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LEAD INDUCED MORPHOLOGICAL AND BIOCHEMICAL FLUCTUATIONS IN KIDNEY OF CIRRHINUS MRIGALA

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ABSTRACT: Heavy metals are major contributors of aquatic pollution. Among them lead is a purely toxic heavy metal widely used in different industries and causes serious damage to aquatic animals like fish. The present study has been undertaken to explore the toxic effects of lead on kidney of fish Cirrhinus mrigala and to detect the spectral biochemical and surface morphological changes. The biochemical changes after lead exposure are studied by Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray spectroscopy (EDX) and Optical absorbance, the currently used method of monitoring animal tissue and their components. The surface morphology of kidney is studied by field emission scanning electron microscopy (FE-SEM). The present study gives a better understanding of lead toxicity to fish.

KEYWORDS: *Cirrhinus mrigala,* lead toxicity, field emission scanning electron microscopy

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1. INTRODUCTION

Deleterious effects on aquatic organism are the results ofdischarge of various chemicals in aquatic environment. Heavy metals are major contributors of aquatic pollution. A variety of natural and anthropogenic activities result in release of heavy metals in aquatic environment [1]. Compared to other animals fish are sensitive to toxicants and are indicators of ecosystem's health [2]. Lead is a naturally occurring heavy metalnot required by any living organism hence it is one of the limited class of purely toxic elements. Significant increase in lead concentration is due to battery manufacturing, old lead plumbing, Pb based paints and leaded gasoline [3]. Leaded paints are the

biggest source of environmental contamination as there are no mandatory standards. The acute and sub acute effects of lead are quite subtle and non-specific but include all body systems. Low levels of lead pollution could cause adverse effects of fish health and reproduction [4]. The toxicity of such metal pollutants is assessed by the extent of histopathological damages induced in the test organism and the degree of cell damage is evident in relation to the concentration of pollutants employed. Kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is responsible for selective reabsorbtion, maintaining volume and pH of blood and body fluids and erythropoieses [5]. The kidney is one of the first organs to be affected by contaminants in the water [6] It is necessary to assess the adverse effects of lead on fishes as fish form a source of human food. *Cirrhinus mrigala* is a commonly cultured major Indian carp, highly esteemed food fish available throughout year, considered as model for the present study. FTIR spectroscopy serves as a important and popular tool to get the quantitative profile of biochemical composition of biological sample with little sample and short preparation. FE-SEM provides surface morphological details. EDS provides compositional analysis of carbon and oxygen. UV visible spectrophotometer is used to check the optical response of biological sample and photoluminescence can be studied by spectrofluorometer. The present study was undertaken to explore the toxicity of lead on kidney of an edible fresh water fish *Cirrhinus mrigala.* The present article seeks to highlight the importance of toxicity testing. Regular monitoring of biochemical, physiological examinations to detect the histological alterations due to toxic agents is done by conventional techniques. Adoption of new, quick techniques into existing protocol will serve to grow the area. Also introduction of these techniques provide better understanding of toxicity in laboratory models. The use of fish as a sentinel benefits in forecasting the toxic effects on human beings. The present study will provide a different perspective for toxicity studies.

2.MATERIALS AND METHODS

Biological material

The live fresh water teleost*Cirrhinus 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 rinsed with water containing little amount of KMnO4 to disinfect them. Animals were then transferred to glass aquarium with continuously aerated tap water for acclimatization. After two weeks acclimatization healthy fishes were identified by general appearance and selected for experimental work. The water was checked for selected Physico chemical parameters (pH 7.2-7.4, Temperature 20-23^{o}C, Dissolved oxygen 6.8 mg/l). Fish were fed ad libitum with groundnut oil cake during acclimatization and exposure to toxicant.

