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Elemental assessment of trace and toxic heavy metals in different parts of three economically important fish species captured from ponds of Kashmore, Sindh, Pakistan

Shahid Ali Jakhrani, Javed Ahmed Ujjan, Mushtaque Ali Jakhrani, Wahid Bux Jatoi, Farkhanda Zaman Dayo and Muhammad Qasim Mazari

Abstract
The aim of present study was to evaluate the biochemical composition and concentrations of heavy metals in *Catlacatla, Cirrhinus mrigala* and *Labeorohita*. Four different weights were captured from Chachar fish pond of Kashmore district. Preferred heavy elements like Iron, Zinc, Copper, Manganese, Nickel, Cadmium and Chromium was analyzed in gills, liver and muscle of economically important fish species found in ponds of Kashmore district. The fish samples were captured by using fish nets with help of professional fishermen of sampling area and were transported to laboratories of Shah Abdul Latif University Khairpur for further procedures like dissection, digestion and analysis. Elemental assessment was carried out by using inductively coupled Plasma Atomic Emission spectrophotometer from Institute of chemical sciences Bahuddin Zakrya University, Multan, Pakistan. Obtained results showed the order for concentration of metals were obtained high in *Catlacatla>Cirrhinus mrigala>Labeorohita* fish organs and maximum concentration of heavy metals was found in gills as compared to rest of organs of these species. Similarly the level of these heavy metals followed the order of concentration as Cu > Fe > Zn >Mn> Ni > Cd > Cr respectively. The concentration of Fe, Zn, Cu, Mn, Ni, Cd, Cr were ranges in *Catlacatla* as (2.4-1.1, 3.5-1.2, 18-9, 0.54-0.12, 0.98-0.18, 0.09-0.02, 0.06µg/g-0.01µg/g, *Cirrhinus mrigala* 2.4-1.2, 3.4-0.4, 17.5-11.3, 0.68-0.15, 0.95-0.47, 0.054-0.011, 0.067µg/g-0.012µg/g and in *Labeorohita*3.4-1.0, 3.2-1.2, 18-10, 0.87-0.10, 0.94-0.11, 0.078-0.012, 0.068µg/g-0.022µg/g. The concentration of these heavy metals was found within the safe limits as “recommended daily allowance (RDA) Guide line proposed by WHO/FAO”.

Keywords: Accumulation, Fish Species, Heavy Metals, Fish Ponds

1. Introduction
The term "fish" most precisely describes any non-tetrapod craniate that has gills throughout life and whose limbs, if any, are in the shape of fins and more than 30,000 species of vertebrates of phylum Chordata found in the fresh and salt waters of the world (*Cleveland P et.al*). Living species range from the primitive, jawless lampreys and hagfishes through the cartilaginous sharks, skates, and rays to the abundant and diverse bony fishes. Most fish species are cold-blooded. Fishes are of interest to humans for many reasons, the most important being their relationship with and dependence on the environment (Dayan, A.D et.al). A more obvious reason for interest in fishes is their role as a moderate but important part of the world’s food supply. Fishes range in adult length from less than 10 mm (0.4 inch) to more than 20 meters (60 feet) and in weight from about 1.5 grams (less than 0.06 ounce) to many thousands of kilograms. Some live in shallow thermal springs at temperatures slightly above 42 °C (100 °F), others in cold Arctic seas a few degrees below 0 °C (32 °F) or in cold deep waters more than 4,000 meters (13,100 feet) beneath the ocean surface (*E. Christopher, Vincent*). The structural and, especially, the physiological adaptations for life at such extremes are relatively poorly known and provide the scientifically curious with great incentive for study. Freshwater fish are those that spend some or all of their lives in fresh water, such as rivers and lakes, with a salinity of less than 0.05%. These environments differ from marine conditions in many ways, the most obvious being the difference in levels of salinity

(*Etherington, L. E.; et al.*). To survive fresh water, the fish need a range of physiological adaptations.41.24% of all known species of fish is found in fresh water. This is primarily due to the rapid speciation that the scattered habitats make possible. When dealing with ponds and lakes, one might use the same basic models of speciation as when studying island bio-geography (*Hartman, K H; et al*).

