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Analytical quantification of copper in frogs (*Rana tigrina*) found from various aquatic habitats

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This study focused on the quantitative determination of Cu metal in various physical parts of frogs by using the Atomic Absorption Spectrometry technique). Cu was measured (2.72±0.77 µg/g) in kidney of *Rana tigrina* captured from industrial site and in *R. tigrina* captured from non-industrial site (28.10±27.02 µg/g). Similarly, 27.56±9.92 µg/g Cu was discovered in liver of *R. tigrina* captured from industrial site and 6.56±3.06 µg/g in *R. tigrina* captured from non-industrial site. From the results of the present study, it may be concluded that frogs are good bio-indicator species to estimate the level of heavy metals pollution of various water sources in some countries.

Key words: Copper, bio-indicator, atomic absorption spectrometry (AAS), aquatic habitat.

INTRODUCTION

Heavy metals are referred to as those elements which have relatively high density and are suspected to be toxic and very much toxic to the animals, humans, plants and aquatic lives at minute concentration levels (Singh, 2005). Amongst these heavy metals, approximately 30 elements are considered to be harmful to humans. Out of these elements, some are crucial for normal functioning of living cells, but they turn into lethal when taken above their recommended cut off limits. However, others can be xenobiotic, yet a bunch of them is very much toxic (Fraústo da Silva and Williams, 1993; Tamás and Martinoia, 2005). In addition, some of these metals are essential for some creatures and are not vital for others in which they may have toxic effects (borderline). Since the requirements of living organisms are concerned, metals and metalloids are distinguished into three classes: essentials, nonessentials and borderline class. Usually, heavy metals are considered non-biodegradable and are buffered to environmental effects for prolonged times and ultimately cause serious pollution tribulations. Some of these harmful elements get access to molecular targets by means of food chain and by respiratory system and subsequently may possibly accumulate in organisms and causing hazards in the long run (Scheifler et al., 2006).

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So, after bioaccumulation, heavy metals become perilous to organisms and their bioaccumulation depends upon their amount taken, their bioavailability, route adopted for their intake and their storage as well as excretion processes. Bioaccumulation of such elements in living organisms is faster than their catabolism at any time (Chapman et al., 1996). Potential toxicity in living organisms caused by exposure to high metal concentrations may follow various kinds of mechanisms. Proteins which have the ability to truss with metals play a critical role in this sense as they turn into a vehicle sources for the cellular metal toxicity (Thirumoorthy et al., 2011; Chiarelli and Roccheri, 2016). Both positive and negative effects on human health and environment are alleged from trace elements. Potentially toxic heavy metals may include Cd, Pb, Hg, Al, As, etc. and essential elements may include Fe, Mn, Cu, Zn and Se (Munoz-Olivas et al., 2001; Jalbani et al., 2007).

Potentially toxic elements are much dangerous when ingested even at very low concentration particularly because of prolonged exposure. The essential elements may also pose hazardous effects when taken beyond their recommended intake (Celik et al., 2007; Pouretedal and Rafat, 2007; Tao et al., 2012; Krebs et al., 2014; Hrabeta et al., 2016). Industrial effluents and agricultural drains are the main causes for the heavy metal contamination of sea water and direct disposal of sewage on waterfront enhances this contamination. Water treatment plants are rarely implanted to avoid this contamination. This heavy metal pollution badly affects the quality of sea water which makes it harmful for biota and human being via sea food chain. Quantitative determination of heavy metal contents in the entire body and tissue levels of marine organisms mostly concerns mollusks and crustaceans, but evidences have also been reported for coelenterates, polychaetes, and echinoderms. However, some aquatic organisms have ability to regulate internal metal levels. For example, mussels have this ability. Oysters have also been reported to have this ability. But instead of having similar feeding channels, former species have more effective abilities than later ones (Reidel et al., 1995; Tyokumbur and Okorie, 2011). In most mining areas, surface water and ground water are usually contaminated and polluted by heavy metals. Sources of the heavy metals in waters can either be natural (geogenic) or anthropogenic (Wong et al., 2003; Adaikpoh et al., 2005; Akoto et al., 2008). Mining and smelting plants are the main anthropogenic sources of heavy metal contaminations in any mining area. The heavy metal contaminations are important due to their potential toxicity for human being and the environments (Lee et al., 2007; Vinodhini and Narayanan, 2008; Nasir et al., 2017).

