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RESEARCH ARTICLE

HEAVY METALS ACCUMULATION IN SELECTED TISSUES OF *ARIUS MACULATUS* FROM UPPANAR ESTUARY, CUDDALORE DISTRICT, TAMILNADU

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ABSTRACT

In this study, Cadmium (Cd), Lead (Pb), Chromium (Cr), Copper (Cu) and Zinc (Zn contents were determined in gill and muscle tissues of fish *Arius maculates* at sub lethal concentration of effluents from Uppanar estuary Cuddalore District, Tamilnadu. Heavy metal concentrations varied significantly in the gill and muscle tissues of *Arius maculates.* The present study shows *Arius maculates* showed higher levels of metal concentrations in gill and muscle which might have resulted tissue damage to the fish. The present study concludes that the presence of metal causes disturbances to the fish.

INTRODUCTION

Environmental pollution is caused by the development of industries, technology and an informal settlement does, however, threaten many freshwater ecosystems. Environmental pollution not only causes a decrease in water quality, but it subsequently affects all living organisms in that system. Therefore, it is necessary to not only identify and manage these pollution sources, but also to maintain their effects on the health of aquatic environment. Human activities are mainly responsible for promoting the pollution in the environment by the way of introducing unwanted toxic compounds. The toxicological effects of pollution are due to their high persistence and accumulation in the organisms (Goyer, 1996). Although suitable concentration of heavy metals plays a vital role in metabolic pathways when their concentration exceeds the threshold level, they act as physiological biochemical and behavioral inhibition in the organisms. Heavy metals enter the aquatic environment naturally through weathering of the earth crust. In addition to geological weathering, human activities have also introduced large quantities of metals to localized area of the sea, in some cases upsetting the natural steady state balance (Forstner and Wittmann, 1983). Presence of these toxic chemicals (pesticides and fungicides) in freshwater media, may cause death or sublethal effects on the freshwater non-target organisms like fishes, snails and crabs Kulkarni and Utkar, (1983). Many investigators reported mass mortality of fishes due to pesticide pollution in temperate waters Mount and Putnicki, (1966); Saunders, (1969); Dacre and Scotti, (1971). Such an incident in Indian waters was also reported by George *et al* (1965). Some of these chemicals also accumulate in animals and plants. Such accumulation of toxic chemicals ultimately creates toxicity to flora and fauna and for the man who eats fish which feed on them. So fishes are the economically important non-target species of freshwater ecosystems that are very much affected by the heavy metal accumulation in an aquatic ecosystem. There are five types of pollutants that affect the estuarine and coastal resources as under: (i) bacterial infection due to the discharge of untreated domestic sewage along with storm water runoff from the cities which can cause epidemics of water borne diseases such as dysentery, typhoid, cholera, Poliomyelitis and hepatitis; (ii) industrial wastes that deplete dissolved oxygen; (iii) toxic chemicals of industrial wastes and land runoff comprising, pesticides and herbicides interfering with the metabolism; (iv) fertilizer's runoff that tend to stimulate growth of some life forms and cause eutrophication and (v) inert chemical sediments and counteracting with the delicate benthos of the estuary.

Uppanar estuary is a good source of fisheries, particularly mullets and catfish. Extensive beds of the edible oyster, silver bellies, horse mackerels, gizzard shad, whiting, *Etroplus, Therapon, Gerres, Chanos chanos, Elops saureus, Polynemus, Teuthis* and jewfish are inhabiting in smaller numbers almost throughout the year. Prawns and crabs form about 5.0 and 2.5%, respectively of the total catches. The town of Cuddalore is endowed with three rivers: the uppanar, Gaddilum and Ponnai River, of which the Uppanar has been degraded as a 'black spot' in spite of the fact that it was once known as the centre piece of the 'garden town'. Industrial effluents consist of a variety of substances of either known or unknown lethality. Most water sources will receive a number of industrial effluents either directly or indirectly.

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Fishes are important in water pollution studies as they are sensitive to a wide variety of toxicants in water (Mathur, 1969; Holden, 1972; Julin and Sanders, 1977) and are used as pollution indicators in water quality management. A major objective of today's environmental research is to improve the quality of our environment to prevent the pollution by the extensive use of pesticides which were proved to be highly toxic not only to the fishes (Chambers and Yarbrough, 1974) but also to other organisms which form food of the fishes (Butler *et al.*, 1970). Fish gills, which serve as the primary uptake site in fish for trace metals, represent the most important targets when exposed to elevated levels of ambient metals (Newman and Jagoe, 1994). The gills are the first target organs in the heavy metal accumulation because they are directly in contact with water (Dubale and Shah, 1979). The gills, which serve as the primary uptake site in the fish for trace metals, represent the most important targets when exposed to elevated levels of ambient metals (Newman and Jogoe, 1997).