However fishes are to be considered as great part of food potential for human population in worldwide and its products constitutes an important role in the human diet to upgrade their nutritional standards. Actually their biochemical composition and uptake of minerals available in fish food shows their nutritional values in routine diet (Hartman, K.H *et al*. 2004) [12]. They are also known to be the chief source of essential trace metals and amino acids mainly the (lysine) which is not much abundant into the cereals. Freshwater fishes are also considered as treasure of exceptional type of protein, minerals, and vitamins and also having valuable lipids and fatty acids. But unfortunately dietary sources are main path of exposure to heavy metals and also other hazardous factors that can be accumulated into such medium where these fishes are found. It is a common fact that polluted fishes could be dangerous dietary sources for such toxic components or heavy metals (Pauly, Daniel, 2004) [26].

The bio-accumulation of heavy metals in living organisms and bio-magnifications describes the processes and path-ways of pollutants from one trophic level to another. Many fish species which are widely used as bio-indicators of certain heavy metals contamination in aqua culture. The acidic conditions of aquatic environment might be responsible for creating free divalent ions hazardous elements that are routinely absorbed by fish gills and shows significant signs of aquatic pollution in respect of these heavy metals. Certain inorganic fertilizers are commonly used in water culture and causes significant disturbance in natural environment of water dweller organisms (Clevelan P *et al*. 2001) [6]. Most of agricultural water wastes that containing pesticides and fertilizers combines with industrial runoffs in addition to sewage effluents supply the water bodies and sediment with huge quantities of inorganic anions in many water resources where fishes are cultivated. Moreover these heavy metals can be absorbed through their food chain that ultimately disturbs their normal physiological state. Once accumulates in any medium in even trace levels may not be decomposed by passing hundreds of years and may shows critical effects to members of certain bio-ta. Although the abundance of Fe, Zn and Cu in trace levels may not harm the organisms due to their biochemical role in the life processes of all aquatic plants and animals and considered to be essential metals in the aquatic and terrestrial environment in trace amounts (Tim Cashion, 2016) [33].

In present research, upper and lower level of trace, toxic and essential heavy metals from gills, liver and muscle tissues of three economically vital fresh water fish species were evaluated in prescribed study area. These mentioned species were consumed in large quantity by the common men of that area and also from nearby cities like Buxapure, Badani, Kandhkot, Ghotki, Ghouspure, Karampure and also from nearby trade markets Sukkur and Sadiqabad people. Not only their consumption impact these fishes having importance as a source of employment, earning and trade of local individuals. Evaluation of such health hazards elements consumed through their diet in excessive amount may causes some serious blonder to respective society. So it is now necessary to manipulate these results to local government authorities and fish farmers to take efforts for possible reduction of such pollutants in local fresh water resources.

2. Materials and Methods

2.1. Description of Study Area

District Kashmore is present in northern area of Sindh and its lies with Ghotki, Jacobabad, Shikarpur and Sukkur within Sindh. District is connected with the borders of Baluchistan on one side and another side with Punjab province (Sindh population surges, 2016). The latitude of Kashmore, Pakistan is 28° 25' 58.78 92" N and the longitude is 69° 35' 1.35 60" E (NRB: Local Government Elections, 2012). River Indus flowing. Therefore along with the Eastern side of Kashmore. Major source of irrigation is through the Guddu barrage and there is excess availability of water in this area due to this fish is to be considered as a cash product of this area (Geography: The rivers of Pakistan, 2017).

