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Tissue Specific Heavy Metals Uptake in Economically Important Fish, *Cyprinus carpio* at Acute Exposure of Metals Mixtures

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Abstract.- The main objective of this study was to check the tissue-specific accumulation of heavy metals (Pb, Co and Cd) in the form of mixtures in 180-day old *Cyprinus carpio* (common carp) during the 96-h exposure period. The four waterborne heavy metals mixtures viz., Pb-Cd, Pb-Co, Co-Cd and Pb-Co-Cd were selected for these experiments to assess the acute toxicity (in terms of 96-h LC_{50} and lethal concentrations) and bio-accumulation patterns in various tissues of *C. carpio*. Toxicities of four metals mixtures followed the order: Co-Cd > Pb-Cd > Pb-Co > Pb-Co-Cd; Co-Cd > Pb-Co > Pb-Cd > Pb-Co-Cd for 96-h LC_{50} and lethal concentration exposures, respectively. Significant differences were observed for the accumulation of metals in selected fish tissues. Amassing of heavy metals in various tissues of fish followed the order: kidney > liver > gills > skin > muscles. In conclusion, results revealed higher level of all selected metals in kidney and liver of *C. carpio* while muscles showed least tendency for accumulation. However, the overall load of metals mixtures for their accumulation in fish followed the order: Pb-Co-Cd > Pb-Co > Pb-Cd > Co-Cd. Therefore, it is necessary to control the use of heavy metals because they are posing hazardous impacts to aquatic fauna which ultimately leads to serious human health concern.

Key Words: Metals mixtures, acute toxicity, bioaccumulation, common carp.

INTRODUCTION

Heavy metals *viz*., Cu, Pb, Cr, Mn, Fe, As, Hg, Cd, Zn, Ni and Co are common river pollutant in Punjab province, causing adverse health effects on the indigenous fish fauna of Pakistan (Javed, 2012a). Environmental scientists and eco-toxicologists use the word "Heavy Metals" to characterize those metals that have caused environmental problems (Singare *et al*., 2012). Toxic effects of heavy metals have been reviewed, including their bio-accumulation in fish (Rasmussen and Anderson, 2000; Adami *et al*., 2002; Waqar, 2006). The danger of heavy metal is aggravated by their persistent behavior, because they are non-biodegradable in environment, can only be transformed from one state to another (more toxic and complex form). Because of their chemical stability, metals tend to accumulate into the different tissues of aquatic organisms (Uysal *et al*., 2008). Indiscriminate use of heavy metals in different industries has resulted an enormous production of waste water and its discharge into aquatic environment, presenting a major threat to the fish fauna of Pakistan (Hussain *et al*., 2010). Heavy metal contamination can alter the ecological balance of the recipient environment and population of aquatic organisms (Farombi *et al*., 2007), due to their toxicity and accumulative behavior.

Aquatic ecosystems are usually exposed to a mixture of heavy metals that can exhibit significant interactions among them (Bu-Olayan and Thomas, 2008; Hussain *et al*., 2010). Heavy metals exposure may modify the fish behavior, metabolism, physiology, growth and reproduction (Ali *et al*., 2003; James *et al*., 2003). Heavy metals are also known to induce genetic alterations and teratogenesis in fish (Bagdonas and Vosyliene, 2006). Aquatic organisms have specific ability to regulate essential metals up to a certain level however; this ability is disrobed under continual exposure to initiate metals accumulation in the body organs beyond their permissible limits (Jabeen *et al*., 2012). Because fishes are among the major components of aquatic habitat therefore they act as a bio indicator of heavy metals level in the aquatic environment. The main routs of heavy metals accumulation over time by fish are through the skin, gills and food (Wong *et al*., 2001). Therefore, conservation of fish in their natural environment make it essential to determine their growth potentials and ability to bio-accumulate metals

