UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Vol. 6, Issue 2, Pp: (1-7), June, 2022
Email: editor .usjas@usindh.edu.pk
Website: http://usindh.edu.pk/index.php/USJAS
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro
(USJAS)

ESTIMATION OF MANGANESE CONCENTRATION IN *PUNTIUS TETRAZONA* (TIGER BARB) FROM MULTAN

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ARTICLE INFORMATION

Article History:
Received: 23rd April 2022
Accepted: 28th August 2022
Published online: 27th June 2022

Author's contribution
All authors contribute equally.

Key words:
Heavy metals, atomic absorption, Morphometry, *Puntius tetrazona,* Condition factor.

ABSTRACT

Heavy metals are the most significant contaminants in aquatic ecosystems. The majority of heavy metals tend to concentrate in tissues as well as other organs of the fish's body after drinking polluted water, resulting in fish poisoning. These metals can interfere with reproduction and growth, as well as damage the immune system and cause pathogenic repercussions. Water in Multan city may be polluted with several heavy metals. The usage of this water given to the aquarium may have an effect on the growth and weight of the Tiger barb (*Puntius tetrazona*). The effect of heavy metal (Manganese) on this fish has not been determined in the Multan city region. Fish samples were gathered from the Multan city region. Each sample of fish (*Puntius tetrazona*) had passed through Morphometric procedure then calculated the wet and dry weight of each sample. All samples were dried in microwave oven and were grinded to powder. After grinding, samples were subjected to ash formation by Muffle furnace. Then ash of each sample was dissolved in 1%HNO_3 to form solution. Then elemental analysis was determined by Atomic Absorption Spectrum. Presence of Manganese was confirmed and its effects were determined on *Puntius tetrazona* by different statistical techniques.

1. INTRODUCTION

From the past 45 years, aquaculture has been the fastest food production sector provides half of fish eaten by humans all around the world. Fish, crabs and mollusks are all used in aquaculture to provide food.

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The meat of fish is considered as useful source of protein for good human health. In survey of 2009, the meat of fish account for 16.6% of the population of world intake of protein of animals as well as 6.5% of all protein consumed. To meet the demand of protein from fish and other aquaculture for economic development, it is needed to enhance the factor contributing to aquaculture (Farkas, *et al.,* 2003).

The both developing and developed countries are facing problems related water pollution. Water pollution may affect the fish physiological function that may lead to high mortal rate and eventually decline of biota of fish (Wim, *et al.,* 2007). In Pakistan, industrial effluents, domestic wastes as well as runoffs of agriculture is directly released into canals, ponds, streams, rivers, lakes and as well as other water resources (Samantha *et al.,* 2005). Heavy metals are regarded as important in this respect because these are easily taken into the food chain as well as the process of bioaccumulation. Few heavy metals are important for normal physiological activities of fish but these become toxic when these metals accumulate in the body tissues of fish and are not able to metabolize (Fazio *et al.,* 2014).

Increased concentrations of heavy metals into the water bodies have serious consequences on aquatic biota and in order to conserve the native fauna it is essential to check their tolerance limits against metallic ion toxicity (Al-Akel and Shamsi, 1996). As (Arsenic), Hg (Mercury), Pb (Lead), particularly Mn (Manganese) are much harmful, poisonous, toxic and even in range of parts per billion (ppb) (Witeska and Kosciuk, 2003).

Manganese is heavy metal which is most widely distributed as well as abundant naturally. During the life span of organisms, the amount of manganese is required in trace amount. In the body of living organisms, it controls normal body function as well as development. Actually, it plays important role in different non-metabolic as well as metabolic regulatory functions (Santanaria, 2008). Manganese functions like a part of metallo-enzymes or like an activator of enzyme as Arginase, pyruvate carboxylase, cytosolic enzyme, the manganese superoxide dismutase (Crowley *et al.,* 2000). Manganese is the latest emerging pollutant of the environment which is caused by a wide variety of industrial sources like that of welding, mining, processing of goods and production of alloy, may be liberated in to the marine water which becomes an unseen toxic metal in the marine ecosystem. The rates of accumulation of manganese (Mn), and its liberation, are usually fast-regulating processes. The high levels of exposure of manganese prove toxic as well as decrease the fitness of the body living organisms (Wang *et al.,* 2013).

