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SPECTROSCOPIC INVESTIGATIONS OF BRAIN OF *CIRRHINUS MRIGALA* AFTER MERCURY EXPOSURE

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ABSTRACT

The natural aquatic ecosystems may extensively be contaminated with heavy metals released from domestic, industrial and other manmade activities. Mercury (Hg) is a highly toxic, nonessential, persistent, immutable and nonbiodegradable heavy metal and is highly toxic and cause death and sublethal pathology of aquatic animals. It is critical to look at alterations in nervous system as it is the primary target organ of mercury action. The present study has been undertaken to explore the toxic effects of mercury on fish brain and to detect the spectral changes after the exposure. The samples of desired tissue were prepared after a chronic exposure of *C. mrigala* to sub lethal concentrations ($1/10^{th}$ and $1/20^{th}$ of LC_{50} values 0.0402 ppm and 0.0206 ppm) of mercuric chloride for a period of 30 days. The samples were characterized for their structural study using X-ray diffraction (XRD), which shows the amorphous nature. IR spectroscopy has been used to study vibrational states and bonding of different molecules in brain tissue. Field emission scanning electron microscopy (FE-SEM) technique has been used to study the surface morphology of C. *mrigala* brain. The gas chromatography mass spectrometry (GCMS) has been performed for structural assessment or probable molecules in brain tissue. The spectroscopic techniques are distinguished among the currently used method of monitoring animal tissues and their components. The XRD, IR, FE-SEM, GCMS were preferred as little preparation of samples is necessary and changes in the tissue chemistry produced by metal exposure are evident. Significant differences in absorbance areas and intensities between control and metal exposed tissues showed the alterations in biochemical content. 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; XRD; FE-SEM; IR, GCMS spectroscopy; pathology; toxicity

1. INTRODUCTION

Metallic elements work as structural elements or components of biological structures or activators of biological systems. Some metals are essential metals while others serve no biological purpose, while still others produce environmental hazards [1]. The natural aquatic systems are contaminated extensively with heavy metals released from domestic, industrial and other manmade activities [2]. Industrial units frequently discharge toxic wastes into different water bodies, which adversely affect the fresh water ecosystems. Although aquatic ecosystems are equipped with a variety of physico-chemical and biological mechanism to eliminate or reduce adverse effects of toxic substances, toxicants may evoke changes in development, growth, reproduction and behaviour or may cause death of freshwater organisms [3].

Fish are an integral component of the aquatic ecosystems. In addition to being a source of protein to humans, they play important roles as indicator of trace element pollution [4]. Fish, as living bioindicator species, play an increasingly important role in monitoring of water pollution as they respond with great sensitivity to changes in the aquatic environment. Fishes are sensitive indicators of aquatic pollution and accumulate mercury [5]. The various studies carried out on different fish species have shown that heavy metals may change the biochemical composition and physiological activities of fish [6]. It is important to know the harmful effects of heavy metals for healthy fish production [7].

Mercury is among the heavy metals that are toxic to organisms at very low concentrations and is never beneficial to living beings [8]. Mercury (Hg) is a highly toxic, nonessential, persistent, immutable and nonbiodegradable heavy metal and cause death and sublethal pathology of aquatic animals. Mercury has received significant attention from scientific community due to its toxicity to human and wildlife [9]. It is critical to look at alterations in nervous system as it is the primary target organ of mercury action [10]. There are still little data on mercury exposure and its effect in different organs in tropical fish [11]. It points up the need for more studies on effects of mercury on brain. The present study has been undertaken to explore the toxic effects of mercury on fish brain and to detect the spectral changes after the exposure.

In the present paper, we focus our attention on the mercury induced changes in the brain of fresh water fish *Cirrhinus mrigala* using XRD, IR, FE-SEM, GCMS and optical study such as absorbance, transmittance and photoluminescence (PL).

2. Materials and methods

The freshwater juvenile *Cirrhinus mrigala* (length 19-20 cm and weight 75 ±2g) were collected from a freshwater reservoir and acclimated to the laboratory conditions for 15 days. The laboratory water was analyzed for selected physico-chemical parameters such as pH, dissolved oxygen, total hardness, phosphates and nitrates. The water was also tested for mercury as mercuric chloride was used as a toxicant. The LC_{50} value for mercuric chloride was determined by static bioassay and was found to be 0.412 ppm. The acclimated test animals in a group of 10 were exposed to $1/20^{th}$ and $1/10^{th}$ of LC_{50} concentration (0.0206 ppm and 0.0402 ppm) of mercuric chloride for a period of 30 days. The test solution was renewed once after 24 hours by replacing the test solution. The fish were given ad-libitum access to food during the experiment. A control group in similar conditions was kept simultaneously with only seasoned water. All experiments were conducted under a constant 12:12 LD photoperiod. After the chronic exposure of 30 days to sub lethal concentration of mercuric chloride the test animals were sacrificed. The whole brain was removed and prepared for further spectroscopic analysis. The brain was blotted and dried in oven at $60^{o}C$ for 72 hr. The samples were then ground in agate mortar and pestle in order to obtain brain powder.

