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ANALYSIS ON HEAVY METALS IN THE COMMON CARP *CYPRINUS CARPIO* (LINNAEUS, 1758) AND WATER SAMPLES FROM TANDA DAM LAKE, DISTRICT KOHAT – PAKISTAN

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ABSTRACT

The present research study was designed to analyze six heavy metals including copper (Cu), nickel (Ni), manganese (Mn), iron (Fe), cadmium (Cd) and zinc (Zn) concentrations in common carp, *Cyprinus carpio* collected from Tanda fish hatchery located in Kohat (KPK) district, Pakistan. Fish and water samples were collected during April-2018 to February-2019 on seasonal basis such as summer (April-May), monsoon (July-August) and winter (December-February). All samples were analyzed by Analyat 700 USA Atomic Absorption spectrophotometer. The concentrations ranges of trace elements in fish hatchery water were found as follows: Cu, 0.01-0.04 µg l^{-1}; Ni, 0.00-0.02 µg l^{-1}; Mn, 0.03-0.08 µg l^{-1}; Fe, 0.02-0.08 µg l^{-1}; Cd, 0.00-0.01 µg l^{-1}; Zn, 0.02-0.06 µg l^{-1}. The concentration ranges of trace elements in *C. carpio* muscles were found as follows: Cu: 0.34 – 2.22 µg g^{-1}; Ni: 0.11-0.92, µg g^{-1}; Mn: 0.01 – 0.45 µg g^{-1}; Fe: 1.23 – 6.98 µg g^{-1}; Cd: 0.00 – 0.06 µg g^{-1}; Zn: 2.10 – 6.89 µg g^{-1}. Metal concentrations in muscle tissues of *C. carpio* were decreased as Fe > Zn > Cu > Ni > Mn > Cd. All metal accumulations in muscle tissues of *C. carpio* and water samples collected from Tanda Dam were under the permissible range.

Key-words: Heavy metals, *Cyprinus carpio,* Tanda Fish hatchery, Khyber Pakhtunkhwa (KPK)

INTRODUCTION

Metals are unrelenting contaminants and they accumulate in soil, water, sediments and mostly in trophic chains (Swaileh and Sansur, 2006). Water pollution in waters is a problem in terms of water ecosystem pollution in developed and developing countries due to increasing population, technology, and increasing industrialization (Mansour and Sidky, 2002). Heavy metals from both natural and anthropogenic sources are constantly released into aquatic ecosystems. These metals pose a serious threat to living organisms due to their toxicity, long-term persistence, bioaccumulation and bio magnifications in nutrient cycling (Pandy *et al.,* 2003).

The absorption of heavy metals in submerged organisms can have a long-lasting effect on the food cycling in the ecosystem. Heavy metals affect the growth rate of especially major carp (Hayat *et al.,* 2007). Freshwater organisms, fish and crustaceans accumulate metals from accumulation in water and sediments to much greater concentrations (Al-Weher, 2008; Mahboob *et al.,* 2014).

Fish are widely used to assess the health of aquatic ecosystems because pollutants constitute the food chain (Farkas *et al.,* 2002). Two main ways for heavy metals to enter the aquatic food chain are to consume water and food directly from the non-dietary pathways between the digestive system and permeable membranes such as the muscle and gills (Nhiwatiwa *et al.,* 2011).

Concentrations of heavy metals in the organs of fish are primarily determined by the level of water and food pollution. Therefore, it is an indicator of the environmental pollution level (Philips, 1980; Salanki and Salama, 1987). Common carp are quite resistant to water pollution (Vinodhini and Narayan, 2008).

Pakistan is a country of diverse landscapes, wildlife, and ecosystems. This variety of landscapes and habitats are especially prominent in its North West frontier province. The rivers and lakes in Pakistan provide habitat for fish, amphibians and reptiles including few outsider extraordinary fish species which are generally refined in incubation facilities of KPK region.