This research work was conducted to use frogs found from various aquatic habitats to access heavy metal contamination of various water sources. For this purpose, liver and kidney of frogs were evaluated, as these organs are suspected to bio-accumulate heavy metals in them. Hence, frogs were found to be good bio-indicators of heavy metal contamination of various water sources.

MATERIALS AND METHODS

Samples collection

Three aquatic habitats sewage, fish pond and canal water were selected to collect frogs. A total of 75 frogs were captured from these habitats during August to September 2012 at 40°C. All the sample collection areas were non-industrial. Twenty five frogs were captured from each site. Canal water samples of frogs were captured from Nawabpur canal situated in Multan city, Pakistan. Five equidistant sites of canal were used to get five frogs from each site. Sewage water samples of frogs were captured from the effluents of officer residence colony, Bahauddin Zakariya University, Multan, Pakistan. Similar pattern of almost equidistant five sites was taken in consideration and fish pond water samples of frogs were captured from five various fish ponds which were located in the area of Matti Tal Road, near Multan City, Pakistan (Figure 1). Five frogs were taken from each pond. All samples were weighed and preserved properly till sample preparations.

Samples preparation

Frogs were dissected and liver and kidney tissues were obtained from all the samples. After weighing, tissue samples were preserved in 70% ethyl alcohol solution and all these samples were stored at -20°C. The solution of tissue samples was prepared using aqua regia. Each tissue sample (0.1 to 0.2 g) was mixed to a volume of 3 ml of aqua regia and then this volume was subjected to reflux for half an hour at 150°C. Later on, this solution was cooled to room temperature and then 10 ml volume of deionized water was added to each sample. These solutions were further filtered by using Whatman No. 42 filter papers. All samples were further diluted after filtration by adding 25 ml of deionized water and these samples were stored at room temperature before analysis.

Standard preparation

A standard solution of copper (heavy metal) was prepared by using 0.1000 g of Cu wire in a final volume of 100 ml of nitric acid (corresponding to a concentration was 1000 ppm). Various working standard solutions of Cu were prepared by using dilution formula $N_1V_1=N_2V_2$. These working standard solutions were of the concentrations (100, 10, 0.3, 0.5, 1, 1.5, and 2 ppm) and these standards were properly tagged.

Copper analysis

An instrument of Atomic Absorption Spectrometer model A-1800 by Hitachi, Japan, was used for the quantitative analysis of Cu metal in various samples. Before starting the analysis, the instrument ofAtomic Absorption was warmed up for 30 min. To ensure the correct measurements, a blank was always run for each sample prior to its analysis. For the purpose of calibration and to evaluate the performance of the described instrument, working standard solutions were run on the instrument. Later on, the solutions of sample were aspirated to measure their absorbance. Blank runs were used to ensure cross contamination and interferences. The quantitative analysis of analyte was done using calibration curves and statistics were completed on spreadsheets. The optimum

