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RESEARCH ARTICLE

Study of mercury induced morphological and biochemical changes in liver of Cirrhinus mrigala

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Abstract

The aquatic animals are exposed to elevated levels of heavy metals continuously.Mercury (Hg) is highly toxic, nonessential, persistent, immutable and nonbiodegradable metal and is highly toxic to animals and cause death and sub lethal pathology of aquatic animals. The present study has been undertaken to explore the toxic effects of mercury on fish liver and to detect the spectral changes after the exposure. The biochemical changes after mercury exposure are studied by Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray spectroscopy (EDX), Optical absorbance and photoluminescence. The surface morphology of liver is studied by field emission scanning electron microscopy (FESEM).The spectroscopic techniques like FTIR, EDX, Absorbance and PL can be used as a firm tool to detect the impact of toxic elements. The present study can be used to correlate the overall biochemical status of the tissues with histopathological changes undergone at cellular level after chronic exposure to mercury.

INTRODUCTION

All forms of metals used in any industry are discharged to aquatic resources like estuaries, rivers, streams, and lakes [1]. These metals dissolve in water, transfer into food chain and are easily absorbed by fish and other aquatic organisms. It then brings about ill effects on the organism. Pesticides, heavy metals, are bioaccumulating substances, can adversely affect the biota and abiota [2]. In the aquatic environment, heavy metals are natural trace components, but their levels have increased because of increased agricultural, industrial and mining anthropogenic activities. The aquatic animals are exposed to elevated levels of heavy metals continuously [3, 4]. Contamination of fresh water with a wide range of pollutants has hence become a matter of concern since last few decades.

Mercury (Hg) is one of the oldest toxicants known [5] with considerable risk factors of environment and food chain [6]. Mercury, cadmium and lead are among the heavy metals that are toxic to organisms at very low concentrations and are never beneficial to living beings [7].

The fish liver is a vital organ concerned with basic metabolism and is the major organ of accumulation, biotransformation and excretion of contaminants in fish [8]. Impact on aquatic ecosystems can be evaluated by measuring biochemical parameters in the liver of fish that respond specifically to the degree and type of contamination [9]. The liver is particularly susceptible to damage from a variety of toxicants. Liver is responsible to clean pollutants from the blood so it is considered as indicator of aquatic environmental pollution [10].

Spectroscopic tools like Fourier Transform Infrared spectroscopy, photoluminescence, are being used extensively to probe quantitative biochemical information in biological samples [11]. Also the optical absorbance and photoluminescence are discussed by many workers [12-13]. FE-SEM provides surface morphological details, compositional analysis of carbon and oxygen is studied by EDS, optical response of biological sample is studied

by UV visible spectrophotometer and photoluminescence can be studied by spectrofluorometer. In the present paper, we focus our attention on the mercury induced biochemical changes in the liver tissues of freshwater fish *Cirrhinus mrigala*.

2. Materials and methods

Specimens of *C. mrigala* were collected from a reservoir at Kalambe near Kolhapur, M. S. India. Animals were acclimatized to laboratory conditions for 15 days. Fish were fed ad libitum with groundnut oil cake and water was changed daily. Fish with 70-75 g weight and 18-20 cm length were selected for experimental work. Chemicals-Analytical grade Mercuric chloride (BDH) was used without further purification.

The sub lethal concentration selected for chronic toxicity experiment were $1/20^{th}$ of LC_{50} and $1/10^{th}$ of LC_{50} ($1/20^{th}$ and $1/10^{th}$ of LC_{50} values 0.0206 ppm and 0.0402 ppm) concentration of mercuric chloride. The acclimated test animals in a group of 10 were exposed to the sub lethal concentration for a period of 30 days. A control set was run simultaneously. The water with toxicant renewed daily and fish were fed ad libitum during the period. The fish were sacrificed after 30 days and the desired tissue was pulled out. Sample preparation -The liver tissue was blotted and dried for 72hrs in oven at 60^0C.and then ground in mortar and pestle to obtain liver powder. The powder was used as a sample for further analysis.

The vibrational analysis of liver of C-mrigala have been studied using the perkin elmer, USA, Fourier transform infrared spectroscope (FTIR). The surface morphology has been studied using the Mira 3, Tescan, chez republic, field emission scanning electron microscope. Energy dispersive spectroscopy has studied using the Mira 3 tescan and oxford instrument, United Kingdom. Absorption spectra were recorded at room temperature and near to normal incidence using a UV-1800 Shimadzu, Japan. Photoluminescence has been studied using the fluoromax-4, horiba instrument PVT, Japan.