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during acute and chronic exposure of waterborne metals mixtures. This will help in formulating the strategies for sustainable conservation of fish species and to predict hazardous potential of these persistent metals in the aquatic habitats of Pakistan (Javed, 2012a). *Cyprinus carpio* (Common carp or European carp) is a widespread freshwater fish, economically important and cultured in many parts of the world (Ahmad, 2011). Carp can grow to a maximum weight of over 37.3 Kg and an oldest recorded age of 65 years. Common carp is reported to be highly resistant to aquatic pollution (Narayanan and Vinodhini, 2008). The objective of this study was to quantify the accumulation of metals mixtures *viz*., Pb-Co, Pb-Cd, Co-Cd and Pb-Co-Cd in selected tissues of *C. carpio* at acute toxicity exposures (96-h LC_{50} and lethal concentrations).

MATERIALS AND METHODS

Metals mixtures acute toxicity assay

This experiment was conducted in wet laboratory at Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan. The 180-day old fingerlings of *C. carpio* were collected from Government Fish Seed Hatchery, Faisalabad. Fish were transported to the laboratory in polythene bags with appropriate care and handling. After transportation, fingerlings were acclimatized to laboratory conditions by using dechlorinated tap water in cemented tanks for one week. During the acclimatization period, fish were fed to satiation with crumbled feed (30% digestible protein and 3.00 Kcal/g digestible energy) twice a day. However, the fish were not fed during the acute toxicity tests. Prior to start experiment, all the aquaria and glassware were thoroughly washed with water. Ten individuals of *C. carpio* with similar weights and lengths were shifted from cemented tanks to glass aquaria and exposed separately to different concentrations of each mixture treatments for the duration of 96 h. The acute toxicity assay was conducted to determine the 96-h LC_{50} and lethal concentrations of Pb-Co, Pb-Cd, Co-Cd and Pb-Co-Cd mixture for fish. Each experiment was repeated three times. Appropriate quantity of chemically pure chloride compounds of Pb, Co and Cd were separately dissolved in deionized water and stock solutions prepared for the required metal mixture preparations on ion equivalence basis for binary (1:1 ratio) and tertiary (1:1:1 ratio) mixture. For the determination of 96-h LC_{50} and lethal concentrations, the concentration of each mixture was started from zero with an increment of 0.05 and 5 mgL^{-1} (as total concentration) for low and high concentrations, respectively. However, to avoid sudden stress to experimental fish, mixture concentration in aquarium water was increased gradually and 50% test concentration was maintained within 3.5 h and total toxicant concentration in 7 h. Water temperature (30°C), total hardness (225 mg L^{-1}) and pH (7.75) of test media were kept constant during the experiment. Water temperature was maintained through electric heaters. The chemicals ***i.e.*** $CaSO_4$ and EDTA were used to increase and decrease water hardness, respectively. Dissolved oxygen of the test media was maintained with the help of aerator. During each acute toxicity trials (96-h LC_{50} and lethal concentrations), observations on fish mortality were made at 12-h intervals and dead fish were taken out from the test media and slightly blotted dry.

Bioaccumulation assay

Dead fish obtained from acute toxicity trials were disserted and their tissue samples *viz*., gills, kidney, skin, muscles and liver were separated for heavy metals analyses, rinsed with distilled water and blotted with blotting paper. These tissue samples were digested in HNO_3 and $HClO_4$ (3:1 V/V) by placing flasks on hot plate, until a clear solution was obtained (S.M.E.W.W., 1989). After this digestion, samples were cooled, diluted, filtered and then checked for respective metal concentration by using Atomic Absorption Spectrophotometer (Aanalyst-400 Perkin Elmer, USA). Calibration standards for each metal were made by serially diluting stock solutions with reagent grade water and checked standards were run along with samples.

Statistical analyses

Mean values of 96-h LC_{50} and lethal concentrations were calculated for each mixture treatment with 95% confidence intervals by using the Probit analyses method (Ezeonyejiaku and

Obiakora, 2011) with the help of MINITAB computer package. The data obtained from this experiment was statistically analyzed by using MICROSTAT package of the computer by following Steel *et al*. (1996). The data on various parameters were analyzed statistically by using the Analysis of Variance (ANOVA) and Factorial design (RCBD). The mean values were compared by using Tuckey's Student Newman-Keul test and a value of $p<0.05$ was accepted as statistically significant.