The samples were characterized for their structural study using X-ray diffraction (XRD). IR spectroscopy has been used to study vibrational status and bonding of different molecules in brain tissue. Field emission scanning electron microscopy (FE-SEM) technique has been used to study the surface morphology of C. *mrigala* brain. The gas chromatography mass spectrometry (GCMS) has been performed for structural assessment or probable molecules in brain tissue. The spectroscopic techniques are distinguished among the currently used method of monitoring animal tissues and their components. The XRD, IR, FE-SEM, GCMS were preferred as little preparation of samples is necessary and changes in the tissue chemistry produced by metal exposure are evident.

3. Results and discussion

3.1 X-ray diffraction (XRD) study

The XRD is important technique to find out the structures of compound [12, 13]. In the present investigation we have studied the XRD diffraction as a tool to analyze nature of sample (amorphous or crystalline). XRD patterns were obtained with the help of copper target having wavelength Ka=1.5418 Aº. The brain tissues of *C. mrigala* were slowly scanned between 20 to 80º. The Fig. 2 shows the amorphous nature of *C. mrigala* brain tissues of control. Being an organic compound, brain shows amorphous nature and has many functional groups. XRD study reveals that *C. mrigala* brain is amorphous.

3.2 IR spectroscopy

The present study was carried out to analyze the toxic effects of mercuric chloride on brain of *C. mrigala*. The brain is an organ that serves as the center of the nervous system in all vertebrate and most invertebrate animals [14]. Fish are a source of high quality essential minerals with vitamins, and protein. The brain is a virtually unique, rich source of omega-3 long-chain poly-unsaturated fatty acids (PUFA) [15]. Fig.1 shows the IR spectra of brain of control animal, and animal exposed to 0.0206 ppm and 0.0402 ppm concentration of mercuric chloride. The main absorption bands and their assignments are defined in Table 1. It

shows the amines, alkynes, alkenes, primary amines, aromatic amines, alkyl halides, aliphatic amines, alkyl halides groups. The IR spectrum of brain is complex and contains several bands formed from different functional groups belonging to protein, lipids and carbohydrates. The decreased band areas at different stretching modes of amide bands, in the brain of mercury exposed *C. mrigala* suggest the decrease in composition of proteins. The decrease is positively correlated with the concentration of toxicant.

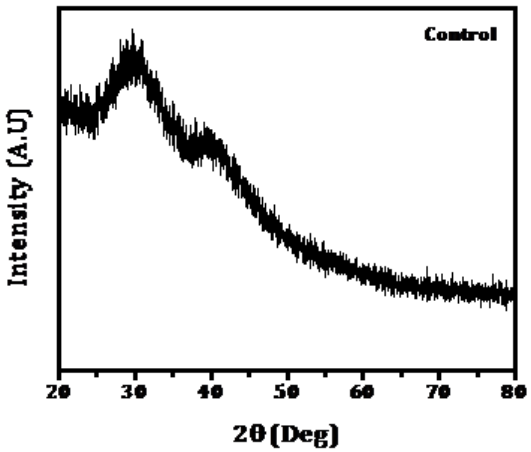


Fig.2 X-ray diffraction pattern of the *C. mrigala* fish brain (a) Control, (b) mercuric chloride 0.0206 ppm (c) mercuric chloride 0.0402 ppm

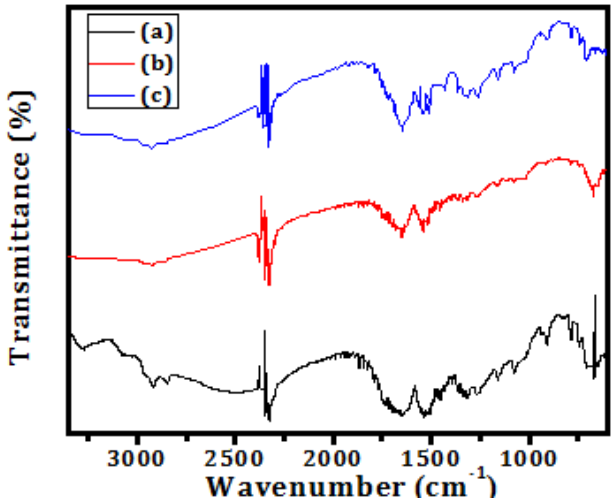


Fig. 2 IR spectra of the *C. mrigala* fish brain (a) Control, (b) mercuric chloride 0.0206 ppm (c) mercuric chloride 0.0402 ppm

