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Effect of lead on morphological and biochemical profile of fish liver

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Abstract
Fresh water ecosystems have been contaminated alarmingly through a number of heavy metals. Lead is a purely toxic heavy metal widely used in different industries and causes serious damage to aquaculture species such as fish. The present work explores the toxic effects of lead on liver of fish *Cirrhinus mrigala* and the spectral and surface morphological changes in liver. Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray spectroscopy (EDX) and Optical absorbance are the currently used method of monitoring animal tissue and their components. The biochemical profile of liver was explored by FTIR, EDX and optical absorbance. The surface morphology of liver is studied by field emission scanning electron microscopy (FE-SEM). The present study gives a manifold confirmation on the alterations at cellular level after chronic exposure to lead.

Keywords: Lead, *Cirrhinus mrigala*, Liver

Introduction
Scientific community has widely attracted to heavy metals like mercury, lead and chromium as their toxicity is responsible for potential human health hazards (Farombi *et al*. 2007). The ecological balance of any ecosystem is severely interfered by heavy metal pollution which intern produces devastating effects on environmental quality (El-Moselhy *et al*. 2014). The heavy metal pollutants present in water may induce severe ecological consequences and effect aquatic ecosystems integrity (Vosylienė and Jankaitė 2006) [19]. On the fish itself however, it may decrease fecundity leading reproductive decline and eventual extinction of this important natural resource (Burger and Gochfeld 2005) [4].
Lead is not required by any living organism hence it is one of the limited class of purely toxic elements. The biggest source of environmental contamination of lead is use of leaded paints as there are no mandatory standards in its disposal. The acute and sub acute effects of lead are quite subtle and non-specific but include all body systems. Recent reports indicate lead as a pollutant causes various neurological, reproductive, immunological, gastrointestinal and histochemical changes in the animals (Abdallah *et al.* 2010) [1] (Angeles *et al.* 2016) [3]. Fish, as a living bioindicator species, monitor water pollution, because they respond with great sensitivity to changes in the aquatic environment (Palaniappan and Vijayasundaram). Fish tissue especially the liver and kidney is endowed with a defense system to protect them from oxidative stress caused by metals (Patricia Morcillo, Héctor Cordero, José Meseguer, María Á. Esteban 2015) [17]. The biochemical profile of fish liver indicates the impact of metals as it responds specifically to the degree and type of contamination (Akkas *et al.* 2007) [2]. The liver shows serious morphological alterations after exposure to toxicants due to its susceptibility to various toxicants (Palaniappan and Vijayasundaram).
Spectroscopy is being used as a powerful method for studying molecular structure and intra molecular interaction in biological tissues and cells. The present paper focuses on application of spectroscopic methods for structural analysis and biochemical characterization of fish liver. The present study is an effort to explore the toxic effect of lead on morphology and biochemistry of fish liver.

2. Materials and methods
Healthy Specimens of *C. mrigala* were collected from a local reservoir near Kolhapur, M. S. India. Animals were acclimatized to laboratory conditions for 15 days.
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 14.1 ppm and 28.2 ppm) concentration of lead acetate. The acclimated test animals were exposed to the sub lethal concentration for a period of 30 days in a group of 10. A control set was arranged simultaneously. The water with toxicant renewed daily and fish were fed ad libitum during the period. The desired tissue was pulled out from the fish after its chronic exposure of 30 days to the toxicant. Sample preparation -The pulled liver tissue was blotted and dried for 72 hrs 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 Fourier transform infrared spectroscope (FTIR) was used for vibrational analysis of liver of *C. mrigala*. The Mira 3, Tescan, chez republic, field emission scanning electron microscope was used to reveal surface morphology of liver. 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.

3. Results and Discussion
3.1 Fourier Transform Infrared Spectroscopic study (FTIR)
The Fig. 1 shows the FTIR spectra of control and lead exposed *C. mrigala* liver. The general band assignments of the FTIR spectra of control and lead exposed *C. mrigala* liver have been mentioned in Table. 1. This is shown in fig.1. All the peaks mention in Table.1 belong to O–H stretch, H–bonded, N–H stretch, C–H stretch, –C=C– stretch, N–O asymmetric stretch, C–H rock, C–N stretch, and –C≡C–H: C–H bends. Also these peaks are Alcohols, Phenols,

1˚,2˚Amines, Amides, Alkanes, Alkenes, Nitro compounds, Alkyl halides, Aliphatic amines and Alkynes functional group (Gough *et al.* 2003; Le Naour *et al.* 2009; Zohdi *et al.* 2013) [10, 12, 20].

