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DISCRIMINATING AND BIO- ACCUMULATION OF MERCURY AND LEAD CONTAMINATION IN FRESH WATER FISH OF TILAPIA *OREOCHROMIS MOSSAMBICUS* (PETER, 1973).

Biological Science

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ABSTRACT

Environmental pollution is a worldwide problem as heavy metals belong to the most important pollutants. The progress of industries has led to increased emission of pollutants into ecosystems. The present study highlighted the objective of this work was to determine the acute toxicity and bioaccumulation of mercury and lead and biochemical content in tilapia (*Oreochromis mossambicus*) from the two different water bodies Thamirabarani river and estuarine river in Kanyakumari District. The average concentration of both metals in water ranged from 4.3 to 44.1µgL^{-1} followed by 8.41 to 16.37µgL^{-1} mercury and Lead respectively. Acute toxicity tests showed that the mercury and Lead concentration that caused toxicity to tilapia ranged from 67.25µg Lead L^{-1}, and 45.39µg HgL^{-1} at 24hrs 21.34µgL^{-1} and to 60.51µgL^{-1} in the 96 hrs respectively. When measured Lead and mercury concentration in various tissues for mercury and Lead described the significant correlations found among the various concentrations in all experimental tissues. The median lethal concentration (LC_{50}) of mercury and Lead in *O. mossambicus* for 24, 48, 72 and 96hrs of exposure periods having LC_{50} values such as 8.325, 7.201, 6. 906, 5.128ppm for mercury and 7.249, 7.131, 6.106, 5.165 for lead treated fishes respectively. Furthermore, the results were showed that more lead and mercury was accumulated in the intestine, stomach and liver of fish than in muscles. The order of tendency to accumulate Pb and Hg in Tilapia tissues was intestine > stomach > liver > gill> muscle. While the macromolecular analysis clearly showed the maximum protein and lipid content was observed in Thamirabarani river fishes than the estuarine river. From the present research showed the overall conclusion is whenever the non-essential metals concentrations are increased in the fresh water, the target organs of the living organism especially *O. mossambicus* accumulated rate and affected in main organs also been remarkably increased in fish as a result consuming candidate definitely affected the many adverse effects them body.

KEYWORDS

Acute toxicity, Bio- accretion, *Oreochromis mossambicus*, Biological indicators

INTRODUCTION

The contamination of fresh water with a wide range of pollutants has become a matter of concern over the last few decades (Burton and Boltt. 1975; Vosyliene and Jankaite, 2006). Augmented detoriaration leads to the environmental pollution by means of various anthropogenic activities. Industrial effluents that contain toxic substances like heavy metals, pesticides and other chemicals are discharged in to the water bodies. Mercury is a very low amount in our environment as a onsequence of both anthropogenic and natural process Naidu *et al*., 1984; Koch *et al*., 1993 and Mason *et al*., 2000). It is ubiquitous but is potentially a toxic trace element. Inorganic as well as organic forms of Hg and Pb are present in the environment and the former seems to be more toxic and slightly more accumulated in some fresh water aquatic species than the later (Chaurisia *et al*., 2007). However, mercury through the processes of bioaccumulation and biomagnification, it will become a toxin to higher level organisms reported on journal of young scientist investigation 2009. Mercury is the most potential biocide known for its toxicity and widespread contamination (Dallas and Day, 1993). There are few reports were described the toxic effects of mercuricidal compounds in fresh water fishes (Urmila and Radhakrishnan, 1990). Free metal ions are the bioavailable chemical species which determine the lead and mercury toxicity to the fish in their liver and brain. Metal cations including mercury and lead bind towards the negative sites of fish, brain and gills (Pagenkopf, 1983; Dallinger *et al*., 1987). Acute toxicity has determined the concentration of a test material or the level of the agent that produces a deleterious effect on a group of test organisms during a short term exposure under controlled conditions (Cossarini, 1987; Health, 1991). Still, there are no other works have been done the similar research, hence the present study designed the following objectives such as; to determined the acute toxicity and bioaccumulation of mercury and lead in tilapia (*O. mossambicus*) from the two different water bodies in Kanyakumari District. In addition to estimated the biochemical content and quantity of the macromolecules of an experimental fishes.

