Study of mercury induced morphological and biochemical changes in gill of *Cirrhinus mrigala*

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Abstract:

Pollution due to heavy metals is a major ecological concern due to its impact on human health through food chain. 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 gill 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 gill 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.

Keywords – *C. mrigala*; mercuric chloride; toxicity; spectroscopy

1. Introduction

Increased anthropogenic activities release non essential heavy metals to the resources. A wide range of pollutants like industrial effluents and wastes, agricultural pesticide runoff, domestic garbage dumps and mining activities cause contamination of resources and make matter of major concern around the globe [1, 2]. Metals are of special concern due to their diversified effects and the range of concentrations that could cause toxic effects to fish [3]. Metals like mercury, lead are non essential and cause a serious concern because of their persistence, bioaccumulation and biomagnifications. Mercury is a metal pollutant of high toxicity to aquatic animals including fish. In an aquatic ecosystem, fish are considered as heavy metal indicator because heavy metal readily accumulates in the fish body [4]. Fish form important group of vertebrate animals and they are widely consumed in

many parts of the world as they are highly nutritious and contain a high quality proteins. The fishes are adversely affected by pollutants like metals hence they are considered to be the most relevant organism for assessing the pollution in the aquatic ecosystems [5].

During all contamination processes in fish, heavy metal cross biological barrier, the gill epithelium and may cause accumulation in metabolically active tissues like gills. This causes disturbances in vital processes and changes the biochemistry which ultimately reduces their food quality [6]. Gills are the first organ to which the pollutant comes into contact. The gills are hence more vulnerable to damage than any other tissue.

FTIR spectroscopy is a technique which provides quantitative details of biochemical composition of biological sample. It is an important and popular tool which needs a little sample, short preparation and gives sharp results with high sensitivity. 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. There are still little data on mercury exposure and its effect on different organs of tropical fish other than histological technique. In the present study spectroscopic analysis was done to explore the mercury effects on gills of a freshwater fish *Cirrhinus mrigala*.

2. Materials and methods

2.1 Biological material

The live fresh water teleost *C. mrigala* of average length 18-20 cm and average body weight 70-75 g were collected from a reservoir at Kalambe near Kolhapur, M. S. India. Animals were disinfected with KMnO4 and acclimatized to laboratory conditions for 15 days in glass aquarium with continuously aerated tap water. Healthy fishes were identified by general appearance and selected for experimental work. The water was checked for selected Physico chemical parameters. Fish were fed ad libitum with groundnut oil cake.

2.2 Exposure

Chemicals-Analytical grade Mercuric chloride HgCl2 (BDH) was used without further purification. The heavy metal mercury was used in the form of mercuric chloride for the present study. Stock solution of mercuric chloride (HgCl2) was prepared by dissolving analytical grade mercuric

chloride in double distilled water. The desired concentration of mercuric chloride ($1/20^{th}$ of LC_{50} - 0.0206 ppm and $1/10^{th}$ of LC_{50} - 0.0402 ppm) was prepared from the stock solution. The acclimated test animals in a group of 10 were exposed to these sub lethal concentration for a period of 30 days. Each group consisted of three tanks which served as a triplicates. A control set was run simultaneously. The water with toxicant renewed daily and fish were fed ad libitum during the period. The toxicant was renewed completely by replacing fresh solution of same concentration. All bioassays were performed in triplicates for 30 days. The fish were sacrificed after 30 days and the desired tissue was pulled out. Sample preparation - The gill tissue was blotted and dried for 72hrs in oven at 60^0C.and then ground in mortar and pestle to obtain gill powder. The powder was used as a sample for further analysis.

