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Changes due to the effect of the Heavy Metals ($Hgcl_2$ and $Znso_4$) concentration on the marine fish, *Tilapia Mossambica* (Peters, 1852)

V.Sethuraman, Badhul Haq, A.Chezhian, S.Shanker*, D.Senthamil Selvan

Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai, Tamilnadu, India

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ABSTRACT

Pollution is the major problem in the estuarine environment. The fishes in this environment have more gambles to get accumulated with the metals in their body. This study is to check the changes in the body of the fishes(Tilapia Mossambica), when their body gets accumulated with Mercuric chloride, Zinc sulphate and also with both the two metals. The phiso-chemical parameters in the estuarine water is checked and seemed it in normal level. The LC_{50} value for the meals was also found out. The blood glucose rose to +1555.529 in $HgCl_2$ increase, +20.929 in $ZnSo_4$ and 10.12 in both the metals concentration. Protein rise to +47.179 in $HgCl_2$, +29.283 in $ZnSo_4$ and 14.86 in both the metals. The Cholesterol also increased to +87.53, 8.758, and 1.97 in the increase of $HgCl_2$, $ZnSo_4$ and both respectively. Then the DNA raise in $HgCl_2$ and both the metals concentration, the DNA reduces when the $ZnSo_4$ concentration rises. The RNA count decrease in the $HgCl_2$ concentration raise, In $ZnSo_4$ raise in concentration it didn't show any difference in the count and the RNA count increases when the concentration of both the metals raises.

Keywords: Heavy Metals, *Tilapia Mossambica*, LC_{50}, Metal stress and DNA, RNA.

INTRODUCTION

Polluting the aquatic environment by chemicals has become a critical environment problem worldwide. Chemical pollutants are mainly industrial in source. Toxic pollutants including heavy metals are ubiquitous in polluted aquatic environment. Heavy metals are continually released in to the aquatic environment from natural processes such as volcanic activity, weathering of rocks and industrial processes. Many of these metals occur naturally in the environment and are essential for normal metabolism of the aquatic organisms [1,2]. However, Industrial and Agriculture wastes have elevated the natural levels of such metals in the aquatic environment. Among various water pollutants, heavy metals pose a great threat to fishes. They still pose immense health hazards to aquatic organisms.

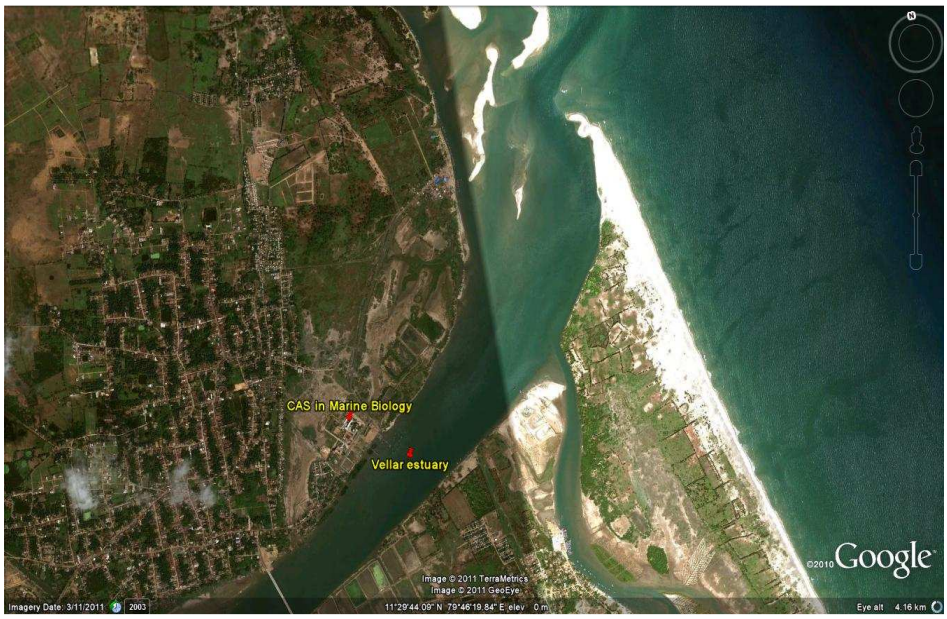
Zinc and mercury receives the largest attention in view of its high toxicity at relatively low concentrations and a long biological half-life resulting in a cumulative effect as pointed out by [3]. Zinc is used in various industrial operations forms and excessive zinc finds its way into lakes and rivers. Although, small quantities of zinc are required for normal development and metabolism of organisms, if the level exceed the physiological requirements, zinc can act as a toxicant. Exposure to excess zinc has been reported to bring about biochemical as well as histological changes in various organs of fishes [4,5]. Number of physiological changes in response to Zinc stress has beenreported in fish such as disturbances in hormone secretion. Thus, the interaction of several stress factors is incomplicate and needs to be studied. Mercury is also found naturally in the environment in the metallic form and in different inorganic forms. Most of the mercury in the atmosphere is elemental mercury vapour and inorganic mercury, while most of the mercury in water, soil, plants and animals are inorganic and organic forms.