Different research work shows that heavy metals can change the biological parameters as well as physiological activities both in blood as well as tissue (Canli, 1995), (Basa *et al.,* 2003). Biological cycles become very slow by contamination of heavy metals. Some elements like as copper (Cu), zinc (Zinc), cadmium (Cd) as well as mercury (Hg) may considered very harmful in the aspect of Eco toxicological effects. Organisms of aquatic environment are capable of accumulating of heavy metals up to concentration which are 10 times to as well as even thousand times higher as the concentration in the environment. In many vital organs heavy metals are accumulated. The supporting structures as well as gills show mostly bioaccumulation waterborne heavy metals, as well as intestines and stomach accumulate elements which are food-associated. Often the highest concentration of heavy metals is found in kidney, gills as well as liver of fish (Golovanova, 2008).

Fish is major organism which is extensively use to determine the condition of health of aqueous environment because of pollutants accumulated in the food chain as well as is responsible for harmful effects and mortality in the aquatic ecosystem. Now a day, aquariums culture is a very beneficial as well as useful tool in certain treatment of psychological disorders both in orphan as well as old people and children (Petrescu-mag *et al.,* 2013). Trade of aquarium fish culture has also negative impacts on the biodiversity. The industry of aquarium culture became a beneficial enterprise for many companies from all over the World, mainly for Southeast Asian companies of aquaculture trading in aquarium farming (Naz *et al.,* 2012).

Tiger barb is very beautiful and small sized aquarium fish. A school of this species in a suitable sized aquarium makes more attractive and awesome display. In aquarium world, this species is most popular, favorite and standard. In past it has also been known as "Sumatrans**".** Tiger barb is easy to keep, due to its small sized body and well suited to aquarists for all level of experience. This fancy fish is quite hardy. So their water is kept clean and requires being change regularly. Due to its prime importance among aquarium fish, it is necessary to determine the factors which affect its growth**".** Naturally Tiger barb is found with dense vegetation and feed on insects, algae, detritus, diatoms and small invertebrates (Subathra *et al.,* 2007).

This research work was planned to evaluate the metal (Manganese) concentration in *Puntius tetrazona* collected from regions of Multan city, effects of heavy metal (Manganese) on morphometric parameters of Tiger barb (*Puntius tetrazona),* effect of Mn on wet and dry weight of *Puntius tetrazona* and bioaccumulation of heavy metal (Manganese) in *Puntius tetrazona.*

2. MATERIALS AND METHODS

An aquarium fish which is commonly called Tiger barb (*Puntius tetrazona*) is collected from region of Multan city. It consists of approximately 35-40 fishes in number. The fish samples are variable in body size and weight. The used chemicals were of highly purified form and were of analytical reagent grade. The chemical used were Chloroform, Methanol and 1% HNO_3. These were purchased from G.M. Scientific store Multan city.

All materials such as Magnifying lens, Vernier calipers, graduated scale, conical flasks, Beakers, Petri dish, Aluminum foil, China crucible, digital scale, microwave oven, Muffle furnace, agate pestle and electric mortar were of high quality.

Morphometry

Each sample of fish (*Puntius tetrazona*) is subjected to Morphometry (external morphology) very carefully for every parameter with the help of graduated scale and Vernier calipers as well as magnifying lens. The measurements of morphometric parameters are made with the help of measuring tape, Vernier calipers, measuring board as well as scales of the nearest 0.1 cm as total body length (TL), length of fork, length of head (HL), standard length (SL), width of head, girth of body, dorsal fin base, dorsal fin length, adipose fin length (2^{nd} dorsal fin length), base of adipose fin (2^{nd} dorsal fin base), length of pectoral fin, length of anal fin, pelvic fin length, base of anal fin, length of tail as well as width of tail. Weight of body of each fish sample is measured for the nearest .01g with digital electric balance. Before the weighing of fish its adhered water as well as the particles are removed from surface of the body of each fish.

The relationship of length-weight (RLW) is determined by the equation: $w= a L^b$, where W= weight (g), L= total body length (cm), b= growth exponent and a = constant. For the determination of linear relationship logarithmic transformation is used as log W=log a + log b L (LeCren, 1951).

Drying

After Morphometry and determination of wet body weight of each sample, the samples are wrapped in pre-weighted aluminum foil and are dried carefully in microwave oven for 8-10 days at $70C^o$ for complete drying. After complete drying all samples are weighted after each 24 hours during three days to obtain constant weight of the samples.

