2002) have critically called the term heavy metals as meaningless and objectionable. Heavy metals are natural constituents of freshwater and marine system and are found in very trace amounts. Human activities are responsible for the increment of their amount in the environment and consequently metals pollution is increasing in water resources (Chiarelli et al., 2016). Essential and beneficial metals have cut off values; low and high concentration beyond the cut off values will also be dangerous for life. Some elements have essential role in human being. Physiology of the target species and geochemical behavior are affected due to heavy metals toxicity. The following factors are considered the most important; chemical speciation of metals in aquatic environment, presence of other metals or toxicants, environmental conditions, condition of the organisms tested, adaptation of the organism to the absorption of metals. Bioassays of sediments involving benthic organisms and barytes were carried out at Aberdeen University. Results showed the bioaccumulation of Ba, Zn, Pb and Mn from barytes spiked marine sediments (Ansari et al., 2004).

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Lead is associated with damages to the nervous system (Finkelstein et al., 1998). Hazardous effects of mercury to human include damage to brain and ultimately mercury causes damages to central nervous system (Yoshino et al., 1966). High dose of chromium causes liver and kidney damages, and the circulatory system is also affected (Krishna et al., 2004). The most dangerous effect of selenium exposure to human is breakdown of human circulatory tissues and long term exposure of selenium is believed to damage nervous system (Boadi et al., 1991). Sources of Zn consist of galvanized metal for example wire cages and wire mesh staples. Other sources include cosmetics, skin lotion, shampoos, fertilizers, paints, fungicide and industrial chemicals. In most species, Zinc poisoning is regarded as being rare, but it is the second most common type of heavy metal poisoning in birds (Beyer et al., 2004). Zinc is necessary for standard growth, development and normal functions for all animal species. Death, tumor growth and some chromosomal aberrations is caused by Zinc deficit (Hrabeta et al., 2016; Krebs et al., 2014). It must be mentioned here that lead, cadmium, chromium, mercury etc. are highly toxic heavy metals and do not have any safe limits (Saeed et al., 2005); they have accumulation effects when ingested through food or water and cause various health problems like anemia, kidney diseases, nervous disorder (Gilani et al., 2015), high blood pressure, etc.

Frog belongs to carnivorous group of amphibians. 88% of amphibian species belong to the order *Anura*. These species are around 6645 in number belonging to 33 families. According to an estimate, one third species of frogs have become extinct since the 1980s (Stuart et al., 2004). They are main components of aquatic and terrestrial ecosystems. So they have an important link between human and ecosystem health. Mostly adult amphibians and toads feed on invertebrates and have an energy-efficient trophic link between insects and other vertebrates. So, they are linkers between terrestrial and aquatic habitats and are sensitive to environmental changes both because they have highly semi-permeable skins and different life cycle stages. A little study and information is available on the effects of environmental contamination on frogs and toads.

Kimball (2010) performed a study on the trace element concentrations (P, Ca, Mn, S, Na, Mg, Ba and Zn) in *Rana kl. esculenta* bones and on phalanges. So, concerned studies in this regard will be the best sources to check the pollutants, especially metals but limited studies have been done in this regard. Especially, in developing countries like Pakistan, there is need of time to check these pollutants by using different bio indicators and micro analytical methods like atomic absorption spectrometry etc. Welz and Sperling (1998) analyzed the whole body of *Rana catesbeiana* tadpoles and found the highest concentration of different heavy metals (cadmium, chrome, manganese, arsenic, mercury).

Grillitsch and Chovanec (1995) performed a study on heavy metals in tadpoles (*Rana dalmatina*, *Bufo bufo* and *Rana ridibunda*). Similar study was done by Puky and Oertel (1997) in which they evaluated metals concentration variation depending upon different development stages. These authors found the higher heavy metal concentrations in adults, which may be caused by changes in feeding during development, since they are carnivorous and the detritivorous diet may be richer in metals.

