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ANALYSIS OF SOME HEAVY METAL CONCENTRATION IN COMMON CARP (FISH) COLLECTED FROM KALPANI RIVER

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Abstract

Heavy metal concentrations were analyzed in water sediments and different tissue samples of fish collected from Kalpani stream. Least concentration was shown in water and different tissue samples of fish. The results of the present research work revealed that most of the metals are particulate bound and least bioaccumulation occurred in fish. Thus metal accumulation in Common carp is within the permissible limits of FAO, WHO and U.S Recommended daily allowance (1989) which reflects low metal load in Kalpani Stream.

Introduction

Kalpani Stream is considered as the life line of part of Nowshera and Mardan Districts. It originates at a height of about 1520 feet in Malakand Agency and LandiKhwar nearby, Chanchanokhat of Districts Mardan. Its direction of flow is from North to South, and has a running length of about 110 kilometers from its origin. Variation in topography, land use, wetland, soil composition and several other natural processes affect the distribution of different fish species in different habitats (Koel 1997). Change in altitude, soil composition, soil texture, beds of water bodies etc give

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unique pattern of distribution to fish fauna. Changes in environmental conditions can potentially affect reproduction, feeding and survival value of population, behavior, physiology and genetics of fish (Shoener 1974; Southwood 1977). The entire fish species of Kalpani Stream belong to South Asian sub region of the Oriental Region however, *Heteropneustusfossilis,* species of *Puntius* and *Chana punctatus* are from Indus River. Various efforts have been made by researchers to explain the different fish species of Kalpani. The different fish species of Kalpani and its offshoots have been considered and studied at different places and times by different researchers including Mirza, (1980), McClelland, (1839) and several other research workers.The Kalpani Stream is the main reservoir of the waste disposal as well as industrial effluents in District Mardan. Municipal treatment arrangements along with industrial waste prior to their discarding to Kalpani Stream are meager. The situation has become severe with the increasing anthropogenic activities therefore, Kalpani Stream has been considered as dirtiest streams of KP (Directory Industrial Establishment 2007).



Fig 1:Map of Kalpani Nala

Cyprinus carpio commonly known as Common carpis considered as one of the ancient and important cultural fish species for food. Its culture first began in China and later on was introduced in European countries, from where its domesticated farms were introduced in several other countries. Being the most important fresh water fish, it is also considered as prized food in Europe, Asia and Middle East countries (Eddy and Underhill 1974).

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Fig 2: A photograph of Common Carp Fish found in Kalpani Nala

Heavy metals have gained much consideration among the non-degradable noxious substance owing to their poor consequences on water inhabiting fauna and flora (Dirilgen 2001). Heavy metals are the most toxic substances because of their varied effects. Those metals which are highly soluble in water are easily absorbed into the biotic components of an ecosystem. Metals with higher concentration are known to cause harmful effects on blood and organs in fish. They form metal compound when react with enzymes, DNA, RNA and cellular proteins (Akahori et al.1999).Keeping in view the present study was undertaken to know the seasonal effect on heavy metals bioaccumulation in water, sediment and different organs of Common carp fish of Kalpani river (Nala).

Materials and Methods

Water samples collection

Water samples were collected in pre-cleaned and acid washed polypropylene bottles. These water samples were filtered through filter paper and conserved by adding concentrated nitric acid (HNO_3).

Digestion

Water samples were digested according to the method described in APHA (1985). According to this method the water samples were taken in separate beakers along with a mixture of 15mL of concentrated HNO_3 and 5mL of $HClO_4$. The beakers were covered with Pyrex glass cover and placed on hot plate for boiling. The samples were allowed to evaporate until 2-3ml volume is left which indicate that digestion has been completed. After digestion samples were cooled, filtered, and were diluted to 100ml distilled water by

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proper rinsing of the digestion beakers. Samples were stored in properly washed glass bottles until the metal concentration could be determined.

Sediment sample collection

Sediment samples were collected in pre-cleaned, acid washed scoop or grab, transferred to clean polythene bags and transported to the laboratory. These samples were then dried in oven at 110^0C for 5 to 6 hours and ground to powder form in a glass mortar. After that samples were stored in pre-cleaned polythene bags.