The vibrational analysis of gill of *C. mrigala* has 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 transforms infrared spectroscopic study (FTIR)

The present study has been carried out to understand the molecular structure and molecular composition of the gill of the *C.mrigala* using FTIR spectroscopy [7]. The FTIR spectra have been studied in the range of 400-4000 cm-1. The spectrum shows the several bands due to functional groups proteins, lipids, nucleic acids and amides and these observed frequencies, bonds and functional groups are mentioned in Table.1. The FTIR peaks at 3463 cm-1, 3437 cm-1 and 3403 cm-1 for control, exposed to 0.0206 ppm concentration of HgCl2 and 0.0402 ppm concentration of HgCl2, respectively. The peaks belong to O-H stretch, H-bonded with alcohol and phenol as functional group. These peaks represent proteins. The peaks at 2950 cm-1, 2921 cm-1 and 2921 cm-1 represent control, and tissue samples of fish exposed to 0.0206 ppm and 0.0402 ppm HgCl2, respectively.

These peaks represent alkenes of lipids, proteins and carbohydrates.

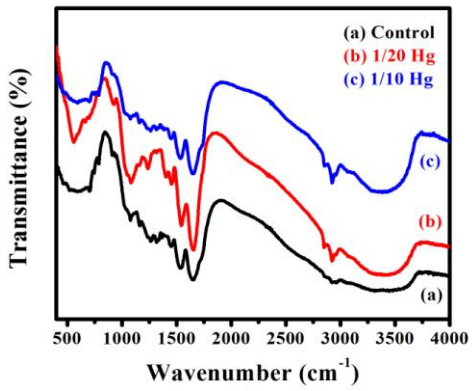

Fig.1 FTIR spectra of control and mercury exposed *C-mrigala* gill (a) control, (b) 1/20th $HgCl_2$, (c) 1/10th $HgCl_2$

The peak at 2844 cm^{-1} for all samples represents the C-H stretch for alkanes which indicates the lipids. The FTIR peaks at 1650 cm-1, 1658 cm-1 and 1641 cm-1 indicate the –C=C– stretch for alkanes of structural proteins. The N-O asymmetric stretch for nitro compounds of the structural proteins is indicated by 1531 cm-1, 1539 cm-1 and 1531 cm-1 for control, and exposed fish tissue samples respectively. The peak for 0.0402 ppm concentration of HgCl2 is at 1446 cm-1 represents the C–H bend for alkanes of the fatty acids present in fish gill. The observed peak at 1311cm-1 for control sample belongs to C–N stretch and C–O stretch for Aromatic amines Alcohols, Carboxylic acids, Esters, Ethers etc for Glycogen. But this peak is not observed after the HgCl2 exposure. The peaks for 1161 cm-1 and 1237 cm-1 indicate the nucleic acids. But again this peak is vanished in the tissue samples of fish exposed at 0.0402 ppm concentration of HgCl2. The peak at 709 cm-1 is belongs to –C≡C–H: C–H bend for alkynes. It represents the carbohydrates, proteins, lipids. Our results are well agreed with Senthamilselvan et al. [8]. The peak at 540 cm-1 for all samples belongs to C-Br stretch for Alkyl halides. This is due to KBr used for FTIR analysis. The shift in peaks position, change in transmission intensity, vanished peaks after exposure to 0.0402 ppm and 0.0206 ppm concentration of HgCl2 indicate that a chronic exposure to mercury causes biochemical alterations in proteins, lipids, carbohydrates and nucleic acids of gill of fish *C.mrigala*. The biochemical change in gill is further may cause functional deformity.

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

Sr. No.	Frequency (cm^{-1})			Bonds	Functional group
	Control	1/20	1/10		
1	3463	3437	3403	O-H stretch, H-bonded	Alcohols, Phenols
2	2950	2921	2921	C-H stretch	Alkanes
3	2844	2844	2844	C-H stretch	Alkanes
4	1650	1658	1641	–C=C– stretch	Alkenes
5	1531	1539	1531	N–O asymmetric stretch	Nitro compounds
6	---	1446	---	C–H bend	Alkanes
7	1311	---	---	C–N stretch C–O stretch	Aromatic amines Alcohols, Carboxylic acids, Esters, Ethers
8	1161	1237	----	C–N stretch	Aliphatic amines
9	709	---	---	–C≡C–H: C–H bend	Alkynes
10	540	540	540	C–Br stretch	Alkyl halides