Pavel and Kucera (1986) analysed the accumulation of copper, manganese, zinc and iron in the whole body of *R. esculenta* adults. Zhang et al. (2007) performed a study that the increasing of Cd and Pb concentrations increase the ATP activity in *Bufo raddei* tadpoles. Stolyar et al. (2008) performed a study on heavy metals in the liver of the *R. ridibunda* from a river in Western Ukraine. They have reported concentrations of the metals in the order: Fe>Cu and approximately Zn>Mn>Cd. Simon et al. (2010) analyzed the trace element concentrations (P, Ca, Mn, S, Na, Mg, Ba and Zn) in *R. esculenta* bones and on phalanges. Tao et al. (2012) found that Zn and Cu are the most likely to be accumulated in all aquatic organisms of different tropic levels. Like all these studies, the present study is also a step towards the assessment of extent of metal toxicity in various water habitats.

Atomic absorption spectrometry has wide range application and reliable analytical technique for metal analysis (Welz and Sperling, 2008). For detecting metals and metalloids in ecological samples, flame atomic absorption spectrometry (FAAS) is a very familiar system. It is very consistent and simple to utilize. The aim of study was to evaluate Zn metal concentration in tissues (kidney and liver) of *Rana tigrina* from different habitats (canal water, fish pond and sewage water) and to assess the extent of heavy metal pollution in these habitats using *R. tigrina* as bio-indicator, as limited reports exist from Pakistan point of view.

MATERIALS AND METHODS

Equipment and glassware

The following equipment and glassware were used during this research: Electrical balance (AW220, Shimadzu, Japan), atomic absorption spectrophotometer (A-1800, Hitachi, Japan), heating oven (Gallen Kamp, England), reflux condenser (Quick Fit), personal computer and calibrated pyrex glassware.

Chemicals

Deionized water and analytical grade chemicals were used. Others included the nitric acid (HNO_3), hydrochloric acid (HCl), zinc granules (Zn), Copper metal (Cu) and manganese chloride ($MnCl_2 \cdot 2H_2O$). All these chemicals were of Merck analytical grade.

Sample collection

A total of 75 *R. tigrina* were captured from three different aquatic

Table 1. Statistical data for different parameters of *Rana tigrina* taken from different habitat.

S/N	Location	Parameter	Range	Mean	STDEV
1	Canal water	Total body weight (g)	54.26-98.49	71.266	10.6871
2	Fish pond water	Total body weight (g)	57.20-67.30	60.800	2.6042
3	Sewage water	Total body weight (g)	69.30-89.50	78.556	6.1792
4	Canal water	Kidney weight (g)	0.1324-0.1931	0.1638	0.02080
5	Fish pond water	Kidney weight (g)	0.1158-0.1987	0.1596	0.02580
6	Sewage water	Kidney weight (g)	0.1104-0.1961	0.1614	0.02600
7	Canal water	Zn Conc. (µg/g) in kidney	52.46-179.230	102.6983	3.20470
8	Fish pond water	Zn Conc. (µg/g) in kidney	0.1773-135.34	68.7054	3.25480
9	Sewage water	Zn Conc. (µg/g) in kidney	74.18-277.360	143.1836	4.31720
10	Canal water	Liver weight (g)	0.1246-0.1931	0.1638	0.02080
11	Fish pond water	Liver weight (g)	0.1158-0.1987	0.1594	0.02576
12	Sewage water	Liver weight (g)	0.1291-0.1961	0.1614	0.02600
13	Canal water	Zn Conc. (µg/g) in liver	98.75-232.16	176.2632	3.69134
14	Fish pond water	Zn Conc. (µg/g) in liver	109.64-228.28	164.7224	3.42362
15	Sewage water	Zn Conc. (µg/g) in liver	132.54-231.71	171.2524	3.14737

habitats such as Canal Water, sewage and fish pond during August to September 2012. Among them, 25 frogs were taken from each location. *R. tigrina* from Canal Water were captured from Nawab Pur Road Canal Water, Multan City, Pakistan. Five *R. tigrina* were captured from each of five various sites at equal distance of this Canal Water. *R. tigrina* from sewage water source were captured from sewage water from residence colony of Bahauddin Zakariya University (B.Z.U.), Multan, Pakistan while, *R. tigrina* from fish pond water were captured from a fish pond located in area of Matti Tal Road, Multan City, Punjab, Pakistan. Each was weighed and preserved till sample preparation.