Exposure

Chemicals-Analytical grade lead acetate (BDH) was used without further purification. The heavy metal lead was used in the form of lead acetate for the present study. The LC50 values of lead were determined by static bioassay of 96 hours and the sub lethal concentrations of toxicants were selected for the chronic toxicity tests of long-term exposures. (See supplementary information). A prolonged (chronic) period of 30 days was selected for the present study. The sub lethal concentrations of toxicants i.e. lead acetate selected for the chronic toxicity study (30 days) were $1/20^{th}$ and $1/10^{th}$ concentrations of LC50 values. These values were 14.1 and 28.2 ppm respectively. The acclimated test animals in a group of 10 were exposed to these sub lethal concentration for a period of 30 days. The toxicity study was run in triplicates. A control set was run simultaneously. Fish were fed ad libitum during the study 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 kidney tissue was blotted and dried for 72hrs in oven at 60^0C.and then ground in mortar and pestle to obtain kidney powder. Further spectroscopic analysis was done with the powder.The vibrational analysis of kidney of *C. mrigala* has been studied using the Perkin elmer, USA, Fourier transform infrared spectroscope (FTIR). Field emission scanning electron microscope (Mira 3, Tescan, chez republic), was used to study the ultrastructural details of fish kidney. Energy dispersive spectroscopy has been studied using the Mira 3 Tescan an oxford instrument, United Kingdom. By using a UV-1800 Shimadzu, Japan absorption spectra were recorded at room temperature and near to normal incidence. Photoluminescence has been studied using the fluoromax-4, horiba instrument PVT, Japan.

3. RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopy (FTIR)

Fig.1 shows the FTIR spectra of *C. mrigala* kidney. All observed peak, bond and functional group are mentioned in Table.1. The peak at 3369 cm-1, 3459cm-1 and 3289 cm-1 for control and lead exposed at 14.0 ppm and lead exposed at 28.2 ppm are assigned for N–H stretch for 1˚, 2˚ amines, Amides etc. The peak at observed at 2929 cm-1 for control and lead exposed samples represents the O–H stretching in carboxylic acids. The at 2929 cm-1 is a band typical for the oleifinic groups of lipids and fatty acids and four bands attributed to the asymmetric and symmetric stretching vibrations of the CH2 and CH3 groups of lipids and proteins also [1]. The peak at 2850 cm-1 for all samples assigned for alkenes and C–H stretch, also this peak mainly assigned for proteins, lipids and triglycerides [7].

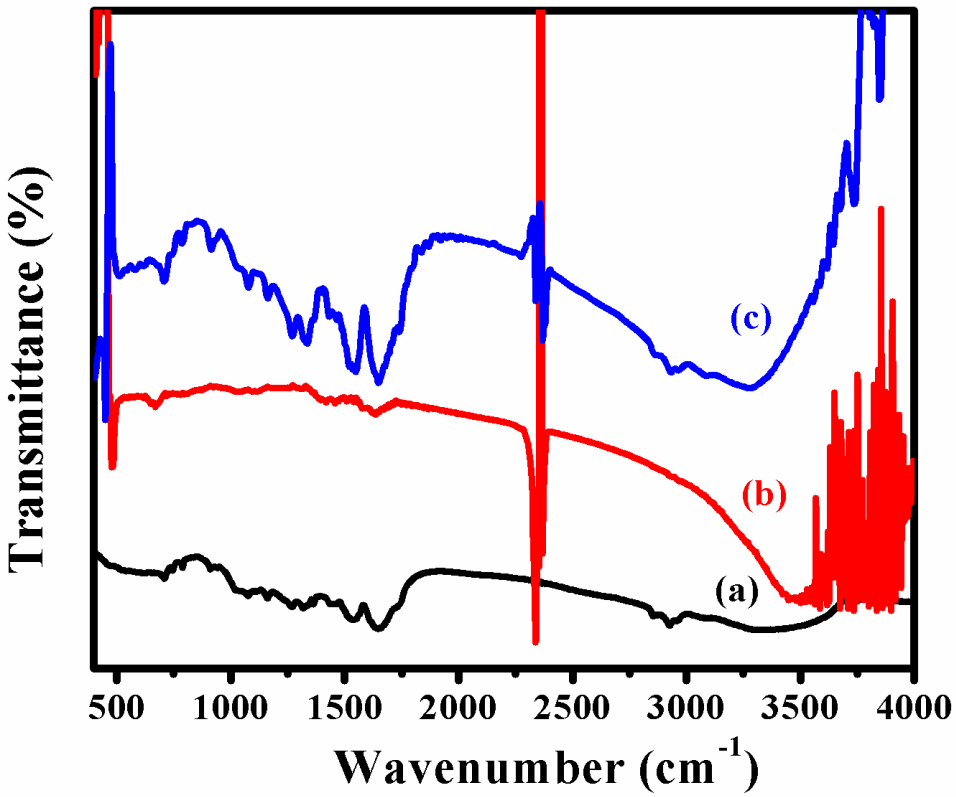


Fig.1 FTIR spectra of control and lead exposed *C-mrigala* kidney (a) control, (b) 14.1ppm lead exposure, (c) 28.2 ppm lead exposure

