Relative Sensitivity of Chinese Carps (*Ctenopharyngodon Idella* and *Hypophthalmichthys Molitrix)* to Acute Exposure of Metals Mixtures

S. Naz and M. Javed

Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

The present study was conducted to measure acute toxicity (96-hr LC_{50} and lethal concentrations) of water-borne metals mixture (zinc, iron, nickel, lead and manganese) on two fish species, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* at constant pH (7), water temperature (30°C) and total hardness (200mgL^{-1}). The two fish species demonstrated inconsistent tolerance limits, while concerning 96-hr LC_{50} and lethal concentrations against 19 mixtures of five metals. The mixture of five metals (Fe+Zn+Pb+Ni+Mn) caused significantly higher toxicity to the fish, with lethal concentrations of 41.81±14.93 and 69.10±15.22 mgL^{-1}(96 hr LC_{50}), respectively. However, least toxicity to the fish was caused by Zn+Ni and Zn+Pb mixtures, with the mean LC_{50} and lethal concentrations of 81.92±7.75 and 134.45±31.79 mgL^{-1}, respectively. Regarding overall sensitivity of two fish species, *Ctenopharyngodon idella* depicted least sensitivity to metals mixtures with a mean LC_{50} and lethal concentrations of 77.86±13.90 and 122.40±23.74 mgL^{-1}, respectively. *Ctenopharyngodon idella* was less sensitive to metal mixtures compared to *Hypophthalmichthys molitrix* in the present study.

Key words: *LC_{50}, Lethal concentrations, Metals mixtures, Ctenopharyngodon idella, Hypophthalmichthys molitrix.*

INTRODUCTION

Unintended or processed spillage and relevance of sewage mire to agricultural land, dumping of industrial and urban wastes, as well as mining and smelting of natural ores, and deterioration of natural resources due to controlled or unrestrained human activities are the foremost environmental concerns in current epoch (Ghosh and Singh, 2005). Water is most imperative for mankind amongst natural resources, as its quality is in a straight line allied with human welfare (Jabeen, 2012). Together with the explosive and petroleum products, phenol, textile dyes and other perilous wastes as well as an abundant array of organic and inorganic pollutants are being frequently released into ecosystem. Due to their non-biodegradable nature, heavy metals are the main element of inorganic pollutants as they persist in environment and can enter into the body through food, air and contaminated water, (Jadhav et al., 2010). Growth abnormalities, macro-DNA damage, structural deformities mostly vertebral deformities, chromosomal aberrations, respiratory disorder, and behavioral anomalies are major toxic effects of heavy metals to fish (Golovanova**,** 2008). Mentioned reasons make it extremely essential to identify at what time they turn out to be toxic, and at what level these substances are present in nature, as they can persist for a long time, bio-magnify and bio-accumulate in aquatic ecosystem (Azmat and Javed, 2011).

Corresponding author
sam_ali_3@yahoo.com

To gauge the toxicity of metals to the fish, delicate methods are applied, as effects of pollutants on aquatic organisms can be assessed promptly by doing so (Azmat et al., 2012). Digestive and reproductive problems, slowed growth, damage in nervous system and brain can come into being due to elevated levels of lead in the fish bodies (Crandall and Goodnight, 1963; Katz, 1979). For hemoglobin and myoglobin development in fish, iron is the most vital element as it also plays an important part for the whole aquatic organism's growth. Unluckily, raised industrial wastes polluted the natural ecosystem and this nutrient (iron) enlarged at momentous contamination stage (Hussain et al., 2011). Nickel and manganese are naturally existing micro-nutrients in the aquatic environment (Hayat et al., 2007). As a trace element for electron transfer in catalytic reactions, zinc is very necessary. On the other hand, surfeit ingestion of metals chronically escort towards bioaccumulation in the body organs (Nussey et al., 2000). A grey-listed metal, nickel (Mason, 1996), for numerous animal species, is an element of less importance (Phipps et al., 2002), and if it is present in abundance, than the endurance, augmentation, behavior, and reproduction of aquatic animals is affected by (Wong et al., 1993) owing to bioaccumulation in higher trophic levels in the terrestrial ecosystem are considered. Numbers of commotions in human beings are caused by it also (Sreedevi et al., 1992), even if its effects are not likely. To assess the health of an aquatic ecosystem, fish are extensively used. Pollutants upsurge in the food chain, are liable for unfavorable effects and fatality of aquatic organisms (Farkas et al., 2002). Fish may concentrate mass quantity of metals from the water as they are frequently at the top of aquatic food chain (Mansour and Sidky, 2002).