RESULTS

Acute toxicities of metals mixtures

C. carpio was tested for acute toxicity (in terms of 96-h LC_{50} and lethal concentrations) of metals mixtures *viz.*, Pb-Co, Pb-Cd, Co-Cd and Pb-Co-Cd. The data presented in Table I shows that 96-h LC_{50} and lethal concentrations of metals mixtures for *C. carpio* varied significantly at $p<0.05$.

The toxicity of four metals mixtures varied significantly for the fish. *C. carpio* showed more tolerance towards ternary mixture Pb-Co-Cd while it exhibited more sensitivity to binary mixture of Co-Cd. The mean 96-h LC_{50} of Pb-Co-Cd was 42.82±0.10 mgL^{-1} obtained with 95% confidence interval range of 36.68–47.53 mgL^{-1} while for Co-Cd mixture the LC_{50} value was calculated as 15.93±0.06 mgL^{-1} with confidence interval value of 12.88 – 17.94 mgL^{-1}, respectively. Toxicities of four metals mixtures followed the order: Co-Cd > Pb-Cd > Pb-Co > Pb-Co-Cd.

Similar to 96-h LC_{50}, *C. carpio* also showed least sensitivity towards Pb-Co-Cd mixture in terms of 96-h lethal concentration as evident from its mean value of 74.58±0.12 mgL^{-1} having confidence interval range of 66.96±88.44 mgL^{-1}. Toxicities of four mixtures varied significantly at $p<0.05$, for fish. Among four mixtures, Co-Cd showed higher toxicity towards fish followed by that of Pb-Co, Pb-Cd and Pb-Co-Cd.

Tissue specific metals bioaccumulation

Table II shows the mean values of metal accumulation in different tissues of *C. carpio* at 96-h LC_{50} and lethal concentration exposures. There were significant ($p<0.05$) differences for the accumulation of metals (Pb, Co and Cd) in various tissues of fish.

Pb-Co

At 96-h LC_{50}, exposure of Pb-Co mixture to the fish caused significant amassing of Pb in liver (78.45±0.18 μgg^{-1}), followed by that of kidney (70.26±0.11 μgg^{-1}) and gills (43.55±0.10 μgg^{-1}) while muscles exhibited least tendency to accumulate this metal. The accumulation of Co was found significantly higher (86.15±0.05 μgg^{-1}) in the kidney and lower (19.22±0.05 μgg^{-1}) in the muscles of *C. carpio*. However, under the 96-h lethal concentration exposure of Pb-Co mixture, accumulation of Pb was minimum in muscles followed by skin and gills, whearse liver (108.52±0.05 μgg^{-1}) showed higher ability to amass Pb. The concentration of Co was found higher in the kidney (98.74±0.12 μgg^{-1}) of *C. carpio*. Overall abilities of five organs revealed significantly higher accumulation of both metals (LC_{50} = 78.21±11.24 μgg^{-1}; lethal concentration = 98.88±0.20 μgg^{-1}) in kidney and lower in muscles (LC_{50} = 14.87±6.15 μgg^{-1}; lethal concentration = 16.09±6.41 μgg^{-1}). However, overall means showed that at 96-h LC_{50} exposure, accumulations of Co was higher in all selected tissues of fish than that of Pb, however, this order was inverted at lethal exposure.

Pb-Cd

The binary metals mixture of Pb and Cd caused significantly higher accumulation of both these metals in the fish kidney with the mean concentration of 78.94±0.07 and 82.22±0.16 μgg^{-1} while these were lower in muscles as 9.81±0.04 and 11.69±0.04 μgg^{-1}, respectively at 96-h LC_{50} exposure. However, at lethal concentration exposure, Pb burden in selected tissues of *C. carpio* followed the sequence: liver > kidney > gills > skin > muscles and for Cd this order was: kidney > liver > gills > skin > muscles. Regarding overall abilities of five organs to concentrate both these metals (Pb and Cd), kidney exhibited higher tendency for bioaccumulation both at LC_{50} and lethal concentration exposures. However, overall accumulation of Pb and Cd showed non-significant differences at 96-h LC_{50} exposure while at lethal concentration exposure amassing of Cd was higher

Table I.- Mean 96-h LC_{50} and lethal concentrations (mgL^{-1}±SD) of waterborne metals mixtures for the fish *Cyprinus carpio*.