The peaks for C≡N stretching in nitriles are assigned at 2339 cm-1 for all samples. The peak at 1648 cm-1 for control and lead exposed samples assigned for N–H bending for 1˚ amines. Also this band is assigned for peptides and protein [8]. The peak at 1532 cm-1, 1532 cm-1 and 1535 cm-1 are belongs to control lead exposed to 14.1 ppm and lead exposed to 28.2 ppm, respectively. These peaks are assigned for N–O asymmetric stretch for nitro compounds. The peak at 1258 cm-1 and 1332 cm-1 for control and 28.2 ppm lead exposed sample are C–H wag (–CH_2X) bend for alkyl halides. The peak at 1169 cm-1 for control and lead exposed at 14.1ppm sample and peak at 1157 cm-1 assigned for C–H wag (–CH2X), C–N stretching, also they represents the alkyl halides and aliphatic amines functional group. The peak at 1055 cm^{-1} for control and 14.1 ppm lead exposed samples as well as peak at 1047 cm-1 for 28.2 lead exposed sample are assigned for C–N stretching for Aliphatic amines functional group [9]. The peak at 704 cm-1, 665 cm-1 and 708 cm-1 for control, lead exposed at 14.1 ppm and lead exposed at 28.2 ppm, respectively for C–Cl stretching assigned for alkyl halides functional group.

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

Sr. No.	Frequency (cm^{-1}) Control	14.1ppm	28.2ppm	Bonds	Functional group
1	3369	3459	3289	N–H stretch	1˚, 2˚ amines, Amides
2.	2929	2929	2929	O–H stretch	Carboxylic acids
3.	2850	2850	2850	C–H stretch	Alkanes
4.	2339	2339	2339	C≡N stretch	Nitriles
5.	1648	1648	1648	N–H bend	1˚ amines
6.	1532	1532	1535	N–O asymmetric stretch	Nitro compounds
7.	1258	--	1332	C–H wag (–CH2X)	Alkyl halides
8.	1169	1169	1157	C–H wag (–CH2X), C–N stretch	Alkyl halides, Aliphatic amines
9.	1055	1055	1047	C–N stretch	Aliphatic amines
10.	704	665	708	C–Cl stretch	Alkyl halides

Field emission scanning electron microscopic study (FESEM)

Kidney is the major organ of detoxicification and excretion of metabolic waste. A homogeneous intact arrangement of uriniferous tubules in control samples is seen. Fig. 2 (A1 and A2) Histologically a normal fish kidney shows its functional units with glomeruli, proximal and distal tubules. Evenly distributed haemopoietic tissue fills the intertubular spaces. The glomeruli are formed by capillaries surrounded by Bowman's capsule, tall columnar epithelial cells with round nuclei at the periphery and brush border in the lumen. Scanning Electron Microscopy of kidney from Pb treated *C. mrigala* in the present study revealed impairment in Bowman's capsule and also shown abnormalities and changes in architectural pattern of uriniferous tubules. The surface morphological study has been carried out for kidney of control and lead exposed fish. Various histological alterations observed by light microscopy at low resolutions are confirmed by SEM images. The Fig. 2 (B1 and B2) shows necrosis in haemopoetic tissue and altered arrangement of renal tubules after exposure to 14.1 ppm concentration of lead acetate. Dilated renal tubules hypertrophied epithelial cells and vacuolation leading to degeneration of cells are the prominent structural alterations in kidney of *C. mrigala* after chronic lead exposure. Further disintegration like dissociated epithelial lining, shrinking of glomerulus and enlargement of Bowman's capsule is observed in kidneys of fish exposed to and 28.2ppm lead acetate concentration.

