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BIOACCUMULATION OF HEAVY METALS IN SELECTIVE TISSUES OF THE FISH OREOCHROMIS MOSSAMBICUS (PETERS) EXPOSED TO WATER SAMPLE COLLECTED FROM PALLIKARANAI WETLAND ECOSYSTEM, CHENNAI, TAMILNADU.

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ABSTRACT

Aquatic environment forms the basis for living organisms. Now a day, the aquatic ecosystems are getting polluted by various anthropogenic activities. Among the various kinds of pollutants that can adversely affects the quality of the aquatic ecosystem, heavy metals play a prime role. In the present investigation the bioaccumulation of such heavy metals in general and on the major target organs in particular were assessed in the freshwater fish, *Orechromis mossambicus*. The water sample from the Pallikaranai wetland ecosystem was collected and the further experiment was conducted in the laboratory. Experimental analyses clearly revealed that there was a great deal of heavy metal accumulation in the target organs of the candidate fish species *O. mossambicus*. This study warrants that there is an urgent mitigation required to save the Pallikaranai wetland ecosystem and also the biodiversity of the such habitat will be prevented from deterioration.

KEYWORDS: Pallikaranai wetland ecosystem, heavy metal, environmental pollution, Oreochromis mossambicus.

INTRODUCTION

Currently pollution is one of the major threats in the aquatic ecosystem. Because of unplanned urbanization, hasty industrialization, insecticides, pesticides and herbicides (Subburaj *et al.* 2018) are undergoing complicated problems in aquatic ecosystem. This

pollution contains toxic and nontoxic heavy metals. Hence, water molecules repeatedly stores variety of xenobiotics and reduced water quality. This heavy metals cause the physiological and biochemical changes in the fish (Kuppusamy *et al.* 2016). Fish plays an important role in monitoring of aquatic pollution, because they respond the great sensitivity and it's a bioindicator to made changes in the aquatic environmental pollution (Anusiya Devi *et al.* 2016). The fish mainly absorb waterborne metals from water through their gills; hence, gills are the first object of xenobiotics (Aldoghachi *et al.* 2016). When the heavy metals get inside in to the organism and accumulate the fish organs like liver, kidney and gills and significantly changed the biochemical parameters (Pretto *et al.* 2011).

Heavy metals are simply dumped to the environment from a variety of natural and anthropogenic sources (Abumourad *et al*. 2014; Gaber *et al*. 2014; Zaki *et al*. 2014; Bauvais *et al*. 2015). In aquatic environments, heavy metal pollution results from direct atmospheric deposition, geologic weathering or through the discharge of agricultural, municipal, residential or industrial waste products, also via wastewater treatment plants (WWTPs) (Maier *et al*. 2014; Dhanakumar *et al*. 2015; Garcia *et al*. 2015). The gill and liver of fish is a highly sensitive and accurate way to monitoring in biochemical and enzymological studies of heavy metals accumulation and the liver is particularly vulnerable to damage from heavy metal toxicants (Ramachandiran *et al.* 2017). In the present investigation, an assessment of the acute toxicity by exposing the fish to sublethal concentrations of the polluted water collected from the Pallikaranai wetland ecosystem (Chennai, Tamilnadu, India), and pursuing an extensive assessment on the biochemical attributes of the test fish, *O. mossambicus* inhabiting in that niche was intended. The objective of the present study was total carbohydrate, protein and lipid contents of gill, liver, muscle and kidney of the fish *Oreochromis mossambicus*.

MATERIAL AND METHODS

Live fresh water fish fingerlings of *Oreochromis mossambicus* ranging 8 to 10g in weight and 5 to 6cm in length were obtained from river of Cauvery, Mohanoor, Namakkal district of Tamilnadu, India by local fisherman. The fish fingerlings were carried to the laboratory in well aerated polythene bags and acclimatized to the laboratory conditions in 120 liters capacity plastic container for a week prior to experiment. The plastic container was previously fumigated with potassium permanganate to prevent from fungal infections. The water was renewed every day to provide freshwater rich in oxygen along with waste feed and

feacal matter in order to maintain a healthy environment for the fish. After a week the fish were introduced into separate groups (Group I, II and III) each group had 20 fish fingerlings of sublethal concentrations of 1% and 3% (Group I: control (tap water free from chlorine); Group II: 1% (1liter of tap water with100ml of experimental water) and Group III: 3% (1liter of tap water with 300ml of experimental water)) for a maximum period of 30 days. The experimental water collected from Pallikaranai wetland, Chennai, Tamilnadu, India.

