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RESEARCH PAPER

Cd2+ and Pb2+ Induced Structural, Functional and Compositional Changes in The Liver and Muscle Tissue of Crucian Carp (Carassius auratus gibelio): an FT-IR Study

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Abstract

Contamination of aquatic ecosystems with toxic metals such as Cd^{2+} and Pb^{2+} is a serious issue in the industrialized world, which can affect freshwater fish even at low concentrations. The aim of the present study was to investigate the effect of Cd^{2+} and Pb^{2+} alone or in combination on the biochemical constituents of liver and muscle in Crucian carp using Fourier Transform Infrared Spectroscopy (FT-IR). Results from the spectral analysis revealed significant decline in protein and increase in lipids in the two tissues with marked effect caused by the combined exposure of Cd^{2+} and Pb^{2+}. In case of liver, alteration in the intensity and band area at amide I resulted in a differential response of structural protein for the exposed groups. Furthermore, a decrease in the α-helix and alterations in the nucleic acids content was also observed in both liver and muscle of the exposed fish. Moreover, biochemical alterations in the vital tissue of freshwater fish due to toxic contaminants can be used as a marker of environmental pollution with the help of FT-IR spectroscopy.

Keywords: FT-IR, Crucian carp (Carassius auratus gibelio), Liver, Muscle, Cd2+, Pb2+, Biochemical changes.

Introduction

Heavy metals are serious environmental pollutants because of their persistency, non biodegradability and higher accumulative tendency in the living tissue (Begum *et al*. 2005, Gupta and Karthikeyan 2016). Among heavy metals, cadmium (Cd) and lead (Pb) are the prominent toxic metals (Lim *et al*. 2008, Senthil Kumar *et al*. 2008) with no known nutritive values (Poole *et al*. 2005, Xu *et al*. 2008) and are abundant in nature. The common sources of Cd and Pb pollution are contaminated soils, sediments and waters due to natural and anthropogenic activities which cause their entry into the food chain and generating various adverse effects in animals and humans (Chai *et al*. 2014, Chakraborty *et al*. 2012, Khan *et al*. 2014). Though freshwater ecosystems have certain physico-chemical and biological mechanisms to counteract or eliminate the adverse effects of pollutants; however, toxicants may induce changes in normal growth, reproduction and behavior or may be fatal to freshwater organisms (Rand *et al*. 2003).

Cadmium has the ability to disturb various cellular functions and can damage the structures of different cellular compartments (Nemmiche *et al*. 2007), because of the higher affinity of Cd^{2+} ions to biological structures consisting of sulfhydryl (-SH), carboxyl and phosphate groups. This may cause the inhibition of numerous enzymes and disturbance of important metabolic processes including lipid metabolism (Krishnakumar *et al*. 2012, Murugavel and Pari 2007). On the other hand, Pb toxicity may occur through the ionic Pb^{2+} replacement with certain divalent ions such as Zn, Fe and by calcium mimicry (Tellis *et al*. 2014) causing neurological disorders, genotoxicity, muscular spasms, haematological alterations, paralysis and mortality in the exposed freshwater fish (Martinez *et al*. 2004, Grosell *et al*. 2006, Monteiro *et al*. 2011). However, the mechanism of Cd and Pb induced molecular alterations in the tissues and cells are still not clear.

Fish play an important role in balanced and nutritious diet containing a vital source of proteins and long chain polyunsaturated fatty acids with high quantity of fat soluble vitamins. However it can also be a source of trace metal exposure due to excessive amount of elements they can contain, in which some are highly toxic to human (Carvalho *et al*. 2005). The nutritive value of fish greatly depends on their

biochemical constituent which is affected by polluted water because of their direct contact with the toxicant in contaminated waters (Burger *et al*. 2002). Metals usually accumulate in the high fatty tissues of muscle, or in some specific organs, based on the lipophilic nature of the toxic chemical and how they are metabolized by the organism. Once enter to an organism, metals tend to remain in various tissues and may continuously accumulate with subsequent exposures. The bioaccumulation of metals mostly depends on the species, feeding habits and life style of the exposed fish. Although fish muscle is not an active part in accumulating heavy metals, there is evidence that certain metals in the fish muscles exceeded the acceptable range in some polluted regions. Therefore, studies on metal toxicity of fish are of vital importance in terms of food safety perspectives (Palaniappan and Renju 2009, Uysal *et al*. 2008).