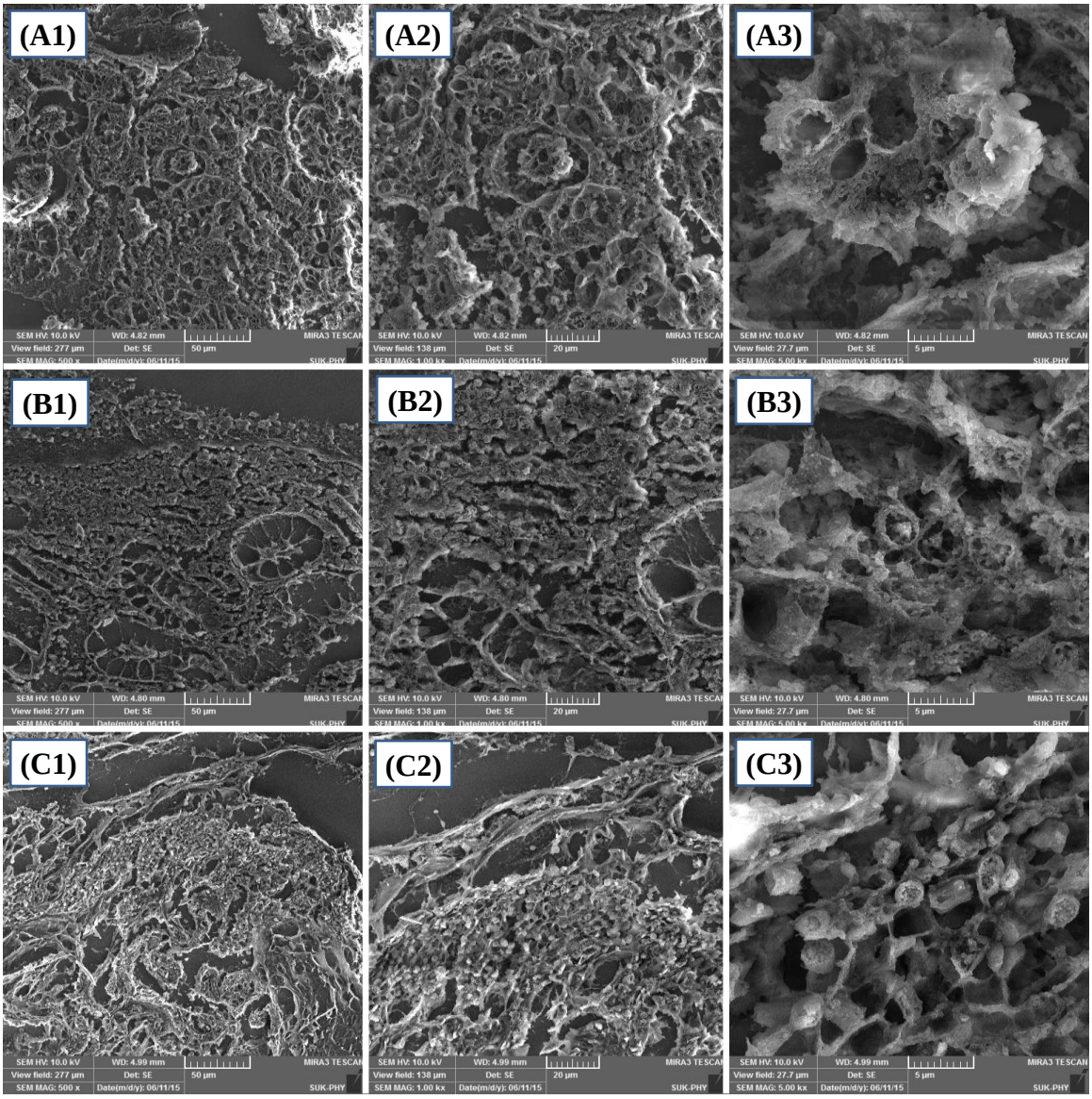


Fig.2 FE-SEM images of control and lead exposed *C-mrigala* kidney (A1) Control X=500x, (A2) Control X=1kx, (A3) Control X=5kx, (B1) 14.1 ppm lead acetate X=500x, (B2) 14.1 ppm lead acetate X=1kx, (B3) 14.1 ppm lead acetate X=5kx, (C1) 28.2 ppm lead acetate X=500x, (C2) 28.2 ppm lead acetate X=1kx, (C3) 28.2 ppm lead acetate X=5kx

These alterations are seen in Fig.2 (C1 and C2). In the present study toxicity induced kidney alterations are revealed by FESEM. The present study suggests that lead induces haemolyticanaemia in initial stages which further causes alterations in all parts of kidney. The degree of structural degenerations is directly proportional to concentration of toxicant. The transformation may lead to functional alterations and disturb the fundamental function of kidney.