Fig 1: Map of Kashmore district including our sampling area

Fig 2: A view of study area fish pond near Chachar village

2.2. Sample Collection and Preservation

360 total samples of economically important fishes were obtained through professional fishermen by using different size of fish nets from Chachar fish ponds. Fresh fish samples of three selected verities of fish species *Catlacatla, Cirrhinus mrigala and Labeorohita* of variable weights i-e 250g, 500g, 750g and 1000g were studied. Fresh fish samples were immediately stored in an ice box (at 4 °C) and shifted to the laboratory for further procedures, such as dissection, digestion and analysis. The different specimens of fish samples were obtained by dissection with sterilized stainless steel cutter equipment s. required grams of muscle, gills and liver specimen's samples from three varieties of fishes were accurately weighed by electronic balance.

2.3. Dissection and Digestion of fish's samples

Fresh fish samples were weighed with the help of electronic balance before dissection and extracted specimens were completely dried at 100°C until and unless constant weight was obtained. Moisture free samples were further grained and later on tri-acid mixture namely Nitric acid, Sulphuric acid and Perchloric acid were added with ratio of 10:4:1 respectively in each sample. Each of them was heated at 130 ^{0}C through the heating digester with air condenser in fuming cup board until clear solution was obtained. After this solution was filtered through Whatman 42# filter paper and added 50mL of de-ionized water and then made up required volume of that solution. Determination of Fe, Cr, Cd, Cu, Ni, Mn, Pb and Zn were made on each solution by using Inductively Coupled Plasma Atomic Emission Spectroscopy *(*ICP-AE*S*), Analytical blanks were prepared in the same way as the samples were homogenized. For Elemental analysis the standards were prepared in the same acid medium by using standard solutions. The required standard solutions and chemicals were of proper analytical grade was obtained from Merck.

3. Results

Results obtained were statically analyzed and were expressed as mean of triplicate values. The concentrations of Iron, Zinc, Copper, Manganese, Nickel, Cadmium and Chromium in Gills, Liver, Muscle of four different weighs of *Catlacatla*, *Cirrhinus mrigala* and *Labeorohita* fish species are given in Table-01, Table-02 and Table-03 respectively.

Highest level of Iron in *Catlacala* fish species was analyzed in gills of Cc-2 (2.2µg/g) and maximum concentration of Zinc, Copper. Manganese. Nickel, Cadmium and Chromium were noted in gills Cc-2 (3.5µg/g), gills Cc-3 (18.4µg/g), gills Cc-1 (0.54µg/g), gills Cc-2 and muscle of Cc-4 (0.98µg/g), gills Cc-2 (0.09µg/g) and gills of Cc-2 as (0.06µg/g) mentioned in Table-01 and Fig-03 The maximum concentration of these heavy metals *Cirrhinus mrigala* fish species were found in Fe, Zn, Cu, Mn, Ni, Cd and Cr were analyzed in liver of Cm-4 (2.4µg/g), gills of Cm-4 (3.4µg/g), gills of Cm-4 (17.5µg/g), gills of Cm-3 (0.68µg/g), muscles of Cm-4 (0.95µg/g), muscle of Cm-3 (0.05µg/g) and gills of Cm-1 (0.07µg/g) which are shown in Table-02 and Fig-04 Highest level of Fe, Zn, Cu, Mn, Ni, Cd and Cr in *Labeorohita* fish species were observed as in gills of Lr-4 (3.4µg/g), gills of Lr-4 (3.2µg/g), muscles of Lr-4 (18µg/g), gills of Lr-3 (0.87µg/g), gills of Lr-1 (0.98µg/g), gills of Lr-3 and muscle of Lr-4 (0.07µg/g) and finally in gills of Lr-3 (0.07µg/g) that are mentioned in Table-03 and Fig-05.Concentration of trace and toxic heavy metals in gills, liver and muscle of *Catlacatla* fish species mentioned with the analytical ranges in Table-04 and Fig-06.The level of trace and toxic metals in various specimens of *Cirrhinus mrigala* are given with certain analytical ranges in Table-05 and Fig-07. Accumulation of heavy metals in biological specimens of *Labeorohita* are mentioned with minimum and maximum ranges in Table-06 and Fig-08. Concentration of these heavy metals in all three fish species namely *Catlacatla*, *Cirrhinus mrigala and Labeorohita* with analytical averages and RDA limits proposed by WHO mentioned in Table-07 and Fig-09.