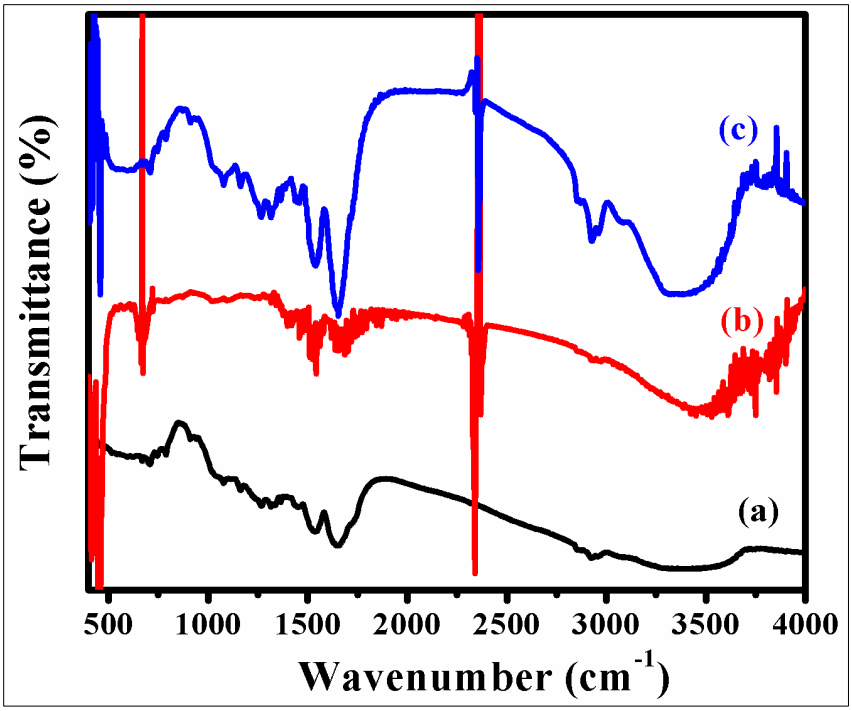

Fig 1: FTIR spectra of control and lead exposed on liver (a) control, (b) lead exposed at 14.1 ppm, (c) lead exposed 28.2 ppm.

S. No.	Frequency (cm^{-1})			Bonds	Functional group
	Control	14.1ppm	28.2ppm		
1	3394	3459	3362	O–H stretch, H–bonded, N–H stretch	Alcohols, Phenols, 1˚,2˚Amines,
2.	2923	2923	2923	C–H stretch	Alkanes
3.	2850	2850	2850	C–H stretch	Alkanes
4.	1648	1654	1656	–C=C– stretch	Alkenes
5.	1536	1543	1526	N–O asymmetric stretch	Nitro compounds
6.	1321	1380	1315	C–H rock	Alkanes
7.	1260	1260	1260	C–H wag (–CH2X)	Alkyl halides
8.	1057	1057	1057	C–N stretch	Aliphatic amines
9.	706	674	708	–C≡C–H: C–H bend	Alkynes

3.2 Field emission scanning electron microscopic study

The SEM study of liver of control fish showed normal histology of liver with sheets of radially arranged well marked nuclear hepatocytes and intervening sinusoids (Fi.2 A1, A2, A3). The liver of fish exposed to both sublethal concentration of lead ($1/20^{th}$ and $1/10^{th}$ of LC_{50} values 14.1 ppm and 28.2 ppm) showed loss of liver architecture, parenchymal disorganization, cell death and dilation of vennules. Degeneration of hepatocytes resulted in decreased cell population (Fig.2 (B1, B2, B3, C1, C2 and C3)). Similar histological observations were reported after induced fluoride toxicity in rabbits by Shashi *et al*, (Shashi and Thapar 2001) [18], in the liver of fish *Channa punctatus* by Haque *et al*, (Haque *et al.* 2012) [11]. Elsie *et al*, (Elsie M. B. Sorensen, Ruben Ramirez-Mitchell, Charles W. Harlan 1980) [7] reported vacuolation of most polygonal fish liver cells and their marked degeneration after chronic environmental arsenic exposure. Similar histological findings were reported by Chavan *et al*., (Chavan and Muley 2014) [5] in fish *Cirrhinus mrigala* after induced metal toxicity. Histological studies are needed to add by spectroscopic analysis and fine structural studies by SEM. The present work helps to investigate the effect of heavy metal on the biochemical composition and fine structure to evaluate the potentiality of toxicant to fish as a sentinel.

