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Monitoring the Effect of Water Pollution on Four Bioindicators of Aquatic Resources of Sindh Pakistan

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Abstract: This investigation was aimed to estimate the heavy metal pollution in marine and fresh water and their acute toxicity and its toxicological affects on survival, physiological and biochemical parameters of the widely consumed fresh water and marine water fishes of Sindh. Four bioindicators, two from marine water (*Liza subviridus* and *Johnius belengerii*) and two from fresh water species (*Cyprius carpio* and *Pomodasy argyrew*) were collected to study the species-site interaction. Water samples from both stations collected to analyze the essential and non essential metals and muscles of fish were sampled for metabolic parameters and persistent metals pollutant. Total lipids, proteins, amino acids and glycogen were estimated by Spectrophotometry whereas Atomic Absorption Spectrophotometry was used for metals detection. Results showed that interaction of metal pollutants vary specie to specie. This showed that pollutants act by changing the structural or biological function of bioindicator. High concentrations of contaminants were found in tissues of fishes collected from marine water as compared to fresh water fishes.

Key words: Heavy metals, marine water fish, fresh water fish, metabolic parameters

INTRODUCTION

Untreated wastes of industrial, technological and agricultural origin containing various metallic compounds often contaminate natural waters. Heavy metals due to their bio-accumulative and non-biodegradable properties constitute a core group of aquatic pollutants. These metals particulates enter the aquatic medium through effluents discharged from tanneries, textiles, electroplating, metal finishing, mining, dyeing and printing industries, ceramic and pharmaceutical industries etc. (Azmat and Talat, 2006). They concentrate in the tissues of aquatic biota and are known to produce cumulative deleterious effects (Cosson, 1994).

Furthermore, there is an increased public awareness regarding pesticides, fertilizers, agricultural products and metals that might endanger our indigenous fish populations and aquatic ecosystems. This is mainly because humans use these natural resources as food and water supplies, are therefore also exposed to produce polluting these resources (Evans *et al*., 2000). Of particular concern is the exposure of bio-organisms to metal pollution, as it is known that metals act as mutagenic/genotoxic compounds, interfere with xenobiotic metabolic pathways and may also affect glycolysis, the Krebs cycle, oxidative phosphorylation, protein, amino acid metabolism as well as carbohydrate and lipid metabolism (Drastichová *et al*., 2005; De la Torre *et al*., 2000). Knowledge of acute toxicity of a xenobiotic often can be very helpful in predicting and preventing acute damage to aquatic life in receiving waters as well as in regulating toxic waste discharges. A perusal of the available literature reveals that studies on the acute effects of toxic metals on the biochemical constituents of fishes are scanty (El-Demeerdash and Elgamy, 1999; El-Naga *et al*., 2005).

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Heavy metals concentration in the tissues of fish enter into human beings through food chain and due to there cumulative action causes potential health hazards sometimes even lethal (El-Shehawi *et al.*, 2007). The toxic effects may result from the bio concentration of metals and their consequence binding with biologically active constituents of the body such as lipids, amino acids and proteins (Smedes and Thomson, 1996; Thangam and Sivakumar, 2004; Vutukuru, 2005).

Chuddar *et al.* (2002) report that heavy metal, nickel effects biochemical component like glycogen of gill, digestive gland and whole body of freshwater bivalve, *Parreysia cylindrical* under studied. The significant decrease in total glycogen content of gill, digestive gland and whole body was observed due to pollution stress caused by nickel.

In this context, an attempt was made to investigate bioaccumulation of heavy metals in marine and fresh water fishes and their acute effects on some biochemical profiles to show an important link in the aquatic food chain. Biochemical profiles in fish and other aquatic organisms under heavy metal stress serve as important bioindicators in the monitoring of aquatic environment.