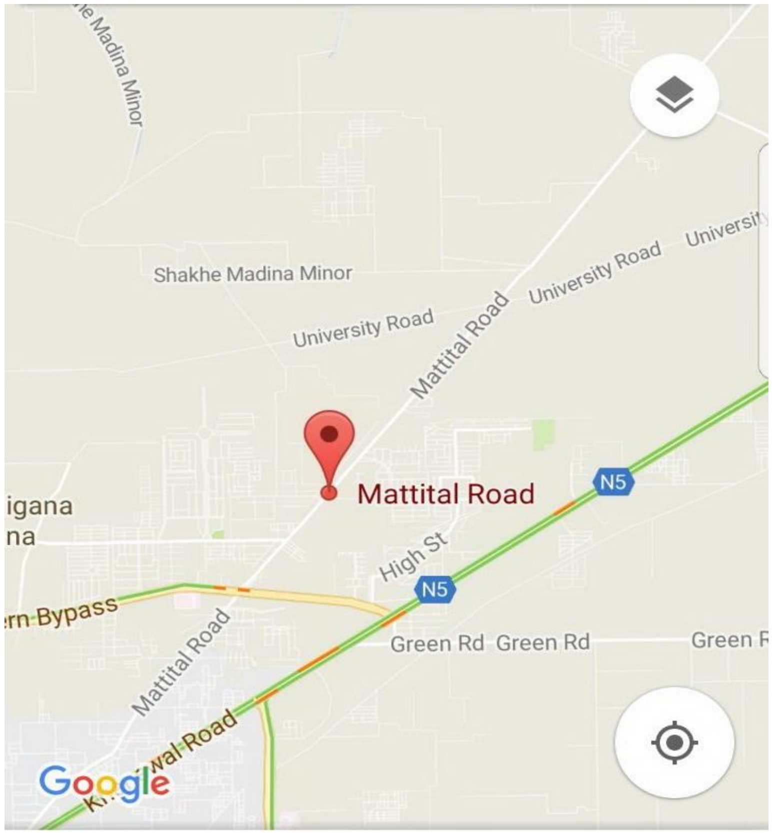

Figure 1. The study area (Mattital Road, Multan, Punjab, Pakistan).

conditions of the Atomic Absorption instrument were: lamp current 7.5 mA, slit width 1.3 nm, burner height was 7.5 mm, pressure of fuel was 0.30 kg/cm^3, λ_{max} found for Cu was 324.8 nm and limit of detection (LOD) was found to be 0.04 mg/L. Flame composition consisted of the mixture of C_2H_2 and air. Calibration range used was 0.3 to 5.0 mg/L.

RESULTS AND DISCUSSION

In the present study, various tissues, for example kidney and liver, of frogs were analyzed to quantify Cu concentrations in them, as the ultimate aim was to govern the heavy metal contamination level of various aquatic habitats. All frog tissue samples and standards were analyzed in triplicate. Cu concentrations in the samples are summarized in Table 1.

The results of Cu metal concentrations in liver tissues of frogs collected from the canal water, fish pond and sewage waters compared with the results of Cu metal concentrations in kidney tissues of frogs collected from the same habitats have been summarized in Table 1. The accumulation of Cu in liver tissues of frogs from different habitats is found to be greater as compared to kidney tissue. The range of total body weight of frogs found in canal water was recorded as 54.26 to 98.49 g and mean was 71.27±10.69 g as shown in Table 1 whereas range of total body weight of frogs found in fish pond water was recorded as 57.20 to 67.30 g and the mean was 60.80±02.60 g as described in Table 1. The range of total body weight of frogs found in sewage water was 69.30 to 89.50 g, and the mean was 78.56±06.20 g as described in Table 1.

The range of total kidney weight of frogs found in canal water was 0.13 to 0.19 g, mean is 0.16±0.02 g as described in Table 1. The range of total kidney weight of frogs found in fish pond water was 0.12 to 0.20 g and the mean was 0.16±0.03 g as described in Table 1.

The range of total kidney weight of frogs found in sewage water was 0.11 to 0.20 g, mean is 0.16±0.03 g as described in Table 1. The range of Cu concentration (µg/g) in kidney of frogs found in canal water was 0.23 to 2.71 µg/g, and mean was 1.28±0.60 µg/g as described in Table 1. The range of Cu concentration (µg/g) in kidney of frogs found in sewage water was 0.09 to 3.99 µg/g, and mean was 1.54±1.10 µg/g as described in Table 1. The frogs found in sewage water have greater Cu concentration in kidney than frogs found in canal water as described in Table 1. It is clear from Figures 2 and 3, that as the weight of the kidney increased the accumulation of Cu decreased. As the kidney and total body weight of frogs increased the accumulation of Cu decreased in the kidney. It means that there is an inverse relationship

Table 1. Statistical data for different parameters of frogs taken from different habitats.