Pakistan is one of the countries facing freshwater pollution, mainly due to untreated discharge of industrial wastes into rivers. Reportedly only 1% of industrial wastewater is being treated before its discharge into freshwater resources (streams and rivers) (Govt. of Pakistan Position paper) (Khan *et al.,* 2012). The aim of the study was to determine the heavy metal concentrations (Cu, Ni, Mn, Fe, Cd and Zn) in water and muscle of *C. carpio* from Tanda

fish hatchery (KPK). The obtained results from this study would provide information on level of heavy metals in the water of the Tanda Dam Lake and *C. carpio* fish species. It shall enable the effective monitoring of both environmental quality and the health of the organisms inhabiting the lake ecosystem.

MATERIALS AND METHODS

Study Area

Tanda dam is a small dam located in Kohat district of Khyber Pakhtunkhwa (KPK) Pakistan. This dam is mainly used for irrigation and fisheries purpose. And the Tanda Dam Lake is mostly used as reservoir, fisheries and the picnic purpose. The dam supplies water for irrigation to Jurma, Shahpur and many villages by means of canals from Tanda Lake. The lake is home to migratory birds from Siberia and the Caspian during winter. There are two major season winter (November to March) and summer (May to September) found in this area. Peshawar is not situated in the monsoon region, unlike the other northern parts of Pakistan, but occasionally monsoon currents make it as far as Peshawar causing downpours. The winter rainfall due to western disturbances shows a higher record during the months of February and April. The highest winter rainfall has been recorded in March, while the highest summer rainfall in the month of August. Accordingly, rainfall occurs in summer and winter, respectively.

Collection of water and fish samples for heavy metal analysis

Water and fish samples (*C. carpio*) were collected during summer (April-May), monsoon (July-August) and winter (December-February) from Tanda Dam fish hatchery located in district Kohat (KPK) in April-2018 to February-2019. Water sampling was performed by rinsing the bottles three times and immersing about 10 cm below the water surface. Water samples were kept in PET (polyethylene terephthalate) bottles, which were acid-washed with 10% concentrated nitric acid HNO_3 (v/v) and rinsed thoroughly with distilled deionized water. 500 mL of water samples were taken, and samples were kept in ice box and transported to the laboratory (Öztürk *et al.,* 2009). The fish samples were collected from the Tanda dam fish hatchery were washed using deionized water and place in separated labeled polyethylene bags and kept in ice box. The samples were kept in freezer at -20°C until ready for analyses.

Sample preparation for heavy metal analysis

The water samples were filtered through 0.45 µm micropore membrane filter and acidified with concentrated HNO_3 (65%) to a pH less than 2. The samples were kept at 4°C before treatment. Approximately 9 mL of concentrated HNO_3 were added to filtered water sample and heated gently at 70°C until the solution become transparent (APHA AWWA WEF, 2005). The samples were allowed to cool and filtered using 0.45µm micropore membrane filter. The solutions were then add up with ultra-pure of water to 100 mL and analyzed for trace metal concentration.

Five (5) gram boneless muscle tissue were removed using surgical scalpels, homogenized in a blender and were digested to a strong acid digestion ($H2O_2$ + HNO_3 conc.) mixture at 1: 3 ratios (Dural *et al.,* 2010) at 150°C for 20 minutes and the samples were allowed to cool at room temperature. For Samples were then diluted to a 50 mL with ultra-pure water and filtered through 0.45 µm micropore membrane filter paper for analyses. All glassware and equipment were acid–washed before using.

Table 1. Lenght and weight of fish *C. carpio* were collected from Tanda fish Hatchery during April-2018 to February-2019.