Fourier Transform Infrared (FT-IR) spectroscopy is a non-perturbing and sensitive analytical technique with practical advantages. Application of FT-IR to biological sample is started at the mid of this century. Recently, this technique has become an independent and advance modality in terms of high sensitivity in detecting changes in the functional groups belonging to the specific components of tissue such as proteins, lipids, carbohydrates and nucleic acids (Karthikeyan and Easwaran 2013). Because of high sensitivity, this technique is capable of providing a strong insight on the structural and functional changes induced by various factors (Lu *et al*. 2011, Palaniappan and Renju 2009, Staniszewska *et al*. 2014). The liver and muscle tissue of fish under toxic metal exposure draw much of our attention due to detoxification and accumulation of metals (Khan *et al*. 2014). Crucian carp is an important food fish and a good experimental model, indicating the effects of organic and inorganic pollutants in different studies (Zhang *et al*. 2007, Shao *et al*. 2010, Khan *et al*. 2014). However, there is limited information about the individual and combine effect of water born Cd and Pb on the biochemical alteration of liver and muscle of Crucian carp. In the present study an attempt was made to elucidate the structural, functional and compositional changes induced by environmentally relevant Cd and Pb using FT-IR spectroscopy.

Materials and Methods

Chemical and Reagents

Cadmium chloride and Lead nitrate of purity > 99%, Nitric acid, Acetic acid (conc. glyacial), Sodium thiaosulphate, EDTA (disodium salt of EDTA), Potassium iodide crystal, Megnisum sulphate, Erichrome black T, Ammonium chloride and Ammonium hydroxide were purchased from Sinopharm Chemical Reagents Co., Ltd (Beijing, China). The deionized water used for preparation of reagents and elemental stock solutions were passed through Millipore purification apparatus (Millipore, MA, USA) to a resistivity higher than 18.2 MΩ·cm. ICP-Multi-element certified reference materials (CRM) were obtained from PerkinElmer No.N9300281, 1 Shelton, Connecticut, USA. All the chemicals were analytical grade and used without any further purification.

Fish Acclimation and Experimental Condition

Crucian carp with mean body weight 92±4.2 g and mean length 12±2.6 cm were obtained from a freshwater fish breeding base in Wuhan, China and immediately transported to the laboratory in plastic container. On arrival, fish were released to 200 L plastic tank having dechlorinated tap water with continuous supply of oxygen. Tap water was dechlorinated by exposure to light followed by one day aeration with stone aerators before release of fish. Water quality was regularly monitored prior and later during experimentation according to the standard methods of APHA (1992). The optimum condition (total hardness 156.32±4.43 mgL^{-1} as $CaCO_3$, temp. 22.41±2.11 ^oC, pH 7.6±0.31 , dissolved oxygen 8.26±0.68 mgL^{-1}) for water quality was maintained till the end of the experiment. Fish were acclamated to the laboratory condition for a peroid of 1 week in a laboratory tanks (50 cm × 30 cm × 30 cm) under natural photoperiod. During acclimation fish were fed with artificial feed once a day until a day before termination of acclimation period. Half of the aquarium water was renewd everyday to clean the residual feed and ammonia produced by fish. All the experiments were carried out according to the guidelines of Chinese Law for Animal Health Protection and Instructions for Granting Permits for Animal Experimentation for Scientific Purposes [Ethics approval No. SCXK (YU) 2005-0001].

Exposure to Cd^{2+} and Pb^{2+}

All the acclimated fish were randomly divided into four different groups: control group, Pb group, Cd group and Cd+Pb group without making any distinction between sexes. Control group was kept under similar experimental condition but without any addition of test chemical while Pb group was exposed to 30 μgL^{-1} Pb as $Pb(NO_3)_2$. The Cd group was exposed to 100 μgL^{-1} Cd in the form of $CdCl_2$, whereas Cd+Pb group was exposed to a combination of the two test chemicals at the same rate. The exposure duration was 21days for all the groups. The respective concentrations were closely monitored in the aquarium at 2 days interval by ICP-OES to maintain the desired concentration in the tanks. The exposure concentration of Cd and Pb was selected on the basis of previous studies (Khan *et al*. 2015a, Khan *et al*. 2015b, Qu *et al*. 2014), which suggested that

exposure to these concentrations might significantly inhibit the activity of antioxidant enzymes and induce a pro-oxidant condition in the various tissues of freshwater fish. Moreover, these concentrations are somewhat related to the contamination levels of rivers and lakes in China (An *et al*. 2010, Bing *et al*. 2013, Li *et al*. 2013, Wang *et al*. 2012, Yang *et al*. 2009, Zhou *et al*. 2007). At completion of the exposure period, fish from all the groups were sacrificed and tissues like liver and white muscles were isolated and stored at -80 °C until analysis.

Sample Preparation

The liver and muscle tissues were lyophilized for 12h to remove its water content completely. The samples were then ground with the help of an agate mortar and pestle to bring it in powdered form. Finely powdered tissues were mixed with pre-dried potassium bromide in a ratio of 1:100 respectively and subjected to a high pressure (3000 Psi) for 5 min in an evacuated die to produce a transparent sample pellet of 1 mm thickness and 13 mm diameter for use in FTIR spectrophotometer.