Treatments	96-h LC_{50} (mgL^{-1})	95% Confidence interval (mgL^{-1})	Lethal concentrations (mgL^{-1})	95% Confidence interval (mgL^{-1})
Pb-Co	33.71±0.04 **b**	28.21 – 37.64	56.45±0.20 **c**	50.51 – 68.14
Pb-Cd	31.25±0.07 **c**	25.21 – 35.62	58.34±0.06 **b**	51.44 – 71.86
Co-Cd	15.93±0.06 **d**	12.88 – 17.94	27.82±0.03 **d**	24.80 – 34.01
Pb-Co-Cd	42.82±0.10 **a**	36.68 – 47.53	74.58±0.12 **a**	66.96 – 88.44

Means with similar letters in single column are non-significant at p<0.05.

Table II.- Metals accumulation patterns (μgg^{-1}±SD) in different tissues of *Cyprinus carpio* during the 96-h acute exposure.

	Organs					
	Gills	**Kidney**	**Skin**	**Muscles**	**Liver**	**Overall Means**
96-h LC_{50}						
Pb-Co						
Pb	43.55±0.10 **c**	70.26±0.11 **b**	35.19±0.12 **d**	10.52±0.03 **e**	78.45±0.18 **a**	**47.59±27.43 b**
Co	68.35±0.14 **c**	86.15±0.05 **a**	48.81±0.07 **d**	19.22±0.05 **e**	75.63±0.13 **b**	**59.63±26.39 a**
Means±SD	**55.95±17.54 c**	**78.21±11.24 a**	**42.00±9.63 d**	**14.87±6.15 e**	**77.04±1.99 b**	
Pb-Cd						
Pb	57.24±0.04 **c**	78.94±0.07 **a**	44.33±0.04 **d**	9.81±0.04 **e**	66.84±0.98 **b**	**51.43±26.51 a**
Cd	50.16±0.05 **c**	82.22±0.16 **a**	41.09±0.09 **d**	11.69±0.04 **e**	72.44±0.13 **b**	**51.52±27.74 a**
Means±SD	**53.70±5.01 c**	**80.58±2.32 a**	**42.71±2.29 d**	**10.75±1.33 e**	**69.64±3.96 b**	
Co-Cd						
Co	50.45±0.12 **c**	71.62±0.09 **a**	31.93±0.08 **d**	3.70±0.03 **e**	67.39±0.11 **b**	**45.02±27.91 b**
Cd	46.70±0.19 **c**	77.67±0.13 **a**	30.32±0.17 **d**	8.43±0.03 **e**	72.18±0.08 **b**	**47.06±28.90 a**
Means±SD	**48.58±2.65 c**	**74.65±4.28 a**	**31.13±1.14 d**	**6.07±3.34 e**	**69.79±3.39 b**	
Pb-Co-Cd						
Pb	52.85±0.10 **c**	86.47±0.19 **a**	47.15±0.12 **d**	13.68±0.04 **e**	71.45±0.20 **b**	**54.32±27.53 b**
Co	42.94±0.18 **d**	82.48±0.30 **a**	50.57±0.44 **c**	11.87±0.03 **e**	59.80±0.16 **b**	**49.57±25.76 c**
Cd	73.56±0.15 **c**	91.35±0.14 **a**	60.24±0.08 **d**	15.16±0.05 **e**	84.62±0.15 **b**	**64.99±30.25 a**
Means±SD	**56.45±15.62 c**	**86.77±4.44 a**	**52.65±6.79 d**	**13.57±1.65 e**	**71.96±12.42 b**	
96-h Lethal Concentrations						
Pb-Co						
Pb	70.45±0.08 **c**	99.02±0.03 **b**	67.34±0.10 **d**	11.55±0.06 **e**	108.52±0.05 **a**	**71.38±37.88 a**
Co	72.93±0.08 **c**	98.74±0.12 **a**	65.98±0.09 **d**	20.62±0.03 **e**	84.52±0.10 **b**	**68.56±29.54 b**
Means±SD	**71.69±1.75 c**	**98.88±0.20 a**	**66.66±0.96 d**	**16.09±6.41 e**	**96.52±16.97 b**	
Pb-Cd						
Pb	80.42±0.18 **c**	92.23±0.13 **b**	76.64±0.09 **d**	18.87±0.03 **e**	100.02±0.03 **a**	**73.64±32.00 b**
Cd	82.91±0.07 **c**	111.25±0.04 **a**	78.20±0.06 **d**	22.68±0.04 **e**	97.59±0.05 **b**	**78.53±33.81 a**
Means±SD	**81.67±1.76 c**	**101.74±13.45 a**	**77.42±1.10 d**	**20.78±2.69 e**	**98.81±1.72 b**	
Co-Cd						
Co	74.25±0.05 **c**	88.56±0.11 **a**	71.63±0.06 **d**	6.82±0.02 **e**	84.21±0.05 **b**	**65.09±33.31 b**
Cd	76.54±0.06 **d**	94.12±0.08 **a**	84.32±0.05 **c**	11.98±0.04 **e**	90.56±0.06 **b**	**71.50±33.94 a**
Means±SD	**75.40±1.62 d**	**91.34±3.93 a**	**77.98±8.97 c**	**9.40±3.65 e**	**87.39±4.49 b**	
Pb-Cd-Co						
Pb	80.24±0.09 **c**	95.16±0.05 **a**	68.02±0.06 **d**	15.96±0.03 **e**	83.77±0.07 **b**	**68.63±30.99 c**
Co	89.17±0.03 **d**	113.45±0.05 **a**	91.89±0.05 **c**	17.44±0.05 **e**	110.36±0.04 **b**	**84.46±38.99 b**
Cd	103.91±0.05 **c**	138.65±0.05 **a**	88.75±0.07 **d**	18.41±0.02 **e**	110.85±0.03 **b**	**92.12±45.00 a**
Means±SD	**91.11±11.95 c**	**115.75±21.84 a**	**82.89±12.97 d**	**17.27±1.23 e**	**101.66±15.50 b**	