Season	Length (cm)	Min-Max	Weight (g)	Min-Max
Summer (April to June)	12.61 ± 2.76	8.00-16.00	24.33 ± 8.00	13.00-38.00
Monsoon (July to Aug)	13.61 ± 2.24	10.50-17.80	27.33 ± 6.98	17.00-42.00
Winter (December to February)	21.75 ± 3.90	16.00-28.00	59.77 ± 18.39	34.00-86.00
Total (All Data)	15.99 ± 5.10	8.00-28.00	37.14 ± 20.15	13.00-86.00

Heavy Metal Analysis

Analyat 700 USA Atomic Absorption Spectrophotometer was used in present study from Centralized Science laboratory, University of Karachi (CSL). The absorption wave lengths (λ) used for the determination of various metals are as follows: Cu, 324.70 nm; Ni, 232.00 nm; Mn, 279.50 nm; Fe, 248.30 nm; Cd, 228.80 nm and Zn, 213.90 nm. Due to the lack of a reference standard material, accuracy of the analysis and the effect of the matrices in the media were controlled with the standard addition method. All studied elements were tested with standard addition method for 3 randomly selected samples. Approximately 20 ml filtered water and fish sample solution was taken for heavy metal analysis.

Data Analysis

One-way Analysis of Variance (ANOVA) was used to determine significant differences ($p \leq 0.05$), while posthoc Tukey's (HSD) test was used to separate means where there were significant differences.

RESULTS AND DISCUSSION

The length (cm) and weight (g) of fish samples for element analyses are given in Table 1. Metal concentrations in water and muscle tissues of *C. carpio* are presented in Tables 2 and 3, which include mean concentrations with associated standard deviation, minimum and maximum values and the results from the literature reported elsewhere.

The concentrations ranges of trace elements in water were found as follows: Cu, 0.01-0.04 µg l^{-1}; Ni, 0.00-0.02 µg l^{-1}; Mn, 0.03-0.08 µg l^{-1}; Fe, 0.02-0.08 µg l^{-1}; Cd, 0.00-0.01 µg l^{-1}; Zn, 0.02-0.06 µg l^{-1}. Metal concentrations in the Tanda Dam fish hatchery water were decreased as follows; Fe > Mn > Zn > Cu > Ni > Cd. Cu and Ni show highest accumulation in monsoon season; Mn and Zn in summer season, and Cd and Fe in winter season (Table 2).

The mean concentrations of metals in the muscle tissues of *C. carpio* are given in Table 3. The concentration ranges of trace elements in muscles tissue of *C. carpio* were found as follows: Cu: 0.34 – 2.22 µg g^{-1} ; Ni: 0.11-0.92, µg g^{-1}; Mn: 0.01 – 0.45 µg g^{-1}; Fe: 1.23 – 6.98 µg g^{-1}; Cd: 0.00 – 0.06 µg g^{-1}; Zn: 2.10 – 6.89 µg g^{-1}. Metal concentrations in muscle tissues of *C. Carpio* were found in the order as follows; Fe > Zn > Cu > Ni > Mn > Cd. The highest mean concentration of Fe (5.35 ± 0.48 µg g^{-1}) and Zn (4.25±0.73 µg g^{-1}) were found in the monsoon and winter season (Table 3).

Table 2. Heavy metal concentrations in water of Tanda Dam fish Hatchery collected during April-2018 to February-2019.

		Cu	Ni	Mn	Fe	Cd	Zn
ThisStudy							
Summer		0.023	0.009	0.087	0.029	0.006	0.069
Monsoon		0.043	0.021	0.038	0.031	0.001	0.021
Winter		0.010	0.002	0.045	0.088	0.015	0.056
All Season	Mean ± SD	0.03 ± 0.00	0.01 ± 0.00	0.06 ± 0.01	0.05 ± 0.01	0.01 ± 0.00	0.05 ± 0.01
	Min-Max	0.01-0.04	0.00-0.02	0.03-0.08	0.02-0.08	0.00-0.01	0.02-0.06
Heavy metals spectrum in water bodies inhabited by carp elsewhere							
WHO, 2017							4
Kır *et al*., 2016		0.042	0.041	BDL	0.351	BDL	0.024
Aissaoui *et al*., 2017		0.415	-	-	-	0.039	1.212
Saygı and Atasagun, 2012		0.0010	0.0034	0.0782	0.0458	-	0.0271
Benzer *et al*., 2013		-	-	-	1.492	-	-

Min: minimum levels; Max: maximum levels; SD: Standard deviation; BDL: Below Dedection Limit