Digestion

Sediments samples were digested according to the method described in APHA (1985). According to this method, oven-dried samples of sediments were passed through a 2mm sieve. The *aqua regia* method involving15mL of conc. HNO_3 and 5mL of $HClO_4$ (APHA, 1985) was used as the standard method to digest the sample. 2gm of oven dried and marter grounded sample was placed in a beaker by adding 15mL of conc. HNO_3 and 5mL of $HClO_4$.The beaker is then placed on hot plate and mixture is heated at 80 to 90 ^{0}Cin a fume hood until the dark color disappears. The dense white fume from the beaker after brown fumes is the indication of completion of the digestion process.

When the residue is completely dissolved, the container is cooled, and the solution is diluted and transferred quantitatively to a 100 mL volumetric flask by adding distilled water and passing through (Watman 42) filter paper. Samples were stored in properly washed glass bottles until the metal concentration could be determined.

Fish sample collection

To get information about contamination and bioaccumulation of heavy metals in common edible fresh water fish species of Kalpani Stream twelve samples were collected from different parts of the Kalpani Stream. Fishing was done with the help of local fishermen. For this purpose Gill net with a cork line or floats at the top and metal line or sinkers with the bottom rope made up of nylon threads were used as fish Gears. Fish samples were preserved in ice container and were carried to the laboratory for further analysis.

Fish identification

Fish identification was conducted in the PCSIR laboratory complexes Peshawar, following the method of Talwar and Jhingran (1991) and Mirza and Sandhu (2001).

Morphometric parameters necessary for fish identification like length, weight eye diameter, number of barbles etc. of each fish were noted.

Fish dissection

After morphometric study fish was dissected. Tissue samples were taken from the liver, muscles, skin, gills

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and intestine. These tissues were washed with distilled water, weighted and shifted to properly mark sterilized polythene bags and stored at -20 ^{0}C for further analysis.

Tissue digestion

For heavy metals estimation the tissue digestion was carried out in the PCSIR Laboratory complexes Peshawar. Tissue samples were thawed, rinsed in distilled water and blotted with blotting paper. After blotting the samples were shifted to 100 volumetric flasks. All the flask were washed properly and rinsed with distilled water, before transferring the tissue samples. Then the known weight of each tissue was transferred to these volumetric flasks.

Samples digestion was carried according to the methods presented by Van Loon (1980) and Duefreez and Steyn, (1992). A slight modification was made in the procedure (Yousafzai and Shakoori 2006) instead of putting 10ml nitric acid (55%) and 5ml per chloric acid (70%) at the time of digestion, 5ml nitric acid (55%)and 1ml per chloric acid (70%) were added to each flask and the flask were then kept for overnight.

Next day a second dose of 5ml nitric acid (55%) and 4ml per chloric acid (70%) was added to each flask. The flask were kept on hot plate, covered with Pyrex glass cover and allowed to digest at 200C^o to 250C^o until a clear transparent solution was observed. Initially dark brown fumes appeared followed by white fumes. The dense white fumes from the flask, after brown fumes was an intimation of completion of the digestion process. By this method digestion was accomplished in about 30 minutes instead of 3 to 4 hours as described by Van Loon 1980. After digestion, samples were cooled, filtered through (Watman 42) filter paper and diluted to 100 ml with distilled water by proper rinsing of the digestion beakers. Samples were stored in properly washed glass bottles until the metal concentration could be determined.

Heavy metals analysis

Determination of heavy metals was done through atomic absorption spectrophotometer in the PCSIR Laboratory Complex Peshawar. Atomic Absorption Spectrophotometer (Spectra AA 2000) was used for determination of heavy metals concentration like Zn^{2+}, Ni^{2+},Cu^{2+},Cr^{3+}, Hg^{2+}, Cd^{2+} and Pb^{2+} in water, sediments and tissue samples of each fish. A range of analytical standards for each metal was prepared from E Merck stock solution. Standard curves were prepared and obtained data were calibrated against the standard curves to know the concentration of heavy metals present.

Statistical analysis

Mean and standard error of the mean of data was taken.

Results

Metals concentration in water and soil of Kalpani stream

Toxic metals in rivers water and sediments not only affect water but also compromise the quality of aquatic food. Seasonal variations in concentration of different

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heavy metals in water and soil sediment of Kalpani stream are presented in tables 1 and 2 respectively. Water analysis showed the presence of Cu and Cd while the metals like Zn, Ni, Cr and Pb were not detected in stream water throughout the year (Table 1). Concentration of Cd remained constant (0.02 ± 0.01µg g^{-1}) in water samples throughout the year 2012. A rise in concentration of Cu in the stream water was noted in the month of April, 2012 while in the rest of the year the concentration of Cu remained almost constant (0.03 ± 0.001 µg g^{-1}) as given in table 1.