MATERIALS AND METHODS

***Procurement of fish*:** Live specimens of *Arius maculatus* with an average length of 8.5 ± 0.50 cm and weight of 15.0 ± 0.5 g were collected from Uppanar River by operating cast net. The fish were acclimatized in the aquaria of 120 liters capacity containing well aerated sea water (salinity 28 ppt; pH 7.69; oxygen content 4.32 mg/l and water temperature (32.6°C) for a period of one week prior to experiment. During acclimatization, the fish were fed on chopped prawn and clams. Food was withheld one day before the' commencement of the experiment. The water was changed along with waste feed and faecal matter every 24 hours. Fish collected from Perumal Lake were used as control and Uppanar brackish water. Live fish were also collected from the experimental station, which carried effluents or discharges from the surrounding industries. The fish were transported to the laboratory and maintained in the same way as the control fish collected from Uppanar river. The water was renewed once in two days.

Experimental design: Fishes were divided into two equal groups each comprising of 10 fishes. Each group was kept in separate plastic tanks. The first group was kept as negative control; the fishes were maintained in water containing normal water without any treatment. The fishes of two groups were exposed to a sub-lethal concentration of effluent s (1% and 3 %) added in the water for 30 days. Solutions were renewed once daily after exposure period, animals (n=20/group) were sacrificed and the tissues was removed, homogenized and stored at −80 °C for further biochemical analyses. After experiment, the fish each from the respective experimental as well as control groups were sacrificed. The gill and muscle were isolated from the fish and used for various study. Solutions were renewed once daily after exposure period, animals (n=20/group) were sacrificed and the gills and muscle tissues were removed, homogenized and stored at −80 °C for further biochemical analyses.

Analysis of bioaccumulation of heavy metals in tissues: Heavy metals such as Cadmium, Lead, Chromium, Copper and Zinc were determined in gill and muscle using Atomic Absorption Spectrophotometer - model 2380 Perkin - Elmer, VB.A., (Singanan and Somasekhara, 1996). The experimental fish was exposed to sublethal concentrations (1% and 3%) of the effluent for a maximum period of 30 days. Subsequently the organs were dissected out and 5 mg of each tissue was taken in a 125 ml Erlenmeyer flask and glass beads were added with 25 ml of deionised water along with 10 ml of (1: 1) mixture of concentrated nitric acid and perchloric acid. The samples were boiled until the solution was clear and transferred to a 100 ml volumetric flask and diluted with deionized water and the readings were taken using standard solutions. The results obtained were expressed in µ/g.

Statistical analysis: Statistical comparisons were made using ANOVA, One way ANOVA (Multiple range test) with Statistical package for social science (SPSS Package).

RESULTS

Level of cadmium concentration in various tissues: Table 1 shows cadmium content in the gill of the control sample tissues was 0.020 µg/g at two different periods of exposure (30 days). At 1% and 3% sublethal concentrations, the cadmium accumulation was observed to be 0.250 and 0.1751µg/g at different periods of exposure. The level of cadmium in the muscle of the control sample was found to be 0.023 µg/g for the two different periods of exposure (30 days) and in experimental muscle for the same period at 1% sublethal concentration, the level of cadmium was observed to be and 0.625 µg/g. The same trend was also noticed at 3% (0.725 µg/g) sublethal concentrations as well Muscle exposed to sublethal concentration effluent (for 30 days).

Level of lead concentration in various tissues: Table 2 shows the rate of accumulation of lead in the gill of the control sample was found to be and 0.032 µg/g for the two different periods of exposure. The accumulation for 7 and 30 days of exposure at 1% concentration revealed a value of 11.750 µg/g. At higher concentration of 3% the rate of accumulation increased to 12.225 µg/g for 30 days of exposure. The accumulation of lead in the control sample of muscle was found to be 0.022 µg/g for an exposure of days. At 1% sublethal concentration for the exposure periods of 30 days, the lead accumulation was 5.200 µg/g. At 3% concentration, for the same periods of exposure, the accumulation rate was 5.400 µg/g.

Level of chromium concentration in various tissues: Table 3 shows accumulation of chromium in the gill showed a control value of 0.043. At 1% sublethal concentration of effluent during 30 days of exposure, the rate of accumulation was 1.055 µg/g. The rate of accumulation was slightly higher at 3% sublethal concentration during 30 days of exposure as 1.083 µg/g. The net accumulation of chromium in the control muscle was 0.030 µg/g for 30 days of exposure. The rate of chromium accumulation at 1% and 3% for 30 days of exposure showed an enhanced value (1.420 and 1.433 µg/g.

