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Assessment of heavy metals in *Catla catla* reared under different treated waste water dilutions at water and sanitation agency (WASA) treatment plant chokera, Faisalabad, Pakistan

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Abstract

Accumulation of heavy metals in fish organs of *Catla catla* was assessed after rearing them in different waste water dilutions i.e. 20%, 40%, 60%, 80%, 100% at Water and Sanitation Agency (WASA) Treatment Plants Chokera, Faisalabad, Pakistan. Each aquarium was filled with respective concentration. One aquarium was kept as control and no waste water was added to it. A Single breed of *Catla catla* was kept in respective aquaria maintaining a constant temperature (30°C) and pH (7.00). After the 90 days trial, the fish from each aquarium was dissected and tissue samples were digested in aqua regia for the analysis of heavy metals under atomic absorption spectrophotometer. The results indicated that the order of accumulation of heavy metals was Zn> Co> Cu> Ni> Cd. It was noticed that metal accumulation in various organs of fish varies significantly, ranging from highest in the liver to the lowest in muscles and fins i.e. metals accumulated in pattern: liver> kidney > gills bones > skin> muscles > fins. The amount of metal accumulation also depends on the concentration of waste water made for the experimental trial. The maximum amount of heavy metals was accumulated in 100% waste water and the least amount of heavy metal was accumulated in control medium which was devoid of waste water.

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Introduction

Being a critical life sustaining factor, the quality of water concerns a lot towards the economy as well as environmental issue in the developing countries (Utangand Akpan, 2012). Pakistan's current population of about 160 million, impacts directly on the water sector for meeting the domestic, industrial and agricultural needs. In such a crucial condition, the water supply planners must find new ways and means to meet the water supply demand (Crafford and Avenant-Oldewage, 2010).Water reuse is an important way to be adopted to fulfill the water supply demand. This process consists of wastewater treatment and water recycles (Madaeni and Ghanei, 2006).

Water that has been contaminated by anthropogenic resources is termed as wastewater. it usually comprises of liquid waste (produced by domestic, commercial, industrial and/or agricultural activities) having much amounts of potential contaminants and concentrations (Modasiya *et al.*, 2013) of which heavy metals are of greatest concern because they damage the physiological, biochemical and cellular processes of both plants and animals, thus deteriorating the life sustaining quality of water (Pallavi and Neera, 2006; Semsettin *et al.,* 2007; Shukla *et al.,* 2007; Yoon *et al.,* 2008). Many metals are the natural components of freshwater environments. Of which some are important for life, others are necessary for life but still others are harmful to aquatic life. The concentrations, at which the role of metals may be considered significant, vary; as some are essential in low concentrations while others show toxicity at higher concentrations (Javed, 2004). Some of the metals like Cu, Fe, Mn, Ni and Zn are needed as micronutrients for metabolism in plants and microorganisms, while many other metals like Cd, Cr and Pb have no known physiological activity, but they prove to be harmful beyond a certain limit (Bruins, *et al.* 2000), which is very much narrow for elements like Cd (0.01 mg/L), Pb (0.10 mg/L) (ISI, 1982) and Cu (0.050 mg/L) (Kar *et al.,* 2008).

Heavy metals are generally not removed even after the treatment of wastewater at sewage treatment plants and cause the risk of heavy metal contamination in the food chain (Fytianos *et al.,* 2001). Fish are on the top of aquatic food chain. These are constantly exposed to chemicals and heavy metals in polluted and contaminated waters. Heavy metals are very harmful due to their non-biodegradability, solubility, bioaccumulation and long half-life (Choudhary *et al.,* 2012). Even at low concentrations, heavy metals have damaging effects to man and animals as they are not eliminated from the body. Studies have also indicated that fish can accumulate and retain heavy metals from their environment depending upon their exposure concentration and duration as well as other factors such as salinity, temperature hardness and metabolism of the animals (Karthikeyan *et al.,* 2007). The main effect of heavy metals is on growth performance of fish, decreased survival and reproductive Intake of heavy metals through the food chain by human populations has been widely reported throughout the world (Muchuweti *et al.,* 2006). Fish bio-accumulate heavy metals from water and diet, and contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in the water, sediment and food (Labonne *et al.*, 2001; Goodwin *et al.,* 2003; Osman *et al.,* 2007). The aim of this study is to assess the potential viability of accumulation of heavy metals in the organs of *Catla catla* by using different dilutions of waste water taken from Wastewater Treatment Plants of Water and Sanitation Agency, Faisalabad.