Table 1: Concentration of trace and toxic elements in different biological specimens of *Catlacatla* fish samples found in ponds of Kashmore, Sindh, Pakistan

Specimens	Codes	Fe µg/g	Zn µg/g	Cu µg/g	Mn µg/g	Ni µg/g	Cd µg/g	Cr µg/g
Gills	Cc-1	1.4	2.4	14.2	0.54	0.82	0.08	0.05
	Cc-2	2.2	3.5	10.1	0.32	0.98	0.09	0.06
	Cc-3	2.0	2.4	18.4	0.41	0.37	0.06	0.03
	Cc-4	1.2	2.9	16.5	0.49	0.93	0.07	0.05
Liver	Cc-1	1.4	1.2	14.2	0.12	0.14	0.03	0.02
	Cc-2	1.0	1.5	9.02	0.14	0.16	0.02	0.02
	Cc-3	1.1	1.4	17.3	0.19	0.18	0.04	0.03
	Cc-4	2.4	1.2	17.5	0.19	0.15	0.03	0.02
Muscle	Cc-1	1.9	1.8	14.4	0.18	0.85	0.05	0.03
	Cc-2	2.2	2.4	17.0	0.15	0.91	0.07	0.02
	Cc-3	1.7	1.4	14.8	0.12	0.45	0.05	0.02
	Cc-4	1.2	3.4	16.1	0.28	0.98	0.08	0.03

Table 2: Concentration of trace and toxic elements in different biological specimens of *Cirrhinus mrigala* fish samples found in ponds of Kashmore, Sindh, Pakistan

Specimens	Codes	Fe µg/g	Zn µg/g	Cu µg/g	Mn µg/g	Ni µg/g	Cd µg/g	Cr µg/g
Gills	Cm-1	1.6	1.8	12.5	0.38	0.84	0.03	0.07
	Cm-2	1.6	2.4	15.6	0.58	0.47	0.03	0.06
	Cm-3	1.2	1.2	13.0	0.68	0.92	0.04	0.04
	Cm-4	1.8	3.4	17.5	0.62	0.69	0.02	0.05
Liver	Cm-1	1.0	1.2	14.8	0.21	0.56	0.01	0.02
	Cm-2	1.5	1.4	12.3	0.15	0.58	0.02	0.03
	Cm-3	2.2	1.8	11.3	0.24	0.47	0.01	0.05
	Cm-4	2.4	1.9	15.3	0.19	0.52	0.01	0.04
Muscle	Cm-1	1.6	2.3	14.5	0.41	0.94	0.03	0.03
	Cm-2	1.8	1.5	11.4	0.34	0.87	0.02	0.06
	Cm-3	1.2	3.0	15.6	0.21	0.56	0.05	0.05
	Cm-4	1.8	2.3	16.2	0.48	0.95	0.02	0.06

Table 3: Concentration of trace and toxic elements in different biological specimens of *Labeorohita* fish samples found in ponds of Kashmore, Sindh, Pakistan