Level of copper concentration in various tissues: Table 4 shows gill of *Arius maculatus* were exposed to sublethal concentrations (1% and 3%) for two different exposure periods 30 days). The copper level in the control gill tissue was found to be 0.013 µg/g for 30 days of exposure. The sublethal concentration of 1% revealed 2.425 µg/g during 30

Table 1. Accumulation of cadmium (µg/g) in the tissues of *Arius maculates* exposed to sublethal concentrations (% 96 hrs LC_{50}) of the effluent

Exposure Periods	Concentration levels (% 96 hrs LC_{50})			
	Tissues	Control	1%	3%
30 Days	Gill	0.020 ± 0. 008	0.350 ± 0. 058*	0.575 ± 0. 096*
	Muscle	0.023 ± 0. 013	0.625 ± 0. 096*	0.725 ± 0. 082*

Values represents mean ± SE. * significance 5% level of significance (ANOVA)

Table 2. Accumulation of lead (µg/g) in the tissues of *Arius maculates* exposed to sub-lethal concentrations (% 96 hr LC_{50}) of the effluent

Exposure Periods	Concentration levels (% 96 hrs LC_{50})			
	Tissues	Control	1%	3%
30 Days	Gill	0.032 ± 0.001	11.750 ± 0.129*	12.225 ± 0.096*
	Muscle	0.022 ± 0.001	5.200 ± 0.082*	5.400 ± 0.082**

Values represents mean ± SE. * significance 5% level of significance (ANOVA)

Table 3. Accumulation of chromium (µg/g) in the tissues of *Arius maculates* exposed to sublethal concentrations (% 96 hrs LC_{50}) of the effluent

Exposure Periods	Concentration levels (% 96 hrs LC_{50})			
	Tissues	Control	1%	3%
30 Days	Gill	0.061 ± 0.002	0.928 ± 0.010*	1.073 ± 0.010*
	Muscle	0.030 ± 0.002	1.420 ± 0.008*	1.433 ± 0.010*

Values represents mean ± SE. * significance 5% level of significance (ANOVA)

Table 4. Accumulation of copper (µg/g) in the tissues of *Arius maculates* exposed to sublethal concentrations (% 96 hrs LC_{50}) of the effluent

Exposure Periods	Concentration levels (% 96 hrs LC_{50})			
	Tissues	Control	1%	3%
30 Days	Gill	0.022 ± 0.001	2.425 ± 0.096*	2.725 ± 0.096*
	Muscle	0.035 ± 0.001	3.450 ± 0.129*	3.700 ± 0.082*

Values represents mean ± SE. * significance 5% level of significance (ANOVA)

Table 5. Accumulation of zinc (µg/g) in the tissues of *Arius maculates* exposed to sublethal concentrations (% 96 hrs LC_{50}) of the effluent

Exposure Periods	Concentration levels (% 96 hrs LC_{50})			
	Tissues	Control	1%	3%
30 Days	Gill	0.074 ± 0.001	12.325 ± 0.096*	15.450 ± 0.129*
	Muscle	0.048 ± 0.001	15.725 ± 0.096*	16.325 ± 0.096*

Values represents mean ± SE. * significance 5% level of significance (ANOVA)

days of exposure and 3% concentration showed 2.725 µg/g. The level of copper concentration in the muscle of the control sample was 0.035 µg/g for 30 days of exposure. Bioaccumulation of copper ranged from 3.450 µg/g at 1 % concentration to 3.700 µg/g at 3% concentration for 30 days of exposure.

Level of zinc concentration in various tissues: Table 5 shows accumulation of zinc in the gill of the control sample was 0.074 µg/g for 30 days of exposure. At 1% sublethal concentration the accumulation level of zinc was 12.325 µg/g. and at 3% level, the accumulation of zinc was higher showing a value of 15.540 µg/g for and 30 days of exposure. The accumulation of zinc in the muscle of the control sample was 0.048 µg/g for 30 days of exposure. The accumulation of zinc for 30 days of exposure at 1% and 3% concentrations, the zinc accumulation was 15.725 and 16.325 µg/ g.