Materials and methods

For the "Assessment of heavy metals in different organs of *Catla catla* reared under treated waste water dilutions", an experiment was conducted at Water and Sanitation Agency (WASA) Sewage Stabilizing Treatment Plant at Chokera, Faisalabad. Fingerlings of C*atlacatla* were acclimatized for one week by keeping under laboratory conditions prior to the start of experiment. During that period, fish was fed, to satiation, a control diet (35 % digestible protein and 2.90 Kcalg^{-1} digestible energy) twice a day.

After acclimation, wet weights, fork and total lengths were measured and transferred to aquarium at the ratio of 10 fishes per aquarium, having 50-liter water capacity. In each aquarium, constant air was supplied through the experimental period of 90-days with an air pump through capillary system. Each growth trial consisted of three replications. Ordinary tap water was kept as a medium for control fish. *Catla catla* was exposed to different treated wastewater dilutions and the effect of heavy metals on it was determined by monitoring the growth performance. The desired pH (7.00) and temperature (30˚C) were maintained throughout the experimental period. The water quality parameters viz. dissolved oxygen, carbon dioxide, sodium, potassium, calcium, magnesium, total ammonia and electrical conductivity were monitored throughout the experimental period on daily basis by following the methods of APHA (1989). To maintain the desired pH value of the test mediums, NaOH (to increase pH) and HCl (to decrease pH) were added as required. Electric heaters were used in each aquarium to maintain the water temperature. The increase/decrease in fish weights, fork and total lengths and feed conversion ratio (FCR) were monitored weekly. The length-weight relationships and condition factor of each fish species were also calculated. Fish was fed to satiation twice a day with the feed (2.90 kcalg^{-1} digestible energy and 35% protein). During 90-day exposure of fish to treated wastewater dilutions, the growth performance of C*atla catla* (+ or -) in terms of wet weights, fork and total lengths, condition factor (K), feed intake, feed conversion ratio (FCR) was evaluated on weekly basis. After obtaining the data, fish was released back into their respective aquarium. The test medium was changed on weekly basis to maintain the desired wastewater dilution level, metal concentrations and availability of fish food organisms (planktons).

The water quality parameters viz. water temperature, pH, dissolved oxygen, total hardness, calcium, magnesium, sodium, potassium, total ammonia, carbon dioxide and electrical conductivity were monitored throughout the trial by following the methods devised by APHA (1998).

Determination of heavy metals in fish and water

The organs of fish and water samples were digested in acids. Their volumes were prepared by following SMEWW (1989). After that, the concentrations of copper, cadmium, cobalt, nickel and zinc were determined by running the fish and water samples through Atomic Absorption Spectrophotometer.

Determination of Physico-chemical Parameters

Water temperature and pH was observed using electronic multi-meter (HI-9828: HANNA). Sodium, potassium and calcium were determined with the help of Flame Photometer (PFPI) by using the method Nos. -10a and -11a of "Hand Book-60" respectively (Richards, 1954). Calcium was estimated according to standard methods of APHA (1998). At the start and end of each trial, water samples from each aquarium were taken and tested for the determination of metal concentrations through the methods of APHA (1998) by the Atomic Absorption Spectro-photometer, (Analyst 400).

Statistical Analyses of Data

The data obtained was analyzed statistically by following the methods (Steel *et al.,*1996) through microcomputer (Minitab version 15). The comparison of mean values of parameters and interactions were computed by using ANOVA (Analysis of Variances) and Duncan's Multiple Range tests. To find out relationships among various parameters understudy, regression and correlation analyses were performed.