Variation in the concentrations of Zn, Ni, Cr, Cu, Cd and Pb in sediment of Kalpani stream was found throughout the year (from January to October, 2012) as shown in table 2. Sediment samples showed highest concentration of Ni as compared to the other heavy metals. Mean concentrations of heavy metals in most of the samples were highest in the months of October, 2012 (table 2). Most of the sediments samples showed decrease in concentration of metals in April, 2012. The concentration of Zn and Cd were higher in samples collected in the month of July, 2012.

Table 1: Heavy metal accumulation in water samples collected from Kalpani Stream Mardan in 2012.

Month of the year	Heavy metals concentration (µg g^{-1}) ± Standard Deviation					
	Zn	Ni	Cr	Cu	Cd	Pb
January	N.D	N.D	N.D	0.011 ± 0.001	0.02 ± 0.01	N.D
April	N.D	N.D	N.D	0.032 ± 0.001	0.02 ± 0.01	N.D
July	N.D	N.D	N.D	0.011 ± 0.001	0.02 ± 0.01	N.D
October	N.D	N.D	N.D	0.012 ± 0.001	0.02 ± 0.01	N.D

Table 2: Heavy metal accumulation in sediments samples collected from Kalpani Stream Mardan (Jan - October, 2012).

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Month of the year	Heavy metals concentration (µg g^{-1}) ± Standard Deviation					
	Zn	Ni	Cr	Cu	Cd	Pb
January	1.78 ± 0.14	3.36 ± 0.24	0.83 ± 0.01	0.47 ± 0.02	0.012 ± 0.001	0.041 ± 0.003
April	1.49 ± 0.24	2.92 ± 0.14	0.65 ± 0.27	0.40 ± 0.10	0.011 ± 0.002	0.040 ± 0.001
July	1.91 ± 0.36	3.27 ± 0.49	0.64 ± 0.11	0.49 ± 0.03	0.013 ± 0.001	0.032 ± 0.002
October	1.80 ± 0.02	3.75 ± 0.73	1.10 ± 0.23	0.57 ± 0.06	0.012 ± 0.001	0.091 ± 0.003

Heavy metals concentration in different parts of *Cyprinus carpio* fish

Concentration of various heavy metals in different organs of *Cyprinus carpio* fish netted from Kalpani stream Mardan during 2012, are given in tables 3, 4, 5, 6 and 7. Variation in concentration of Zn, Cr, Cu and Cd in muscles of *Cyprinus carpio* was found at different times of the year 2012 (table 3). Presence of Ni and Pb were not detected in muscles of the fish throughout the year while Cd was only detected in January 2012. Concentration of Cr in fish muscles was much higher (0.60 ± 0.10µg g^{-1}) in the month of January as compared to the other month of the year.

Variation in concentrations of heavy metals in intestine of *Cyprinus carpio* during 2012 is given in table 4. Mean concentration of Zn was much higher as compared to the other heavy metals in intestinal samples collected throughout the year 2012. It was noted that Zn and Ni were in higher concentration in January as compared to the other months of the year. While Cr, Cu and Cd showed higher concentration during April. Lead was not detected in the intestine of fish throughout the year 2012 (Table 4).

The table 5 shows concentration of different heavy metals in the skin of *Cyprinus carpio* netted from Kalpani stream, Mardan. Concentration of Zn, Ni, Cr and Cu in skin of the fish ranges between 2.93 - 1.31, 0.10 - 0.02, 0.60 - 0.11 and 0.09- 0.05 µg g^{-1}respectively (table 5). Cd and Pb were not detected in the skin samples of *Cyprinus carpio* throughout the year.

Liver samples of the *Cyprinus carpio* showed variation in concentration of Zn, Ni, Cr, Cu and Cd (table 6). Highest mean concentration of Zn and Cu in fish liver was detected in January as compared to the other months. The liver samples demonstrated highest mean concentrations of Ni, Cr and Cd during the month of April, July and October respectively (table 6).