Larger stress is placed by metal mixture on the activities and fish growth rates as compared to single metals. It was observed that the developmental stages of common carp to a greater extent, were not being affected remarkably by single metal, on the other hand a variety of alterations in its development stages caused by metal mixture (Ramesha et al., 2003). Metal mixture effect is more toxic than that of the effect of single metal and differs in its toxicity on living organisms. Specific composition, concentration and duration of exposure of metal mixture on the fish are major factors for toxicity (Javed, 2012). This requires the risk evaluation of metal mixtures on fish as toxicity of metals in a mixture form is increased due to interactions of different metals and metallic ions contest for their binding sites in an organism (Otitoloju, 2003). That is why, in line to protect freshwater fisheries in the Punjab province, it is important to disclose their lenience limits against mixtures of lead, nickel, iron, zinc and manganese under delicate exposures in the laboratory.

MATERIALS AND METHODS

At constant pH (7), water temperature (30°C) and total hardness (200mgL^{-1}), an experiment on acute toxicity (96-hr LC_{50} and lethal concentrations) of water-borne metals mixture including five metals (zinc, iron, nickel, lead and manganese) to the two fish species viz. *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*, was performed by means of static water system in glass aquaria. *Ctenopharyngodon idella* of average weight (5.01±1.67g), average fork length (71.31±12.29mm) and average total length (81.01±12.02mm) were utilized for this experiment while *Hypophthalmicthys molitrix* used, had an average weight, fork and total lengths of 4.84±11.79g, 66.07±11.57 and 75.92±11.73mm, respectively. By using a pump fixed with capillary system, fresh air was provided to all the aquaria used in the experiment. After preparing stock solutions of iron, zinc, lead, nickel and manganese, the following 19 mixtures were prepared on metallic ion equivalence basis by dissolving required amounts of various stock solutions of metals.

50 liter de-chlorinated tap water of pH (7), total hardness (200mgL^{-1}) and water temperature (30^{0}C) was filled in meticulously cleaned metal free aquaria. To conduct the acute toxicity tests (96-hr LC_{50} and lethal concentrations) against each metal mixture at constant hardness, temperature and pH, each fish species was independently tested. Each test dose was estimated by means of three replications, moreover, the fish mortalities were observed twice a day. The concentration of metal mixture inside the experimental aquaria was steadily increased from zero to 50% in 6 hours to finally 100% in 8 hours to keep away instant stress from the fish. For both LC_{50} and lethal concentration tests with each species of fish, the test concentrations for all metals mixtures and fish species were started from zero with an augmentation of 0.05 and 5 mgL^{-1} (as total concentration on metallic ion basis) for low and high concentrations, respectively. During 96 hours of each acute trial, feed was not given to the fish. To find out the acute responses throughout the period of 96 - hour, a group of 10 fish of each species were tested against metal mixture. The dead fish were separated instantly at the time of mortality whereas no mortality was observed among the control fish reared under metal free water surroundings

Mixture #	Mixture	Mixture #	Mixture
1	Fe+Pb	11	Fe+Zn+Pb
2	Fe+Ni	12	Fe+Zn+Ni
3	Fe+Mn	13	Fe+Zn+Mn
4	Fe+Zn	14	Zn+Pb+Ni
5	Zn+Pb	15	Zn+Pb+Mn
6	Zn+Ni	16	Pb+Ni+Mn
7	Zn+Mn	17	Fe+Zn+Pb+Ni
8	Pb+Ni	18	Fe+Zn+Pb+Mn
9	Pb+Mn	19	Fe+Zn+Pb+Ni+Mn
10	Ni+Mn		