Materials and Methods

Sample collection and Preparation

Details about the sampling are shown in Table. 1. Tilapia *Oreochromis mossambicus* were collected from two different places of TBR and ESR in K.K District. After the fish were stocked in the ponds, we used nylon nets to collect the samples of stocked tilapia from the fish ponds. Every time three to five hundred mL water samples per pond were collected. One litre polyethylene bottles cleaned with 10% nitric acid and then rinsed with deionised water were used as containers for the collection of water samples. The collected water was filtered through a 125µm nylon mesh to remove large suspended particles and macro invertebrates immediately affected collection and then was acidified by adding 5mL of 1N HNO_3 before Hg and Pb analysis.

Acute Toxicity Assay

Laboratory bioassays were conducted to determine the 24h, 48h, 72hr and 96hr LC_{50} values for tilapia exposed to Hg and Pb. The experimental design and calculations for the acute toxicity were based on well known procedures given by Finney (1978).

Estimation of Total Protein and Lipid

Total protein content was determined by the method of Lipid (Lowry *et al*., 1951; Jeyaraman, 2006). The total protein and Lipid content is expressed as mg/100 mg of tissue.

Macromolecular Estimation (DNA and RNA)

The DNA concentration was estimated by the method of (Ceriotti,1955) concentration is expressed as mg per g wet tissue.

RESULT

Acute toxicity study

Lc_{50} values for 24h, 48h, 72h and 96h are listed table 1. The LC_{50} became progressively smaller as the duration of exposure increased. a limited number of studies have investigated Hg and Pb toxicity to tilapia. This experimental results 96hrs LC_{50} 26.34mgL^{-1} followed by increasing the LC_{50} when duration of the exposured time was decreased 35.21, 53.60, and 69.06mg-1 such as 72, 48 and 24 hrs respectively. To obtain the concentration at which 50% of the test population can live for an indefinite time, to estimate incipient Lc_{50}.

Table-1. Lethal Concentration (LC_{50}) of *O. mossambicus* with 24 to 96 hrs experiment

Time	Lc_{50} (MgL^{-1})
24	67.25 (65.03- 71.38)
48	53.60 (49.74 – 56.03)
72	35.21 (31.41-7.90)
96	26.34 (23.47- 30.11)

Table 2: Acute toxicity test of mercury based stress tolerance on *O. mossambicus* fish

Exposure period	LC50 ppm	Correlation co-efficient	Probit Regression equation	Slope function	Confidence limit		Chi square analysis	Table value
					Upper	Lower		
24	8.235	0.936	Y=4.135+ .306X	1.217	9.03	7.290	4.143	**9.46**
48	7.201	0.920	Y=2.61+6.13X	1.291	8.07	7.029	76.134	
72	6.960	0.916	Y=140+6.13X	1.313	6.11	6.004	6.004	
96	**5.128**	**0.906**	**Y=0.30+6.26**	**1.412**	**5.20**	**5.202**	**5.022**	

Acute toxicity of mercury and lead on fresh *O. mossambicus* (P.) was experimented and obtained results were tabulated (Table 2 and 3). It proves that the percentage of mortality was directly proportional to the concentration of the treated heavy metals (Mercury and Lead) while the percentage of mortality in control was effectively nilled. The present results were showed that the median lethal concentration (LC_{50}) of mercury and lead in *O. mossambicus* for 24, 48, 72 followed by 96hrs of exposure periods having LC_{50} values such as 8.325, 7.201, 6.906, 5.128ppm for mercury and 7.249, 7.131, 6.106, 5.165 for lead treated ppm respectively. Apart from the present results were clearly showed that when the exposure period was inversely proportional to LC_{50}values.