Means with similar letters in single row and overall column are non-significant at p<0.05.

than that of Pb as evident from their overall mean values of 78.53±33.81 and 73.64±32.00 μgg^{-1}, respectively.

Co-Cd

An exposure of Co-Cd mixture caused significantly variable amassing of both these metals in tissues of fish. *C. carpio* showed significantly least tendency to accumulate cobalt in muscles as evident from its mean value of 3.70±0.03 μgg^{-1}. The 96-h LC_{50} exposure caused significantly higher accumulation of Cd in fish kidney (77.67±0.13 ugg^{-1}) followed by that of liver (72.18±0.08 ugg^{-1}) however, accumulation of this metal was significantly lower in the fish muscles (8.43±0.03 ugg^{-1}). Muscle is not an active tissue for metals accumulation, may be due to low levels of binding proteins in them. However, at lethal concentration exposure, amassing of Co followed the order: kidney > liver > gills > skin > muscles while accumulation of Cd was higher in kidney followed by that of liver, skin, gills and muscles with statistically significant differences. Among all investigated tissues, the rate of metals accumulation was found maximum in kidney and liver of exposed fish. The overall accumulation of mixture in fish body at LC_{50} and lethal concentration exposures followed the pattern: kidney > liver > gills > skin > muscles and kidney > liver > skin > gills > muscles, respectively. Results revealed that, the overall accumulation of Cd was higher both at LC_{50} and lethal concentration exposures.