MATERIALS AND METHODS

Two marine water species (*Liza subviridus* (L.) and *Johnius belengerii* (J)) from Ireland Manora (Fig. 1). Karachi and two fresh water fishes ((*Cyprius carpio* (C) and *Pomodasy argyrew* (P)) from Halegyy Lake (Fig. 2). Sindh were collected and selected for monitoring the integrated effect of water born metal pollution. Water sample from above site were also collected for determination of heavy metal pollution in both aquatic resources. Collection of water was made during fishing by fisher man

Fig. 1: Manora Island Karachi where the collection of marine water fish were made

Fig. 2: Haleji lake of Sindh where collection of fresh water fish were made

in the depth of 7 to 9 feet in both aquatic resources. Ten sample of each fish were analysed for essential and non-essential metal and their biochemical profile were estimated for nutritional quality.

Metal Analysis was done after collection of fishes by dry ashing. Equal weight of four fishes was put into crucibles. The crucibles were placed in the oven for 2 h at 135°C. After that the samples were mineralized at 400°C in the chemical oven for 24 h then 2 mL of nitric acid was added and sampled were dehydrated at 450°C. To each sample 10 mL of hydrochloric acid was added and then make up to 50 mL with double distilled water. Water sample were digested in the same manner for the detection of pollutant toxic metals.

Macronutrient elements were determined by flame photometry in laboratory and reported in mg kg^{-1}. The content of heavy metals were determined by atomic absorption Spectrophotometry (AAS) along with standards (As a reference), supplied by the Agilent Technologies and results were given in μg kg^{-1}. Hg was analyzed on AAS by using mercury lamp, along with standard reference. All data obtained, subjected to statistical analysis on Excel 2000.

Biochemical Analysis

Glycogen content was measured by Anthrone reagent as described by Carroll *et al.* (1956). Results were expressed in mg g^{-1}.

Total lipids contents were extracted from tissues by using phosphovanilline method and were determined according to Smedes and Thomson (1996) using diagonastic kit. Protein and amino acids were estimated by Sawhney and Singh (2005) methods.

RESULTS AND DISCUSSION

The toxicity tests are necessary in water pollution evolution because chemical and physical measurements alone are not sufficient to asses potential effects on aquatic biota.

Analysis of metals in water samples from marine and fresh water resources under study showed that there is a significant difference in concentration of pollutants that were higher in marine water as compared to fresh water (Table 1, 2). In addition it is an important step to detect the level of toxicants and their effects in the marine organism. Such effects might lead to integrated effects on metabolic functions such as behavioral, growth, reproduction and survival. This can result in changes in fish health and reproduction that may alter fish population and community structure. The values of pollutants were compared with Current National Recommended Water Quality Criteria of priority pollutant which indicates that except as all metals having higher concentration in both aquatic resources.

Table 1: Macronutrients metal analysis of aquatic resources of Sindh Pakistan

	Macronutrients (mg kg^{-1})			
Aquatic resources	Na	K	Mg	Ca
Marine water	165±32	57±12.3	111±18.2	95±23.0
Fresh water	65±14	39±10.2	23±9.0	25±7.5

Table 2: Trace metal analysis of aquatic resources of Sindh Pakistan

	Trace metals (μg kg^{-1})				
Aquatic resources	As	Pb	Cd	Hg	Zn
Marine water	50±12.6	10±2.0	18±4.5	7±1.2	58±15.0
Current national recommended water quality criteria of priority pollutant	36	3.1	8.8	0.94	90
Fresh water	23±5.6	12±2.3	5±1.2	2±0.5	13±2.8
Current national recommended water quality criteria of priority pollutant	150	9	0.25	0.77	120

Table 3: Analysis of macronutrients of four bioindicators of Sindh Pakistan (mg kg^{-1})

Treatments	Na	K	Ca	Mg
Fresh water of fish				
Cyprnius carpio	61±1.50	38±0.70	27±1.70	19±1.06
Pomadasy agyrew	50±1.20	39±1.41	23±1.50	16±1.24
Correlation	0.996	0.0	0.605	-0.83
Paired difference (t)	34.78	-0.632	3.25	3.523
Marine water fish				
Johnius belangeri	59±1.51	40±2.5	24±1.48	18±2.12
Liza subviridus	52±1.20	40±2.8	22±1.15	19±1.67
Correlation	0.982	0.209	-0.91	0.972
Paired difference (t)	18.5	0.00	0.845	-1.581
Literature	2.89-3.58 mg g^{-1} Mohammady *et al.* (2005) 360 ppm Azmat *et al.* (2006) 100 mg/100 g fish meat Badawi (1972)	3.33-4.13 mg g^{-1} Mohammady *et al.* (2005) 220 ppm Azmat *et al.* (2006)	1.94-2.55 mg g^{-1} Mohammady *et al.* (2005) 160 ppm Azmat *et al.* (2006)	2.16-3.13 mg g^{-1} Mohammady *et al.* (2005) 190 ppm Azmat *et al.* (2006)