3. Results and discussion

3.1 Fourier Transform Infrared Spectroscopy (FTIR)

The present study is carried out to analyze the toxic effects of mercury in the liver tissues of fresh water fish *C. mrigala* by using FTIR spectroscopy. The areas of transmittance in FTIR spectrum are directly related to the concentration of the molecules [14]. The Fig.1 shows the FTIR spectra of control and mercury exposed liver tissues of *C. mrigala* in the range of 400 to 4000 cm^{-1}. The spectrum shows a complex of several bands formed due to different functional groups of proteins lipids and others. From fig.1 the spectrum shows transmittance arising due to alcohols, phenols, amines, amides...etc. The transmittance 3361 cm^{-1} for all samples is generally assigned to O–H stretch, H–bonded and N–H stretch from Alcohols, phenols, 1˚, 2˚ amines, amides of proteins. The area of 3361 cm-1 peak is decreased from control to mercury exposed. The peak at 2929 cm-1 for all samples represents the C-H stretch from alkanes of lipids. The decrease area of the transmittance for 2929 cm^{-1} is due to functional groups of lipids. The peak at 1726 cm^{-1} for control sample is indicates C=O stretch for aldehydes, saturated aliphatic due to proteins. The absence of these peaks in mercury exposed samples indicates functional deformity of protein. The peak at 1650 cm^{-1} for all samples is arising due N–H bend for 1˚ amines for secondary structures of proteins. The peak at 1531 cm^{-1} for all samples for N–H bends for 1˚ amines functional group of proteins. The decrease in area of peak at 1650 and 1531 cm-1 in intoxicated samples suggest the reduction in proteins. The peak at 1031 cm^{-1}, 1082 cm^{-1} and 1152 cm^{-1} for control, 0.0206 ppm and 0.0402 ppm mercuric chloride exposure, respectively represents C–N stretch, C-O stretch for Aliphatic amines of polysaccharides. The peak at 709 cm-1 and 785 cm-1 for control and 0.0206 ppm exposed to mercuric chloride for C-Cl stretch of Alkyl halides for proteins, lipids and carbohydrates. The absence of this peak after 0.0402 ppm mercuric chloride exposure reflects the loss of all essential components in liver tissue due higher dose. [15, 16]

3.2 Energy dispersive x-ray spectroscopic study

The EDX analysis has been carried out to reveal the effect of mercury on carbon oxygen percentage of liver tissue. The EDX spectrum reveled carbon and oxygen. For the control sample the weight and atomic percentage of carbon is 83.38% and 86.99%, respectively. While, that for oxygen is 16.62% and 13.01%, respectively this is shown in fig.2 (a). The weight and atomic percentage of carbon for liver of fish exposed to 0.0206 ppm concentration of mercury cholride is 74.05% and 79.17%, respectively, while, that for oxygen is 25.95% and 20.83%, respectively this is shown in fig.2 (b). After the chronic exposure to sublethal concentration of 0.0402 ppm mercuric chloride, the weight and atomic percentage of carbon is 81.22% and 85.21%, respectively. Besides, oxygen weight and atomic percentage is 18.78% and 14.79%, respectively this is shown in fig. 2(c). The change in atomic and weight percentage of carbon is significant and due to mercury exposure. The gradual decrease in weight and atomic percentage of oxygen is due to excessive stress of toxicant and excessive use of oxygen by liver of *C. mrigala.*

3.3 Field emission scanning electron microscopic study (ESEM)

The surface morphological study has been carried out for liver of control and mercury exposed fish. The Fig. 3 (A1 and A2) reveals arrangement of hepatic cells in control samples. The Fig. 3 (B1 and B2) shows branching pattern and round elevations, sinusoid dilations and lipid droplet accumulation after exposure to sublethal concentration of mercuric chloride (0.0206 ppm) The abundance of cell is observed to be decreased after mercury exposure. Further decrease in cell population and drastic morphological alteration are seen after exposure to 0.0402 ppm this is shown in fig.3 (C1 and C2). FESEM study reveals significant metal exposure induced alterations in liver. Severe morphological transformation of hepatic cells transformation leads to functional alterations and disturb the fundamental function of liver. SEM study of fish liver have been reported by Noskor et al. [17]

3.4 Optical absorbance

Optical absorbance of liver of *C. mrigala* has been studied using UV-vis spectrophotometer [18]. The optical absorbance has been studied after dissolving the liver powder in methanol. The optical absorbance has been observed at 354 nm shown in fig. 4. Changes in the absorbance of liver were observed after the mercury exposure.

3.5 Photoluminescence

It is interesting to study the continuous emission of biological systems. [19, 20]. To understand the emission of liver of *C. mrigala*, the photoluminescence technique is used. The emission of liver of *C. mrigala* has been studied with external excitation of 335 nm. The emissions have been observed at 378 nm for liver and this is shown in Fig. 5. The Stokes shift has been observed to be 24 nm for liver of *C. mrigala*..