Fish are the richest source of an essentially healthy diet. They are however, endangered by water borne pollutants transferredalong the food chain [6]. From the view point of fish culture, *Tilapia mossambica* is the most extensively cultivated species globally.

The work was designed to determine the survival of the mullet fish *Tilapia mossambica,*exposed to 96h. Median lethal concentration (LC_{50}) of metals (mercury chloride, zinc sulphate) and combined metals (mercury chloride and zinc sulphate) to fish and the median lethal time (LT_{50}) of fish in the combined metals and to find out the impact of the metal toxicity on some biochemical components of the fish *Tilapia mossambica* during acute exposure.

Study area

Figure I. Map of Vellar estuary showing sampling stations



MATERIALS AND METHODS

The healthy fishes of the same size were collected and were transported to laboratory in Polythene bags containing Oxygenated water and were stocked in a large rectangular cement tank (4x6x3), previously soap washed, disinfected with potassium permanganate and thoroughly rinsed thrice prior to filling with water. Fishes were acclimatized to laboratory conditions before

being used for experiments. The fish were fed with dry fish, flour pellets and ground dried shrimp.
Since the most environmentally relevant metal/H^+ interactions takes place in soft waters, it is worthwhile first to consider the origin and character of such waters [7]. In the present study, all experiments were performed with estuarine water free from chlorine.

Toxicant
Mercury Chloride plus Zinc Sulphate was used for the present investigation they purchased in anhydrous dried chloride form. Mercury chloride and zinc sulphate (analytical grade) were obtained from Qualigens Fine Chemicals. Glasxo India Limited, Bombay, India (No. 17584).

Experiments
Temperature of the water was monitored by a thermometer. pH value was determined by a pen type pH meter (pH Scan 1, Eutech Cybernetics PTE Ltd., Singapore). Dissolved oxygen content was estimated by Winkler's method using starch indicator. Total alkalinity was determined using methyl orange indicator. Salinity was measured by Mohr's method using potassium chromate indicator. Total hardness was estimated using erichrome black-T' indicator and calcium level was determined using murexide indicator, while magnesium content was calculated by subtracting calcium hardness from total hardness.

Assessment of metals stress
The survival/mortality of fish was recorded up to 24 hours. Dead fish was counted, recorded and removed. Mortality was not encountered in control. Dead fish were removed immediately from the experimental tubs. Fishes were judged to be dead when they lost their equilibrium, floated belly up, became immobile, complete cessation of ventilatory and mouth movements and inability to respond to any stimulus.

Analysis of Bio-chemical
At the end of every 24 hours, 2 fishes were collected from each experimental tank and blood was drawn from the heart region by cardiac puncture with heparin as an anticoagulant and centrifuged at 9000 rpm for 20 min and preserved for the analysis of glucose [8], protein [9] and cholesterol [10].