Pb-Co-Cd

Regarding the tissue specific bioaccumulation of Pb, Co and Cd at LC_{50} exposure, the kidney of *C. carpio* exhibited higher ability to accumulate these metals as obvious from their mean values of 86.47±0.19, 82.48±0.30 and 91.35±0.14 μgg^{-1}, respectively. At lethal concentration exposure, load of Pb, Co and Cd in selected tissues of *C. carpio* were in the order: kidney > liver > gills > skin > muscles; kidney > liver > skin > gills > muscles and kidney > liver > gills > skin > muscles, respectively. Regarding overall tendency of five organs, kidney amassed significantly higher amounts of metals (Pb, Co and Cd) at 96-h LC_{50} and lethal concentration exposures while muscle tissue contained significantly least metals concentration. At 96-h LC_{50}, the overall metals accumulation pattern was: Cd > Pb > Co while at lethal concentration exposures this order was: Cd > Co > Pb. This order actually represents the higher load of Cd in fish during the acute exposures.

Table III.- Uptake and accumulation of metals by the fish during acute concentrations.

Metals mixtures	Acute toxicity exposures	
	96-h LC_{50} (μgg^{-1})	**Lethal concentrations (μgg^{-1})**
Pb-Co	107.23 **b**	139.93 **c**
Pb-Cd	102.95 **c**	152.16 **b**
Co-Cd	92.08 **d**	136.60 **d**
Pb-Co-Cd	168.88 **a**	245.21 **a**

Means with similar letters in single column are non-significant at $p<0.05$.

Comparison of mixtures for bioaccumulation

At 96-h LC_{50}, the exposure of four mixtures showed higher amount of Pb-Co-Cd followed by Pb-Co, Pb-Cd and Co-Cd as evident from their sum values of 168.88, 107.23, 102.95 and 92.08 μgg^{-1}, respectively in *C. carpio.* Amassing of Pb-Co-Cd mixture was also observed higher at lethal concentration exposure. However, at lethal concentration exposure overall accumulation pattern of four mixtures followed the order: Pb-Co-Cd > Pb-Cd > Pb-Co > Co-Cd (Table III).

DISCUSSION

The acute toxicities of waterborne metals mixtures *viz.*, Pb-Co, Pb-Cd, Co-Cd and Pb-Co-Cd to *C. carpio* were evaluated in terms of 96-h LC_{50} and 96-h lethal concentrations at constant temperature, total hardness and pH of water. The present investigations revealed that the toxicities of four metals mixtures varied significantly for fish. *C. carpio* showed resistance towards Pb-Co-Cd while it exhibited more sensitivity towards Co-Cd mixture as evident from their mean LC_{50} values of 42.82±0.10 mgL^{-1} and 15.93±0.06 mgL^{-1}, respectively. Sindhe and Kulkarni (2004) conducted the toxicity tests for Hg, Cd and their mixture (Hg+Cd). The order of toxicity in terms of 96-h LC_{50} was: Hg > Hg+Cd > Cd. Rugmony *et al*. (2005) observed that salmonids were more sensitive to higher level of Cd. However, when fish exposed to the mixture of Cd and Pb (Cd+Pb), the accumulation of both these metals was significantly increased. Similarly, Rashed (2001) reported that *Oreochromis niloticus* showed low tolerance against mixture of metals than a single metal. In the present findings, Co-Cd mixture showed higher toxicity towards *C. carpio* followed by that of Pb-Co, Pb-Cd and Pb-Co-Cd in terms of 96-h lethal concentrations. Khangarot (2006) observed the toxicities of Ni+Zn, Ni+Cu and Zn+Ni+Cu mixtures to *Lebista reticulatus* (freshwater teleost) and found that Ni+Cu mixture produced more than an additive toxicity. Ramesha *et al*. (1997) reported that metals in the form of mixtures exerted greater toxic stress to the developmental stages of *C. carpio* than that of individual metals. Similarly, Khangarot *et al*. (2006) also reported the mixed toxicity of Zn+Cu mixture to *C. carpio* (common carp). Their results concealed that, even the low concentration of metal mixture showed additive effects while at higher concentration a synergistic effect was pronounced. Similarly, effects of heavy metals model mixture of Cu, Ni, Zn, Cr, Cd, Pb and Mn on embryos of *Oncorhynchus mykiss* was investigated by Vosyliene *et al*. (2003). Their findings revealed that long-term exposure to the sub-lethal concentrations of heavy metals model mixture can affect the growth, development, behavior, physiology, haematology and morphology of larvae.