Specimens	Codes	Fe µg/g	Zn µg/g	Cu µg/g	Mn µg/g	Ni µg/g	Cd µg/g	Cr µg/g
Gills	Lr-1	1.2	2.1	11.8	0.65	0.98	0.05	0.05
	Lr-2	3.3	1.8	14.3	0.54	0.82	0.04	0.03
	Lr-3	2.1	2.6	13.0	0.87	0.65	0.07	0.07
	Lr-4	3.4	3.2	14.0	0.63	0.94	0.06	0.06
Liver	Lr-1	1.3	1.4	12.2	0.12	0.21	0.03	0.02
	Lr-2	1.2	1.2	9.30	0.15	0.11	0.01	0.03
	Lr-3	2.4	1.4	10.2	0.13	0.35	0.02	0.01
	Lr-4	2.5	1.7	15.6	0.14	0.12	0.03	0.03
Muscle	Lr-1	1.5	2.4	17.2	0.42	0.71	0.05	0.02
	Lr-2	1.2	1.4	15.5	0.21	0.87	0.06	0.03
	Lr-3	2.3	2.3	16.1	0.65	0.65	0.03	0.02
	Lr-4	2.5	1.8	18.0	0.37	0.83	0.07	0.04

Table 4: Analytical ranges of trace and toxic elements in *Catlacatla* fish samples found in ponds of Kashmore, Sindh, Pakistan

Elements	Gills			Liver			Muscle		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Fe µg/g	1.2	2.2	1.7	1.2	2.2	1.7	1.1	2.4	1.6
Zn µg/g	2.4	3.5	2.8	1.2	1.5	1.4	1.4	3.4	2.4
Cu µg/g	10	18	14	9	17	13.5	14	17	15.5
Mn µg/g	0.32	0.54	0.43	0.13	0.20	0.16	0.12	0.28	0.20
Ni µg/g	0.45	0.97	0.72	0.21	0.18	0.19	0.45	0.98	0.71
Cd µg/g	0.06	0.09	0.07	0.02	0.04	0.03	0.05	0.08	0.06
Cr µg/g	0.03	0.06	0.05	0.01	0.03	0.02	0.02	0.03	0.02

Table 5: Analytical ranges of trace and toxic elements in *Cirrhinus mrigala* fish samples found in ponds of Kashmore, Sindh, Pakistan

Elements	Gills			Liver			Muscle		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Fe µg/g	1.2	1.8	1.5	1.3	1.9	1.6	1.0	2.4	1.7
Zn µg/g	1.2	3.4	2.3	0.4	1.4	0.91	1.4	2.3	1.8
Cu µg/g	12.5	17.5	15	11.3	15.3	13.4	11.4	16.2	13.7
Mn µg/g	0.38	0.68	0.51	0.15	0.24	0.19	0.21	0.48	0.35
Ni µg/g	0.47	0.92	0.64	0.47	0.58	0.61	0.56	0.95	0.72
Cd µg/g	0.02	0.03	0.02	0.01	0.02	0.01	0.02	0.05	0.03
Cr µg/g	0.04	0.06	0.05	0.01	0.03	0.02	0.02	0.04	0.03

Table 6: Analytical ranges of trace and toxic elements in *Labeorohita* fish samples found in ponds of Kashmore, Sindh, Pakistan

Elements	Gills			Liver			Muscle		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Fe µg/g	1.2	3.4	2.3	1.0	2.5	1.7	1.2	2.5	1.8
Zn µg/g	1.8	3.2	2.5	1.2	1.7	1.4	1.4	2.4	1.7
Cu µg/g	11.8	14.3	13	10.0	15.6	12.8	16.1	18.0	17
Mn µg/g	0.54	0.87	0.71	0.10	0.15	0.12	0.21	0.65	0.43
Ni µg/g	0.65	0.94	0.81	0.11	0.35	0.23	0.70	0.87	0.73
Cd µg/g	0.04	0.07	0.05	0.01	0.03	0.02	0.03	0.06	0.05
Cr µg/g	0.03	0.06	0.04	0.01	0.03	0.02	0.02	0.04	0.03

Table 7: Average ranges of concentration of trace and toxic elements in various specimens of economically important fishes found in Ponds of Kashmore, Sindh, Pakistan