The analyzed data was adequately corresponds to the required metallic ion concentrations in the test media. The estimation of 96-hr LC_{50} and lethal concentrations at 95 % confidence intervals was made by using the Probit analyses method (Hamilton et al., 1977). Analysis of variance and Tukey's /Student Newnan-Keul tests (Steel et al., 1996) were applied to observe and determine the statistical differences among the variables specific for this study.

RESULTS AND DISCUSSION

Ninety days old two fish species, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* were exposed, separately, to 19 mixtures.

i. *Ctenopharyngodon idella:* *Ctenopharyngodon idella* showed significantly variable HMM tolerance, in terms of both 96-hr LC_{50} and lethal concentration, as evident from Table 1. This fish had significantly lowest mean 96-hr LC_{50} value of 51.60±0.87 mgL^{-1} against HMM # 18 that was statistically similar to the mean value of 52.36±1.17 mgL^{-1} determined for HMM # 19 (Fe+Zn+Pb+Ni+Mn). Therefore, this fish showed significantly more sensitivity to the mixture # 18 (Fe+Zn+Pb+Mn) and 19 (Fe+Zn+Pb+Ni+Mn). This fish was significantly least sensitive to a mixture of lead and manganese (92.10±0.39 mgL^{-1}). The lethal concentrations of HMMs, varied significantly between a maximum mean value of 165.33±1.16 and a minimum of 79.86±1.22 mgL^{-1} determined for mixture # 9 and 19, respectively. This fish was significantly more sensitive to a mixture of Fe+Zn+Pb+Ni+Mn, followed by that of HMM # 18 (Fe+Zn+Pb+Mn). The difference between these two mean values was statistically significant at $p<0.05$ (Table 1).

ii. *Hypophthalmichthys molitrix:* Table 1 represents sensitivity levels of *Hypophthalmichthys molitrix* towards 96-hr LC_{50} and lethal concentrations of 19 mixtures. This fish exhibited significantly variable tolerance limits against mixtures of different metals. Fish were significantly more sensitive to five metal mixture (Fe+Zn+Pb+Ni+Mn) with the mean LC_{50} and lethal concentrations of 31.25±1.24 and 58.34±0.58 mgL^{-1}, respectively while fish were significantly least sensitive to Zn+Ni mixture (HMM # 6) with the mean LC_{50} and lethal concentrations of 76.44±0.95 and 120.14±0.95mgL^{-1}, respectively.

The overall tolerance limits of two fish species determined in terms of 96-hr LC_{50} against 19 mixtures are also presented in Table 1. While comparing the two fish species, *Hypophthalmichthys molitrix* showed significantly higher sensitivity to HMMs with a mean value of 59.63±11.01 mgL^{-1} while *Ctenopharyngodon idella* were significantly least sensitive (77.86±13.90 mgL^{-1}). The overall sensitivity of the two fish species against various HMMs varied significantly. Fish were significantly more sensitive to mixture # 19 (Fe+Zn+Pb+Ni+Mn), followed by that of 18 (Fe+Zn+Pb+Mn) with statistically significant difference while it was significantly least due to HMM # 9 (Pb+Mn).

Table 1 also shows the lethal concentrations of two fish species against 19 mixtures. *Hypophthalmichthys molitrix* were significantly more sensitive to metals mixtures with the mean metallic ion concentration of 97.85±16.20 mgL^{-1} while *Ctenopharyngodon idella* exhibited significantly least sensitivity with the mean value of 122.40±23.74 mgL^{-1}. The differences between the two fish species, for their ability to tolerate 19 mixtures, varied significantly. Fish were significantly less sensitive to HMM # 5 (Zn+Pb) while they showed significantly higher sensitivity for a mixture of five metals (Fe+Zn+Pb+Ni+Mn) with a mean lethal concentration of 69.10±15.22 mgL^{-1}, followed by that of mixture # 18 (Fe+Zn+Pb+Mn) with statistically significant difference (Table 1).

