Original Article

Synergistic Effect of Mercury and Chromium on the Histology and Physiology of Fish, *Tilapia Mossambica* (Peters, 1852) and *Lates calcarifer Calcarifer* (Bloch, 1790)

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ABSTRACTS

Fingerlings of estuarine fishes, *Tilapia mossambica* and *Lates calcarifer* were exposed to sub-lethal concentration of mercury and chromium (2.8 ppm) for a period of 28 days. When these fish were exposed to metals concentration, severe gills alterations were observed. But the alteration was less in fish *T. mossambica* when compared to that of *L. calcarife*. The fish *L. calcarifer* exposed to mercury plus chromium, showed lifting up of the epithelium, swelling, hyperplasia, hypertrophy, proliferation of chloride cells, but in mercury treatment, lamellar fusions, fused secondary lamella and necrosis were observed, whereas in *T. mossambica* the gills disintegration of epithelial cells, desquamated epithelium, hemorrhaged and exhibited complete damage of epithelial cells of lamellae. The Na^+, K^+-ATPase activity of both gills and plasma showed significant reduction throughout the experiment period in both fishes. The enzyme activity was more drastic in the case of plasma. The results are discussed in relation to the significance of the above enzyme as non-specific biomarkers against environmental stress.

Key words: Hg and Cr, histopathology, K^+-ATPase, Na^+, *T. mossambica, L. calcarife*

INTRODUCTION

Contamination of the marine environment by metals has risen in recent years due to the global population increase and industrial development. The littoral and estuarine zones are more exposed to this problem than the oceans because of their proximity to the sources of pollution. Water harbors all types of biotic and abiotic components including metals essential for life processes. With the agricultural and industrial revolution that has over taken most of the world, most of the water resources are getting wastes discharged from industries situated near these resources, besides agricultural run-off and corrosion in the pipes. Human activity has continuously disturbed the nature's environment, particularly the aquatic ecosystems.

Many of these metals, such as Cu, Mn, Fe, and Zn are essential micronutrients, but can become toxic at concentrations higher than the amount required for normal growth.[1] Other heavy metals such as Cd, Hg and Pb, have so for unknown roles in living organisms, and are toxic even at very low concentrations.[1,2] Since many heavy metals can be very toxic and thus may threaten the health of organisms, studies have been conducted to investigate heavy metal levels in environmental samples as well as heavy metal accumulation and its effects on organisms and factors affecting heavy metal accumulations by various organisms.[3] These include studies on the effect of toxic metallic substances such as Pb, Hg, Cr, Zn, Cu, Fe, Mn, Be, Cd,Co,Cr, Pb, Ni, Se, Sn and Zr.

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Heavy metals are the most dangerous contaminants of aquatic biota. They cause stress response in fish.[4] The effects of heavy metals and trace elements in water are well known since the episodes of the Minimata and Nigata by incidents and with the existence of itai-itai disease caused due to mercury pollution in Japan. One of the most dangerous pollutants that can be found in waters is mercury. Mercury uptake by fishes is accumulative process resulting from bioaccumulation of the metal with age and biomagnification through food web. The hazards of heavy metal pollution have received much attention all over the world in recent years, both processes result in an increasing Hg concentration with size and tropic level.[5]

The results of survival tests indicate that metal mixtures are usually more toxic than single metal solutions and their action is synergistic.[6] However, the results obtained by[7] showed that various mixtures of zinc, chromium and nickel were toxic to striped dwarf catfish *Mystus vittatus* in a synergistic or antagonistic way. Antagonistic and synergistic effects of heavy metals are well documented in a variety of aquatic species.[8] This is especially true in the case of mercury, selenium, mercury, copper, zinc, glutathione and chelating agents such as EDTA. Separate investigations indicate that some heavy metal mixtures are distinguished by antagonistic effects.[9,10] In some authors' opinion the effect of the mixtures does not depend upon the ratio of toxicants in the mixture, whereas others advance evidence that it does.[11]

The gills play a primary role in osmoregulation in fishes. Many studies of teleost gills have described the morphological and functional characteristics of gill epithelial cells. These cells participate in various functions, such as gas exchange, maintenance of blood acid–base balance and ionic regulation.[12] The effects of aluminum and low pH on gill development of rainbow trout (*Oncorhynchus mykiss*, *Walbaum*) Larvae, had been studied by,[13] who summarized the main structural damage to the gills by aquatic irritants, including some of the toxic metals. The work on combined toxicity of mercury and chromium is very scantly, hence in the present study is to determine the toxicity of mercury plus chromium toxicity on the histology and Na^+, K^+- ATPase of the fish, *Tilapia mossambica* and *Lates calcarifer*.