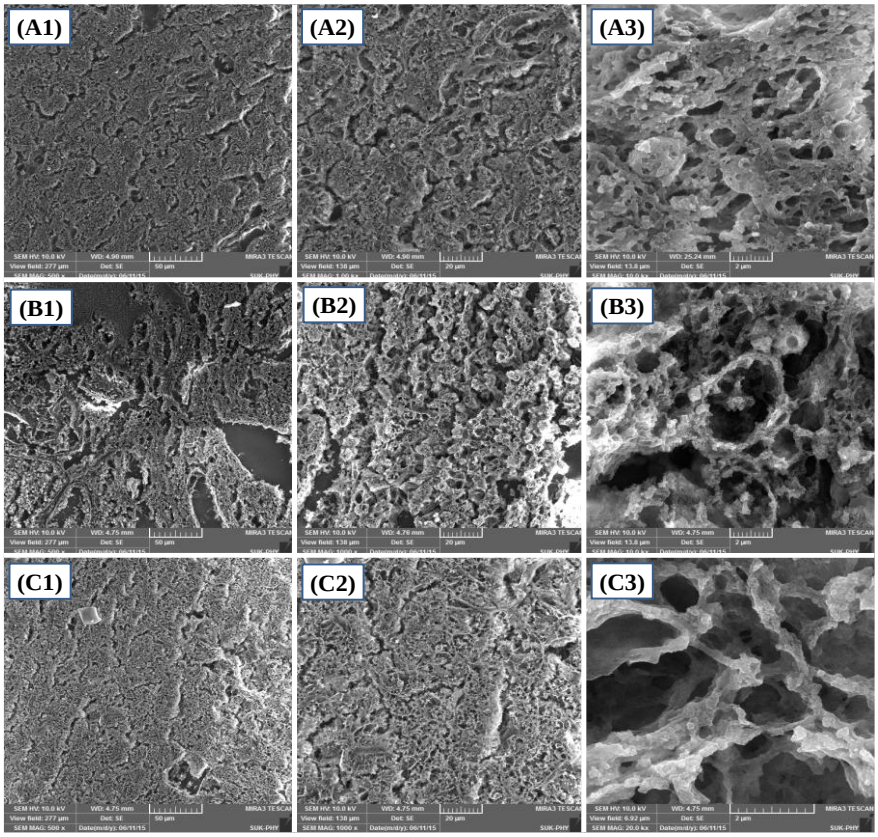

Fig 2: FESEM images of control and lead exposed on liver, (A1) control X500, (A2) control X1000, (A3) control kX10, (B1) lead exposed at 14.1 ppm X500, (B2) lead exposed at 14.1 ppm X1000, (B3) lead exposed at 14.1 ppm kX10, (C1) lead exposed at 28.2 ppm X500, (C2) lead exposed at 28.2 ppm X1000, (C3) lead exposed at 28.2 ppm kX10

3.3 Energy dispersive X-ray spectroscopic study (EDS)

Fig. 3(A) (a, b, c) shows the EDS spectra of control and lead exposed *C. mrigala* liver. The EDX analysis has been carried out to reveal the effect of lead on carbon oxygen percentage of liver tissue. The observed weight and atomic percentage of carbon, oxygen and nitrogen have been mentioned in Fig .3(A). In the control sample nitrogen is not observed. But in lead exposed samples nitrogen is detected and this result is consistent with FTIR results. After the lead exposure (14.1 ppm) along with nitrogen and oxygen, carbon has been detected in EDS spectrum. The change in weight and atomic percentage of carbon, oxygen and detection of nitrogen is due to lead exposure.