The toxicity of a variety of metals to the fish alters broadly for the purpose of their various physicochemical characteristics (Arora et al., 2012). A bulk of investigations has been performed on the toxic impacts of single metal species. However, in natural waters organisms are characteristically exposed to the mixtures of metals. Therefore, with the intention of presenting data sustaining the convenience of freshwater fish as bio-marker of heavy metal's pollution, the acute toxicity of mixture of five metals viz. iron, zinc, lead, nickel and manganese has been conducted for the two commercially imperative fish species viz. *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix.*

Static bioassay tests were executed to evaluate the acute toxicity of mixture of iron, lead, zinc, manganese and nickel, independently, on 90-day old *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix.* The present research divulges that the two fish species demonstrated significantly inconsistent tolerance limits, in terms of 96-hr LC_{50}, against mixture of five metals. Amongst five fish species, significant differences were scrutinized for their sensitivity to mixture of metals (Mebane et al., 2012; Azmat et al., 2012). Amongst 19 treatments, a mixture of five metals (Fe+Zn+Pb+Ni+Mn) caused significantly elevated toxicity to the fish, in terms of 96-hr LC_{50} and lethal concentrations. Concerning overall sensitivity of two fish species, *Ctenopharyngodon idella* were significantly less sensitive than *Hypophthalmichthys molitrix*. The present outcomes are assenting with the results of Hua and Qixing (2009). They conducted an experiment to foretell the secluded and collective effects of metals (Cd and Zn) on grass carp (*Ctenopharyngodon idella*). The calculated LC_{50} (96-hr) values signified that acute toxicity of zinc was significantly lower than cadmium. The 96-hr LC_{50} of cadmium and zinc were 26.86 and 33.14 mgL^{-1}, respectively. Metals mixture revelation for 96 hours was synergistic while it was antagonistic due to 48 hour exposure duration. Acute techniques allow us to assess swiftly the effects of toxicants on the organisms. The decisive factor of lethal toxicity is mortality, the ultimate response of an organism to a toxic effect. With reference to these processes, the sensitivity of organisms of diverse phylogenetic ranks and a range of developmental stages to toxicants has been frequently compared (Bellas et al., 2001; Abdullah and Javed, 2006; Javed and Abdullah, 2006; Azmat, 2011; Kakinen et al., 2011). Jackson et al. (2005) evaluated the acute toxicity of heavy metal mixture (Zn+Pb) to *Callianassa Kraussi* was in hard water. The mixture of zinc and lead emerged out as more toxic than the individual metals with appreciably additive effects on fish tolerance limits (Mebane et al., 2012).

Significant variations in the tolerance limits of three fish species (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*), were determined by their lethal concentrations against metals mixture (Hussain et al., 2011; Azmat and Javed, 2011). The overall sensitivity of the two fish species against various HMMs varied significantly. Fish were significantly more sensitive to mixture # 19

Table 1 Tolerance limits of two fish species, as 96-hr LC_{50} and lethal concentrations (μgL^{-1}), against various metals mixtures.