Elements	RDA/WHO Limits	*Caltacatla*			*Cirrhinus mrigala*			*Labeorohita*		
		Min	Max	Average	Min	Max	Average	Min	Max	Average
Fe µg/g	0.2-11mg-day	1.1	2.4	1.7	1.2	2.4	1.8	1.2	3.4	2.3
Zn µg/g	8-9mg- day	1.2	3.4	2.3	1.2	3.4	2.3	1.2	3.2	2.2
Cu µg/g	0.5-1mg day	9.0	18.0	13.5	11.3	17.5	14.4	9.3	18.0	13.6
Mn µg/g	0.2-1mg-day	0.12	0.54	0.33	0.19	0.68	0.42	0.10	0.87	0.46
Ni µg/g	1.6-2mg-day	0.14	0.98	0.56	0.41	0.95	0.83	0.12	0.98	0.55
Cd µg/g	0.01-0.2mg limit in food	0.06	0.09	0.07	0.01	0.05	0.03	0.01	0.07	0.04
Cr µg/g	0.01-0.3mg limit in food	0.01	0.06	0.3	0.01	0.06	0.03	0.01	0.06	0.03

3.1 Graphical representation of heavy metals concentration in various fish organs

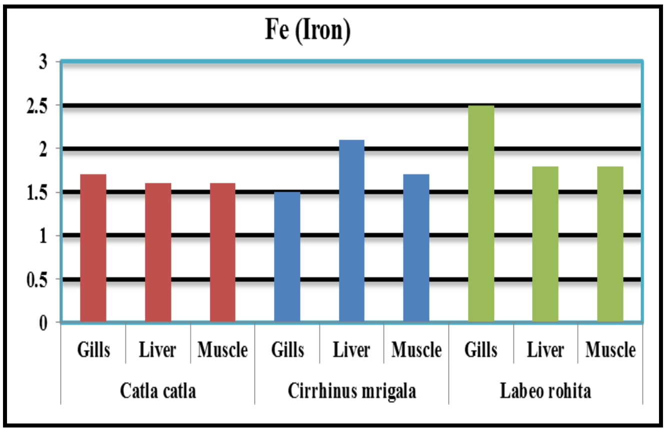

Fig 3: Level of Iron in different fish organs

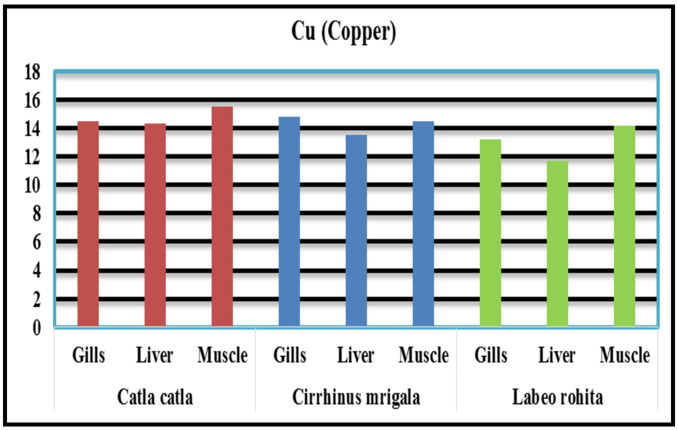

Fig 4: Level of Copper in different fish organs

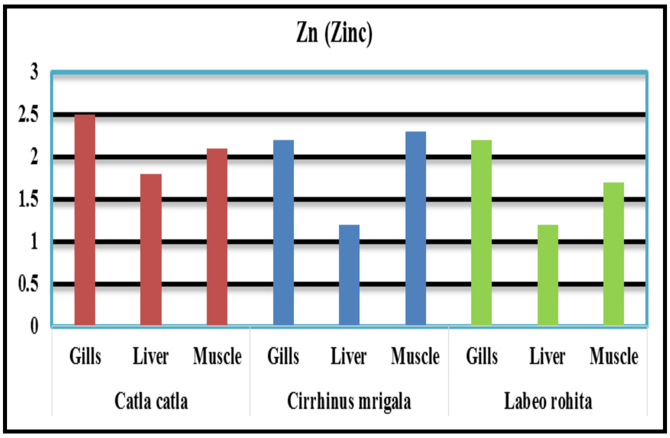

Fig 5: Level of Zinc in different fish organs

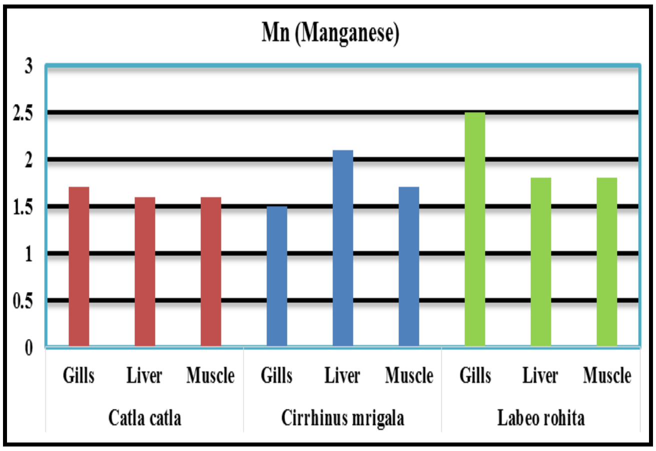

Fig 6: Level of Manganese in different fish organs

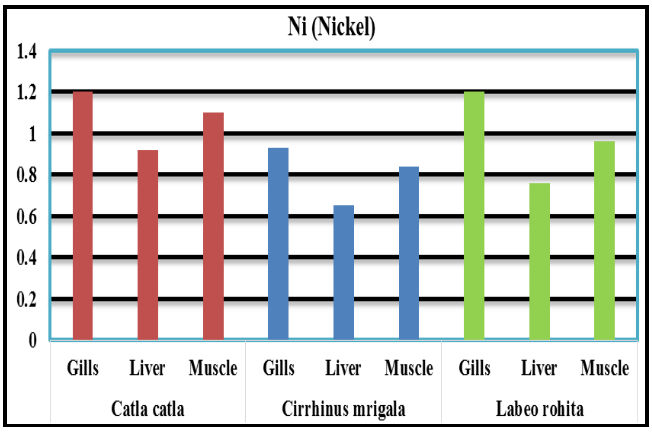

Fig 7: Level of Nickel in different fish organs

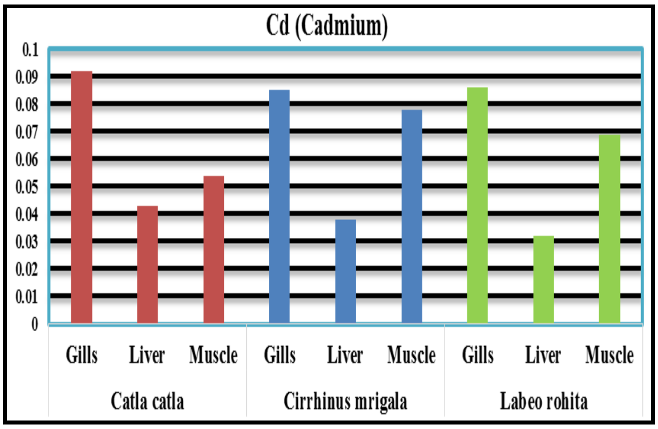

Fig 8: Level of Cadmium in different fish organs

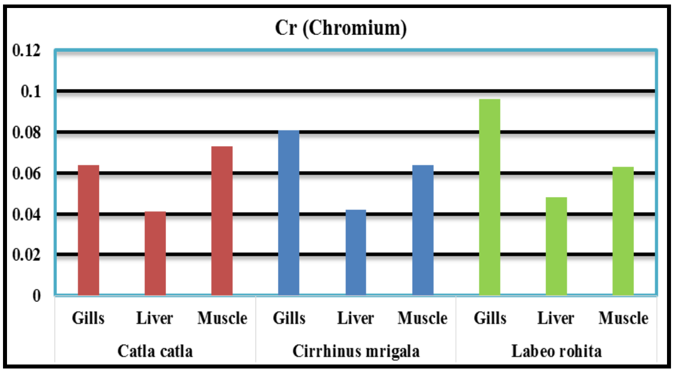

Fig 9: Level of Chromium in different fish organs

3.2. Discussion

Fig-03 determines the average level of concentration in gills, liver and muscle of three economically important fish species i.e *Catlacatla, Cirrhinus mrigala* and *Labeorohita* of four different weighs 250g, 500g, 750g and 1000g respectively. The graphical representation shows that in Cirrhinuismrigala maximum amount of Iron was noted in liver while in case of the *Labeorohita* maximum amount was found in gill as compared to rest of fish species under investigation. Many researchers have studied accumulation of metals in the fish tissues. Different factors like temperature, seasonal changes, physico-chemical status of water might cause metal accumulation in the tissues of fish.