Table 3 showed the concentration of macronutrients in marine and fresh water fish in ranged of 61-52 mg kg^{-1} for Na, 38-40 mg kg^{-1} for K, for Ca 22-27 mg kg^{-1} and Mg 16-19 mg kg^{-1} which is approximately same for four bioindicator of Sindh region. This showed that although there is significant difference in concentration in of these ions (Table 1) in both aquatic resource but rate of accumulation these elements were same as the Na, K, Ca and Mg are very important minerals elements and found insoluble salts in the sacroplasm of the muscular cells, inter cellular fluid, blood and plasma (Azmat *et al*., 2006). The values of macronutrients compared with literature showed decline in concentration which may effects fish health because these elements are also play an important role in physiological processes involves in structure of several organic compounds (Oilvereau *et al*., 1981). An increase in concentration of K, Na or Mg contents in sea-water (Table 1) may alter the morpho-functional changes in fishes. These changes include the increase in the height and the diameter of the micli of pinealcytes, the increase being followed by apocrynic secretion in the cells which may disturb the ionic balance of internal miles (Deane and Woo, 2005).

Table 4 showed bioaccumulation potential of heavy toxic metals in four species under investigation and compared with international literature. Pb concentration in muscles of two biomarkers from fresh water (*Cyprius carpio* (0.6 μg kg^{-1}) and *Pomodasy argyrew* (5.8 μg kg^{-1}) and two from marine water (*Liza subviridus* (7.4 μg kg^{-1}) and *Johnius belengerii* (7.6 μg kg^{-1}) showed significant difference. *Johnius belengerii* showed averaged highest value (7.6 μg kg^{-1}) whereas *Pomodasy argyrew* showed averaged lowered value (5.8 μg kg^{-1}). It indicated that interaction of heavy toxic metals with biomarkers vary specie to specie and more prominent in marine water fish. Extensive clinical and experimental evidence support the significance of Pb-Ca interaction which is apparent in current study that *Liza subviridus* has lowest concentration of Ca (22 mg kg^{-1}) and that of Pb 7.4 μg kg^{-1} and *Johnius belengerii* (7.6 μg kg^{-1}) while Ca 24 mg kg^{-1}. These interactions occur at the cellular and molecular level and are the abilities of Pb to displace Ca during specific physiological process (Table 3, 4). It is likely that Pb blocks Ca efflux from cells by substituting Ca in Ca^{++}/Na adenosine triphosphate (Simons, 1986).

Hg concentration in muscle of two fresh water species was higher as compared to marine water fish. *Pomodasy argyrew* showed highest averaged value (5 μg kg^{-1}) whereas *Liza subviridus* showed lowest (3.2 μg kg^{-1}). In addition there was a significant difference among the average concentration of Hg in two stations. This data indicate that different species have various capabilities to accumulate and

Table 4: Heavy metals level in four bioindicators of Sindh Pakistan (µg kg^{-1})