Analysis of DNA and RNA
Nucleic acid was extracted from gill according to the method [11]. De-oxy Ribonucleic acid (DNA) was estimated by the following the protocol [12] using highly polymerized Calf-Thymus DNA as standard. Ribonucleic Acid (RNA) was estimated according to the method of [13] by using yeast RNA as standard. All the values were analyzed statistically.

Different range of concentration of Metals
Wide range of concentration of selected metals mercury chloride, zinc sulphate, mercury chloride + zinc sulphate 5,10,15,20 and 25 ppm of were prepared in non-chlorinated water separately. Then, ten fishes were introduced in each plastic container containing 10 liters of water with the required amount of the above detent metals-concentration of preliminary observation showed that beyond 15 ppm of the above metal, mixture the test fishes were died. Therefore, the concentration of all metals, falling with in 15 ppm, respectively were prepared and test fishes were separately prepared and test fishes were introduced in a narrow range concentration of 8,9,10,11.12,13,14 and 15 ppm.

RESULTS

The phiso-chemical parameter of the estuarie water is shown in table-1. The temperature is maintained around 38^0C, pH is maintained at 8.5, Dissolved oxygen is at maximum level, Salinity was kept at 35ppt, Total hardness of the water is around 7, and Calcium and magnesium levels in the water is 4.3 and 3.8 respectively. The values are shown in table-1.

Table-1. Hydrobiological features of the water used for the present investigation

S. No	Parameters	Levels
1	Temperature (C°)	38.9 ± 0.2
2	pH	8.5 ± 0.01
3	DO(ml/l)	5.4 ± 0. 2
4	NH_3 (mg/l)	0.03 ± 0.4
5	Salinity ‰	35 ± 0.2
6	Total Hardness (g/l)	7.74±0.02
7	Ca (g/l)	4.03 ± 0.1
8	Mg (g/l)	3.8± 0.3

Values are mean ±SE for five individual observations

The mortality/survival of the fish *Tilapia mossambica* exposed to wide and narrow range concentration of Mercury chloride, Zinc sulphate and both Mercury chloride + Zinc sulphate is shown in table 2-7. Using these tables we can come to a conclusion of the LC_{50} of the wide and narrow range of concentration of these metals.

Appropriate amount of mercuric chloride and zinc sulphate dissolved in seawater freshly every time to prepare a stock solution. All these Results shows that the concentration of the metals is more, the mortality is faster and quicker.

Table-2. Mortality of *T. mossambica* exposed to wide range concentration of mercury chloride at different duration of exposure

Concentration (ppm)	Duration of Exposure in hours			
	24	48	72	96
3	-	-	-	-
6	-	-	-	-
9	-	-	-	-
11	-	-	-	*
13	-	-	*	-
16	*	-	-	-

**Denotes the LC_{50}arriving point*
Empty column denotes no mortality

Table-3 Mortality of *T.mossambica* exposed to different Narrow range concentration of

Concentration (ppm)	Duration of Exposure in hours			
	24	48	72	96
10	-	-	-	-
11	-	-	-	*
12	-	-	*	-
13	-	-	*	-

Mercury chloride at different duration of exposure
**Denotes the LC_{50}arriving point*
Empty column denotes no mortality

Table-4. Mortality of *T. mossambica* exposed to different concentration of Wide range zincSulphate at different duration of exposure

Concentration (ppm)	Duration of Exposure in hours			
	24	48	72	96
8	-	-	-	-
10	-	-	-	-
12	-	-	-	-
14	-	-	-	*
16	-	-	*	-
18		*	-	-

**Denotes the LC_{50}arriving point*
Empty column denotes no mortality

Table-5. Mortality of *T.mossambica* exposed to different concentration of Narrow range trail zincsulphate at different duration of exposure.