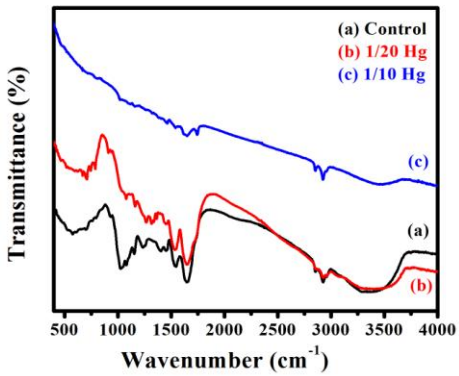
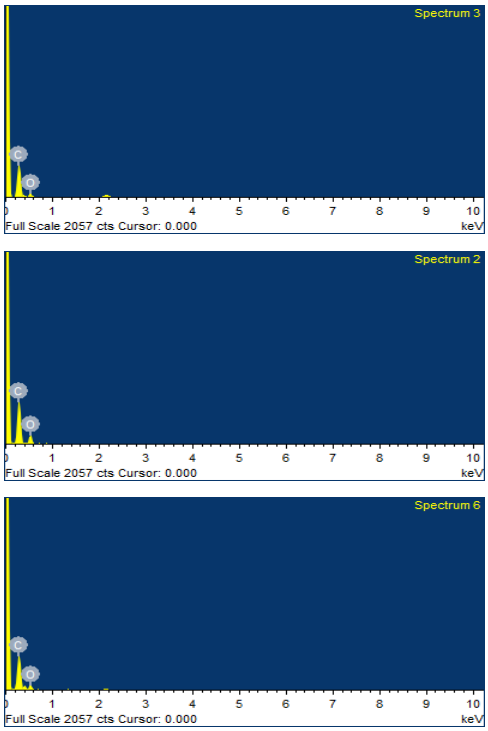


Fig.1 FTIR spectra of control and mercury exposed *C. mrigala* Liver (a) control, (b) $1/20^{th}$ $HgCl_2$, (c) $1/10^{th}$ $HgCl_2$



Element	Weight%	Atomic%
C K	83.38	86.99
O K	16.62	13.01

Element	Weight%	Atomic%
C K	74.05	79.17
O K	25.95	20.83

Element	Weight%	Atomic%
C K	81.22	85.21
O K	18.78	14.79

Fig. 2 EDX spectra of control and mercury exposed *C. mrigala* Liver (a) control, (b) 1/20th $HgCl_2$, (c) 1/10th $HgCl_2$

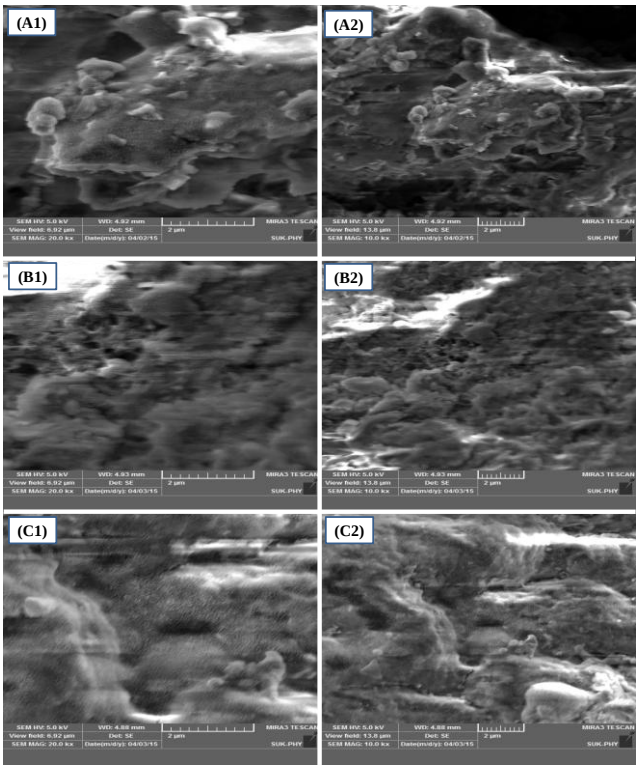


Fig. 3 FE-SEM images of control and mercury exposed *C. mrigala* Liver (A1) Control X=50kx, (A2) Control X=25kx, (B1) 1/20th $HgCl_2$ X=50kx, (B2) 1/20th $HgCl_2$ X=25kx, (C1) 1/10th $HgCl_2$ X=50kx, (C2) 1/10th $HgCl_2$ X=25kx.