In the study area, The metal concentration but insignificantly among various seasons ($F = 0.294$($p < 0.850$) and no significant difference was found in between metals and season in fish samples also - Cu ($F = 0.048$, $p < .954$), Mn ($F = 1.202$, $p < .327$), Fe ($F = 2.701$, $p < .0.100$), Cd ($F = 0.462$, $p < .657$), Zn ($F = 1.283$, $p < .306$) except Ni ($F = 21.451$, $p < .0001$). Metal accumulations in water and fish tissues showed differences with the values of metal accumulation reported in the literature (Tables 2 and 3). Metal accumulation in different fish species depends on the bio-available metal concentration in the abiotic components of their habitats, nutritional habits, ecological needs, metabolism, age and size of the fish (Ajima *et al.*, 2015).

Cu values are lower than reported data from literature (Benzer *et al.,* 2013), while Cu values are higher than reported data from some studies of the literature (Khan *et al.,* 2012; Yousafzai *et al.,* 2017). Ni values are higher than reported data from literature except Kaya and Turkoglu (2018). Mn values are higher than reported data from literature except Khan *et al.* (2012). Fe values are reported data from literature except Yousafzai *et al.* (2017). Zn values are lower than reported data from literature except Yousafzai *et al.* (2017) and Khan *et al.* (2012). (Table 3).

Table 3. Heavy metal concentrations in muscle of *Cyprinus carpio* collected from Tanda Dam fish Hatchery during April-2018 to February-2019.

		Cu	Ni	Mn	Fe	Cd	Zn
This Study							
Summer	Mean± SD	0.92 ± 0.08	0.33 ± 0.07	0.15 ± 0.06	3.55 ± 0.55	0.02 ± 0.00	3.65 ± 0.54
	Min-Max	0.69-1.23	0.11-0.67	0.02-0.45	2.21-5.89	0.00-0.06	2.33-5.79
Monsoon	Mean± SD	0.98 ± 0.26	0.51 ± 0.13	0.08 ± 0.04	3.69 ± 0.75	0.27 ± 0.00	4.25 ± 0.73
	Min-Max	0.48-2.22	0.11-0.92	0.01-0.23	1.23-6.74	0.01-0.06	2.10-6.89
Winter	Mean± SD	0.90 ± 0.23	0.52 ± 0.87	0.06 ± 0.01	5.35 ± 0.48	0.02 ± 0.00	2.95 ± 0.40
	Min-Max	0.34-1.89	0.13-0.77	0.01-0.13	3.46-6.98	0.00-0.04	2.11-4.56
All Season	Mean± SD	0.94 ± 0.11	0.78 ± 0.42	0.09 ± 0.03	4.20 ± 0.39	0.02 ± 0.00	3.62 ± 0.34
	Min-Max	0.34-2.22	0.11-0.92	0.01-0.45	1.23-6.98	0.00-0.06	2.10-6.89
Heavy metals spectrum in muscles of carp reported elsewhere							
WHO, 1989		30	-	-	100	0.5	40
FAO, 1983		30	-	-	-	-	30
Kaya and Turkoglu, 2018		-	0.221	0.331	-	0.004	-
Rajeshkumar and Li, 2018		0.21	-	-	-	0.12	-
Aissaoui *et al.,* 2017		1.307	-	-	-	0.797	6.770
Yousafzai *et al.,* 2017		0.016	-	-	0.067	-	0.018
Kır *et al.* 2016		-	1.86	0.17	17.42	0.05	10.15
Iqbal and Shah, 2014		1.293	-	0.487	9.835	0.745	24.23
Khan *et al.,* 2012		0.042	-	0.020	-	-	0.060
Benzer *et al.,* 2013		15.13	-	125.668	3577	-	13.786

Min: minimum levels; Max: maximum levels;

CONCLUSION

It can be summarized that the contents of Cu, Fe, Cd and Zn concentrations in meat of the studied samples of *Cyprinus carpio* were within the acceptable limits according to FAO and WHO. Hence, the common carp fishes collected from Tanda Dam Lake do not appear to pose any health hazards to human consumption.

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