The experimental fish was exposed on 7, 15 and 30th day, 10 fishes were collected from each group periodically and sacrificed for biochemical analysis of carbohydrate, protein and lipid. 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 deionized 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 with using standard solutions. The results were obtained and expressed in µg/g. Statistical comparisons were made using Two way ANOVA, One way ANOVA and Tukey - LSD test (Multiple range test) with Statistical package for social science (SPSS Package). 100 mg of the different tissues like gill, muscle, liver and kidney were weighed and homogenized by a glass homogenizer using 5 ml of freshly prepared 5% trichloro acetic acid (TCA). The content was centrifuged for 10 minutes at 3000 rpm and the supernatant was collected and mixed with 10 ml of anthrone reagent (500 mg of anthrone and 10 g of thiourea dissolved in one liter of 66% sulphuric acid by volume) and then incubated for 15 minutes in a boiling water bath. Finally, the colour intensity was measured at 620 nm in Bausch and Lomb Spectronic 21 Spectrophotometer.

RESULT

Quantification of carbohydrate

Gill

The total carbohydrate content in the control groups during the exposure period of 7, 15 and 30 days were estimated to be 7.144, 7.068 and 6. 818µg/g respectively. In the lower sublethal concentration (1%) at the different periods of exposure showed a decreased value of 7.048 (for 7 days), 6.143 (for 15 days) and 6.084µg/g (for 30 days). At 3% sublethal concentration, there was a decline in the total carbohydrates during different exposure periods (7, 15 and 30 days) and indicated the values as 6.833, 6.333 and 5.630µg/g respectively (Table 3.1).

Muscle

The total carbohydrate content in the control groups during the exposure period of 7, 15 and 30 days were estimated to be 7.303, 5.99 and 5.38µg/g respectively. In the lower sublethal concentration (1%) at the different periods of the exposure, showed a declined value of 6.678 (for 7 days), of 5.732 (for 15 days) and 5.434µg/g (for 30 days). Similarly, at 3% sublethal concentration there was a decline in the total carbohydrates during the different exposure periods and indicated the values as 6.59, 5.674 and 5.177µg/g on 7, 15 and 30 days respectively (Table 3.1).

Liver

Total carbohydrate content in the control groups during the exposure period of 7, 15 and 30 days was estimated to be 7.166, 7.135 and 6.29µg/g respectively. At the lower sublethal concentration (1%) during the different periods of exposure showed a value of 6. 20 (for 7 days), 6.647 (for 15 days) and 6.179µg/g (for 30 days) respectively. At 3% sublethal concentration, there was a decline in the total carbohydrates during different exposure periods (7, 15 and 30 days) and indicated the values as 6.472, 6.237 and 6.091µg/g respectively (Table 3.1).

Kidney

Total carbohydrate content in the control groups during the exposure period of 7, 15 and 30 days was estimated to be 6.099, 5.825 and 5.558µg/g respectively. In the same way the lower sublethal concentration (1%) at the different periods of exposure showed a declining value of 5.89 (for 7 days), 5.211 (for 15 days) and 4.720µg/g (for 30 days). Similarly, at 3% sublethal concentration, there was a fall in the total carbohydrates during different exposure periods (7, 15 and 30 days) and indicated the values as 5.637, 5.047 and 4.056 µg/g respectively (Table 3.1).

The Two way ANOVA for quantification of carbohydrates in all the tissues showed a significant value at 1% level between the exposure days and the two sublethal concentrations. One way analysis of variance on the muscle showed an insignificant value between the control and experimental concentrations. The Multiple range test for gill and liver showed a significant value at 5% level particularly between control and 3% concentration. Similarly, the kidney showed a significant value at 5% level between control and the two sublethal concentrations. Generally all the tissues showed a significant value at 1% level irrespective of the periods of exposure. Besides, multiple range tests for liver showed a significant value at

5% level.

Quantification of total proteins

Gill

The total amount of proteins estimated in the gill of the control sample revealed a mean value of 78.79, 69.543 and 65.663µg/g for an exposure periods of 7, 15 and 30 days respectively. But experimental sample exposed to lower sublethal concentration of 1% at different exposure periods however, showed a lower value of 62.214 (for 7 days), 58.635 (for 15 days) and 52.355µg/g (for 30 days) of proteins. Similarly at higher sublethal concentration of 3% the values declined in the total proteins during different exposure periods (7, 15 and 30 days) and the values were 57.362, 46.703 and 43.320µg/g respectively (Table 3.2).