Table 1 IR Frequency and their bond as well as corresponding functional group after and before mercuric exposure

Sr. No	Frequency (cm^{-1})			Bond	Functional group
	Control	0.0206 ppm	0.0402 ppm		
1)	3417	-	-	N-H stretch	amines
2)	3275	3333	-	-C≡C-H: CH	alkynes
3)	3055	-	-	C-H stretch	alkenes
4)	3026	-	-	C-H stretch	alkenes
5)	2953	-	-	C-H stretch	alkenes
6)	2920	2924	2938	C-H stretch	alkenes
7)	2859	2846	-	C-H stretch	alkenes
8)	2328	2366	2352	------	-------
9)	1637	1646	1653	N-H bend	primary amines

10)	1530	1540	1547	C-N- stretch	aromatic amines
11)	1326	1307	1456	C–N stretch	aromatic amines
12)	1272	1258	1264	C–H wag (–CH2X)	alkyl halides
13)	1160	1159	1165	C–N stretch	aliphatic amines
14)	1073	1072	1025	C–N stretch	aliphatic amines
15)	908	897	-	N-H stretch	primary secondary amines
16)	786	785	-	C-Cl stretch	alkyl halides
17)	666	658	679	C-Br stretch	alkyl halides

3.3 Field emission scanning electron microscopy (FESEM)

The surface morphology of brain of *C.mrigala* has been studied using FESEM. It shows the porous nature for all three samples. The brain of control animal shows homogenous and cauliflower likes morphology. Significant differences in the superficial appearance are revealed in the brain. The control group shows a homogenous appearance. The brain of fish exposed to both sub lethal concentrations of mercuric chloride showed lifting and increase in roughness in superficial appearance. The FESEM studies suggest structural deformations in brain of *C.mrigala* exposed to 0.0206 ppm and 0.0402 ppm concentration of mercuric chloride.

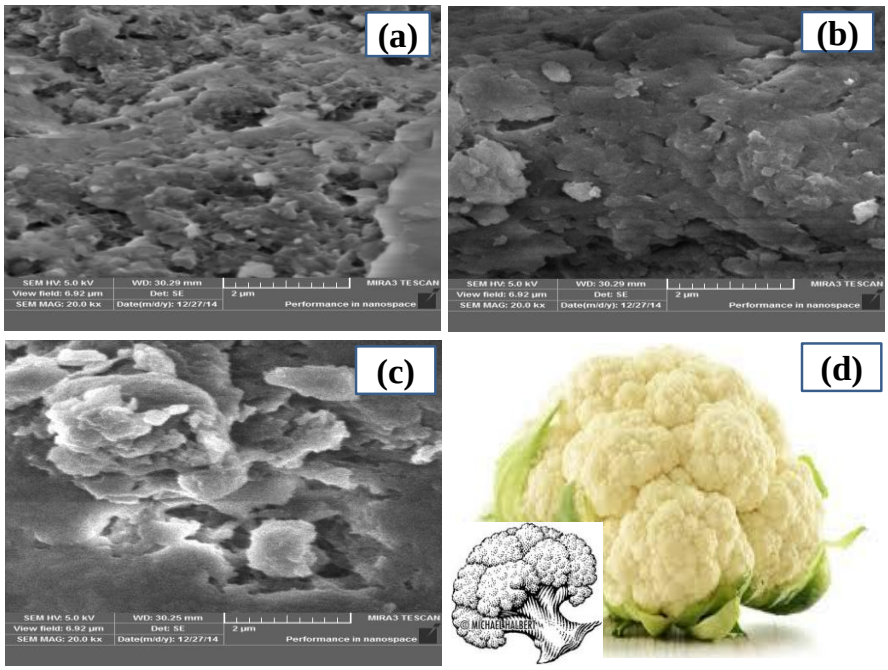


Fig. 2 FESEM images of brain of *C. mrigala* fish a) Control, (b) Mercuric chloride 0.0206 ppm (c) Mercuric chloride 0.0402 ppm, (d) Cauliflower image (inset of (d) sketch of cauliflower)