MATERIALS AND METHODS

Specimens of *Tilapia mossambica* and *Lates calcarifer* an estuarine fish species were collected from the Vellar Estuary, Tamil Nadu (India) and Acclimatized to the laboratory conditions for 20 days. Fish were fed ad libitum with a mixture of rice bran, groundnut oilcake and dry fish, twice a day. Fish ranging from 7-8 cm in length and weighing 8-10 g were selected for experimental purpose. The quality of the water was determined according to[14] and were as follows: Dissolved oxygen 5.4 ± 0.02 mg/l; pH 8.6 ± 0.2; Water Temperature 39.0 ± 2.0°C; Salinity 38 ± 0.07 ppt; Total hardness 8.2±2.0 mg/l; Calcium 5.0 ± 0.1 mg/l; Magnesium 3.0 ± 2.0 and Total alkalinity 16.0 ± 06mg/l.

Preliminary studies were carried out to find out the median lethal concentration (LC_{50}) for 96 h of mercury plus chromium. For this, appropriate amount of mercuric chloride plus chromium chloride were dissolved in seawater freshly every time to prepare a stock solution of 1000 ppm for each toxicant. The median lethal concentration (LC_{50}) of the above toxicant to fish for 96 h of mercury plus chromium (2.8 ppm) were determined in both fishes, respectively and calculated by probity analysis method of.[15] For sub-lethal studies, three glass tanks of 100 l capacity and each with 80 l of water were taken and to the first and second tank, 1/10 of 96 h LC_{50} concentrations of mercury plus chromium (2.8 ppm) was added into each fish tank, respectively, while the 3rd and served as control. Then, 80 fishes were introduced into the each tank and the experiment was maintained for a period of 28 days. Fish were fed ad libitum, and water and toxicants were renewed daily. No mortality was observed throughout the experimental period. At the end of every 7th day, 20 fish from both experimental tanks were selected randomly for collection of blood and tissues for further study. Blood was drawn from the heart region by cardiac puncture using a pre-chilled and heparin moistened disposable syringe. The collected blood was then transferred into small vials containing heparin, an anticoagulant. The whole blood sample was centrifuged at 10,000 rpm for 20 min and clear plasma was collected for further analysis. After collecting blood from control and experimental fish, 200 mg of organs such as gill, liver and kidney were separated one part of 100 mg of gill were taken and homogenized with 2.5 ml of 0.25 M sucrose solution in ice-cold condition and centrifuged for 10 min at 6,000 rpm and clear supernatant was taken for enzyme assay. Both plasma and gill Na^+, K^+ were estimated following the method of.[16] The remaining 100 mg gill tissue was fixed in Bouin's fixative for histopathological studies according to the methods of.[17-19] The significance between he sample mean of control and experiment fish was tested using student's test at 5% level.

RESULT AND DISCUSSION

Plate-A shows the changes in the gills of *Tilapia mossambica* and *Lates calcarifer* exposed to sub lethal concentration of metals for 28 days. In control fish, primary lamella appears normal and mucus free with well defined secondary lamellae branched from them [Figure 1a]. The 7th metals treated *T. mossambica* fish gills showed, lifting up of the epithelium, swelling, hyperplasia, hypertrophy and proliferation of chloride cells were noticed [Figure 1b]. In 14th day treated fish, the gills showed disintegration of

secondary lamellae, attachment of primary and secondary lamellae, dislocation of secondary lamellae, hypertrophy and hyperplasia were noted [Figure 1c]. In 21th day exposed fish gill showed disintegration of secondary lamella attachment and secondary lamellae fish location of Primary and secondary lamellae were noticed [Figure 1d]. After 28th day treatment of gill showed, fused lamellar filaments dis quamated epithelium and completed damage of epithelial cells were noticed [Figure 1e].

In control fish, (*Lates calcarifer*) primary lamella appears normal and mucus free with well defined secondary lamellae branched from them [Figure 2a]. Whereas in fish exposed to the same mixed metal concentration after 7th day treatment gill shows the proliferation of chloride cells, and dislocation of secondary lamellae were noticed [Figure 2b].