Bioaccumulation of heavy metals in fish can be considered as an index of metal pollution in the aquatic environment. Bioaccumulation is the ability of organisms to concentrate an element or a compound from food chain and water to a level higher than that of its environment. Bioaccumulation of metals mixtures may demonstrate the competitive, anti-competitive or non-competitive inhibition, as well as various combinations of these may exhibit enhancement of metal uptake (Eneji *et al*., 2011). Metals enter in the fish body through the contaminated food and water. After entering in fish body, metals accumulates in kidney, liver, skin, gills, fins, muscles, heart, scales, gut and brain (Rauf *et al*., 2009; Kousar and Javed, 2014). However, pattern of metals bioaccumulation in different tissues of fish varied significantly at $p<0.05$. During the present investigations, it was observed that kidney and liver accumulated higher amounts of all selected metals while muscles showed least tendency for accumulation. In most of studies, kidney acts as a target organ for the accumulation of metals. Accumulation of Cd, Hg, Pb, Cr and As were detected by Squadrone *et al*. (2013) in kidney, liver, gill and muscle of *Silurus glanis* (catfish) sampled from the Italian River. Among all selected organs the concentration of metals followed the order: kidney > gill > liver > muscle for Cd, gill > kidney > liver > muscle for Pb, muscle ≥ liver > kidney > gill > for Hg and As while sequence for Cr was: liver > kidney > muscle > gill. Liver have a significant role for the contaminant storage, detoxification, redistribution and transformation and also act as an active site of pathological effects induced by pollutants. Synthesis of metallothioneine (protein) is induced due to the elevated level of heavy metals. The difference in metals accumulation in different tissues of fish might be as a result of these metal binding proteins (Canli and Atli, 2003). Qadir and Malik (2011) observed the amassing of Pb, Cd, Cu and Cr in kidney, liver, gills and muscles of eight edible fish species *viz.*, *Cirrhina reba*, *Labeo rohita*, *Channa punctatus*, *Heteropneustes fossilis*, *Oreochromis niloticus*, *Wallago attu*, *Mystus cavasius* and *Puntlius sophore* collected from the River Chenab, Pakistan. Accumulation of metals was higher in liver followed by that of gills, kidney and muscles. However, the pattern of metals accumulation in selected tissues was: Cr>Pb>Cu>Cd. Their results revealed that the concentrations of Pb, Cd and Cr in muscles of *Labeo rohita*, *Channa punctatus*, *Oreochromis niloticus*, *Wallago attu* and *Puntlius sophore* were above the permissible limits for human consumption showing the potential health risks. Severe damage to the liver and intestinal tissues was observed in *Tilapia nilotica*, due to the contamination of surrounding environment with cadmium chloride (Younis *et al*., 2013). Tekin-Ozan and Aktan (2012) determined the higher level of metals (Cd, Co, Cr, Cu, Mn, Ni, Fe, As and Ba) in liver of *Cyprinus carpio* while this level was lowest in muscle tissues. Exposure of metals mixture (Ni + Co + Cd + Zn + Cu) caused significant accumulation of these metals in that order: Zn > Ni > Cu = Cd > Co in the fish body while the patterns of accumulation in various organs followed this sequence: liver > kidney > gills > skin > muscle > fins > bones that may caused synergistic or antagonistic toxicity to the fish (Javed, 2012a). During the present investigations, the overall burden of metals in selected tissues of fish followed the sequence: kidney > liver > gills > skin > muscles. However, among four metals mixtures, the amassing of ternary mixture i.e. Pb-Co-Cd was significantly higher at 96-h LC_{50} and lethal concentration exposure. Bioaccumulation of Pb, Cd, Zn, Cr, Cu and Ni in the liver, skin, muscles, intestine and gills of *C. carpio* were observed by Yousafzai *et al.* (2012). Overall metals burden was: Zn > Cr > Pb > Cu > Ni > Cd while organs accumulated metals at that order: intestine>skin>liver>gills>muscles. Although muscles showed least tendency to accumulate these metals but Pb, Cd and Ni in muscles exceeds the US, recommended dietary allowances. Similarly, exposure of waterborne and dietary Cu + Cd + Zn + Ni + Co mixture caused significantly higher accumulation of all these metals in fish liver, kidney and gills (Javed and Abdullah, 2004). Ahmed and Bibi (2010) have also recorded the higher accumulation of Pb in liver, skin, intestine, gills and muscles of *Catla catla*. Contrary to that, our findings showed that kidney of *C. carpio* accumulated higher amount of metals as compared to liver. In the present study gills were third in the order for metals bioaccumulation after kidney and liver. According to Reid (1990), gill surface is negatively charged therefore it provides the binding site for the positively charged metals. Skin constitute the single tissue which have maximum surface area and direct contact with exposure medium, the rate of accumulation was less when it was compared to other tissues like kidney, liver and gills. Skin is also consumed by peoples along with muscles therefore, this tissue (skin) is very important for accumulation point of view (Yousafzai and Shakoori, 2006). Skin attained fourth position in our findings but in some treatments it showed non-significant differences with muscle tissues for metals accumulation. Under the routine monitoring of environmental contaminants, muscle is the major tissue of interest because it is consumed by humans. During the present investigation, amassing of metals (Pb, Co and Cd) in muscles were minimum which is also in agreement with findings of Canli and Atli (2003), Azmat *et al*. (2006), Javed (2012b), Yousafzai *et al*. (2012) and Squadrone *et al*. (2013). Muscle is not an active tissue for metals accumulation like other organs, this least ability may be due to low levels of binding proteins in them.