Muscle

The total amount of proteins estimated in the muscle of the control samples were 97.52, 91.523 and 91.69µg/g for an exposure period of 7, 15 and 30 days respectively. Similarly at lower sublethal concentration of 1% for the different exposure periods showed a value of 82.396 (for 7 days), 76.325 (for 15 days) and 74.271µg/g (for 30 days). Contrastingly at higher sublethal concentration of 3% showed a decline in the total proteins during different exposure periods (7, 15 and 30 days) and indicated the values as 73.278, 65.403 and 62.89 µg/g (Table 3.2).

Liver

The total amount of proteins estimated in the liver of the control samples were 83.56, 78.435 and 77.524 µg/g towards the exposure periods of 7, is and 30 days respectively. In the lower sublethal concentration of 1% for the different exposure periods showed a value of 70.403 (for 7 days), 64.291 (for 15 days) and 61.388µg/g (for 30 days). Similarly at higher sublethal concentration of 3% showed a decline in the total proteins for the same exposure periods (7, 15 and 30 days) and indicated the values as 63.352, 54.293 and 54.143µg/g (Table 3.2).

Kidney

The total proteins estimated in the kidney of the control sample revealed a mean value of 70.721, 67.718 and 61.871µg/g for the exposure periods of 7, 15 and 30 days respectively. The lower sublethal concentration of 1% at different exposure periods showed a value of 59.538 (for 7 days), 54.384 (for 15 days) and 52.273µg/g (for 30 days). Similarly at higher sublethal concentration of 3% the total protein content was declined during different exposure

periods (7, 15 and 30 days) and indicated the values as 50.264, 43.320 and 40.548µg/g (Table 3.2).

The Two way ANOVA for quantification of proteins in the tissues showed a significant difference at 1% level between the exposure days and the two sublethal concentrations. The F value confirmed significance of the above values between the two concentrations in all the tissues. Furthermore, it indicated that there was a significant variation within the given concentrations at 5% level. The multiple range test showed a significant value in kidney between 7 and 30 days of exposure. The F value showed an insignificant attribute between the periods of exposure in all the tissues. In the field tissue samples of *O. mossambicus* a significant value in the total protein at 1% level was observed between the control and test samples.

Quantification of lipids

Gill

Analysis of total lipids in the gill of the control sample indicated a mean value of 6.391, 5.382 and 5.666 µg/g for the exposure periods 7, 15 and 30 days respectively. In the lower sublethal concentrations of 1% for the different exposure periods showed a decline in the lipid level as 5.0923 (for 7 days), 4.925 (for 15 days) and 4.698µg/g (for 30 days). At higher sublethal concentrations of 3% for the different exposure periods showed a lower lipid level as 4.483 (for 7 days), 4.419 (for 15 days) and 3.283µg/g (for 30 days) respectively (Table 3.3).

Muscle

Analysis of total lipids in the muscle of the control sample indicated a mean value of 5.822, 5.382 and 5.798µg/g for the exposure periods of 7, 15 and 30 days respectively. At lower sublethal concentrations of 1% for the different exposure periods showed a lower lipid level as 5.333 (for 7 days), 5.177 (for 15 days) and 4.328µg/g (for 30 days). Similar trend was shown at higher sublethal concentration of 3% and the values were 4.875, 4.575 and 3.598µg/g for the different exposure periods (7, 15 and 30 days) respectively (Table 3.3).

Liver

Analysis of total lipids in the liver of the control sample indicated a mean value of 9.214, 8.815 and 8.853µg/g for the exposure periods of 7, 15 and 30 days respectively. Similarly at the lower sublethal concentrations of 1% for different exposure periods showed a decline in

the lipid level as 8.145 (for 7 days), 8.175 (for 15 days) and 8.193µg/g (for 30 days). Similar trend was shown at higher sublethal concentration of 3% and the values were 7.728, 7.353 and 7.255µg/g for the different exposure periods (7, 15 and 30 days) respectively (Table 3.3).

Kidney

Analysis of total lipids in the kidney of the control sample indicated a mean value of 8.802, 8.612 and 8.357 µg/g for the exposure periods of 7, 15 and 30 days respectively. But in the lower sublethal concentrations of 1 % for the different exposure periods showed a decline in the lipid level as 8.217 (for 7 days), 8.124 (for 15 days) and 8.101µg/g (for 30 days). Similar trend was shown at higher sublethal concentration of 3% and the values were recorded as 8.126, 7.444 and 7.278 µg/g for the different exposure periods (7, 15- and 30 days) respectively (Table 3.3).