In 14th day exposed fishes, dislocation, attachment of secondary lamellae and Proliferation of chloride cells were noticed [Figure 2c]. In 21th day exposed fish gills showed proliferation chloride cells, attachment of primary and secondary lamellae and desquamated epithelial cells were noticed [Figure 2d]. After 28th day the gills were complete desquamated [Figure 2e].

Tables 1 and 2 shows the changes in the Na^+, K^+ and ATPase activity in gills and plasma of *T. mossambica* and *L. calcarifer* exposed to sub lethal concentration of metals for 28 days. In *L. calcarifer* the enzyme activity of gills decreased to -23.63% at the end of 7th day treatment. After the 7th day, enzyme activity recovered to +2.42% at the end of 14th day treatment. Again after 14th day, enzyme activity decreased to -12.25% and -10.23%, respectively, but in *T. mossmbica*

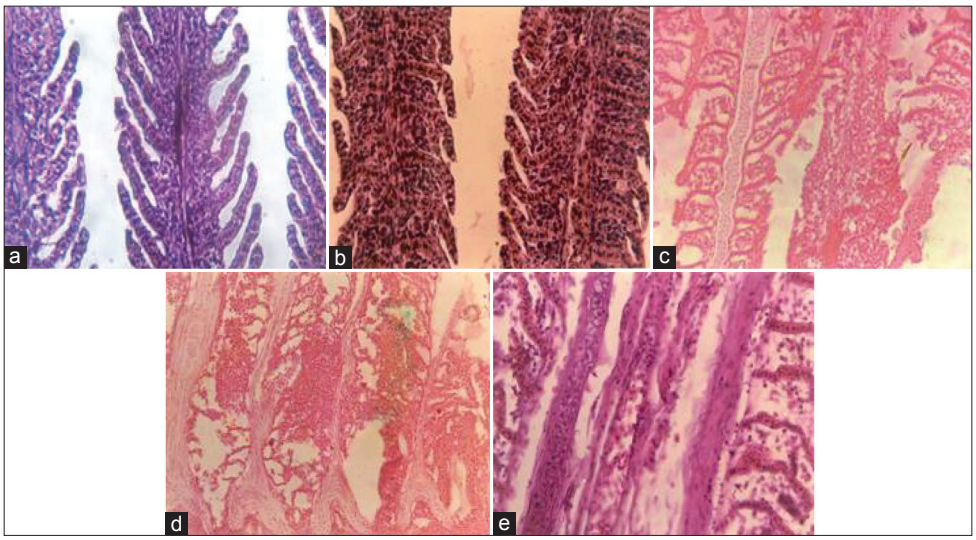

Figure 1: Photographs showing the transvers section of fish, *Tilabia mossambica* exposed to sublethal concentration of mercury plus chromium. (Magnification ×400)

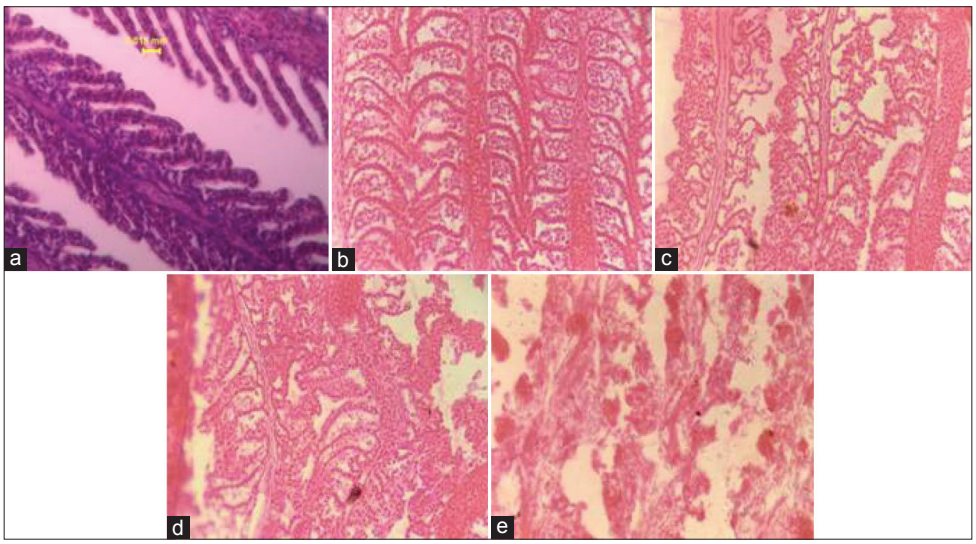

Figure 2: Photographs showing the transvers section of fish, *Lates calcarifer* exposed to sublethal concentration of mercury plus chromium. (Magnification ×400)