Sample preparation

R. tigrina were dissected and four types of tissues were taken from each: skin, pectoral muscle, liver and kidney. Then weighing, length measurement, preservation in 70% alcoholic solution and storage of each part at -20°C was done. The solution of liver and kidney of each *R. tigrina* was prepared in aqua regia. 0.1 to 0.2 g of each sample was added to 3 ml of aqua regia and then refluxed for 30 min at 150°C. The solution was cooled to 25ºC and diluted with 10 ml of deionized water. The medium was filtered by using Whatman No. 42. After filtration, sample was diluted with 25 ml of deionized water and samples solutions were stored at room temperature.

Standard preparation

Zinc standards were prepared as follows: 1000 ppm stock solution of zinc was prepared by dissolving 0.1000 g of Zn granules in nitric acid and final volume was made up to 100 ml .The different concentration of zinc (100, 10, 2, 1.5, 1, 0.5 and 0.3 ppm) were prepared from stock solution by using dilution formula: $N_1V_1=N_2V_2$.

Zinc analysis

Atomic Absorption Spectrometer (A-1800, Hitachi, Japan) was used to determine metal levels. An instrument was warmed up for about half an hour. A blank was run for each metal to correct measurements. The standards of element of interest were run to check out performance of instrument. Finally, sample solution was aspirated to measure its absorbance. For sets of every ten samples, a sample blank was run to check interference and cross contamination. The concentrations of Zn were calculated using calibration curves in spreadsheets. The following optimized instrumental conditions for an atomic absorption spectrometer for zinc analysis were used: lamp current (mA) 10, slit (nm) 1.3, burner height (mm) 7.5, fuel pressure (Kg/cm^3) 0.20, detection limit (mg/L) 0.01, wavelength (nm) 213.8, calibration range (mg/L) 0.3-3.0 and flame composition C_2H_2/Air.

Data analysis

The samples were analyzed in replicates and statistical data obtained using standard statistical methods. The calibration curves were constructed by plotting absorbance (along ordinate) and concentration (along abscissa) of Zn standards. The linear regression method was used to determine slope and intercept for the Zn calibration data. The values of slope and intercept were used to calculate the concentration of Zn in *R. tigrina* samples. Then, Zn concentrations in mg/l, mg/25 ml, µg/25 ml and µg/g of *R. tigrina* sample from canal water, fish pond and sewage water were calculated.

RESULTS AND DISCUSSION

In this study, Zn analysis was carried out to determine Zn concentrations in different tissues (liver and kidney) of *R. tigrina* from different habitats (canal water, fish pond and sewage water). Zinc concentrations in all Zn standards and *R. tigrina* samples were determined in duplicate. Zn concentrations are summarized in Table 1. It clearly demonstrates that liver tissues of *R. tigrina* taken from all the three habitats (canal water, fish pond and sewage water) contain higher levels of Zn than kidney samples (Table 1).

The range of total body weight of *R. tigrina* found in canal water, fish pond water and sewage water were