Mixtures #	Mean 96-hr LC_{50} (μgL^{-1})			Mean lethal concentrations (μgL^{-1})		
	C. idella	*H. molitrix*	Overall Species Means	*C. idella*	*H. molitrix*	Overall Species Means
Mixture 1 (Fe+Pb)	82.29±0.98^{f} (75.55-88.15)	58.34±0.44^{g} (52.23-63.52)	70.32±16.94^{i}	126.65±1.03^{f} (116.57-143.49)	96.42±0.41^{g} (88.01-110.18)	111.54±21.38^{i}
Mixture 2 (Fe+Ni)	89.80±0.77^{b} (81.69-96.36)	52.16±0.58^{i} (45.24-57.58)	70.98±26.62^{i}	135.25±1.42^{cd} (125.04-152.36)	87.09±0.09^{jk} (78.13-104.16)	111.17±34.05^{i}
Mixture 3 (Fe+Mn)	84.60±0.68^{e} (77.23-91.21)	59.47±0.64^{g} (50.91-66.50)	72.04±17.77^{h}	131.19±0.46^{e} (119.90-150.53)	111.27±0.84^{d} (98.34-135.51)	121.23±14.09^{d}
Mixture 4 (Fe+Zn)	91.20±1.42^{ab} (83.75-97.41)	55.06±0.99^{h} (48.47-60.73)	73.13±25.55^{g}	135.04±0.94^{d} (123.82-156.25)	94.79±0.79^{h} (85.29-111.13)	114.92±28.46^{g}
Mixture 5 (Zn+Pb)	81.51±0.65^{f} (69.46-93.38)	71.81±0.23^{b} (65.70-77.06)	76.66±6.85 ^{d}	156.93±0.96^{b} (134.04-205.99)	111.97±1.01^{cd} (103.24-126.08)	134.45±31.79^{a}
Mixture 6 (Zn+Ni)	87.40±0.29^{c} (79.39-94.01)	76.44±0.95^{a} (69.82-82.12)	81.92±7.75^{b}	136.84±0.21^{c} (124.95-158.30)	120.14±0.95^{a} (110.46-136.14)	128.49±11.81^{b}
Mixture 7 (Zn+Mn)	86.47±0.69^{c} (78.69-92.91)	64.18±1.12^{e} (57.13-70.46)	75.33±15.76^{e}	135.61±0.86^{cd} (124.70-153.92)	107.09±0.98^{e} (96.52-125.38)	121.35±20.17^{d}
Mixture 8 (Pb+Ni)	86.36±0.38^{cd} (78.66-92.86)	66.65±0.65^{d} (61.05-71.66)	76.51±13.94^{d}	136.33±0.54^{cd} (124.57-157.02)	102.48±1.46^{f} (94.15-116.30)	119.41±23.94^{e}
Mixture 9 (Pb+Mn)	92.10±0.39^{a} (81.12-103.00)	69.04±0.94^{c} (60.57-75.73)	80.57±16.31^{c}	165.33±1.16^{a} (144.55-206.00)	18.53±0.61^{b} (106.59-140.65)	91.93±103.80^{l}
Mixture 10 (Ni+Mn)	91.92±1.10^{a} (86.51-97.14)	76.28±1.23^{a} (70.17-81.46)	84.10±11.06^{a}	132.65±0.78^{e} (124.45-144.36)	113.30±1.36^{c} (104.54-128.41)	122.98±13.68^{c}
Mixture 11 (Fe+Zn+Pb)	86.85±0.69^{cd} (78.65-93.46)	62.57±0.45^{f} (56.91-67.32)	74.71±17.17^{f}	134.78±0.87^{d} (123.19-155.91)	95.47±1.09^{gh} (87.75-108.66)	115.13±27.80^{f}
Mixture 12 (Fe+Zn+Ni)	71.63±1.14^{g} (63.06-78.49)	56.22±1.08^{h} (49.16-61.77)	63.93±10.90^{k}	114.92±1.02^{h} (103.12-138.63)	90.91±0.39^{i} (82.47-105.87)	102.92±16.98^{j}
Mixture 13 (Fe+Zn+Mn)	84.91±0.99^{de} (78.03-90.39)	64.85±0.36^{e} (58.57-70.32)	74.88±14.18^{f}	123.92±0.90^{g} (114.71-140.37)	102.54±0.89^{f} (92.86-120.34)	113.23±15.12^{hi}
Mixture 14 (Zn+Pb+Ni)	72.41±0.46^{g} (64.15-78.91)	59.47±0.60^{g} (50.91-66.50)	65.94±9.15^{j}	115.50±069^{h} (104.46-136.65)	111.27±0.56^{d} (98.34-135.51)	113.39±2.99^{hi}
Mixture 15 (Zn+Pb+Mn)	56.52±0.77^{j} (48.42-62.24)	53.54±1.50^{i} (47.06-58.91)	55.03±2.10^{n}	91.82±0.79^{j} (83.02-108.88)	86.23±0.97^{k} (77.92-101.05)	89.02±3.95^{m}
Mixture 16 (Pb+Ni+Mn)	64.45±0.94^{h} (57.46-70.01)	58.66±0.58^{g} (51.24-64.49)	61.56±4.09^{l}	96.36±0.44^{i} (88.06-111.55)	95.25±1.12^{gh} (86.28-111.29)	95.80±0.78^{k}
Mixture 17 (Fe+Zn+Pb+Ni)	62.00±0.99^{i} (55.45-67.04)	55.60±0.80^{h} (49.12-60.73)	58.80±4.52^{m}	91.58±0.85^{j} (83.81-106.41)	88.12±0.66^{j} (79.83-103.62)	89.85±2.44^{m}
Mixture 18 (Fe+Zn+Pb+Mn)	51.60±0.87^{k} (45.80-56.41)	41.45±1.03^{j} (36.59-45.61)	46.53±7.17^{o}	85.36±0.90^{k} (77.53-98.72)	67.91±0.80^{l} (61.48-78.78)	76.63±12.34^{n}
Mixture 19 (Fe+Zn+Pb+Ni+Mn)	52.36±1.17^{k} (47.17-56.67)	31.25±1.24^{k} (25.21-35.62)	41.81±14.93^{p}	79.86±1.22^{l} (73.05-91.88)	58.34±0.58^{m} (51.44-71.86)	69.10±15.22^{o}
***Overall Means (Treatments)**	**77.86±13.90^{a}**	**59.63±11.01^{b}**		**122.40±23.74^{a}**	**97.85±16.20^{b}**	