Table 1: Showing the Changes in Na^+, K^+- ATPase activity in gills of fish *Tilapia mossambica* and *Lates calcarifer* exposed to mercury cum chromium metal concentration

Organ	Gill (µg/h/g)							
Exposure period	7th day		14th day		21th day		28th day	
Name of the exposed fish	*Tilapia mossambica*	*Lates calcarifer*	*Tilapia mossambica*	*Lates calcarifer*	*Tilapia mossambica*	*Lates calcarifer*	*Tilapia mossambica*	*Lates calcarifer*
Control	41.386±0.13	42.482±0.11	40.632±0.10	37.720±0.44	33.434±0.11	31.724±0.05	40.122±0.11	39.042±0.26
Experiment	33.427±0.23 (-19.24)	32.445± 0.18 (-23.63)	39.542±011 (-2.68)	38.632±0.03 (+2.42)	28.334±0.12 (-15.25)	27.838±0.02 (-12.25)	36.142±0.12 (-9.92)	35.047±0.04 (-10.23)
t-Test	29.790	46.28	7.005	4.536	40.669	70.289	23.261	154.884

Table 2: Showing the Changes in Na^+, K^+- ATPase activity in plasma of *Tilapia mossambica* and *Lates calcarifer* exposed to mercury cum chromium metal concentration

Organ	Plasma (µg/h/l)							
Exposure period	7th day		14th day		21th day		28th day	
Name of the exposed fish	*Tilapia mossambica*	*Lates calcarifer*	*Tilapia mossambica*	*Lates calcarifer*	*Tilapia mossambica*	*Lates calcarifer*	*Tilapia mossambica*	*Lates calcarifer*
Control	0.031±0.01	0.029±0.02	0.027±0.03	0.026±0.01	0.027±0.20	0.026±0.06	0.030±0.13	0.029±0.08
Experiment	0.026±0.26 (-16.13)	0.024±0.18 (-17.24)	0.024±0.05 (-11.11)	0.023±0.24 (-11.54)	0.019±0.29 (-29.63)	0.018±0.16 (-30.77)	0.016±0.23 (-46.67)	0.015±0.18 (-48.28)
t-Test	1.650	2.808	6.0344	1.0344	2.272	4.790	5.384	7.216

the enzyme activity of gills was decreased to -19.24% and -9.92% at the end of 7th and 28th days, respectively. In both fishes, plasma enzyme activity decreased throughout the experimental period to -11.54% and -11.11% at the end of 14th day. A maximum decrease of -48.28% and -40.67% at the end of 28th day, respectively indicates that the change in enzyme activity in the mixed metal treated fish was significant at 5% level.

In present work we studied some histological damages in gills, like necrosis, chloride cells and hypertrophy in *T. mossambica* and *L. calcarife*. Severe gill damage of fish exposed to high levels of water-borne organic and inorganic Hg has been previously described by.[20,21]. In the present study, lamellar fusion, gill epithelial hyperplasia, and epithelial necrosis were some of the effects of exposure to mercuric chloride and chromium chloride that might cause respiratory and osmoregulatory disorders. These alterations also play a defensive role against contamination more than an irreversible toxic effect.

Found swelling of primary and secondary lamellae, fusion of adjacent secondary lamellae, increased mucus production, progressive loss of micro-ridge pattern, secondary lamellae appeared thickened and shortened with extremely rough surface and considerable mucus in brook trout, *Salvelinus fontinalis* exposed to pH 4.5 and 5.0 for 456 hr.[22] A similar observation was made by[23,24] in brown trout, *Salmo trutta*[25] reported that thickening of the lamellar epithelium increased diffusive distance of the gill. The thickening of the gill epithelium (*via*. cell hypertrophy) is sometimes considered to be an indicator of cell degeneration and eventually necrosis.[26,27] The lifting and hypertrophy of cells greatly increases the diffusion distance (water-blood distance)[28,29] observed epithelial cell lifting, eosinophilic granular cytoplasm, epithelial hypertrophy, mild curvatures of the primary and secondary lamellae, and occasionally epithelial hyperplasia and leukocyte infiltration of gill epithelium changes and also showed elevated gill indices in brown trout, *Salmo trutta* in diluted sewage plant effluents (62 to 205%) exposure.