Concentration (ppm)	Duration of Exposure in hours			
	24	48	72	96
13	-	-	-	-
14	-	-	-	*
15	-	-	*	-

**Denotes the LC_{50}arriving point*
Empty column denotes no mortality

Table-6. Mortality of *T. mossambica* exposed to different wide range concentration of Mercury chloride plus zinc sulphate at different duration of exposure

Concentration (ppm)	Duration of Exposure in hours			
	24	48	72	96
5	-	-	-	-
10	-	-	-	*
15	-	-	*	-
20	*	-	-	-
25	*	-	-	-

**Denotes the LC_{50}arriving point*
Empty column denotes no mortality

Table-7. Mortality of *T. mossambica* exposed to different concentration of Narrow range mercury chloride plus zinc sulphate at different duration of exposure

Concentration (ppm)	During of Exposure in hours			
	24	48	72	96
8	-	-	-	-
9	-	-	-	-
10	-	-	-	*
11	-	-	*	-
12	-	*	-	-

**Denotes the LC_{50}arriving point*
Empty column denotes no mortality

The level of range of narrow concentration is below 15ppm while the wide range of concentration is above 15ppm.

At the end of 96hrs, the survival/mortality of fish in the control and experimental tanks were recorded. The concentration at which 50% survival/mortality occurred after 24hrs. was taken as the median lethal concentration (LC_{50}) for 96hrs.

The changes in the blood glucose, protein and cholesterol levels of *Tilapia mossambica*exposed to mercury chloride, zinc sulphate and mercury chloride + zinc sulphate, found to be increased when there is an increase in the concentration of the heavy metals.

The fish which is exposed to mercury chloride shows an increase in the blood glucose level from 69.100 mg/100ml at the end of 24hrs. to 155.519 mg/100ml at the end of 96hrs respectively. The fish exposed to Zinc sulphate, also shows increase in the blood glucose level from 10.279 mg/100ml to 20.929 mg/100ml at 96hrs. respectively. But in the combined metal toxicity was increased throughout the exposure period showing percent increase of 5.42 and 10.12 at the end of 24hrs. and 96hrs.

In mercury chloride, the protein level increased throughout the experimental period showing an increase of 19.261, 26.376, 46.639 and 47.179 µg/ml, at the end of 24, 48, 72 and 96 hours of treatment, respectively. In zinc sulphate treatment, protein level increased throughout the experimental period showing increase from 9.286 µg/ml at the end of 24h to 29.283 µg/ml at the end 96h, respectively. But in mixed metal toxicity the protein level was initially decrease at 24 and 48 hours. After 48 hours it was increased showing percent increase of 13.78 and 14.86 µg/ml at the end of 72 hours and 96 hours, respectively.

Table 8. Changes in the blood Glucose, Protein and Chlosterol, content of fish, *Tilapia mossambica* exposed to acute concentration of mercury chloride, zinc sulphate and mercury chloride + zinc sulphate

Parameters	Hours	Control	Mercury chloride	Zincsulphate	Mercurychloride+zincsulphate
Glucose (mg/100 ml)	24	129.719±0.580	219.758±0.04* (69.100)	143.054±0.298* (10.279)	136.068±0.346 (5.42)
	48	128.373±0.390	261.877±1.590* (103.997)	138.426±0.240* (7.831)	124.326±0.234 (3.22)
	72	128.373±0.390	290.937±0.646* (+126.634)	149.099±0.348* (+16.145)	133.094±0.344 (3.90)
	96	128.373±0.390	328.018±1.779* (+155.519)	155.241±0.427* (+20.929)	141.536±0.036 (10.12)
Protein (µg/ml)	24	8.442±0.091	10.068±0.076* (+19.261)	9.266±0.016* (+9.286)	8.124±0.013 (-3.76)
	48	8.303±0.007	10.493±0.092* (+26.376)	4.218±0.008* (+11.021)	3.212±0.018 (-61.31)
	72	7.588±0.007	11.127±6.563* (+46.639)	9.682±0.022* (+27.596)	8.634±0.024 (13.78)
	96	7.588±0.007	11.168±0.080* (+47.179)	9.810± 0.030* (+29.283)	8.716±0.034 (14.86)
Cholesterol (mg/100 ml)	24	79.598±0.539	107.940±0.090* (+35.606)	81.757±0.168* (+2.713)	78.764±0.134 (-1.04)
	48	79.282±0.174	118.515±0.230* (+49.486)	85.759±0.156* (+8.169)	80.736±0.166 (1.83)
	72	78.810±0.059	138.426±0.240* (+75.646)	81.015±0.039* (+2.797)	74.0141±0.014 (-6.08)
	96	78.110±0.059	147.889±4.473* (+87.653)	84.951±0.088* (+8.758)	79.642±0.543 (1.97)