(Means with similar Superscripts in a single column and row* are statistically non-significant at $p < 0.05$)

The values within brackets are the 95% confidence intervals (mgL^{-1}) *C. idella*= *Ctenopharyngodon idella* ; *H. molitrix*= *Hypophthalmichthys molitrix*

(Fe+Zn+Pb+Ni+Mn), followed by that of 18 (Fe+Zn+Pb+Mn) with statistically significant difference while it was significantly least due to HMM # 9 (Pb+Mn). The comparative sensitivity of two fish species viz. *Salvelinus confluentus* and *Oncorhynchus mykiss* to zinc and cadmium in single and binary form was determined with the help of paired bioassay. *Salvelinus confluentus* were almost 50 % more tolerant to zinc and twice as tolerant of cadmium as compared to *Oncorhynchus mykiss*. Cadmium caused toxicity equal to that of Cd+Zn mixture, to *Oncorhynchus mykiss* while the toxic effect of Cd+Zn mixture to *Salvelinus confluentus* was higher than cadmium alone (Hansen et al., 2002). Zhou et al. (2011) evaluated the acute toxicity of metallic ions (copper, zinc and cadmium) independently or in mixture form for Chinese minnow (*Gobiocyprius rarus*). Fish showed dissimilar sensitivity to heavy metals that relies on developmental stages and duration of exposure. Synergistic lethal effects were exhibited by Cu+Cd and Cu+Zn mixtures. The improved toxicity of fish implied insertion of metals mixture contemplations in risk evaluation of heavy metals.

Conclusions: In conclusion, the two fish species were significantly more sensitive to mixture # 19 (Fe+Zn+Pb+Ni+Mn), followed by that of 18 (Fe+Zn+Pb+Mn) with statistically significant difference. Of the two fish species, *Ctenopharyngodon idella* were significantly least sensitive towards the 19 metals mixtures both in terms of LC_{50} and lethal concentrations.