Table 7 presents concentrations of different heavy metals in gills samples of *Cyprinus carpio* fish. Mean concentration of Zn was much higher as compared to

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the other heavy metals. Ni and Pb were not detected in the gills of the fish.

Table 3: Concentration of heavy metals in muscles of *Cyprinus carpio* netted from Kalpani Stream Mardan during 2012.

Month of the year	Heavy metals concentration (µg g^{-1}) ± Standard Deviation					
	Zn	Ni	Cr	Cu	Cd	Pb
January	1.31 ± 0.35	N. D	0.60 ± 0.10	0.07 ± 0.03	0.01 ± 0.00	N. D
April	1.53 ± 0.15	N. D	0.06 ± 0.01	N.D	N.D	N. D
July	2.21 ± 1.11	N. D	0.09 ± 0.01	N.D	N.D	N. D
October	1.88 ± 0.25	N. D	0.09 ± 0.02	0.01 ± 0.001	N.D	N. D

Table 4: Concentration of heavy metals in intestines of *Cyprinus carpio* netted from Kalpani Stream Mardan during 2012.

Month of the year	Heavy metals concentration (µg g^{-1}) ± Standard Deviation					
	Zn	Ni	Cr	Cu	Cd	Pb
January	6.28 ± 0.86	0.16 ± 0.140	0.21 ± 0.09	0.011 ± 0.001	0.022 ± 0.002	N. D
April	5.86 ± 0.85	0.13 ± 0.110	0.35 ± 0.01	0.080 ± 0.031	0.024 ± 0.004	N. D
July	4.75 ± 0.96	0.09 ± 0.016	0.32 ± 0.05	0.112 ± 0.023	0.021 ± 0.001	N. D
October	2.44 ± 1.43	0.03 ± 0.005	0.17 ± 0.05	0.091 ± 0.030	0.025 ± 0.002	N. D

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Table 5: Concentration of heavy metals in skin of *Cyprinus carpio* netted from Kalpani Stream Mardan during 2012.

Month of the year	Heavy metals concentration (µg g^{-1}) ± Standard Deviation					
	Zn	Ni	Cr	Cu	Cd	Pb
January	1.61 ± 0.45	N.D	0.60 ± 0.30	0.07 ± 0.03	N.D	N.D
April	1.31 ± 0.35	0.04 ± 0.05	0.44 ± 0.18	0.09 ± 0.06	N.D	N.D
July	2.93 ± 0.74	0.10 ± 0.02	0.45 ± 0.05	0.05 ± 0.04	N.D	N.D
October	2.73 ± 0.88	0.02 ± 0.03	0.11 ± 0.09	0.09 ± 0.02	N.D	N.D

Table 6: Concentration of heavy metals in liver of *Cyprinus carpio* netted from Kalpani Stream Mardan during 2012.

Month of the year	Heavy metals concentration (µg g^{-1}) ± Standard Deviation					
	Zn	Ni	Cr	Cu	Cd	Pb
January	4.62 ± 0.36	0.19 ± 0.040	0.28 ± 0.09	0.05 ± 0.02	0.012 ±	N. D
April	2.76 ± 1.19	0.20 ± 0.010	0.39 ± 0.07	0.01 ± 0.01	0.014 ± 0.002	N. D
July	2.29 ± 0.30	0.09 ± 0.003	0.40 ± 0.08	0.01 ± 0.01	0.011 ± 0.003	N. D
October	3.69 ± 1.90	0.11 ± 0.012	0.32 ± 0.09	0.02 ± 0.03	0.015 ± 0.002	N. D

Table 7: Concentration of heavy metals in gills of *Cyprinus carpio* netted from Kalpani Stream Mardan during 2012.

Month of the year	Heavy metals concentration (µg g^{-1}) ± Standard Deviation					
	Zn	Ni	Cr	Cu	Cd	Pb
January	6.73 ± 0.67	N. D	0.31 ± 0.04	0.07 ± 0.04	0.01 ± 0.00	N. D
April	6.41 ± 0.19	N. D	0.51 ± 0.11	0.08 ± 0.04	0.01 ± 0.01	N. D
July	7.24 ± 2.01	N. D	0.44 ± 0.16	0.09 ± 0.05	0.01 ± 0.00	N. D
October	9.00 ± 0.46	N.	0.41 ± 0.03	0.05 ± 0.00	0.01 ± 0.00	N.