Table 3: Desperate toxicity test of Lead (Pb) based stress tolerance on experimental fish of *O. mossambicus*

Exposure period	LC50 ppm	Correlation co-efficient	Probit Regression equation	Slope function	Confidence limit		Chisquare analysis	Table value
					Upper	Lower		
24	7.249	0.956	Y=4.114+9.912X	1.184	8.005	7.271	4.242	**9.46**
48	7.131	0.938	Y=2.28+9.91X	1.218	7.133	7.029	6.142	
72	6.106	0.924	Y=1.104+8.001X	1.272	1.272	6.112	9.323	
96	**7.065**	**0.917**	**Y=0.339+7.007**	**1.321**	**1.321**	**5.136**	**5.756**	

There was a measured increase in slope function from 1.27 to 1.412 for mercury and 1.184 to 1.321 for lead corresponding to increase the exposure period from 24 to 96hrs. Observation on both upper and lower confidence limits reveals a decreasing trend from 24 to 96h then 9.033 to 5.209 ppm for lead respectively. From the fitted regression equation it was evident that an increase in exposure period results in higher mortality.

Bioaccumulation and Tissue Distribution

Lead and mercury concentrations in Estuarine and Thamirabarani river water in various tissues of tilapia fish. The mean Pb and Hg in pond water concentrations in TBR and ESR were 16.82 ± 2.11 and 43.1 ± 5.09 μgL^{-1} (Table- 3). This study found that the Pb and Hg concentrations were toxic to tilapia in these aquaculture eco-systems. The average lead and mercury concentrations in fish tissues were 29.3, 10.9, 5.37, 5.04 and 3.55 $\mu g\ g^{-1}$ in intestine , stomach, liver, gill and muscle respectively (Table -4). The overall mean BCF of Pb and Hg were found to be highest in the intestine. The other BCFs of the stomach, liver, gill and muscles were 416, 159, and 132 respectively.

Table. 4. Average Lead concentrations (mean ± SD) In pond water ($\mu g\ g^{-1}$ dry wt) in TBR and ESR

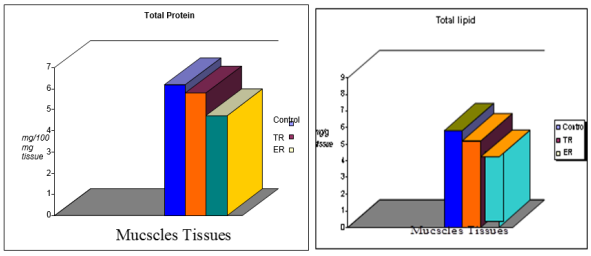
Study site	Lead concentrations					
	Pond water	Gill	Intestine	Liver	Muscle	Stomach
Thamirabarani river	14.8 ± 0.36	3.14±1.70	29.14±11.20	3.00±2.31	3.27±2.95	11.34±3.16
Estuarine river	49.0 ± 3.61	6.13±4.05	34.54±3.80	4.58±2.67	4.58±2.67	15.67±5.13

No statistically significant differences between lead and mercury concentrations in fish and pond water were found ($p<0.05$), yet these appeared to be linearly related ($r^2 = 0.5$). Schendrayatha *et al*., 2002 indicated that the direct accumulation of lead and mercury by tilapia was proportional to the concentrations of these metals in water. Hence, in general Pb and Hg fish tissues increased with Hg, Pb concentration in water results of two way ANOVA show that BCFs differed significantly in various tissues $F = 3.19$, $df = 123$, ($p < 0.05$). No significant variations ($F=1.20$, $df = 123$, $p > 0.05$) between both metal concentrations and individual fish were found. Our results showed that more Pb and Hg were accumulated in the intestine, stomach and liver of fish than in muscle. The order of tendency to accumulate Pb and Hg in Tilapia tissues was intestine > stomach > liver > gill> muscle. The order BCFs was: intestine> stomach> liver~ gill > muscle. Mercury concentrations in all tissues were allometric negatively correlating with fish body weight $r^2 = 0.71 \pm 0.056$ (mean ± SE), $p< 0.05$.