Grinding

After complete drying, each sample is unwrapped and weighted again on digital electric balance very carefully and then is grinded to powdered form in agate pestle and electric mortar until homogenous powder is formed for further analysis. This powdered sample is further used for ashing process and solution formation.

Sample Preparation

For ashing, each pre-weighted powdered sample is shifted to pre-weighted China crucible. China crucible having sample in powdered form is shifted to Muffle Furnace. Each sample is numbered and marked and kept in the furnace in a specific sequence to avoid any mistake of mixing the samples. The temperature of furnace is adjusted at 550 C^o for 24 hours duration. Then all the samples are cooled for approximately 24 hours of duration, all the samples are collected from the furnace carefully. Then each sample is weighted again and recorded [16].

Solution Formation

For the formation of sample solution, 1% HNO_3 solution is prepared for preservation. Ash of each sample obtained from Muffle Furnace is weighted again and measured quantity is dissolved for solution preparation in 25 ml of solution. After filtration of solution and sample mixture, each sample is poured in polyethylene bottle and is allocated proper number (Naeem *et al.*, 2012).

Elemental analysis

Samples are sent to laboratory of Pakistan council of research in water resources (PCRWR) Islamabad for elemental analysis. All samples are subjected to elemental analysis (detection of Manganese) for each sample by Atomic absorption spectrum of Atomic Absorption Spectrophotometer (Hitachi Polarized Zeman AAS, Z 8200, Japans). Then the presence and effect of these heavy metals on sample fish i.e. *Puntius tetrazona* (tiger barb) is determined.

Statistical Analysis

Statistical description of each character is estimated as Mean ± Standard Error. All the analysis by statistics includes analysis by regression as well as calculations for coefficients of correlation; estimation of the standard error is calculated by the use of Minitab as well as MS-Excel. Each condition factor of sample fish is evaluated by Fulton's Condition Factor.

Calculation of relations of variables. The equation for this relation is given as followed:

$$Y = ax + b$$

Y= elemental concentration (µg/g)

X = Length of fish (cm)

(a) And (b) are constants

3. RESULTS AND DISCUSSION

Elemental value of wet body weight Concentration of Manganese is in range of 3.21-143.88 and in mean of 35.67±34.90 which is also highly significant as described in figure 1 and table 1.

Table 1. Grand mean as well as standard error value of concentration of element in wet weight of body in *Puntius tetrazona* (whole sample), (n = 35).

Elements	Concentrations	
	Range	Mean±S.E
	μgg^{-1}	μgg^{-1}
Mn	13.13-190.69	72.86 ±44.22

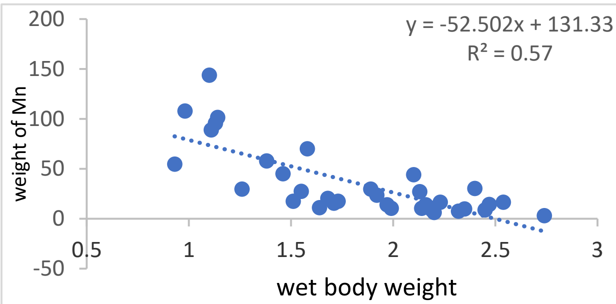

Figure 1. Plot showing weight of Manganese in wet body weight (µgg-1) for *Puntius tetrazona.*

Elemental value of dry body weight

The range value of Manganese is 13.13-196.69 as for as the mean value of Manganese is 72.86±44.22 which is also highly significant as described in Figure 4 and table 2.

Table 2. Grand mean as well as standard error value of concentration of element in dry body weight in *Puntius tetrazona* (whole Sample), (n = 35).

Elements	Concentrations	
	Range	Mean±S.E
	μgg^{-1}	μgg^{-1}
Mn	3.21 - 143.88	35.67±34.49

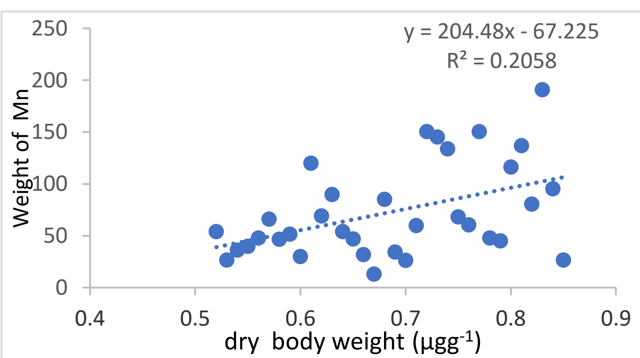

Figure 2. Plot showing concentration of Manganese in dry body weight (µgg-1) for *Puntius tetrazona*.