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Heavy metals transfer factor (TF) from sediment/ water into *Cyprinus carpio* tissues

Transfer factor (TF) of Zn, Ni, Cr, Cu and Cd from sediments into different tissues of *Cyprinus carpio* is given in table 8. Zn demonstrated the highest TF from sediments into the *Cyprinus carpio* tissues as compared to the other metals. The highest transfer factor of Zn was recorded for gills (greater than 3.5) followed by intestine, liver, skin and muscles respectively. Transfer factor of Ni, Cr and Cu were less than one for all the organs throughout the year. Cadmium showed higher transfer factor from sediments into intestine and liver while the muscles and gills demonstrated lower transfer factor (less than one) as given in table 8. Table 9 present transfer factor of Cu and Cd from water into different tissues of *Cyprinus carpio*. Transfer factor for Cu was higher than Cd in all the tissues. Highest TF for Cu was recorded for intestine in the month of July as shown in table 9.

Table 8: Transfer factor (TF) of different heavy metals from sediments into different tissues of the *Cyprinus carpio* netted from Kalpani Stream Mardan during different months of 2012.

Tissues	Month of the year	Transfer factor (TF) of heavy metals from water into tissues					
		Zn	Ni	Cr	Cu	Cd	Pb
Muscles	January	0.74	-	0.72	0.15	0.83	-
	April	1.03	-	0.09	0.00	0.00	-
	July	1.16	-	0.14	0.00	0.00	-
	October	1.04	-	0.08	0.02	0.00	-
Intestine	January	3.53	0.05	0.25	0.02	1.83	-
	April	3.21	0.04	0.54	0.20	2.08	-
	July	2.49	0.05	0.50	0.23	1.82	-
	October	1.36	0.01	0.15	0.16	2.08	-
Skin	January	0.90	0.00	0.72	0.15	-	-
	April	0.88	0.01	0.68	0.23	-	-
	July	1.53	0.03	0.70	0.10	-	-
	October	1.52	0.01	0.10	0.16	-	-
Liver	January	2.60	0.04	0.34	0.11	1.00	-
	April	1.85	0.07	0.60	0.03	1.27	-
	July	1.20	0.03	0.63	0.02	0.85	-
	October	2.05	0.03	0.29	0.04	1.25	-
Gills	January	3.78	-	0.37	0.15	0.83	-
	April	4.30	-	0.78	0.20	0.91	-
	July	3.79	-	0.69	0.18	0.77	-
	October	5.00	-	0.37	0.09	0.83	-

Table 9: Transfer factor of different heavy metals from water into different tissues of the *Cyprinus carpio* netted from Kalpani Stream Mardan during different months of 2012.

Month of the year	Transfer factor (TF) of heavy metals from water into tissues									
	Muscles		Intestine		Skin		Liver		Gills	
	Cu	Cd	Cu	Cd	Cu	Cd	Cu	Cd	Cu	Cd
January	6.36	0.5	1	1.1	6.36	-	4.55	0.6	6.36	0.5
April	0	0	2.5	1.2	2.81	-	0.31	0.7	2.5	0.5
July	0	0	10.18	1.05	4.55	-	0.91	0.55	8.18	0.5
October	0.83	0	7.58	1.25	7.5	-	1.67	0.75	4.17	0.5

Note: The symbol "-" showed no translocation occurred.

Table 10: Correlation between the concentrations of heavy metals in *Cyprinus carpio* fish

	Zn	Ni	Cr	Cu	Cd
Zn	1.00				
Ni	-0.09	1.00			
Cr	0.45	-0.03	1.00		
Cu	0.52	-0.19	0.52	1.00	
Cd	0.53	0.61	-0.19	0.19	1.00

Discussion:

In the present study heavy metals like Zn, Ni, Cr, Cu, Cd and Pb were determined in the water, sediments and different tissues, including muscle, intestine, liver, skin and gills of Common Carp, *Cyprinus carpio* collected from Kalpani Stream Mardan. This study was conducted in different months i.e.

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January, April, July and October of 2012. Muchvariation was shown in pattern of heavy metals accumulation among water, sediments and fish and even among different tissues of fish.