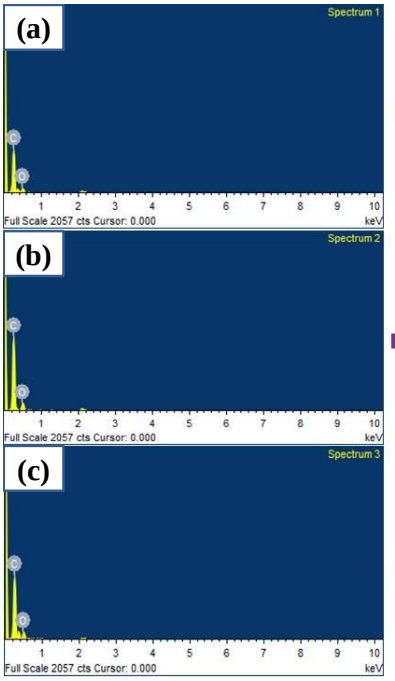
3.4 Energy Dispersive Spectroscopy

The energy dispersive spectrums have been recorded before and after exposure of mercuric chloride (Fig. 3). Carbon and oxygen are observed in brain of *Cirrhinus mrigala* fish. However, carbon is more prominent than oxygen. Carbon contributes about 80 to 83% and oxygen is about 18 to 20%. After the mercuric exposure the change has been observed in weight and atomic percent of brain of *Cirrhinus mrigala* fish.

3.5 Optical absorbance

When a ray of light passes through the material many phenomena are observed such as absorbance, transmittance, reflectance, scattering, refraction, luminescence etc [16-18]. The optical absorbance has been studied for the brain of control and mercury exposed *C.mrigala.* The prepared powder dissolved in dimethyl sulphoxide (DMSO) and used for absorbance and transmittance study. The 0.5 g powder dissolved in 10 ml

DMSO. It shows the strong absorbance at 360 nm. After exposure to sub lethal concentrations of mercuric chloride, change in absorbance has been observed. The absorbance is observed to be decreased (Fig. 4).



Element	Weight %	Atomic %
c	81.54	85.47
o	18.46	14.53

Element	Weight %	Atomic %
c	83.03	86.70
o	16.97	13.30

Element	Weight %	Atomic %
c	80.47	84.59
o	19.53	15.41

Fig. 3 Energy dispersive spectroscopy of brain of *C. mrigala* fish (a) Control, (b) Mercuric chloride 0.0206 ppm (c) Mercuric chloride 0.0402 ppm,

3.6 Transmittance

The optical Transmittance has been studied for the brain of control and mercury exposed *C. mrigala.* The prepared powder dissolved in dimethyl sulphoxide (DMSO) and used for optical transmittance. 90% transmittance is observed for control and a considerable decrease has been observed in brain of mercury exposed fish. For the 0.0206 ppm and 0.0402 ppm of mercuric exposure the transmittance is observed to be decreased to 80% and 59% respectively.

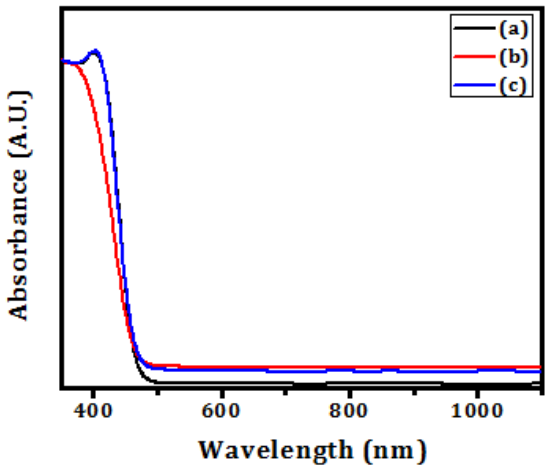


Fig. 4 Optical absorbance of brain of *C. mrigala* fish (a) Control, (b) mercuric chloride 0.0206 ppm (c) mercuric chloride 0.0402 ppm