S/N	Location	Parameter	Range	Mean
1	Canal water	Total body weight (g)	54.26-98.49	71.27±10.69
2	Fish pond water	Total body weight (g)	57.20-67.30	60.80±02.60
3	Sewage water	Total body weight (g)	69.30-89.50	78.56±06.20
4	Canal water	Kidney weight (g)	0.13-0.19	0.16±0.02
5	Fish pond water	Kidney weight (g)	0.12-0.20	0.16±0.03
6	Sewage water	Kidney weight (g)	0.11-0.20	0.16±0.03
7	Canal water	Cu conc. (µg/g) in kidney	0.23-2.71	1.28±0.60
8	Sewage water	Cu conc. (µg/g) in kidney	0.09-3.99	1.54±1.10
9	Canal water	Liver weight (g)	0.13-0.19	0.16±0.02
10	Fish pond water	Liver weight (g)	0.12-0.20	0.16±0.026
11	Sewage water	Liver weight (g)	0.13-0.20	0.16±0.03
12	Canal water	Cu conc. (µg/g) in liver	4.27-46.22	20.51±7.32
13	Fish pond water	Cu conc. (µg/g) in liver	3.38-20.05	11.77±4.99
14	Sewage water	Cu conc. (µg/g) in liver	3.22-28.68	19.00±5.30

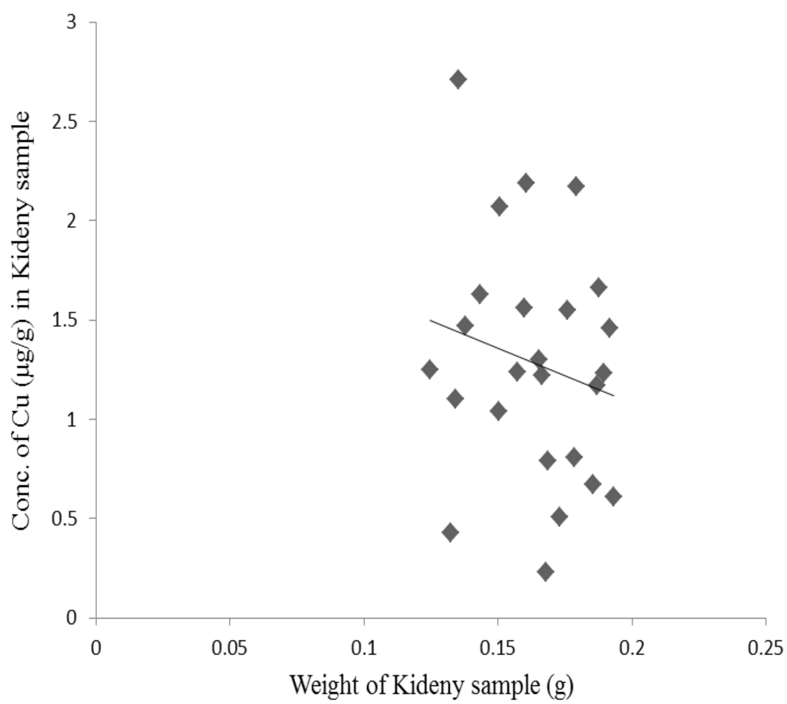

Figure 2. Effect of kidney weight (g) of frogs on the concentration of copper found in canal water habitat.

between kidney, total body weight of frogs and the Cu concentration in kidney. However, Cu concentrations are not quantifiable in kidney tissues of R. tigrina from fish pond water. It may be concluded that the frogs of low weight or small size may get their food from the soil having Cu. As the weight or size of the frogs increases they get their food from the insects or from dissolved organic or inorganic matter in water. It can be concluded from the study that the kidney is the main organ for heavy metals accumulation. R. tigrina shows considerable large amount of Cu. It was slightly high in the liver as compared to other tissues. Kidney is the main organ of heavy metal accumulation in fishes (Hogstrand and Haux, 1991), amphibians (Suzuki and Kawamura, 1984), and mammals (Torra et al., 1994). Metals can enter through the water-permeable skin and the gut and then,