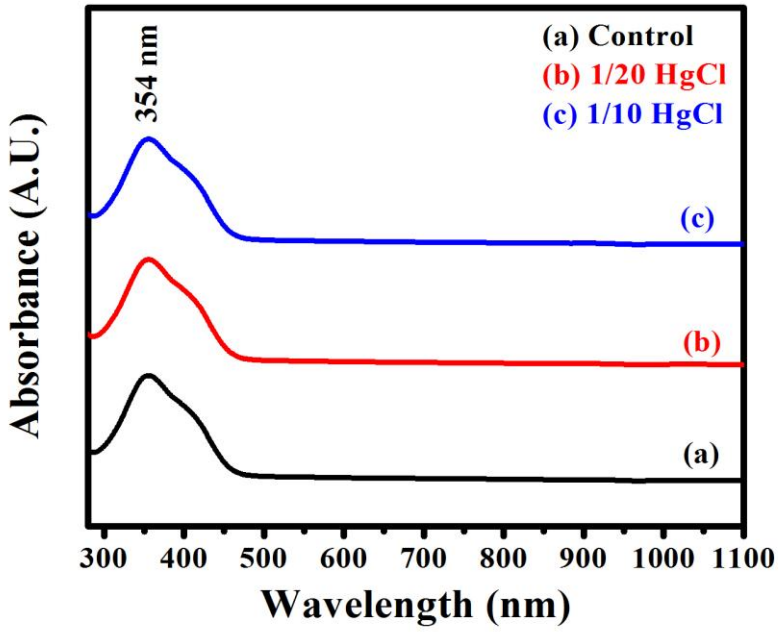


Fig. 4 Optical absorbance spectra of control and mercury exposed *C. mrigala* Liver (a) control, (b) 1/20th $HgCl_2$, (c) 1/10th $HgCl_2$

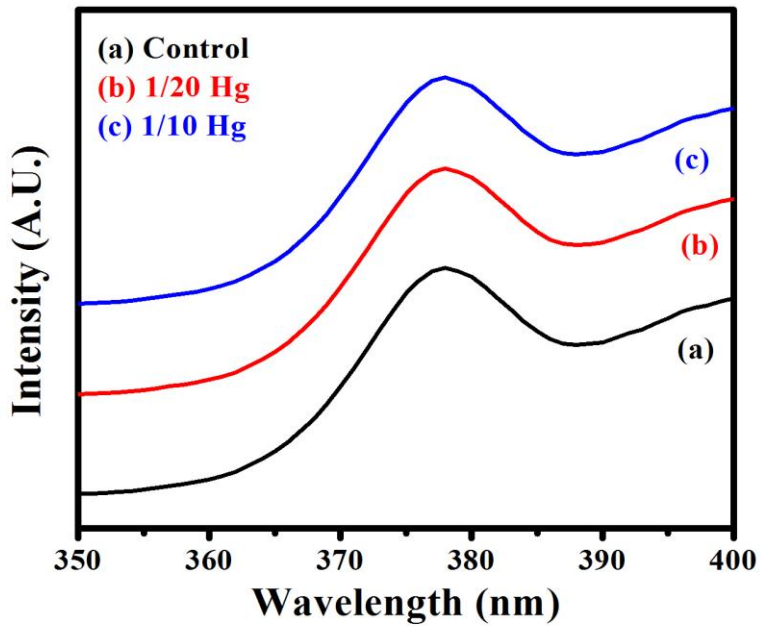


Fig. 5 Photoluminescence spectra of control and mercury exposed *C. mrigala* Liver (a) control, (b) 1/20th $HgCl_2$, (c) 1/10th $HgCl_2$

Table.1 General band assignment of the FTIR spectra of control and mercury exposed C-mrigala Liver

Sr. No.	Control	1/20	1/10	Bonds	Functional group
1	3361	3361	3361	O–H stretch, H–bonded, N–H stretch	Alcohols, phenols, 1˚, 2˚ amines, amides

2	2929	2929	2929	C–H stretch	Alkanes
3	1726	--	--	C=O stretch	Aldehydes, saturated aliphatic
4	1650	1650	1650	N–H bend	1˚ amines
5	1531	1531	1531	N–O asymmetric stretch	Nitro compounds
6	1031	1082	1152	C–N stretch	Aliphatic amines
7	709	--	785	C–Cl stretch	Alkyl halides
8	574	--	--	C–Br stretch	Alkyl halides

4. Conclusions

In the present paper changes in biochemistry of fish liver after mercuric chloride exposure have been discussed. The results of FTIR indicate that liver is a complex of many organic compounds. The surface morphology of liver shows the alterations due to mercuric exposure, which confirms functional alterations in liver. The FESEM studies evaluate the hepatocellular damage. The sharp absorbance has been observed at 354 nm for all samples. The liver of *C.mrigala* shows the strong emission at 378 nm but mercuric exposure is responsible for change in intensity only. The mercury intoxication induced alterations in liver as significant difference in absorbance intensities reflect the alterations in major biochemical components. All results are the index of stress in *C. mrigala* after mercury exposure. The present study provides the baseline information regarding biochemical and morphological alteration and associated health effects from heavy metals ingestion in fish and suggests future assessments.

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