Fig-04 shows that the highest concentration of copper was noted in muscles of *Catlacatla* fish species as compared to its other organs like liver and muscle. In case of the *Cirrhinus Mrigala* highest amount was found in gills as compared to other tissues. The maximum amount of copper was noted in *Catlacatla* fish species as compared to other studied organs which were investigated in present study.

Fig-05 indicates that the maximum level of Zinc was noted in gills of *CatlaCatla* and *Lebeorohita* species as compared to the *Cirhiniusmrigala.* Highest level of zinc was determined in muscle of the *Cirhiniusmrigala* fish specie. The highest concentration among studied species was noted in the gills of *Catlacatla* as compared to *Cirrhinus mrigala and Labeorohita.* According to the gills are the good sites for metal uptake before intering other parts of the organisms.

Fig-06 shows that the highest concentration of Manganese was noted in gills of *Labeorohita* fish species as compared to rest of fish species which were investigated in our present study. in case of *Cirhiniusmrigala* the maximum concentration was noted in liver.

Trace metals into water bodies may have significant effects on aquatic organisms including fishes. Therefore, these factors would be reasons for the concentration of trace metals in fishes.

Fig-07 contains comparative graphical representation of different fish organs which were under investigation. It shows that the maximum concentration of Nickel was noted in gills of all three species *Catlacatla, Cirrhinus mrigala* and *Lebeorohita* fish species. Where as other researcher studied that higher concentration of nickel found in liver and muscle tissues.