3.2 Field emission scanning electron microscopic study (FESEM)

The surface morphological study has been carried out for gill of control and mercury exposed fish. The Fig. 3 (A1 and A2) shows a homogeneous intact arrangement of lamellar cells in control samples. The Fig. 3 (B1 and B2) shows rough and scattered arrangement of lamellar cells in gills of fish exposed to 0.0206 ppm concentration of mercuric chloride. The abundance of cell is observed to be decreased after mercury exposure. Further decrease and morphological alteration are seen in gills of fish exposed to 0.0402 ppm mercuric chloride concentration. This is shown in fig.3 (C1 and C2). FESEM study reveals significant metal exposure induced alterations in gill. Decrease in density of lamellar cells and transformation of their surface structures is proportional to intensity of toxicant. The transformation may lead to functional alterations and disturb the fundamental function of gill. SEM study of fish gill has been reported by Evans et al [9].

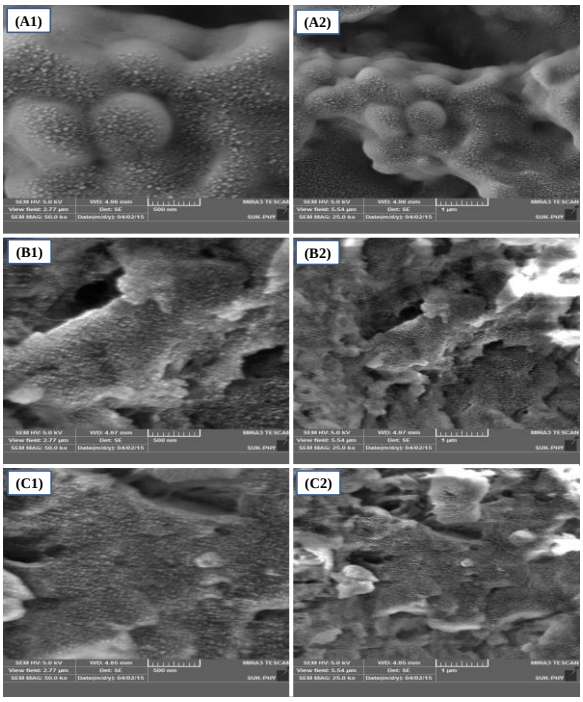

Fig. 2 FESEM images of control and mercury exposed *C.mrigala* gill (A1) Control X=50kx, (A2) Control X=25kx, (B1) 1/20th $HgCl_2$ X=50kx, (B2) 1/20th $HgCl_2$ X=25kx, (C1) 1/10th $HgCl_2$ X=50kx, (C2) 1/10th $HgCl_2$ X=25kx.

3.2 Energy dispersive X-ray spectroscopic study (EDX)

The EDX analysis has been carried out to reveal the effect of mercury on carbon oxygen percentage of gill tissue. The EDX spectrum reveled carbon and oxygen. For the control sample the weight and atomic percentage of carbon is 72.37% and 77.72%, respectively. While, that for oxygen is 27.63% and 22.28%, respectively this is shown in fig.2 (a).

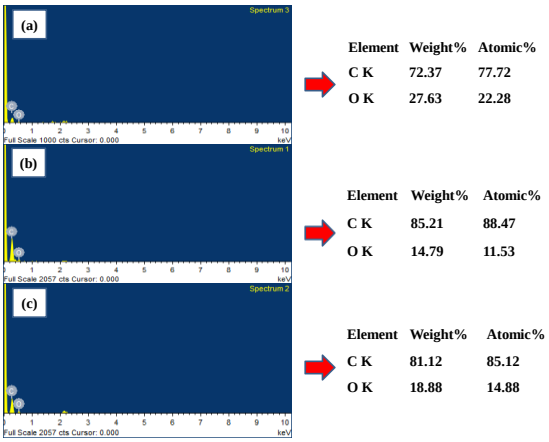

Fig. 3 EDX spectra of control and mercury exposed *C.mrigala* gill (a) control, (b) 1/20th $HgCl_2$, (c) 1/10th $HgCl_2$