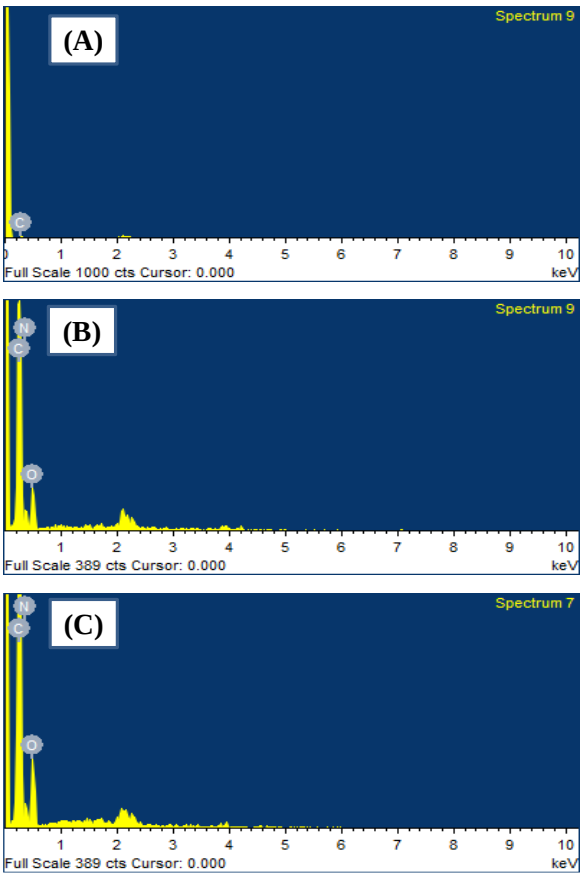
Studies on toxicity are usually done by histological techniques as these are recognized as reliable biomarkers of stress in fish. Histological studies on metal toxicity in fish are done by various workers.

C. mrigala showed necrosis, cellular degeneration and increase in liver transaminases after a chronic lead exposure [10]. Alterations in kidney of various species of *Tilapia* after heavy metal exposure were described by Ribeiro et al. [11]. Alterations like hypertrophied epithelial cells of renal tubules, reduction in tubular lumen, and contraction of glomerulus in fish kidney were observed by Mishra and Mohanty [12]. The observed results in the present study are consistent with [13]. Histological alterations in various components of teleost fish kidney after heavy metal exposure are described by Ortiz [13]. *C. mrigala* exposed to fenvalterate showed severe necrosis, vacuolation leading to degeneration in renal tubules [14]. Reports on metal toxicity in fish by FESEM are very few. SEM studies of fish brain after mercury toxicity revealed change in surface morphology and spectroscopic studies showed fluctuations in absorbance areas and intensities to detect biochemical changes [15]. Toxic responses to mixture of trace metals by SEM reported structural and functional alterations in fish gills [16]. Cadmium induced alterations in fish gill include hypertrophy, necrosis and fusion of secondary lamellae resulting in functional impairment [17]. The present study explores the stress in fish kidney due to metal toxicity by FESEM.

Energy Dispersive X-ray Spectroscopic Study (EDS)

Fig. 3 shows the EDS spectra of control and lead exposed *C-mrigala* kidney. The EDX analysis has been carried out to reveal the effect of lead on carbon oxygen percentage of kidney tissue. The observed weight and atomic percentage of carbon, oxygen and nitrogen have been mentioned in Fig .3. For the control sample nitrogen has been not observed. But in lead exposed samples nitrogen is detected and this result is consistent with FTIR results. The observed weight and atomic percentage of carbon is 31.85 and 38.36, respectively. Similarly, the weight and atomic percentage of oxygen is 68.15 and 61.64, respectively. This is shown in Fig. 3(a). The Fig.3 (b) shows EDS spectrum for 14.1 ppm lead expose samples. After the lead exposed at 14.1 ppm along with nitrogen and oxygen, carbon has been detected in EDS spectrum. The weight and atomic percentage of carbon is 43.64 and 49.68, respectively. The weight and atomic percentage of oxygen is 38.65 and 33.03, respectively. Also, the weight and atomic percentage of nitrogen is 17.71 and 17.29, respectively. The observed weight and atomic percentage of carbon oxygen and nitrogen for lead exposed at 28.2 ppm have been mentioned in Fig.3 (c). The weight and atomic percentage of carbon is 70.45 and 75.69, respectively. The weight and atomic percentage of oxygen is 25.13 and 20.16, respectively. The weight and atomic percentage of nitrogen is 4.40 and 4.05, respectively. Investigators have demonstrated that lead induces cell damage by dramatic increase in free radicals or reactive oxygen species. Metal toxicity is characterized by oxicative stress. The mechanism by which lead induces oxidative stress is not fully understood [18]. The relative decrease in oxygen percent of lead exposed fish kidney indicates

oxidative stress which may be due to decrease in antioxidant defense mechanism.