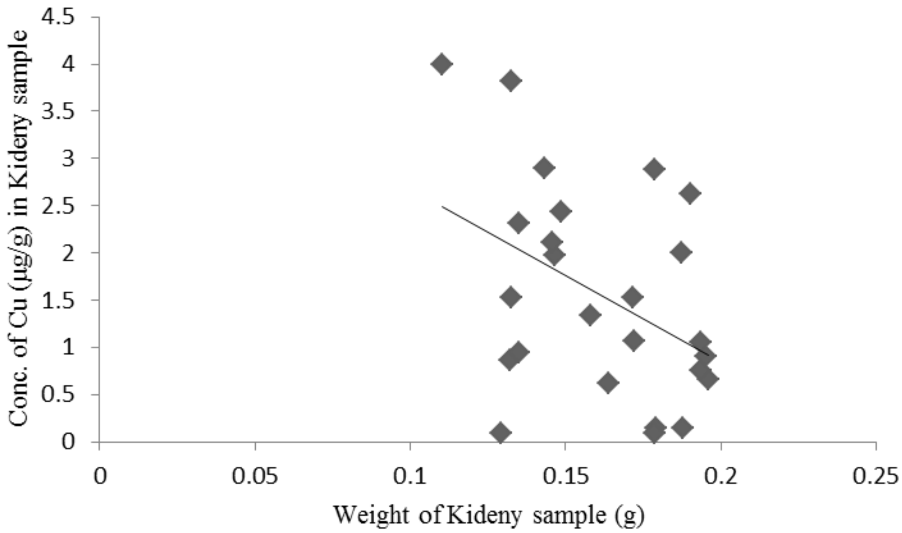

Figure 3. Effect of kidney weight (g) of frogs on the concentration of copper found in sewage water habitat.

via the blood circulation, accumulate in the liver and other tissues (Papadimitriou and Loumbourdis, 2003).

The weight of liver of frogs that live in canal water ranged from 0.13 to 0.19 g and the mean was 0.16±0.02 g as described in Table 1. The weight of liver of frogs that live in fish pond water ranged from 0.12 to 0.20 g; mean is 0.16±0.026 g as described in Table 1. The weight of liver of frogs that live in sewage water ranged from 0.13 to 0.20 g, and the mean was 0.16±0.03 g. The frogs found in canal water have greater average weight as 0.1638 g, than frogs found in fish pond and sewage water. The range of Cu concentration (µg/g) in liver of frogs that live in canal water was 4.27 to 46.22 µg/g, and the mean was 20.51±7.32 µg/g as described in Table 1. The range of Cu concentration (µg/g) in liver of frogs that live in fish pond water was 3.38 to 20.05 µg/g, and the mean was 11.77±4.99 µg/g as described in Table 1. The range of Cu concentration (µg/g) in liver of frogs that live in sewage water was 3.22 to 28.68 µg/g, and the mean was 19.00±5.30 µg/g as described in Table 1. The frogs found in canal water had greater Cu concentration in liver than frogs found in fish pond and sewage water. The frogs that lived in sewage water had Cu concentration in liver greater than frogs found in fish pond water. As shown in Figures 4, 5 and 6, as the weight of liver increased, the Cu concentration accumulation in liver decreased. It means that there was an inverse relationship between liver weight of frogs and the Cu concentration. It may be concluded that the frogs of low weight or small size may get their food from the soil having Cu. As the total body weight or size of the frogs increases the concentration of accumulation of Cu in liver increases. A comparative study of the results of bioaccumulation of Cu metal concentrations (µg/g) in liver and kidney tissues of frogs collected from the canal water, fish pond and sewage waters indicate that Cu levels in liver tissues are comparatively higher than in kidney tissues. However, Cu concentrations are not quantifiable in kidney tissues of frogs from fish pond water.