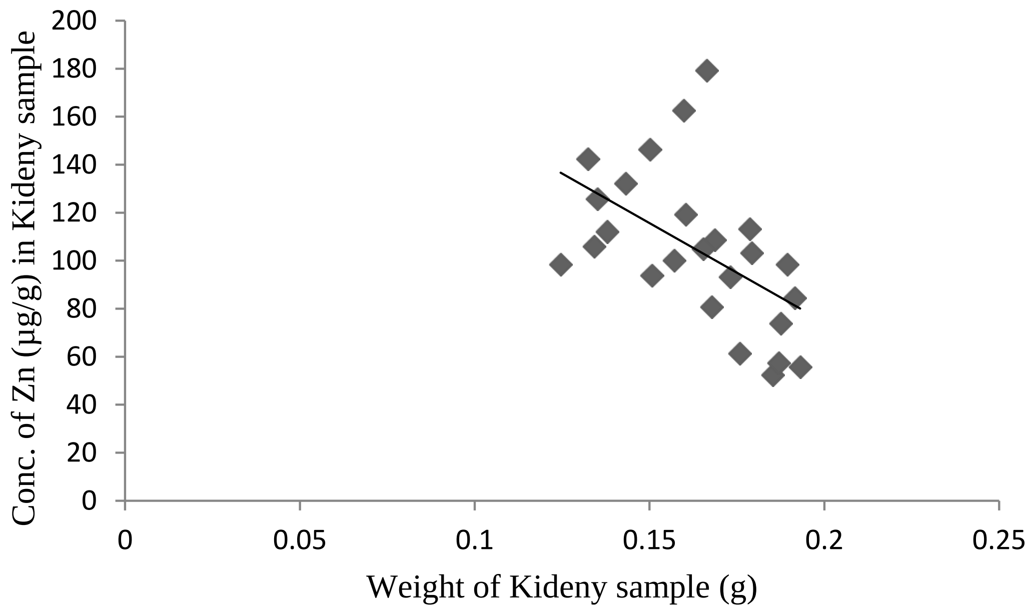

Figure 1. Effect of kidney weight (g) of *Rana tigrina* on the concentration of zinc found in canal water habitat.

recorded as 54.26-98.49, 57.20-67.30 and 69.30-89.5 g, respectively. In case of total body weight, the *R. tigrina* found in canal water has greater standard deviation than *R. tigrina* found in fish pond water and sewage water. The range of total kidney weight of *R. tigrina* found in canal water, fish pond water and sewage water were 0.1324-0.1931, 0.1158-0.1987 and 0.1104-0.1961 g, respectively (Table 1). In case of total kidney weight, the *R. tigrina* found in sewage water has greater standard deviation than *R. tigrina* found in canal water and fish pond water as shown in (Table 1). The range of total weight of liver of *R. tigrina* found in canal water, fish pond water and sewage water were 0.1246-0.1931, 0.1158-0.1987 and 0.1291-0.1961 g, respectively. The *R. tigrina* found in canal water has a bit greater liver average weight of 0.1638 g than that of *R. tigrina* found in fish pond and sewage water.

The range of zinc concentration in kidney of *R. tigrina* found in canal water, fish pond water and sewage water were 52.46-179.230, 0.1773-135.34 and 74.18-277.360 µg/g, respectively. The *R. tigrina* found in sewage water had greater zinc concentration in kidney than *R. tigrina* found in canal and fish pond water as shown in Table 1. It is clear from Figures 1 to 3 that as the weight of the kidney increased the accumulation of zinc decreased. It means that there is inverse relationship between the kidney weight and the zinc concentration. As the total body weight of *R. tigrina* increased the accumulation of zinc decreased in the kidney. It means that there is an inverse relationship between total body weight of *R. tigrina* and the zinc concentration in kidney. It may be concluded that the *R. tigrina* of low weight or small size may have gotten their food from the soil having high concentration of zinc. As the weight or size of the *R. tigrina* increased they got their food from the insects or from dissolved organic or inorganic matter in water.

The range of zinc concentration in liver of *R. tigrina* found in canal water, fish pond water and sewage water was 98.75-232.16, 109.64-228.28 and 132.54-231.71 µg/g, respectively. The *R. tigrina* found in canal water had greater zinc concentration in liver than *R. tigrina* found in fish pond and sewage water. As shown in Figures 4 to 6, as the weight of liver increased the zinc concentration accumulation in liver decreased. It means that there was inverse relationship between liver weight of *R. tigrina* and the zinc concentration. It may be concluded that the *R. tigrina* of low weight or small size may have gotten their food from the soil having high concentration of zinc. Similar trend of metals accumulation in fish was studied by Geffen et al. (1998). They found that tissue concentration of some metals declined exponentially with fish size. Qu et al. (2014) exposed freshwater fish to waterborne zinc under different pH levels and found increased hepatic Zn deposition.