FT-IR Analysis

FT-IR spectra were recorded on NEXUS 470 spectrophotometer installed at Central Lab. of Food Science and Technology College, Huazhong Agricultural University. The pellets were scanned at room temperature in the spectral range of 4000~500 cm^{-1} at a resolution of 4 cm^{-1}, with air as the background. Special care was taken during pellet preparation by taking equal amount of sample and applying same pressure to maintain the same thickness of pellets. Thus the spectra possibly related to the intensities of the absorption bands and to the concentration of the corresponding functional groups (Cakmak *et al*. 2006, Dogan *et al*. 2007). All the spectra obtained were analyzed by ORIGIN 9.0 software (Origin Lab CO., Northampton, MA, USA).

Statistical Analysis

Statistical analysis was performed by SPSS 16 software, Chicago USA. All the experiments were replicated 3 times. One way analysis of variance followed by Duncan Multiple Range Test (DMRT) was performed to differentiate the corresponding band area values of control and experimental animals in each group. A probability level (*P*-value) of less than 0.05 was regarded as statistically significant.

Results

The present study was conducted to explore the structural, functional and compositional changes in the liver and muscle tissues of Crucian carp exposed to environmentally relevant Cd^{2+} and Pb^{2+} for 21days using FT-IR spectroscopy. The representative FT-IR spectra of control, Pb^{2+}, Cd^{2+} and Cd^{2+}+Pb^{2+} exposed fish liver and muscle in the region of 4000 to 500 $cm^{-1}$are given in Figure 1 a,b. Shifts in peak positions, changes in intensities, and band areas of the infrared spectrum were exploited to get important structural and functional information about the studied tissues (Hayashi *et al*. 2007). The observed peak positions of the spectra for the studied organs and their assignments according to the previous literature are presented in Table 1 (Palaniappan and Renju 2009, Senthil Kumar and Rajkumar 2014, Sivakumar *et al*. 2014).

As the spectra of the two tissues with multiple bands originate from the functional groups of various biomolecules including proteins, lipids, polysaccharides and nucleic acids, the detailed spectral features were investigated in two distinct regions for liver (3700 to 3000 cm^{-1} and 1800 to 800 cm^{-1}) and three distinct regions for muscle (3600–3100 cm^{-1}, 3050–2800 cm^{-1} and 1800–800 cm^{-1}) as shown in Figure 2 a,b and Figure 3 a,b,c respectively. Structural variations in the studied tissues were monitored with help of changes in the frequency of the respective bands, while the compositional changes

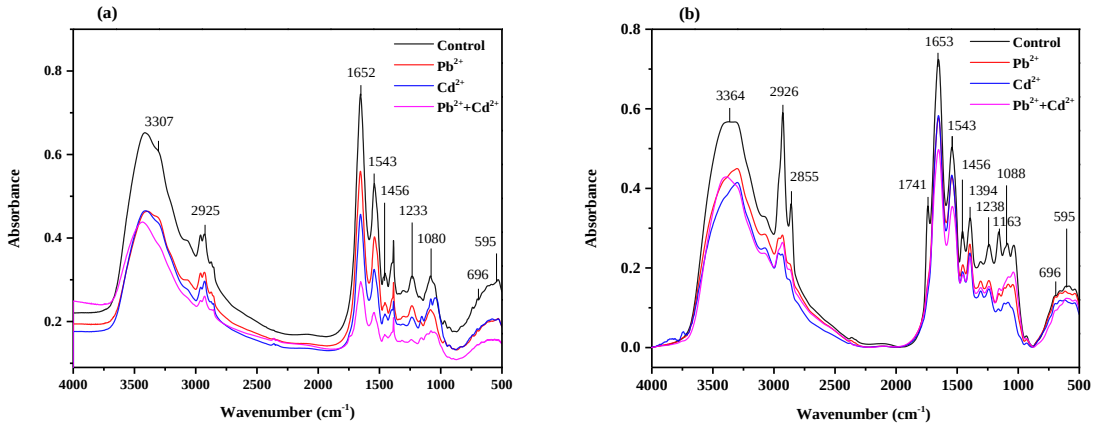

Figure 1. The average FT-IR spectra of Crucian carp's liver (a) and muscle (b), representing the control, Pb2+, Cd2+ and Pb2++Cd2+ exposed groups for 21days in the region of 4000-500 cm-1.