Parameters of Regression of weight of wet body versus body burden element

Concentration of Manganese is **0.753** which is also highly significant as describe in table 3. For Manganese t value is -52.192, standard error value is 7.910 as described in table 3.

Table 3. Parameters of Regression of wet body weight (g) versus elemental body burden (µg) in *Puntius tetrazona* (Tiger barb), (n = 35). [Elemental body burden (µg) = a + b weight (g) of wet body]

weight of Wet body (g)	**Elements**	**R**	**A**	**B**	**S.E (b)**	**t-value (b=1)**
	Mn	**0.753*****	130.22	- 52.066	7.91	- 52.192

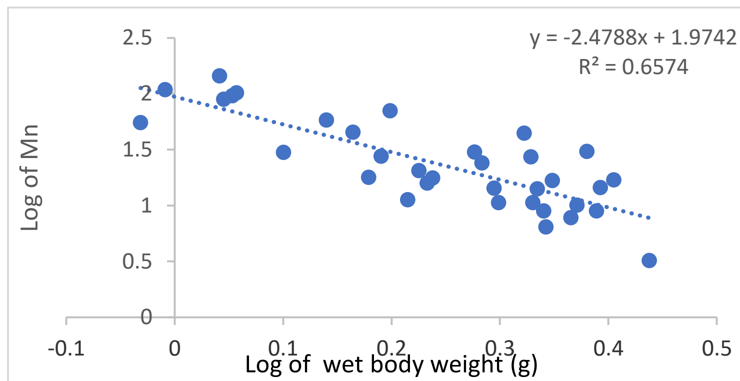

Figure 3. Plot showing log of wet body weight (g) versus elemental (Mn) body burden (µg) for Puntius tetrazona

Table 4. Parameters in Regression of log of weight (g) of wet body versus log of elemental body burden (µg) in *Puntius tetrazona* (n = 35) [Log of elemental body burden (µg) = a + b Log of weight (g) of wet body]

weight of Wet body (g)	**Elements**	**R**	**A**	**B**	**S.E (b)**	**t-value (b=1)**
	Mn	**0.697*****	294.79	-54.766	9.801	- 57.766

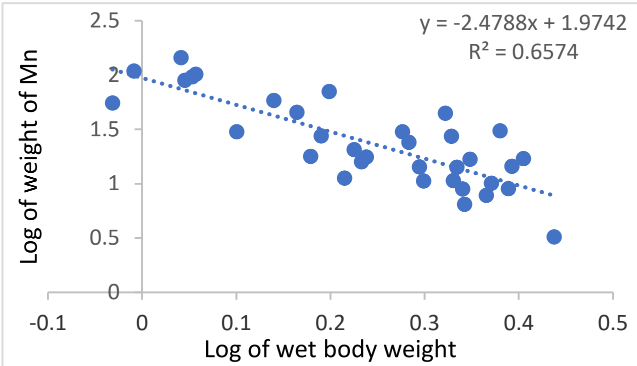

Figure 4. Plot showing log of weight of Manganese versus log of wet body weight for *Puntius tetrazona.*

Regression value of log of weight of wet body versus log of body burden element

The concentration of Manganese is also significant in value of 0.813 as shown in table 4. Manganese t value is -5.7044 and standard error (S.E) value is 0.311 as described in table 4.

Table 5. Parameters in Regression of log of weight (g) of wet body versus log of elemental body burden (µg) in *Puntius tetrazona* (n = 35) [Log of elemental body burden (µg) = a + b Log of weight (g) of wet body]

weight of Wet body (g)	Elements	R	A	B	S.E (b)	t-value (b=1)
	Mn	**0.722*****	6.100	-7.016	1.171	-10.2742

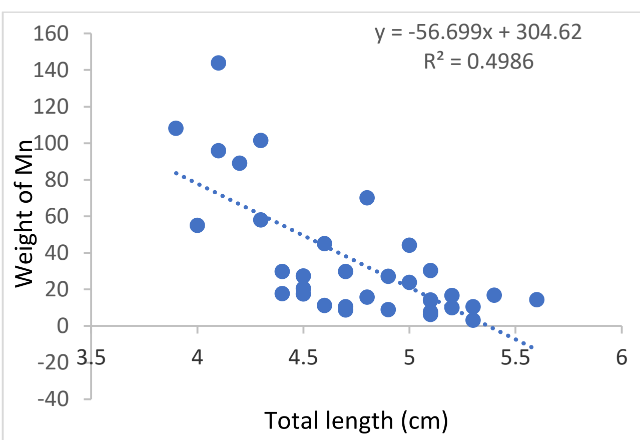

Figure 5. Regression of log of weight (g) of wet body versus log of elemental body burden