Acknowledgements: The author is extremely grateful to the **Pakistan Science Foundation** for giving grant under the research project No.85 (Env) to complete this work as a part of PhD thesis. She is also thankful to the co-authors for their great help and cooperation.

REFERENCES

Abdullah, S., and M. Javed. 2006. Studies on 96-hr LC_{50} and lethal toxicity of metals to the fish, *Cirrhina mrigala*. *Pakistan Journal of Agrcultural Sciences*, 43: 180-185.

Arora, S., M. Jyutika, K. Rajwade, and M. Paknikar. 2012. Nanotoxicology and In Vitro Studies: The Need of the Hour. A Review. *Toxicology and Applied Pharmacology*, 258: 151-165.

Azmat, H. 2011. Studies on 96-hr LC_{50} and lethal responses of major carps in relation to their growth and bioaccumulation of waterborne metals. PhD Thesis, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan.

Azmat, H., and M. Javed. 2011. Acute toxicity of chromium to *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* under laboratory conditions. *International Journal of Agriculture and Biology*, 13: 961-965.

Azmat, H., M. Javed, and G. Jabeen. 2012. Acute toxicity of aluminium to the Fish (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*). *Pakistan Veterinary Journal*, 31: 85-87.

Bellas, J., E. Vazquez, and R. Beiras, 2001. Toxicity of Hg, Cu, Cd, and Cr on early developmental stages of *Ciona intestinalis* (Chordata, Ascidiacea) with potential application in marine water quality assessment. *Water Research*, 35: 2905-2912.

Crandall, C.A. and C.J. Goodnight, 1963. The effects of sublethal concentrations of several toxicants to the common guppy, *Lebistes reticulates*. *Transactions of the American Fisheries Society*, 82: 59-65.

Farkas, A., J. Salanki, and A. Specziar. 2002. Relation between growth and the heavy metal concentration in organ of bream *A. brama* L. populating Lake Balaton. *Archives of Environmental Contamination and Toxicol*ogy, 43: 236-243.

Ghosh, M., and S. P. Singh. 2005. A Review on phytoremediation of heavy metals and utilization of its byproducts. *Applied Ecology and Environmental Research*, 3: 1-18.

Golovanova, I.L. 2008. Effects of heavy metals on the physiological and biochemical status of fishes and aquatic invertebrates. *Aquatic Toxicology*, 1: 93-101.

Hamilton, M.A., R.A.C. Russo, and R.V. Thurston. 1977. Trimmed spearman-karber method for estimating median lethal concentrations in toxicity bioassays. *Environmental Science and Technology*, 11: 714-719.

Hansen, J.A., P.G. Welsh, J. Lipton, D. Cacela, and A.D. Dailey. 2002. Relative sensitivity of bull trout (*Salvelinus confluentus*) and rainbow trout (*Onchorhynchus mykiss*) to acute exposures of cadmium and zinc. *Environmental Toxicology and Chemistry*, 21: 67-75.

Hayat, S., M. Javed, and S. Razzaq. 2007. Growth performance of metal stressed major carps viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* reared under semi-intensive culture system. *Pakistan Veterinary Journal*, 27: 8-12.

Hua, T. and Z. Qixing. 2009. Joint toxic effect of Cd and Zn on grass carp (*Ctenopharyngodon idell*a) and SOD Activity. *Acta Scientific Curriculum*, 29: 312-319.

Hussain, S.M., M. Javed, A. Javid, T. Javid, and N. Hussain, 2011. Growth responses of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* during chronic exposure of iron. *Pakistan Journal of Agricultural Sciences*, 48: 225-230.

Jabeen, G. 2012. Studies on fish species specific metals bio-accumulation patterns in relation to water, sediments and plankton in the river Ravi. PhD Thesis, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan

Jackson, R.N., D. Baird, and S. Els. 2005. The effect of the heavy metals lead (Pb^{2+}) and zinc (Zn^{2+}) on the brood and larval development of the burrowing crustacean, *Callianassa kraussi. Water South Africa,* 31: 107-116.