Cholesterol level in the gill tissues of fish exposed to mercury chloride was increased throughout of the exposure period showing a percent increase of 35.606 mg/100ml to 87.653 mg/1000ml at

the end of 24 hours and 96 hours, respectively. In zinc sulphate, the cholesterol level also increased showing percent increase from 2.713 mg/1000ml to 8.169 mg/1000ml at the end of 24 hours and 48 hours respectively. After 48 hours the cholesterol level slightly declined showing 2.797 mg/1000ml at the end of 72 hours. After 72 hours the cholesterol level once again increased showing percent increase of 8.758 mg/1000ml at the end of 96 hours, respectively. The mixed metal the level was declined initially, showing percent decrease -1.04 mg/1000ml at the end of 24h and then it increases to 1.83 mg/1000ml at the end of 48h, at the end of 96 hours it stands at 1.97 mg/1000ml. These results were shown in table 8.

The changes in the gill DNA and RNA level of *Tilapia mossambica* exposed to mercurychloride, zinc sulphate and mercury chloride+zinc sulphate exposed period. The DNA level was decreased throughout the exposure period showing percent decrease of -7.56 to -15.59 at the end of 24 hours to 96 hours, respectively. Zinc sulphate shows increase in the DNA content as -4.35 µg $Mg^{-1}$to -2.75 µg Mg^{-1} from 24 to 96 hours. But an increase of DNA level was observed in mixed mercury chloride and zinc sulphate treatment from -12.5 µg Mg^{-1} to -9.28 µg Mg^{-1} from 24 to 96 hours.

The total RNA level was also increase throughout the mercury and zinc treatment -5.14 µg Mg^{-1} and -2.10 µg Mg^{-1} at the end of 24h treatment and finally ends with the -15.12 µg Mg^{-1} in mercury chloride and -2.41 µg Mg^{-1} in Zinc sulphate at the end of 96 hours. But in mixed metal treatment the value was decrease from 24h to 96hours showing percent increased from -9.53 to -6.72 µg Mg^{-1}.

Table 9. Shows the changes in the DNA and RNA, content in the gill of fish, *Tilapia mossambica* exposed to acute concentration of mercury chloride, zinc sulphate and mercury chloride plus zinc sulphate

Parameters	Hours	Control	mercury chloride	Zinc sulphate	Mercury chloride+zinc sulphate
DNA (µg Mg^{-1}wet tissue)	24	87.2±3.2	80.6±0.4 (-7.56)	83.4±0.12 (-4.35)	76.3±0.13 (-12.5)
	48	87.2±3.2	78.3±0.8 (-10.20)	86.6±0.9 (-1.14)	80.4±0.4 (-7.79)
	72	87.2±3.2	75.4±0.2 (-13.53)	85.3±0.6 (-2.17)	81.4±0.12 (-6.65)
	96	87.2±3.2	73.6±0.9 (-15.59)	84.8±0.11 (-2.75)	79.1±0.8 (-9.28)
RNA (µg Mg^{-1}wet tissue)	24	95.2±4.2	90.3±0.3 (-5.14)	93.6±0.18 (-2.10)	87.4±0.14 (-9.53)
	48	95.2±4.2	86.5±0.6 (-9.13)	94.3±0.16 (-0.94)	86.4±0.12 (-9.24)
	72	95.2±4.2	82.1±0.2 (-13.76)	95.8±0.13 (-0.63)	90.7±0.14 (-4.72)
	96	95.2±4.2	80.8±0.8 (-15.12)	92.9±0.11 (-2.41)	88.8±0.11 (-6.72)