Results

Accumulations of heavy metals in different organs of fish

The fish accumulates high concentrations of heavy metals depending upon the exposure of heavy metal to the fish and the organ of fish. The higher the concentration of wastewater, the higher is the metal accumulated in the different organs of fish. After the experimental trial, it was noted that amount of metals accumulated in various organs vary significantly, ranging from highest accumulation in liver to that of muscles and gills. The highest amount of metal accumulated is 10.91±10.24 (Zn) in the liver and the lowest amount accumulated is noticed in control media which is not exposed to heavy metal concentration. The order of accumulation of metals is as follows: Zn> Co> Cu> Ni> Cd. The order of organs in which these metals are accumulated is as follows: liver >kidneys> gills> bones> skin> muscles> fins (Table 1-5). Table 1 shows that copper was most accumulated in the liver followed by kidneys and gills respectively. The accumulation was higher most in the treatment 5 containing 100% wastewater, while the metal was least accumulated in control fish.

Table 1. Accumulation of copper (µgg-1) in *Catla catla* during 12-week growth trial.

	Kidney	Liver	Gills	Skin	Fins	Muscle	Bones	*Overall Mean (±S.D)
T1	4.05±0.09b	5.11±1.02a	3.09±0.51c	2.11±0.15d	1.87±0.16f	1.77±0.22g	2.00±0.17e	2.86±1.29e
T2	6.12±0.07b	7.21±1.25a	3.56±0.46c	2.88±0.57d	2.09±0.12f	2.13±0.17e	2.04±0.13f	3.72±2.11d
T3	9.72±1.09b	10.93±1.67a	5.27±1.18c	3.53±0.69d	2.78±1.13e	2.32±0.29g	2.41±0.31f	5.28±3.60c
T4	12.11±1.50b	14.12±2.05a	7.19±1.09c	4.31±0.55d	3.06±1.07f	2.91±0.18g	3.90±0.53e	6.80±4.58b
T5	15.19±1.41b	19.12±2.42a	9.14±1.10c	5.47±0.81d	3.46±1.15f	3.05±0.14g	4.09±0.29e	8.50±6.35a
Control	2.83±0.45b	3.21±0.27a	2.41±0.19c	1.17±0.03d	1.29±0.04f	1.29±0.06f	1.93±0.09e	2.02±0.82f
Overall Mean (±S.D)	8.34±4.83b	9.95±5.98a	5.11±2.62c	3.25±1.54d	2.43±0.81f	2.25±0.67g	2.73±1.00e	

(Means with similar letters in a single row and * column are statistically similar at $p<0.05$).

Table 2. Accumulation of cadmium (µgg-1) in *Catla catla* during 12-week growth trial.

	Kidney	Liver	Gills	Skin	Fins	Muscle	Bones	*Overall Mean (±S.D)
T1	3.17±0.82b	5.12±0.42a	2.09±1.10d	2.48±0.18c	1.15±0.27f	1.03±0.19g	1.75±0.38e	2.40±1.41e
T2	5.44±0.93b	7.14±1.16a	3.89±1.44d	2.72±0.31c	1.40±0.39f	1.29±0.11g	2.22±0.37e	3.44±2.19d
T3	8.37±1.42b	10.28±1.81a	6.43±1.73d	2.98±0.43c	1.52±0.29g	1.49±0.21f	2.81±0.28e	4.84±3.52c
T4	11.07±1.21b	13.39±1.28a	9.29±1.91d	3.04±0.73c	1.74±0.18f	1.70±0.28f	3.06±0.21e	6.18±4.92b
T5	14.92±1.19b	16.90±1.31a	12.15±2.27d	3.19±1.09d	1.97±0.79e	1.91±0.69e	3.41±0.13c	7.78±6.60a
Control	1.90±0.68b	2.29±0.71a	0.92±0.51d	2.22±0.49c	0.61±0.19e	0.67±0.21e	1.10±0.22d	1.39±0.73f
Overall Mean (±S.D)	7.48±4.96b	9.19±5.42a	5.80±4.34c	2.77±0.37d	1.40±0.48e	1.35±0.45f	2.39±0.87e	

(Means with similar letters in a single row and * column are statistically similar at $p<0.05$).

Same trend was observed in the case of metals like cadmium, cobalt, nickel and zinc. i.e the liver accumulated the highest amount of metals and the metal was least absorbed by muscles and fins (Table 2-5).