The maximum Zn concentration in kidney of *R. tigrina* that lives in sewage water were found to be 143.1836 ± 4.31720 µg/g and in liver of *R. tigrina* found in canal water was 176.2632 ± 3.69134 µg/g. Shaapera et al. (2013) reported zinc concentration in the intestine of frog 1.81 ± 0.20 mg/kg, Eneji et al. (2011) reported 18.05 mg/kg of Zn in *T. zilli* and 17.76 mg/kg of Zn in *C. gariepinus* from River Benue. Tyokumbur and Okorie (2011) reported 29.84 ppm of Zn in *R. esculentus* and 32.97 ppm in Crabs from Alaro Stream Ecosystem,

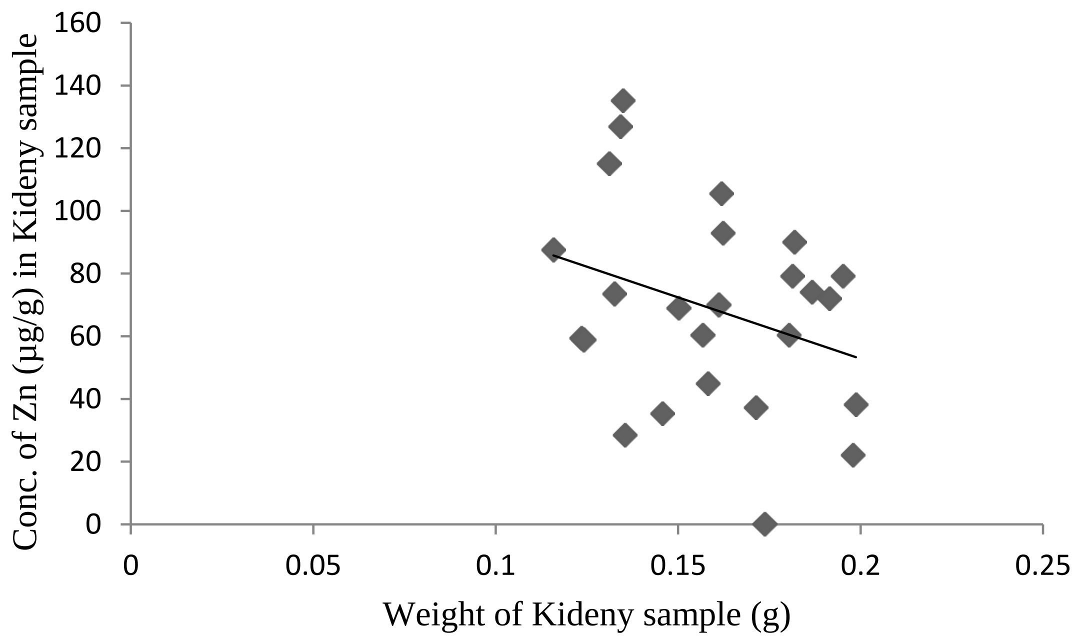

Figure 2. Effect of kidney weight (g) of *R. tigrina* on the concentration of zinc found in fish pond water habitat.