Research on the deleterious effects of metals mixtures on fish is very scarce, so there was a dire need to evaluate the effects of metals which are actually present in aquatic environment in the form of mixtures. Therefore, it is necessary to compare the effects of metals in the form of mixtures, while explaining the interactions between the metals that may induce antagonistic, additive or synergistic effects (Marr *et al*., 1998). Asegbeloyin *et al*. (2010) assess the trace metals viz. Cd, Pb, As, Se and Cr in ten fish species, purchased from the Onitsha market, Nigeria. They conclude that fish samples contained all these heavy metals a little above the recommended safety standards. Similarly, the toxicities of individual metals (Cu, Pb and Zn) and their mixtures (Cu+Pb, Cu+Zn, Pb+Zn and Cu+Pb+Zn) to *Ceriodaphnia dubia* and *Daphnia carinata* were determined by Cooper *et al*. (2009). They calculate the toxic units to characterize the nature of metal interactions. Their results revealed that most of the metal interactions were showed an additive effect.

CONCLUSIONS

Water pollution due to heavy metals has created a critical problem all over the world, because their continuous discharge into the aquatic habitats may soon reach a dangerous level and will pose a serious threat to the health of local communities. Biological monitoring approach help us to predict the potential risk of persistent contaminants (heavy metals) and to formulate the ‘‘safe levels’’ of such bio-accumulative chemicals, having genotoxic potential. Acute and chronic effects of individual metals (Pb, Co and Cd) have been widely studied for different fish species, because these metals are important constituents of industrial and municipal effluents. In aquatic environment, these metals actually occur in the form of different combinations. So, it was necessary to evaluate the effects of these metals in the form of mixtures because the interactions between the several types of metals may results multiple responses. Knowledge about the concentration of metals in fish is important for the managements such as risk of using fish as part of diet and development of strategies for pollution control. The results obtained from this research clearly exposed that it is very necessary to control the use of heavy metals.

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