DISCUSSION

Blood glucose levels have long been used as indicators of stress in fish [14]. Under condition of stress, hyperglycemia may provide additional energy during times of high metabolic need such as a “Fight” or “Flight” response [15]. [16] Reported that alteration of carbohydrate metabolism towards high circulating glucose level and gluconeogenesis are consistent responses of fish to acidic conditions. In the present study, the increased level of glucose may be due to alteration of carbohydrate metabolisms or a physiological response to meet the critical need of the energy under metal exposure.

[17] Reported that in rainbow trout, *Salmo gairdneri* exposed to metal mixture brachial ions decreased. He further reported that loss of branchial ions cause a decline in the osmotic pressure of the plasma and thereby cause an osmotic gradient across cell membranes. As a result the intracellular fluid space expands at the expense of the extracellular fluid [18]. This, intern have three major effects: hemoconcentration, as indicated by decreases in blood volume and increase in hematocrit and plasma protein concentration.

[18] Reported that in rainbow trout, *Salmo gairdneri* exposed to acid condition, whole blood viscosity increased, which reflected a rise in haematocrit and plasma protein concentration. In the present study also the increase in plasma protein level may be due to increased level of blood viscosity or osmotic gradient across cell membrane which reflects an increase in plasma protein concentration.

The DNA damage assay may be used to evaluate effects of genotoxicants, including heavy metal species that are components in metal plating wastewater. Because genetic damage has an impact on the survival of cells and individuals, the use of DNA damage assays using various organisms is pertinent for the study of ecological toxicity assessment [19]. Fish are used as a test organism in which it is possible to detect DNA damage induced by direct mutagens and pro-mutagens in both fresh and salt water [20]. Thus, genotoxic pollutants may lead to the contamination not only of the aquatic organisms themselves but of the entire ecosystem and, finally, of humans through the food chain [21]. The results of the present investigation reveal that the DNA and RNA content reduced in the, of fish exposed to low metal concentrations. The observed decrease in the levels of DNA and RNA content can be best correlated with protein reduction in the mussels exposed to different effluent concentration. Since DNA and RNA synthesis precedes protein synthesis, the reduction in their levels is very well reflected in the protein levels. The binding of metals to the phenylalanine and lysine tRNA should have inhibited their propagation to ribosomes, which caused reduction in the protein content as suggested by [22]. In this study also decreased level of DNA and RNA were observed this may be due to the above reason.

CONCLUSION

- The physico-chemical features of water such as temperature, pH, electrical conductivity, dissolved oxygen, total alkalinity, salinity, total hardness, calcium and magnesium were normal.
- The median lethal concentration of mercury chloride, zinc sulphate and mercury chloride plus zinc sulphate of fish was 11, 14 and 10 ppm, respectively thus showing that the metal was highly toxic to fish.
- The (LC_{50}) value of each concentration of the metals is clearly identified.
- The blood glucose, protein and Cholesterol levels were increased when the metals concentration increase.
- The results of the present investigation reveal that the DNA content is reduced when Mercury chloride concentration is raised and increases when Zinc sulphate concentration is raised. But when both the metals concentration is increased the DNA content increases.
- The RNA content reduces when the Mercury chloride concentration is increased; when the Zinc sulphate concentration is increased the RNA did not show any notable change. But when both the metals concentration is increased the RNA content increases.

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