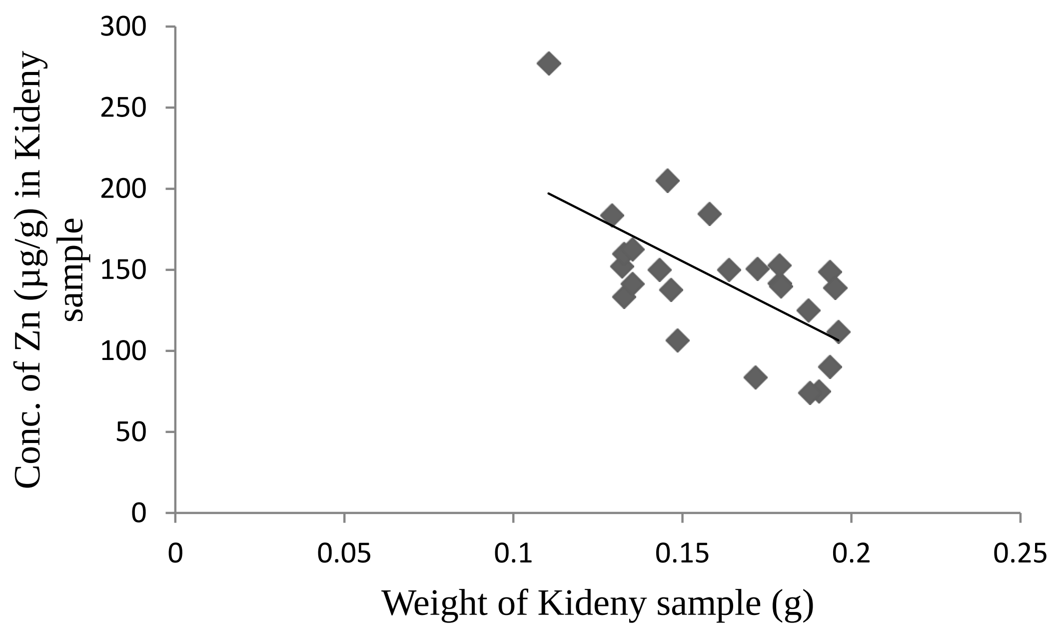

Figure 3. Effect of kidney weight (g) of *R. tigrina* on the concentration of zinc found in sewage water habitat.

Ibadan. Ololade et al. (2008) reported 0.61, 0.23 and 0.10 mg/kg of Zn in crab, fish and periwinkles, respectively. As compared to the reported literature, the present study reported higher concentration of Zinc in various parts of *R. tigrina.* This is because of more heavy metals pollution in various habitats of frogs in Pakistan.

Conclusion

Based on the present study, it can be concluded that liver tissues of *R. tigrina* taken from all the three habitats (canal water, fish pond and sewage water) contain higher level of Zn than kidney. As the weight of the kidney

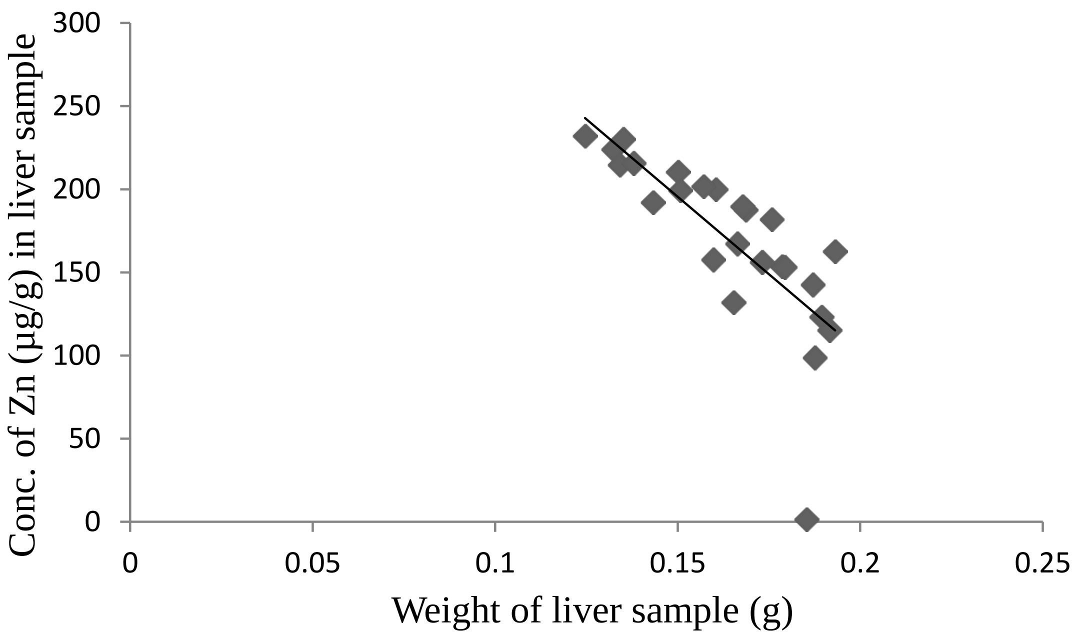

Figure 4. Effect of liver weight (g) of *R. tigrina* on the concentration of zinc found in canal water habitat.