Table 3. Accumulation of cobalt (µgg-1) in *Catla catla* during 12-week growth trial.

	Kidney	Liver	Gills	Skin	Fins	Muscle	Bones	*Overall Mean (±S.D)
T1	3.11±0.22b	4.31±0.77a	3.07±0.18c	1.10±0.07e	1.09±0.03f	1.25±0.12d	1.15±0.06e	2.15±1.32e
T2	7.18±1.43b	8.09±1.46a	7.42±1.17c	1.93±0.21e	1.95±0.22e	2.29±0.16d	1.91±0.32e	4.40±2.98d
T3	10.03±1.71b	12.31±1.45a	11.18±1.42c	2.15±0.37g	2.51±0.31f	3.08±0.18e	3.87±0.71d	6.45±4.50c
T4	14.21±2.07b	16.22±2.11a	13.19±1.51c	3.71±0.22g	3.82±0.34f	4.27±0.11e	5.62±0.41d	8.72±5.55b
T5	18.09±2.12b	21.91±2.81a	15.22±1.72c	4.39±0.32f	4.17±0.28g	5.10±0.08e	7.31±0.53d	10.88±7.3a
Control	2.01±0.29b	2.19±0.09a	0.72±0.07c	0.51±0.11d	0.45±0.08e	0.33±0.09f	0.47±0.09e	0.95±0.79f
Overall Mean (±S.D)	9.11±6.29b	10.84±7.47a	8.47±5.75c	2.30±1.49f	2.33±1.47f	2.72±1.80e	3.39±2.69d	

(Means with similar letters in a single row and * column are statistically similar at $p<0.05$).

Discussion

This experiment was conducted for the assessment of heavy metals on *Catla catla* reared under treated waste water dilutions. During the course of this experiment it was observed that waste water contains many pollutants in which heavy metals are of due significance. These are not completely removed from water even after its treatment. The heavy metals were accumulated in various organs of fish depending upon the waste water concentrations.

Similar results were reported by Kyncl *et al.* (2008), showing that the industrial activities are the source of heavy metals penetrating the environment. The heavy metals are contained in many products and are a part of many waste substances. In the same way, Kadatskaya (2006) showed that wastewater discharged without adequate treatment into surface waters has affected water bodies have created some problems with water resource use. Njoku *et al.* (2012) assessed sewage sludge disposal sites in Abakaliki Southeastern Nigeria for selected heavy metals (Sn, Cu, Fe and Zn). Results showed higher values of heavy metals in sewage sludge disposal sites than non-disposal site. Ada *et al.* (2007) determined that the presence of heavy metals in wastewater is one of the main causes of water and soil pollution. Wang *et al.,* 2005, showed that the contents of Zn and Cu were the highest in sewage sludge, followed by then Cr, Ni and Pb and the content of Cd was the least in it.

Table 4. Accumulation of nickel ($\mu g g^{-1}$) in *Catla catla* during 12-week growth trial.

	Kidney	Liver	Gills	Skin	Fins	Muscle	Bones	*Overall Mean (±S.D)
T1	3.42±0.08b	4.17±0.10a	2.07±0.09c	1.04±0.38d	0.96±0.07e	0.95±0.07e	0.98±0.06e	1.94±1.34e
T2	5.21±0.35b	6.19±0.18a	4.18±0.92c	1.63±0.12d	1.08±0.03f	1.02±0.04f	1.99±0.04e	3.04±2.12d
T3	8.17±1.05b	10.07±1.27a	7.26±1.12c	2.81±0.19d	1.22±0.07f	1.21±0.06f	2.43±0.06e	4.74±3.66c
T4	12.13±1.11b	13.09±1.34a	10.07±1.18c	3.72±0.34d	1.30±0.08f	1.35±0.11f	3.66±0.11e	6.47±5.12b
T5	15.17±1.20b	16.07±1.31a	13.22±1.19c	4.21±0.41d	1.57±0.07f	1.49±0.19g	4.05±0.19e	7.97±6.55a
Control	1.38±0.07b	1.48±0.09a	0.76±0.05c	0.65±0.11d	0.39±0.09f	0.49±0.03e	0.49±0.03e	0.81±0.44f
Overall Mean (±S.D)	7.58±5.29b	8.51±5.55a	6.26±4.82c	2.34±1.46d	1.09±0.40f	1.09±0.35f	2.27±1.42e	

(Means with similar letters in a single row and * column are statistically similar at $p<0.05$).