Total protein content in gills was significantly reduced in both the experimental groups. However the reduced level of protein content was observed in experimental estuarine water fish (Figure 1).

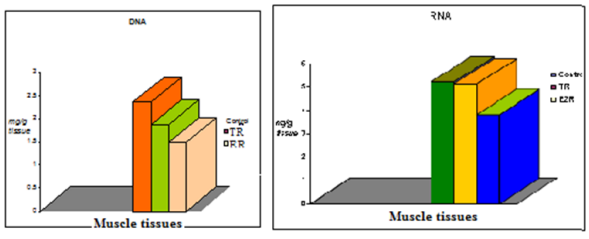
Figure-1: Biochemical content in fresh water fish muscle tissues of *O. mossambicus* from the Thamirabarani and Estuarine River protein (A) Lipid (B).

(A) **(B)**



In muscle tissue of experimental fish total lipid content was significantly reduced in both the experimental fishes like river and estuarine river. Though, the minimum level of lipid content was noticed in an estuarine river fish of *O. mossambicus*. From this result clearly noticed that both experimental fishes consisted lipid level was significantly lower compared with control (Figure1A and B).

Figure-2: Macromolecular quantification (A-RNA and B- DNA) in fresh water fish muscle tissues of *O. mossambicus* from the Thamirabarani and Estuarine River.



When the macromolecular analysis from these two water body fishes significantly maximum DNA content was observed in Thamirabarani river fish followed by ten fold decreased DNA content was noted in estuarine fish (Figure-2A). Meanwhile, more or less similar RNA content also been noticed in both the experimental fishes. When compared with control it was significantly higher RNA level on both groups of water bodies developed fishes (Figure-2B).

DISCUSSION

Toxicity is a qualified property of biochemical which refers to its assets of chemical, which denotes to its effectiveness to induce destructive effects on organisms. It is a function of absorption of the toxicant and the length of exposure of animal (Wilkinson, 1976; Kalay *et al*., 1999). According to the present study, vulnerability to the fresh water teleost *O. mossambicus* increases with an increased the concentration of Hg and Pb concomitantly. From the fitted regression, an increase the exposure period result shows that the higher mortality rate and it was directly proportional to the exposure period. Mason *et al*., (2000) also showed that lead and mercury concentrations are lower in muscle than in other tissues in *O. mossambicus* (Nsikak *et al*., 2007). In the animal

kingdom fishes are most vulnerable to the environmental chemicals having immuno suppressive actions because they cannot escape from their polluted environment. Increasing consideration has been made to the immune system of fish particularly as a bioindicator of xenobiotic pressure (Allen, 1995 and Enane *et al*., 1991). Since healthy cellular and humoral responses are domineering for protection against diseases, metal stressors interfering with the immune system alters the susceptibility of fish to infective diseases (Hodson, 1983; Jain, 2001). According to the present study explained susceptibility of the fresh water teleost fish *O. mossambicus* shows to increase the concentration of Hg and Pb the similar result was also been published by Liang *et al*. (1999); Biegert and Valkovic, 1980). Later, Jha, (1990) indicated that tilapia potentially could be capable to normalize the concentrations of metals in their tissues with time by relating the process of captivation, excretion, decontamination and storage and it could be investigated by analyzing the tissues of individuals exposed to different metal uptake was organisms specific and time dependent in fish. Brain invitro studies investigating effects of CH_3Hg certainly interfere with synaptic diffusion, predominantly by interfering with Ach discharge channels (Atchinson and Narahashi, 1982). The development of invitro fish eco-toxicological assessment tool would greatly enhance the ability to detect the screen the sublethal effects of aquatic pollutants (Atchinson, 1988; Panigrah and Misra, 1980).

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