	As	Hg	Pb	Cd	Zn
Fresh water fish					
Cyprnius carpio	3±0.185	4.4±0.31	6±0.08	3.0±0.22	11±0.22
Pomadasy agyrew	5±0.25	5.0±0.36	5.8±0.13	4.2±0.10	8±0.01
Paired correlation	0.484	-0.272	0.939	0.041	0.957
Paired difference (t)	-9.76	-1.2	2.16	-4.66	13.91
Marine water fish					
Johnius belangeri	5±0.066	3.4±0.21	7.6±0.30	3.0±0.007	11±0.22
Liza subviridus	9±0.0.29	3.2±0.24	7.4±0.18	5.2±0.06	8.0±0.002
Paired correlation	-0.877	0.399	0.886	-0.382	-0.496
Paired difference (t)	-4.00	0.55	0.366	-34.36	45.49
Literature	0.128 µg g^{-1} wet wt. (Emami *et al*., 2005) 0.02-0.23 ppm (Azmat *et al*., 2006) 1.04-1.87 µg g^{-1} (Mohammady *et al*., 2005) 2066±2146 ng g^{-1} (Garg and Ramakrishna, 2006)	1-0.062 ppm (Azmat *et al*., 2006) 0.117 µg g^{-1} wet wt. (Gholam *et al*., 2005) 0.5-5 µg g^{-1} (Gholam *et al*., 2005) 0.03-0.28 wet wt. (Rowe *et al*., 1998) 5.82-8.16 µg g^{-1} (Mohammady *et al*., 2005) 2.94-13.73 µg g^{-1} d.wt. (Canli *et al*., 1998)	0.01-0.02 mg kg^{-1} dry wt. (Medina *et al*. 1986) 0.01-0.02 mg kg^{-1} dry wt. (Windom *et al*., 1987) 0.44-3.24 mg g^{-1} dry wt. (Faverney *et al*., 2001)	0.31±0.02 (Azmat and Talat, 2006)	7.897±0.5 (Iliana, 2006)

store water contaminates independent of their level in water. Same phenomena were observed by De la Torre *et al*. (2000). Hg also interact with the metal binding protein metalothionine (MT), a low molecular weight cytosolic protein protect the biological system by binding metal ions. Higher concentration of amino acids reported in these fish also support Hg amino acid interaction, which may control Hg toxicity.

As concentration in muscles of the four species showed variation in two fresh water and two marine water species *Liza subviridus* showed highest (9 µg kg^{-1}) accumulation of As while *Cyprius carpio* showed lowest value (3 µg kg^{-1}). In addition to this *Pomodasy argyrew* (5 µg kg^{-1}) and *Johnius belengerii* (5 µg kg^{-1}) showed the same pattern of accumulation of As regarding less the marine or fresh water sites. As interact with thiol and effects many functional proteins and its effect is significant on protein contents (Table 5) of reported fish.

There are many similarities between Cd and Zn both are member of group 11B metals having similar tendency to form complexes. Cd has an inhibitory effect on the activity of Zn-containing enzymes. Indeed Cd replace Zn in MT and low protein values in current investigation may result in increased Cd uptake (Gulfaraz and Ahmed, 2001; Funk *et al*., 1987). The lower concentration of Zn in fishes may be related with higher concentration of heavy metal Cd (Table 4) which may be attributed with replacement of Zn with Cd due to chemical similarity.

Cadmium derives its toxicological properties from its chemical similarity to Zn, an essential micronutrient for plants, animals and human. Cd as an ion affects on respiration and binders in exchange of gases (Gulfaraz and Ahmed, 2001). Cosson (1994) reported that Zn ions of metallothionine (MT) molecule were replaced by those of Cd when both metals were combined in the organism. This metal also showed affinity to protein SH group. This may be related with interesting pattern of interaction between metal and biochemical constitutes of these species like protein, amino acids glycogen and total lipids content of these biomarkers (Table 5). In fresh water fish *Cyprius carpio* the highest level of protein was reported (2.36 wet wt. and 3.36 dry wt.) as compared to other fishes,

Table 5: Biochemical analysis of four bioindicators of Sindh Pakistan (g g^{-1})

	Protein				
Treatments	Wet wt.	Dry wt.	Amino acid	Glycogen	Total lipids
Fresh water of fish					
Cyprnius carpio	1.36±0.015	2.36±0.012	14.17±0.86	0.077±0.003	19.20±1.36
Pomadasy agyrew	1.78±0.015	3.25±0.05	30.172±2.10	0.052±0.002	22.86±2.06
Paired correlation	-0.622	-0.035	0.990	-0.134	0.913
Paired difference (t)	21.08	-14.80	12.890	4.47	-3.34
Marine water fish					
Johnius belangeri	1.75±0.015	3.0±0.018	33.00±2.54	0.056±0.003	17.12±1.14
Liza subviridus	2.11±0.015	2.5±0.32	20.152±1.86	0.017±0.002	14.04±1.09
Paired correlation	0.614	0.329	0.928	-0.327	0.913
Paired difference (t)	-25.42	0.947	12.31	7.06	6.29