DISCUSSION

Bioaccumulation is mainly due to the characteristic of different organs, nature of the compound, penetration routes, methods of transports by the blood and so on. The concentrations of metals vary among the animal tissues depending on the route of uptake from the metal and the physiological fate of the metal (Terry and William, 1994). The heavy metals are widespread in the environment. These metals are accumulated in many tissues (Niemi *et al*., 1991; Schweinsberg and Karsa, 1990). The accumulation of metals in aquatic organisms has been linked to decreased survival and reduced reproductive ability (Pelgrom *et al.*, 1995). The amount of bioaccumulations of heavy metals in tissues may vary depending on length and weight of samples (Barghigiani and Ranieri De ,1992; Zyadah,1999). Therefore, the samples were chosen to be around the same weight and length for each station in this study. The amount of bioaccumulations of heavy metals in tissues may vary depending on length and weight of samples (19, 20). Accumulation of toxic metal ions in different organs of marine organisms and their subsequent transfers to the human through the food chain is of great concern. Being non - biodegradable, the ions reach the aquatic environment and remain suspended or partially get dissolved over the water column to become part of biomagnifications. The predominant pathways for heavy metal uptake, target organisms, and organism sensitivity are highly variable and are dependent on factors such as metal concentration, age, size physiological status and growth of fishes (Chapman *et al*., 1996). In the present study an assessment on the level of some heavy metals (cadmium, lead, chromium, copper and zinc) in the different tissues (gill and muscle) of the fish *A. maculatus* was analysed. Similarly high content of metal has been recorded in muscle, gill and this observation that coincides with of fish has been studied by Zhang *et al*. (2007). Kargin, (1996) stated that due to variations in feeding habits, habitats and behaviour of species, the levels of metals found in tissues of the benthic *Mullus barbatus* were always higher than those found in pelagic *S. aurata* throughout the year. Romeo et al. (1999) pointed out that cadmium, copper, mercury and zinc

concentrations in edible muscles of pelagic fish species are lower than for benthic fish species. Although *M. cephalus* and *T. mediterraneus* are both pelagic fish, these species differ from the point of view of living region and feeding behaviour (Yilmaz, 2003). Kalay *et al.* (1999) reported that different fish species contained strikingly different metal levels in their tissues. Abdel-Moniem (1994) reported that metal in many species of fish in different areas: in *M. cephalus* in the Mediterranean Sea); in *Trachurus mediterraneus* in eastern Mediterranean waters. Ilmaz, (2003) reported that in *Mullus barbatus* and *S. aurata* in Üskenderun Gulf. The higher level of copper recorded for *Siganus rivulatus* relative to that in *Sargus sargus* is due to the food habit where the first is herbivorous and the second feeds mainly on crustaceans, molluscs beside small fishes. The same results were previously recorded by Pourang (1995) in two fish species (*Carassius auratus* and *Esox lucius*) collected from Anzali wetland water in Northern Iran.

Moore and Ramamoorthy (1981) who reported that there is generally no correlation between residues and feeding habits. Furthermore, they explained the low level of Pb in fish muscle to the relatively low rate of its binding to SH groups, beside the low solubility of lead salts that restricts movement across cell membranes. The concentration of Pb in muscle on the present fish species was below the recommended level given by the Iranian Standard Bureau for human consumption (Hadjmohammadi, 1988).The high level of zinc in *Siganus rivulatus* confirms obtaining majority of zinc from dietary sources rather than from water (Moore and Ramamoorthy, 1981; Pourang, 1995; Kargin, 1996). The low concentrations of the seven heavy metals under investigation recorded in the two fish species (*Siganus rivulatus* and *Sargus sargus*) indicate that the muscle is not an active tissue in accumulating heavy metals as reported by many authors for some fish species (Carpene and Vasak, 1989; Khan *et al*., 1989).

Gills were also reported as highly Pb-accumulating organs in *Clarias gariepinus* and *Labeo umbratus* by Coetzee et al. (2002), but in *L. rohita*, liver was the main site for Pb accumulation, an observation which conforms to that of Canli *et al*. (1998) for *Chondrostoma regium*. Roesijadi (1992) reported that gills were the target organs in both *L. rohita*, *C. idella* due to their close relation with the external environment as had also been reported by It is in agreement with the observationof Demirak *et al*. (2006). The lowest concentration of Cr in muscles of *L. rohita* was similar with the finding of Mackeviciene (2002) for *Astacus astacus*. In *C. idella*, gills were the major sites for Cr accumulation (0.63 $\mu g\ g^{-1}$), as was also observed in *L. cephalus* by Demirak *et al*. (2006). The present results were in agreement with that of for *Clarias lazera*, but were in disagreement with the values found by Yang et al. (2007) for *G. nanensis*, *Gymnocypris waddellii*, *Ptychobarbus dipogon*, *Schizopygopsis younhusbandi*, *Schizopygopsis microphalus* and *Oxygymnocypris stewartii*, which accumulated higher levels of Zn in muscles. Coetzee *et al*. (2002) reported both the tissues of liver and gills of *C. gariepinus* and *L. umbratus* as highly Zn accumulating. Malik *et al*. (2010) reported that the heavy metal accumulated in the gill and muscle tissues of *L. rohita* and *C. idella*. In conclusion it could be inferred that among the five metals copper, zinc and lead seem to be more toxic than cadmium and chromium, even at low concentrations and induce serious health hazards.

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