In the present study, hypertrophy noticed might be in response to prevention of diffusion across gill epithelium, which finds support from the above authors [30] reported that a layer of edema has the primary effect of increasing the diffusion distance between blood and water and thus reducing the affected area to an essentially dysfunctional state. Gill epithelial swelling, complete desquamated lamellae and blood capillary pillar cells would account for the impairment of oxygen, carbon-di-oxide exchange, and for the hypoxia as reported by.[31] Swelling and desquamated epithelium of gills were observed in the present study, which might have caused impairment of oxygen or carbon-di-oxide exchange.[32] stated that lifting up of epithelium is a protective effect, resulting in increased water blood diffusing distance and hindered toxicant uptake. Lamellar fusion could be protective as it diminishes the amount of vulnerable gill surface area in fish.[13] Fusion of primary lamellae at the distal end and thickened and shortened secondary lamellae observed in the present study may be involved in reducing the impact of effluent toxicity in the present case supporting the observation of the above authors.

Results of present study showed that like many other

species mercuric chloride caused severe damages in chloride cells mainly, and these effects were because of the ion transporting duty of chloride cells more than any other gill epithelial cells. Our findings reveal that, structurally and ultra structurally, the gills were mostly affected by exposure to mercury, plus chromium in terms of both in the severity of the observed alterations to the tissue and the extent of damage. Many of the lesions documented in this study agree with those reported in other fish species under a wide range of exposure conditions, therefore, it seems plausible that these effects reflect physiological adaptation to stress rather than as specific toxic responses to the concentrations of mercury considered here.

The activity of Na^+, K^+ - ATPase in fish gills and possibly in the other animal tissues may be a useful non-specific biomarker as it is easily quantified and affected by a variety of toxicants[33,34] reported that *in vitro* exposure of Na^+, K^+ -ATPase to a variety of organic chloride compounds and metals inhibits this enzyme. ATPase systems were adversely affected in chronic *in vivo* studies with fathead minnows, *Pimephales promelas*, exposed to archolar 1242[35,36] reported a significant decrease of Na^+, K^+-ATPase activity in the microsomas fraction of gill homogenate of rainbow trout exposed to aldrin, chlordane, DDD, DDE, DDT, dicofol, dieldrin, heptachlor, lentane, methoxychlor, perthane, strobane, thiodon, toxaphane, 2,4,5-T and PCBs[37] noted that changes in the membrane lipid content or physical properties of the membrane influenced Na^+, K^+-ATPase activity[38] are of the opinion that membrane disordering effect may be the reason for enzyme inhibition. The loss of ion-specific ATPase could be attributed to the loss of sodium and potassium ions due to cellular leakage into the body fluids. Non-availability of substrates like ATP molecules may also result in the inhibition of these ion-specific ATPases.[39] In the present study, the significant increase in Na^+. K^+-ATPase in both fish gills and plasma may be due to changes in plasma Na^+, K^+ and chloride (Cl) levels. The inhibition of Na^+, K^+-ATPase activity from gills and plasma of *T. mossambica* and *L. calcarifer* during sub lethal treatment may be due to changes in the physical properties of the membrane or disruption of oxidative phosphorylation processes within the cells.

CONCLUSION

Since the *Lates calcarifer* is considered to be one of the chief edible fishes in this region, a continuous assessment with respect to the discharge of effluents into the water bodies are need of the hour. It is of interest to note that a change in severe gill alteration was observed in all treatments but the alteration was less in fish *T. mossambica* when compared to that of *L. calcarife*. That the fish gills show various changes in the gills this may be due to stress cum toxic responses of the exposed fish. The Na^+, K^+-ATPase activity of both gills and plasma showed significant reduction throughout the experiment period in both fishes. The enzyme activity was more drastic in the case of plasma. The results are discussed in relation to the significance of the above enzyme as non-specific biomarkers against environmental stress.

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