which showed *Cyprius carpio* was more tolerant to heavy metal stress. During stress condition, fish needed more energy to detoxify the toxicants and overcome stress. Since fish have a very little amount of carbohydrate, therefore next alternative source of energy is protein to meet increased energy demand. The observed depletion of protein fraction may have been due to their degradation and possible utilization of degraded products for metabolic purpose. Table 5 showed that nutritional composition of marine fish protein was lower than that of fresh water fish under studied which may be related with water body condition. Results obtained from biochemical analysis of these common edible fish can give a useful indication for proper use of biochemical response as biomarkers in monitoring water born pollution by heavy metals.

Increment in free amino acids level may be the results of break down of protein for energy requirement and impaired incorporation of amino acids in protein synthesis (Table 5) which may be attributed to lesser use of amino acids and their involvement of an acid-base balance.

Glycogen content in muscle were investigated as a biological monitoring tool for assessment of effect of heavy metals present in water. Table 5 showed that the value of glycogen content in *Liza subviridus* was (0.017%) which was lowest as compared to the other species. This clearly indicates the change in biological functions due to the highest accumulation of metal in the *Liza subviridus* (Table 4). Glycogen is localized in sarcoplasm under the sarcolemma, in sarcoplasmic reticulum and throughout the sarcoplasmic core. They are defined as a change in biological response that differs from molecular to organisamal level due to metals toxicity. Carbohydrates are the primary and immediate source of energy. In stress condition, carbohydrate reserve depleted to meet energy demand. Depletion of glycogen may be due to direct utilization for energy utilization (Table 5).

Lipid content in muscles were inversely related level of As, Hg and Pb. *Pomadasy agyrew* (22.86) contain highest total lipid concentration as compared to marine fish *Liza subviridus* (14.04) while it was lower in marine water fishes that indicates that marine water fish use more lipids contents due to heavy load of metals (Michael *et al*., 1986; Ozmen *et al*., 2006).

Investigation showed that appreciable decline in the biochemical profiles such as total glycogen, total lipids and total protein contents of the fish in presence of toxins, results in decrease productivity of fish population. However, the decrease in protein content was significant in marine water fish. This study reflects the extent of the toxic effects of toxic metals and the metal induced cumulative deleterious effects at various functional levels in the widely consumed freshwater fish and marine fish. The toxicity of heavy metal caused the glucose level to decrease with increase of pollutants concentration and decrease the glycogen content in muscle as reported by Scott *et al*. (2006).

Statistical data analysis showed significant difference in macro elements of fresh water and marine water fish and order follows (CNa-PNa) > (JNa-LNa) > (CMg-PMg) > (CCa-PCa) > (JMg-LMg) > (JCa-LCa) > (JK-LK). Only one pair of metal i.e., (CK-PK) has no significant difference with marine fish. Similarly for heavy metals order of variation is (JPb-LPb) > (CHg-PHg) > (JHg-LHg) > (JZn-LZn) > (CPb-PPb) > (JCd-LCd) > (CCd-PCd) > (CZn-PZn) > (CAs-PAs) > (JAs-LAs). Whereas biochemical parameters of fish belongs to two different aquatic resources showed variation in proteins, amino acids, glycogen and lipids.

CONCLUSION

On the basis of above investigation it may be concluded that concentration of heavy metals in fish of Sindh region is a matter of serious fact because ultimately its accumulate in human body and can cause damages in human body therefore heavy metals in the tissues of aquatic animals should occasionally monitored. As the heavy metal concentration in tissues reflects past exposure via water or food. It can demonstrate the current situation of the animals before toxicity affects the ecological balance of population in the aquatic environment. Therefore it is suggested that Pakistan coastal metal management smelting facility shell required assessing the potential toxicity of metals-contaminated effluent at its point of discharge to avoid the determining effects of toxic metals in high quality food. Otherwise changes in fish health due to pollution may decline in fish population.

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