Table 5. Accumulation of zinc ($\mu g g^{-1}$) in *Catla catla* during 12-week growth trial.

	Kidney	Liver	Gills	Skin	Fins	Muscle	Bones	*Overall Mean (±S.D)
T1	4.21±0.66b	5.08±0.28a	3.66±0.82c	1.35±0.04d	0.92±0.07f	0.87±0.12g	1.13±0.14e	2.46±1.79e
T2	8.16±1.81b	9.13±1.56a	6.29±0.72c	1.53±0.09e	1.04±0.18g	1.49±0.04f	1.82±0.04d	4.21±3.52d
T3	13.65±2.07b	14.76±2.04a	10.16±0.91c	1.81±0.07f	1.94±0.11e	1.79±0.14g	2.41±0.14d	6.65±5.98c
T4	17.26±2.10b	18.93±2.18a	14.22±2.09c	2.07±0.12f	2.18±0.16e	2.09±0.15f	3.12±0.15d	8.55±7.85b
T5	22.46±2.18b	24.41±2.21a	18.11±1.86c	2.29±0.96f	2.97±0.14e	2.25±0.18g	3.91±0.18d	10.91±10.24a
Control	1.81±0.19b	3.27±0.17a	1.54±0.11c	1.01±0.03d	0.45±0.03f	0.43±0.06f	0.65±0.06e	1.31±1.02f
Overall Mean (±S.D)	11.26±7.95b	12.60±8.24a	9.00±6.37c	1.68±0.47e	1.58±0.94f	1.49±0.71g	2.17±1.22d	

(Means with similar letters in a single row and * column are statistically similar at $p<0.05$).

Metals present in the waste water are accumulated in different organs of fish. After the assessment of amount of heavy metals in the different organs of *Catla catla*, it was noticed that the heavy metals were accumulated in the sequence: Zn>Co>Cu>Ni> Cd. Considering the amount of heavy metals in different organs, it was concluded that maximum amount of these metals were accumulated in the liver while least amount was accumulated in the fins. Similar results were quoted by Taweel *et al.,* 2011, showed that the highest heavy metal concentrations were detected in the liver followed by the gill and the muscle. Vinodhini and Narayanan (2008) studied the bioaccumulation of heavy metals in various organs of the fresh water fish exposed to heavy metal contaminated water system. The order of heavy metal accumulation in the gills and liver was Cd >Pb> Ni >Cr and Pb> Cd > Ni > Cr. Similarly, in case of kidney and flesh tissues, the order was Pb> Cd > Cr > Ni and Pb> Cr > Cd > Ni. According to Staniskiene *et al.,* 2006, the concentration of heavy metals in fishbone was higher than in fish flesh, except for Cr and V, where the concentration in bone and flesh was similar. The largest amount of heavy metals was found in liver 20 freshwater fish (perch, roach, silver bream, semi-bream, chub, smelt, tench and pike). The concentrations of all the metals in the tissues (offal, gills, muscle and liver) of mudfish (*Parachanna obscura*) were higher than the concentrations of the metals in water and indicated bioaccumulation. In another experiment (Crafford andAvenant-Oldewage2010) determined the concentrations of four non-essential elements (strontium, aluminium, lead and nickel) in water, sediment and various fish tissues. The highest non-essential element metal concentrations were generally recorded in gill (filaments and arches), followed by muscle, liver and lastly skin.

Conclusion

The accumulation of heavy metals shows the direct relationship with temperature and pH of water. The factors which modify physico-chemical parameters of metal toxicity are hardness and pH of water. The total ammonia excretion by fish increased with the increase of concentration of waste water. However, the dissolved oxygen contents were higher in control than in treating medium showing significantly more dissolved oxygen consumption by the fish and the concentration of ammonia excreted by the fish increased when the heavy metals of high concentration come in contact with them.

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