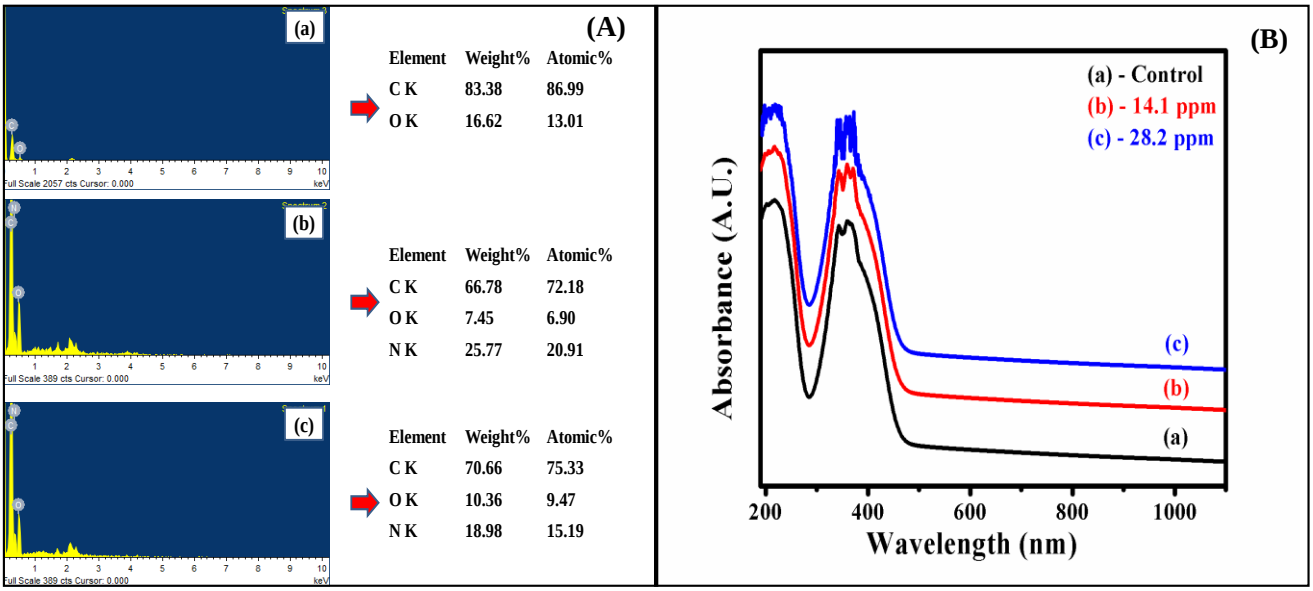

Fig 3: (A) EDS spectra of control and lead exposed on liver (a) control, (b) lead exposed at 14.1 ppm, (c) lead exposed 28.2 ppm. Fig.3 (B) UV-Vis spectra of control and lead exposed on liver (a) control, (b) lead exposed at 14.1 ppm, (c) lead exposed 28.2 ppm

3.4 Optical absorbance

Fig. 3 (B) (a, b, c) shows the optical behavior of liver of *C. mrigala* has been studied using UV-Vis spectrophotometer. This is an important tool to study optical behavior of sample (Lohar *et al*. 2014; G M. Lohar, H D Dhaygude, R A Patil, Y R Ma 2015; Lohar *et al*. 2015a; Lohar *et al*. 2015b). The optical absorbance has been studied with dissolving of prepared gill powder in methanol for control and lead exposed sample. The optical absorbance has been observed to be sharp increased from 487 nm for all samples and the absorbance peak observed at 361 nm. But again absorbances have been decreased from 361 nm to 285 nm. After the 285 nm the absorbance is again increased upto 228 nm and this is shown in Fig. 3(B). This indicate that, this liver of *C. mrigala* have been active in UV region also. The change in absorbance is also indicating the effect of lead exposure

4. Conclusions

The IR studies reveal that the liver of fish is a complex of organic compounds. Further it confirms the biochemical alterations assessing the mechanism of lead toxicity. The FE-SEM studies showed ultra-structural changes in fish liver after an acute and chronic exposure to lead. The ultra-structural changes serve as biomarker for assessing heavy metal pollution. The present study gives a manifold confirmation on significant disturbance and impairment in liver architecture and its vital function.

5. References

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