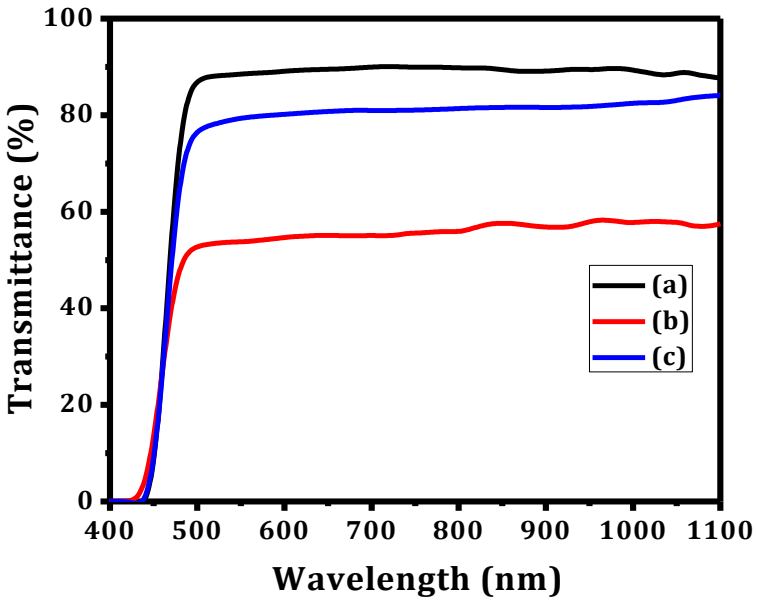


Fig. 4b Optical absorbance of brain of *C. mrigala* fish (a) Control, (b) Mercuric chloride 0.0206 ppm (c) Mercuric chloride 0.0402 ppm

This may be due to exposure to the toxicant. The high transmittance is only in the region of 500 nm to 1100 nm. This increase in transmittance is may be due to the different absorbing and transmitting capacity of different organic compound.

3.7 Photoluminescence

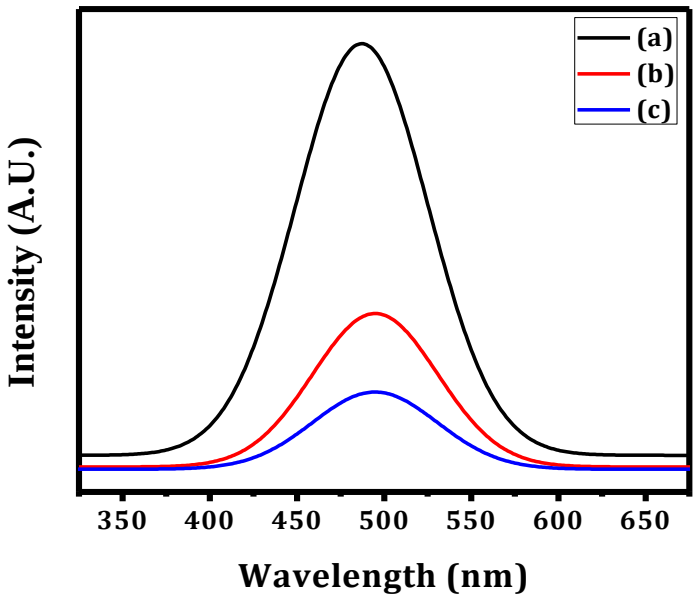


Fig. 4c Optical absorbance of brain of *C. mrigala* fish (a) Control, (b) mercuric chloride 0.0206 ppm (c) mercuric chloride 0.0402 ppm

The photoluminescence has been studied for brain of *C.mrigala.* The brain shows the emission properties. It shows the emission at 473 nm. After the mercuric exposure, the emission intensity of brain is observed to be decreased with red shift. It shows the emission after the mercuric exposure at 478 nm. Similar study has been reported by Jung et al. [19] for rat brain which suggested the emission properties of brain.

4. Conclusions

Changes in the tissue chemistry of *C. mrigala* produced by metal exposure are discussed. The XRD study revealed that brain of *C.mrigala* is amorphous in nature. IR spectroscopy suggests brain as a complex of organic compounds with many functional groups. The FESEM study reveals the hollow nature of brain with a cauliflower like morphology. The present FESEM study clearly reveals that mercuric chloride alters morphological structure of brain which further affects neurophysiologic functions. The sharp absorbance has been observed at 360 nm for all samples. The maximum 90% transmittance has been observed for control. The transmittance is decreased after exposure to sub lethal concentrations of mercury to 82% and 59% respectively. The brain of *C.mrigala* shows the strong emission at 473 nm but a red shift is observed after mercury exposure. Significant difference in absorbance intensities after exposure to toxicant reflects the alterations in major biochemical components. The molecular changes in brain may retard the growth and development of the species. All results are the index of stress in *C. mrigala* after mercury exposure.

The exposures to sub lethal concentrations of mercury may cause serious damage to fish health leading to affect their population. The concentrations of doses used in the present study were too low and comparable with the concentrations in polluted aquatic resources of India. The present study thus, provides evidence for the health status of fish in water bodies. The study will help the regulatory bodies to establish or modify the effluent limits standard for mercury in water bodies for protection of aquatic life. This will aid in maintaining and sustaining the population of fish especially those consumed by humans, in water bodies.

5. Acknowledgement

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