Element	Weight%	Atomic%
C K	57.67	64.48
O K	42.33	35.52

Element	Weight%	Atomic%
C K	60.91	66.39
O K	25.18	20.61
N K	13.91	13.00

Element	Weight%	Atomic%
C K	70.47	75.69
O K	25.13	20.26
N K	4.40	5.05

Fig.3 EDX spectra of control and lead exposed *C-mrigala* kidney (a) control, (b) 14.1 ppm lead acetate, (c) 28.2 ppm lead acetate.

Optical absorbance

Optical behavior of Kidney of *C. mrigala* has been studied using UV-Vis spectrophotometer. This is shown in Fig. 4. It provides an accessible evaluation of absorption of photons, leading to an excitation of an electron from the valence band to the conduction band [19-22]. 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 475 nm for all samples and the absorbance peak observed at 357 nm. But again absorbance has been decreased from 357 nm to 287 nm. After the 287 nm the absorbance is again increased upto 222 nm and this is shown in Fig.4. The absorbance has been observed in UV region also. This indicate that, this kidney of C. mrigala have been active in UV region also. The change in absorbance is also indicating the effect of lead exposure.

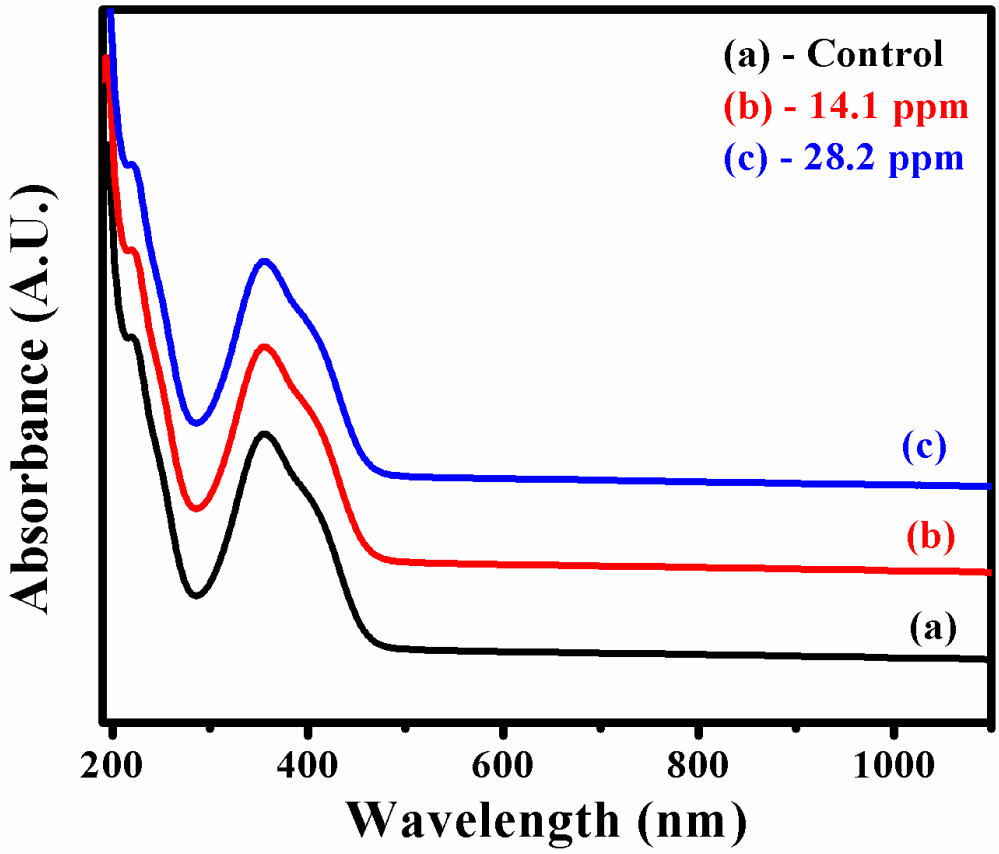


Fig.4 Optical absorbance spectra of control and lead exposed *C-mrigala* kidney (a) control, (b) 14.1 ppm lead acetate, (c) 28.2 ppm lead acetate.

4.CONCLUSION

An attempt is made in this paper to define the effect of lead level that exist in nature as a result of anthropogenic activities by exposing a representative animal to sub lethal level of heavy metal lead. The study suggests that lead induces reduction in volume of haemopoetic renal tissue, disintegration of apical cells, vacuolation and degeneration of tubule cells ultimately leading to functional impairment of kidney. The paper further suggests change in biochemical composition and spectroscopic features of fish kidney due to lead toxicity.

CONFLICT OF INTEREST

The authors declare that no competing financial interests exist.

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