The Two way ANOV A on the - quantification of total lipids in the tissues showed a significant value at 1% level between the exposure periods and the two sublethal concentrations (Table 46). Furthermore the F value confirmed a significant relation between concentration at 1% level in all the tissue. Except liver and kidney, all the tissues showed a significant value for the periods of exposure. Similarly the Multiple range test showed a significant attribute at 5% level in the gill, muscle and kidney between 7, 15 and 30 days of exposure.

Table 3.1. Quantification of protein (µg/g) in the tissues of *Oreochromis mossambicus* exposed to sublethal concentrations (% 96 hr LC_{50}) of the water sample collected from Pallikaranai wetland ecosystem.

Period of exposure	Tissues	Concentration level (% 96 hr LC_{50})		
		Control	1%	3%
7 Days	Gill	78.79	62.214	57.362
	Muscle	97.52	82.396	73.278
	Liver	83.56	70.403	63.352
	Kidney	70.721	59.538	50.264
15 Days	Gill	69.543	58.635	46.703
	Muscle	91.523	76.325	65.403
	Liver	78.435	64.291	54.293
	Kidney	67.718	54.384	43.320
30 Days	Gill	65.663	52.355	43.87
	Muscle	91.69	74.271	62.89
	Liver	77.524	61.388	54.143
	Kidney	61.871	52.273	40.548

Values are mean of five replications.

Table 3.2. Quantification of lipid (µg/g) in the tissues of *Oreochromis mossambicus* exposed to sublethal concentrations (% 96 hr LC_{50}) of the test sample.

Period of exposure	Tissues	Concentration level (% 96 hr LC_{50})		
		Control	1%	3%
7 Days	Gill	6.391	5.0923	4.483
	Muscle	5.822	5.333	4.875
	Liver	9.214	8.145	7.728
	Kidney	8.802	8.217	8.126
15 Days	Gill	5.382	4.925	4.419
	Muscle	5.744	5.177	4.575
	Liver	8.815	8.175	7.353
	Kidney	8.612	8.124	7.444
30 Days	Gill	5.666	4.698	3.283
	Muscle	5.798	4.328	3.598
	Liver	8.853	8.193	7.255
	Kidney	8.357	8.101	7.278

Values are mean of five replications.

Table 3.3. Analysis of Alkaline phosphatase (µ mole PNP/mg) in the tissues of *Oreochromis mossambicus* exposed to sublethal concentrations (% 96 hr LC_{50}) of the test sample.

Period of exposure	Tissues	Concentration level (% 96 hr LC_{50})		
		Control	1%	3%
7 Days	Gill	5.075	4.195	4.615
	Muscle	4.683	4.410	4.198
	Liver	7.825	7.255	6.840
	Intestine	9.768	8.715	8.320
15 Days	Gill	5.075	4.785	4.318
	Muscle	4.655	4.195	3.280
	Liver	7.493	7.228	6.415
	Intestine	9.385	8.665	7.990
30 Days	Gill	5.065	4.235	3.838
	Muscle	4.505	4.068	3.213
	Liver	7.493	6.233	5.950
	Intestine	9.215	8.425	7.385

Values are mean of five replications.

DISCUSSION

Commonly fishes are used to evaluate the health of aquatic ecosystems and their physiological changes help as biomarkers of aquatic pollution. Biochemical analysis is the best way to evaluate the health and functional activities in heavy metal toxicated fishes. In the present study, the level of carbohydrate, protein and lipid was significantly decline on different periods (7, 15 and 30 days) in various concentrations (1% and 3%) in gill, liver,

muscle and kidney of the fish *O. mossambicus*. When the animals require energy in stress condition, carbohydrates utilized the energy through glycolysis in liver. Many investigators described that the quantity of change in biochemical parameter of aquatic organisms in order to toxic reporting (Remia *et al*. 2008; Vijay kumar *et al*. 2009). Bhilave *et al*. (2008) reported that the level of carbohydrate, protein and lipid were considerably decreased in liver, muscle and gill of heavy metal toxicated fish *Cirrhinus mrigala.* Protein plays an important role in cellular metabolisms and regulated intracellular and extracellular media of cell membrane. And also control the growth and differentiations in organisms. So proteins are earliest indicators of heavy metals poisoning in the fish (Dhanalakshmi and Chitra, 2014).

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