The weight and atomic percentage of carbon for samples of fish exposed to 0.0206 ppm concentration of mercuric chloride is 85.21% and 88.47%, respectively, while, that for oxygen is 14.79% and 11.53%, respectively this is shown in fig.2 (b). After the exposure to 0.0402 ppm concentration of mercuric chloride the weight and atomic percentage of carbon is 81.12% and 85.12%, respectively. Besides, oxygen weight and atomic percentage is 18.88% and 14.88%, respectively this is shown in fig.2 (c). The change in atomic and weight percentage of carbon is significant and due to toxicant exposure. The gradual decrease in weight and atomic percentage of oxygen is due excessive stress of toxicant and excessive use of oxygen by gills of *C. mrigala*. As little amount of $HgCl_2$ has been used for the

exposure, this is not observed in EDX of gill.

3.3 Optical Absorbance

Optical behavior of Gill of *C. mrigala* have been studied using UV-vis spectrophotometer [10] The optical absorbance has been studied with dissolving of prepared gill powder in methanol. The optical absorbance has been observed near at 262 nm this is shown in fig.4. It means that gill of *C.mrigala* has been response to UV-light. The slight increase in absorbance in visible region is observed from higher to lower wavelength region. The observed absorbance peak at 262 nm is near UV region. After the mercuric exposure change has been observed in absorbance intensity. The change in absorbance intensity is also indicating the effect of $HgCl_2$ exposure.

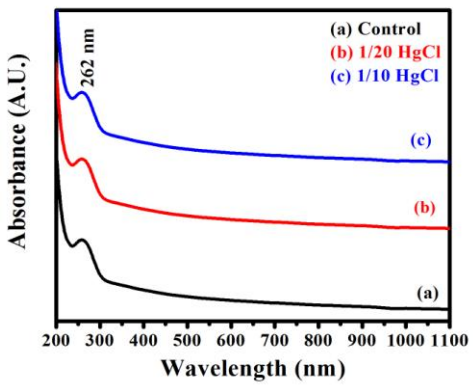

Fig. 4 Optical absorbance spectra of control and mercury exposed *C.mrigala* gill (a) control, (b) $1/20^{th}$ $HgCl_2$, (c) $1/10^{th}$ $HgCl_2$

3.4 Photoluminescence

It is fascinating to observe that biological systems continuously emit weak light [11, 12]. So, spectrofluorometer is tool which helps us to understand the emission of gill of *C. mrigala*. Photoluminescence study has been carried out with the help of spectrofluorometer. The emission of *C.mrigala*. has been studied with external excitation of 260 nm with help of spectrofluorometer. The emissions have been observed at 378 nm and 440 nm for gill of *C.mrigala* this is shown in Fig. 5.

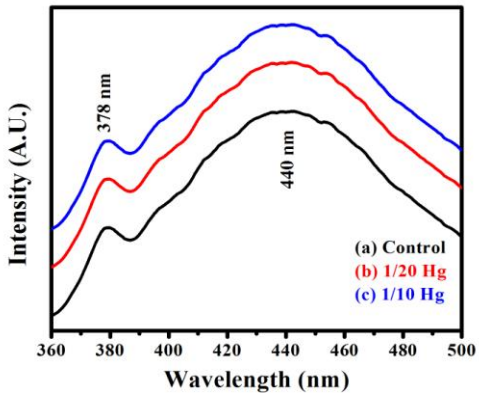

Fig. 5 Photoluminescence spectra of control and mercury exposed *C-mrigala* gill (a) control, (b) $1/20^{th}$ $HgCl_2$, (c) $1/10^{th}$ $HgCl_2$

4. Conclusions

In the present paper changes in biochemistry of fish gill after mercuric chloride exposure have been discussed. The results of FTIR indicate that Gill is a complex of many organic compounds. The surface morphology shows the alterations due to mercuric exposure, which confirms functional alterations in gill. The sharp absorbance has been observed at 262 nm for all samples. The gill of *C.mrigala* shows the strong emission at 378 and 440 nm but mercuric exposure is responsible for change in intensity only. The mercury intoxication induced alterations in gill as significant difference in absorbance intensities reflect the alterations in major biochemical components. The biochemical changes in gill reduce the food quality of fish. All results are the index of stress in *C. mrigala* after mercury exposure.

5. References

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