Water analysis of Kalpani stream showed significant variation with respect to certain heavy metal accumulation. In water samples only Cu and Cd were detected while the rest were below the level of detection. A rise in concentration of Cu in the stream water was noted in the month of April, 2012; The highest concentration during summer could be correlated to increased atmospheric condensation, landslides, earth quake, cyclones, tornadoes and soil erosion in the nearby area of River Kabul which intern affect the water of Kalpani stream.

Sediment samples showed highest concentration of Ni as compared to the other heavy metals. Mean concentrations of heavy metals in most of the samples were highest in the months of October. As several Zn, Cu and Cd using industries are situated the nearby vicinity of river Kabul, therefor, concentration of Zn and Cd were higher in the collected samples. Besides these, runoff water and anthropogenic activities specially mining also participate in metal concentration which is dumped in streams and channels. Most of these heavy metals are deposited in the sediments that are why sediments are considered to be the main reservoir for heavy metal concentration.

As muscles of fish are not in direct contact with water and metabolically less active that is why least concentration is shown by that tissue. Ni and Pb were not detected in muscles of the fish throughout the year, while Cd was only detected in January 2012. In fish the highest Cd concentration occurs in metabolically active tissues while the lowest concentration occurs in muscles (Schmitt and Brumbaugh 1990; Robertson et al.1991). Concentration of Cr in fish muscles was much higher in the month of January as compared to the other months of the year. Previously, Yilmas (2003) has reported 1.46 and 1.48 ppm (wet weight) of Cr in the muscles of *Mugilcephalus and Trachurmediterraneus* respectively collected from Iskenderm Bay. Olaifa (2004) has recorded 0.07 and 0.07 ppm (wet.wt) of Cr in the muscle tissues of *Clariasgariepinus* fish collected from Eleiyele Lake and Zartech pond in Ibandan, Nigeria respectively which is in accordance to the values observed during this study.

One of the routes of entry of metal in fish body is intestine, as common carp is omnivorous so the aquatic fauna and flora taken by this fish as a food may also carry certain heavy metals. Mean concentration of Zn was much higher as compared to the other heavy metals in intestinal samples collected throughout the year 2012. Besides these Cr, Cu and Cd were also detected to some extents in the month of April.Ishaqet al(2011) have reported 6.86 ppm of Zn in the intestine of fish *Clariasgariepinus* collected from Nigeria River which is in accordance to our observed values. High value of Zn was detected in the intestine of sediment treated grey mullet, *Liza macrolepis* as compared to the

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controlled fish group (Hsien Chen and Young Chen, 1999).

The skin of fish is in direct contact with water so metal accumulation in skin occurs due to adsorption which is followed by absorption through several mechanisms. Concentration of Zn, Ni, Cr and Cu was observed in the skin samples of *Cyprinuscarpio* throughout the year. Buhler et al (19770) have presented that high concentration of chromium in the fish skin may be due to increase influx of chromium as compared to elimination

Liver is metabolically more active tissue. The liver samples of the *Cyprinus carpio* showed variation in concentration of Zn, Ni, Cr, Cu and Cd. Highest mean concentration of Zn and Cu in fish liver was detected in January as compared to the other months. The liver samples demonstrated highest mean concentrations of Ni, Cr and Cd during the month of April, July and October respectively. Variation in concentration pattern depends on several factors such as water parameters, environmental conditions, and natural environmental processes such as earth quake, volcanic eruptions, soil erosion, cyclones, tornados etc. occurring in close vicinity of aquatic body.

The main sites of heavy metals uptake in fish are gills (Part and Savnberg 1981). Therefore gill surfaces are the primary site of water borne metals. Highest concentration of Zn is observed in the gill samples collected throughout the year which is followed by Cr, Cu and Cd. As several Zn, Cu and Cd using industries are situated the nearby vicinity of river Kabul, therefor, concentration of Zn and Cd were higher in the collected samples. Besides these, runoff water and anthropogenic activities specially mining also participate in metal concentration which is directly dumped in streams and channels.

Conclusion:

Most of the industries of this zone are operative for few hours of the day because of the shortage of electricity which indirectly leads to low pollutants dumping.Dumped pollutants might have been flushed away by the mighty floods of 2010 which occurred excessively in KPK including Kalpani Stream. Thus these fishes are suitable for human consumption. The water of Kalpani Stream can be used for irrigation and cattle drinking as well.However the effect of heavy metal could not be underestimated, especially when there are nonstop persistent pollutants dumping activities in the stream.

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