Log of Body Burden elements versus total length (cm)

The concentration of Manganese is highly significant which is in value of 0.697 as shown in table 5. For Manganese constant has value of 294.79, constant b is -54.766, standard error (S.E) is 9.801 and t value is -57.766 as described in table 5.

The range of Manganese is 0.722 Both these values are more significant as shown in table 6. For Manganese constant a has value of 6.100, constant b has -7.016, standard error (S.E) value is 1.171, t value is -10.2742 as described in table 6.

Table 6. Parameters of regression of log of weight (g) of wet body versus log of elemental body burden (µg) in *Puntius tetrazona* (n = 35) [Log of elemental body burden (µg) = a + b Log of total length (g)] [Elemental body burden (µg) = a + b condition factor].

weight of Wet body (g)	Elements	R	A	B	S.E (b)	t-value (b=1)
	Mn	**0.722*****	6.100	-7.016	1.171	-10.2742

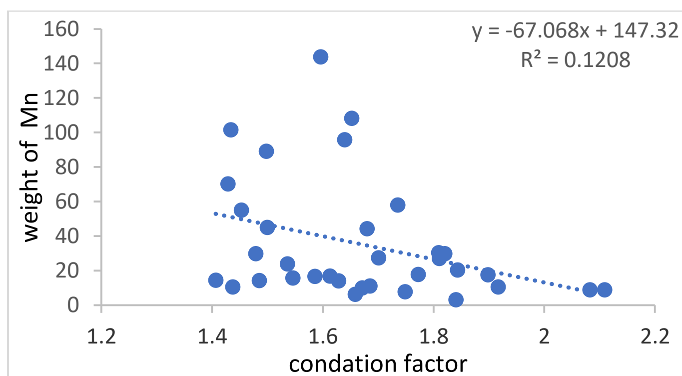

Figure 6. Plot showing log of Chromium versus log of length of body for Puntius

Table 7: Parameters in regression form of condition factor versus concentration of metal (wet weight, $\mu g g^{-1}$) in weight of wet body in *Puntius tetrazona*, (n = 35)

[Elemental body burden (µg) = a + b condition factor]

weight of Wet body (g)	Elements	R	A	B	S.E (b)	t-value (b=1)
	Mn	0.321^{ns}	134.93	59.18	30.38	1.948

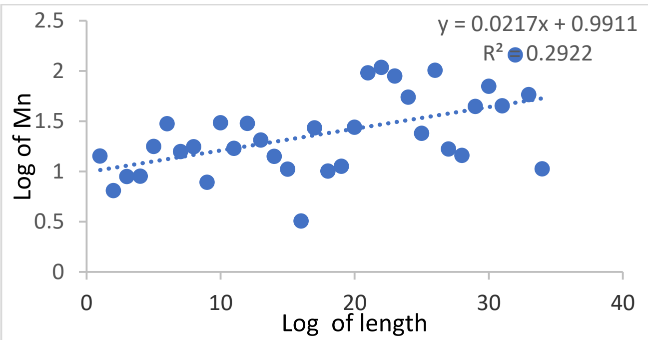

Figure 7. Plot showing log of Manganese versus log of length for Puntius tetrazon

Regression values of metal concentration versus condition factor.

The concentration of Manganese is non-significant in values of **0.321**, range of condition factor is 0.6 to1.02 as described in tables 7. For Manganese a =134.937, b = -59.184, standard error (S.E) = 30.380, t value = -1.94812 as described in table 7.

4. CONCLUSION

Overall, it could be conculcated that majority of heavy metals tend to concentrate in tissues as well as other organs of the fish's body after drinking polluted water, resulting in fish poisoning. These metals can interfere with reproduction and growth, as well as damage the immune system and cause pathogenic repercussions. Water in Multan city may be polluted with several heavy metals. The usage of this water given to the aquarium may have an effect on the growth and weight of the Tiger barb. Present study recommends that control planning should be adopted in order to save this creature.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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