The liver is the chief organ for metal homeostasis, being the site of metalloenzyme production and metal storage as well as excretion via the bile duct. The liver also has the highest metal load compared with other tissues, and so it reveals the bioavailability of metal concentrations in the water bodies (Stolyar et al., 2008). Accumulation of heavy metals affects the metabolic activities resulting in a decrease in body length and body weight (Stolyar et al., 2008). Toxic concentrations of heavy metals increase the biochemical stress in the organisms due to deterioration of metabolic cascades (Hudecova and Ginter, 1992). Balance between production and catabolism of the oxidants is important to maintain biological functioning of organisms. Metabolism activities, however, varies with temperature changes. Frogs are poikilothermic vertebrates, and heat stress can affect their bodily metabolism. Relatively greater concentrations of Cu in the liver were recorded as compared to the kidney in *R. tigrina*. *R. tigrina* showed higher concentration of Cu in the liver. Similarly, Loumbourdis and Wray (1998) also reported greater concentration of Cu in the liver as compared to other organs and highlighted that frog accumulates higher Cu indicating its role in detoxification and storage. Measured higher concentration of Cu could be related to detoxification and storage mechanisms. Liver tissue accumulated higher mean concentrations of heavy metals in comparison to the other body tissues. The liver is a major detoxifying organ of the body, and besides this, it is the main center where hemoglobin breakdown

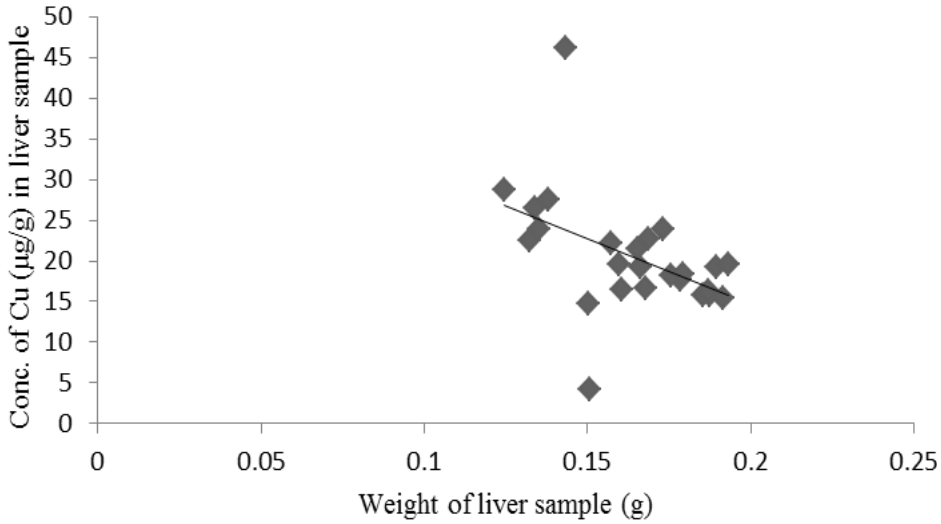

Figure 4. Effect of liver weight (g) of frogs on the concentration of copper found in canal water habitat.

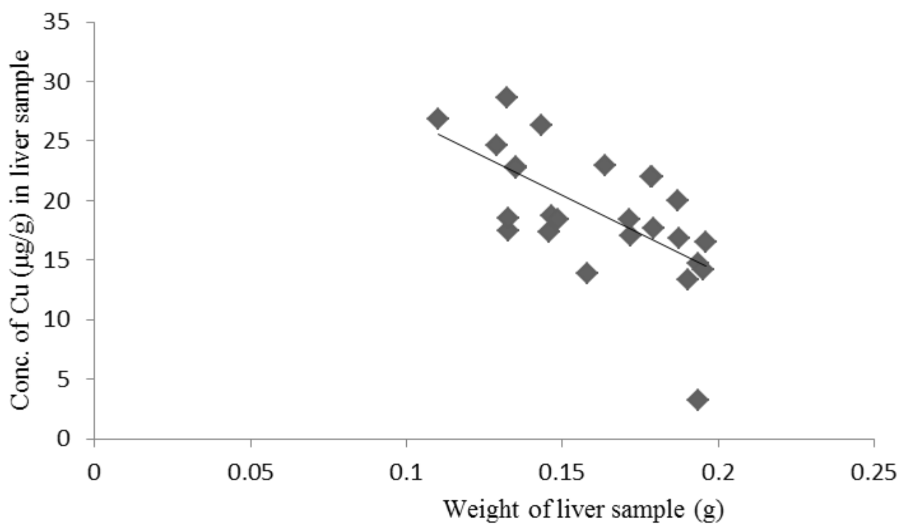

Figure 5. Effect of liver weight (g) of frogs on the concentration of copper found in fish pond water habitat.