Jadhav, G.G., D.S. Salunkhe, D.P. Nerkar, and R.K. Bhadekar. 2010. Isolation and characterization of salt-tolerant nitrogen-fixing microorganisms from food. *Journal of Europe and Asian Biological Sciences*, 4: 33-40.

Javed, M. 2012. Effects of metals mixture on the growth and their bioaccumulation in juvenile major carps. *International Journal of Agriculture and Biology*, 14: 477-480.

Javed, M. and S. Abdullah. 2006. Studies on the acute and lethal toxicities of iron and nickel to the fish. *Pakistan Journal of Biological Sciences*, 9: 330-335.

Kakinen A., O. Bondarenko, A. Ivask, and A. Kahru. 2011. The effect of composition of different ecotoxicological test media on free and bioavailable copper from $CuSO_4$ and CuO nanoparticles: Comparative evidence from a Cu-selective electrode and a Cu-biosensor. *Sensors*, 11: 10502-10521.

Katz, M. 1979. The effects of heavy metals on fish and aquatic organisms in metal pollution in aquatic environments. Forstner, V. and Wittman, G.T., (Editors), Springer-Verlag, Berlin. p. 25.

Mansour, S.A., and M.M. Sidky. 2002. Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. *Food Chemistry*, 78: 15-22.

Mason, C.F. 1996. Biology of freshwater pollution. 3rd Ed. Longman, London, pp. 1-4.

Mebane, C.A., F.S. Dillon and D.P. Hennessy. 2012. Acute toxicity of cadmium, lead, zinc, and their mixtures to stream- resident fish and invertebrates. *Environmental Toxicology and. Chemistry*, 31: 1334-1348.

Nussey, G., J.H.J. VanVuren, and H.H. DuPreez. 2000. Bio-accumulation of chromium, manganese, nickel and lead in the tissues of the moggel, *Labeo umbratus* (Cyprinidae) from Witbank Dam, Mpumalanga. *Water South Africa*, 26: 269-284.

Otitoloju, A.A. 2003. Relevance of joint action toxicity evaluations in setting realistic environmental safe limits of heavy metals. *Journal of Environmental Management*, 67: 121-128.

Phipps, T., S.L. Tank, J. Wirtz, L. Brewer, A. Coyner, L.S. Ortego, and A. Fairbrother. 2002. Essentiality of nickel and homeostatic mechanisms for its regulation in terrestrial organisms. *Environmental Review*, 10: 209-261.

Ramesha, A.M., T.R.C. Gupta, L. Chandrakanth, K.V. Kumar, G. Gangadhara, and K.S. Udapa. 2003. Combined toxicity of mercury and cadmium to the common carp (*Cyprinus carpio*). *Environmental Ecology*, 15: 194-198.

Sreedevi, P., B. Sivarmakrishna, A. Suresh and K. Radhakrishnaiah. 1992. Effect of nickel on some aspects of protein metabolism in the gill and kidney of the fresh water fish, *Cyprinus carpio*. *Environmental Pollution*, 70: 59-63.

Steel, R.G.D., J.H. Torrie, and D.A. Dinkkey. 1996. Principles and procedure of statistics. (3rd Ed.). McGraw Hill Book Co., Singapore. p. 627.

Wong, C.K., K.H. Chu, K.W. Tang, T.W. Tam, and L.J. Wong. 1993. Effects of chromium, copper and nickel on survival and feeding behaviour of *Metapenaeus ensis* larvae and post larvae (Decapoda: Penaeidae). *Marine Environmental Research,* 36: 63-78

Zhou, B., W. Zong-Fan, L. Jun, and W. Gao-Xue. 2011. Single and joint action toxicity of heavy metals on early developmental stages of chinese rare minnow (*Gobiocypris rarus*). *Aquatic Toxicology*, 105: 41-48.