Fig-08 shows that maximum amount of Cadmium was noted in gills of all three species as compared to their liver and muscle. Although the highest concentration of was found in gills of *Catlacatla* as compared to other fish species which were studied.

Fig-09 shows the highest concentration of Chromium in Gills of Cirhiniusmrigala and *Labeorohita* fish species but in case of the Catlacatla higher concentration was noted in muscle tissue and the least amount was found in liver of all species in present study. Many researchers studied that the concentration of higher in muscles of the fish species.

It is observed that the concentration of all the elements found within safe limit. Iron copper and zinc have vital role among heavy metals and are essential for fish metabolism. These fishes have a valuable attention to our employment, earning and diet, deficiency of these metals may cause nausea, fever and many other diseases.

4. Conclusion

The results of present study revealed that the highest accumulation order of these heavy metals were as Cu > Fe > Zn >Mn> Ni > Cd > Cr. The Order of concentration of trace and toxic elements in all three fish varieties were found as C*atlacatla>Labeorohita>Cirrhinus mrigala* fish organs. The concentration of Chromium and Cadmium were noted significantly low even too below than WHO recommended guideline as compared to other trace elements. Bio-accumulation factors among different organs of all three fish varieties indicated that high accumulation of elements in Gills as compared to liver and muscle of fishes.

It was further concluded that level of all parameters which were studied and found significantly below than RDA as proposed by FAO and WHO. Therefore consumption of these fishes belong to study area are strongly recommended safe for Human health purposes.

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