occurs. Cu concentration also turned very high which is probably due to the presence of Cu in the compounds like fungicides and insecticides abundantly used in agriculture practices carried out in the study area. Higher values of Cu in the liver may be due to its detoxification by virtue of metallothionein and other metal-binding proteins (Lance et al., 1995). The uptake and removal patterns of metals depend on multiple factors that scale with body size including surface area to volume ratios. This further suggests *R. tigrina* to be a good bioindicator of water contaminants like the heavy metals (Qureshi et al., 2015).

Conclusion

The present study is relevant to human health because heavy metals are produced by different industries like tanneries for leather processing and added into water reservoirs as a waste. This water enters the food chain of human beings. The heavy metals cause physiological abnormalities. Outcomes of the present study showed that liver tissues of frogs taken from all the three habitats (canal water, fish pond and sewage water) contain higher level of Cu contents than kidney tissues. There is an inverse relation between kidney weight and Cu contents

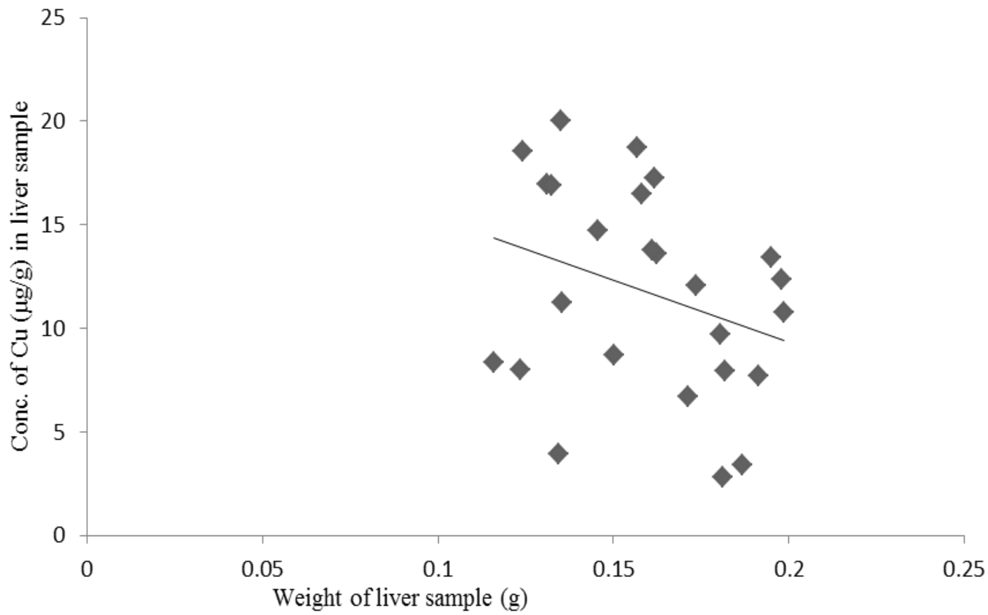

Figure 6. Effect of liver weight (g) of frogs on the concentration of copper found in sewage water habitat.

in it. As kidney weight increases the concentration of Cu decreases. Direct relation has been found between total body weight and Cu contents in kidney. As total body weight of frogs increased the accumulation of Cu increases in kidneys of frogs. Inverse relation has been found between liver weight and Cu contents. As the weight of liver increased, the Cu concentration in liver decreased. A direct relation exists between total body weight of frogs and Cu contents in liver, as the body weight increased the accumulation of Cu in liver increases. Frogs are good biological indicator to assess the heavy metals contamination in aquatic environments.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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