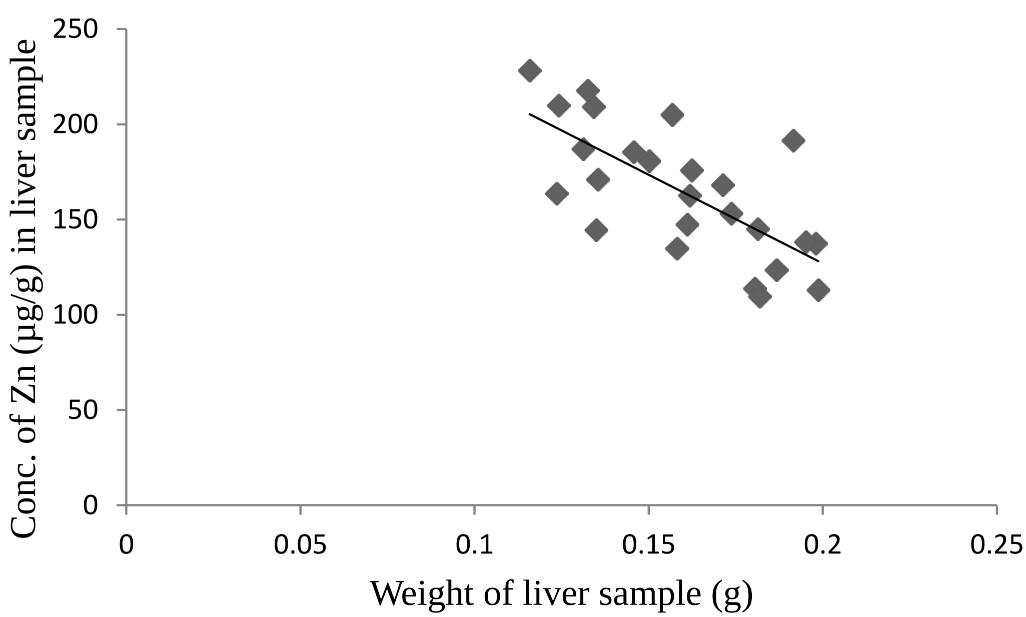

Figure 5. Effect of liver weight (g) of *R. tigrina* on the concentration of zinc found in fish pond water habitat.

increased the accumulation of zinc decreased in the kidney. As the total body weight of *R. tigrina* increased the accumulation of zinc decreased in the kidney. It can also be concluded that as the weight of liver increased the zinc concentration accumulation in liver decreased. As the total body weight or size of the *R. tigrina* increased the concentration of accumulation of zinc in liver increased. *R. tigrina* is a good biological indicator to measure the heavy metals in aquatic environment

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

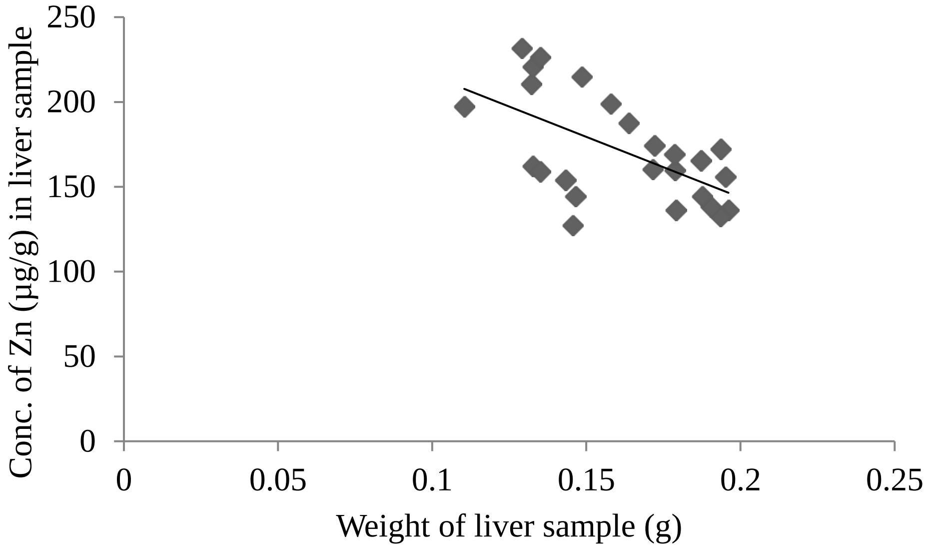

Figure 6. Effect of liver weight (g) of *R. tigrina* on the concentration of zinc found in sewage water habitat.

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