in the corresponding molecules were deduced from the accurate area under the characteristic band (Garip and Severcan 2010, Toyran *et al*. 2006). As shown in Fig 2a, the detail spectra of control and exposed liver tissue in the region of 3700 cm^{-1} to 2400 cm^{-1} consist of two broad bands. The first band at ~3307 cm^{-1} is mainly assigned to amide A: N− H stretching of proteins with little contribution from inter molecular O− H group. The second band at ~2925 cm^{-1} is assigned to asymmetric stretching of CH_2 which mainly corresponds to lipids with little contribution from protein, carbohydrates and nucleic acids. On the other hand, Fig 2b depicts the spectral details of liver tissue in the region of 1800 cm^{-1} to 800 cm^{-1}, representing several bands at ~1652, ~1543, ~1456, ~1233, ~1080, ~696 and ~595 cm^{-1} corresponding to amide I: C=O stretch of α-helix protein, amide II: N−H bending and C−N stretching of proteins, CH_3 bending of lipids with little contribution from proteins, C–O asymmetric stretching of glycogen and nucleic acids, symmetric PO_2− stretching of phospholipids and phosphodiester in nucleic acids, ring breathing mode in DNA basis and O-H deformation respectively. It can be seen from Fig 2a,b and Table 2, the absorption frequencies and band areas of the selected bands were decreased in the exposed groups with the exception of increase in band areas at ~1543 cm^{-1}, ~1456 cm^{-1}, ~1088 cm^{-1} in Pb^{2+}, Cd^{2+}+Pb^{2+} and Cd^{2+}, Cd^{2+}+Pb^{2+} exposed groups respectively.

Fig 3a,b,c shows the detail spectral features of control, Cd^{2+}, Pb^{2+} and Pb^{2+}+Cd^{2+} exposed fish muscles. The absorption band at 3364 cm^{-1} in the spectral region of 3600 cm^{-1} to 3100 cm^{-1} mainly corresponds to amide A and amide B: N–H stretching of proteins (Fig 3a). The bands assigned to ~2926 cm^{-1} in the region of 3050 cm^{-1} to 2800 cm^{-1} belong to asymmetric stretch of CH_2 investigating main<ly lipids with minor contributions from protein, carbohydrates and nucleic acids (Fig 3b). The spectral

Table 1. General vibrational peak assignment of the FT-IR spectra and band position observed for the liver and muscle tissue of Crucian carp after 21 days exposure to Pb^{2+}, Cd^{2+} and Pb^{2+}+Cd^{2+}.

Band	Assignment	Liver	Muscle
3364	Amide A and Amide B: mainly N–H stretching of proteins		+
3307	Mainly N− H stretching of proteins with the little contribution from O− H stretching of polysaccharides and intermolecular H bonding: amide A	+	
2926	CH_2asym. stretch: mainly lipids, with the little contribution from proteins, carbohydrates, nucleic acids	+	+
1741	Ester C=O stretch: triglycerides, cholesterol esters		+
1653	Amide I (C= O stretching of α-helix protein)	+	+
1543	Amide II (N−H bending and C−N stretching of proteins)	+	+
1456	CH_3 bending mainly lipids, with the little contribution from proteins	+	+
1394	COO− symmetric stretch: fatty acids and amino acids		+
1308	CH_3 CH_2stretching of collagen		+
1238	PO_2− asym. Stretch: mainly phospholipids and phosphodiester in nucleic acids	+	+
1163	C–O asym. stretching of glycogen and nucleic acids	+	+
1109	CO–O–C asymmetric stretching: glycogen and nucleic acids		+
1088	PO_2− sym. Stretch: mainly phospholipids and phosphodiester in nucleic acids	+	+
696	Ring breathing mode in the DNA bases	+	
595	O-H deformation	+	

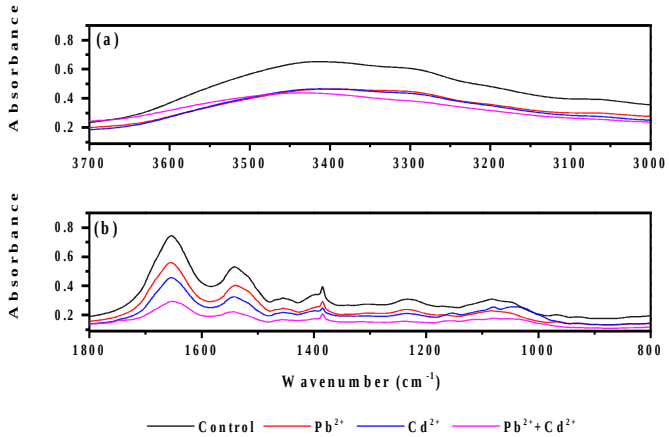

Figure 1. The average FT-IR spectra of Crucian carp's liver, representing the control, Pb^{2+}, Cd^{2+} and Pb^{2+}+Cd^{2+} exposed groups for 21days in the region of 3700-3000 cm^{-1} (a) and 1800-800 cm^{-1} (b).

region from 1800 cm^{-1} to 800 cm^{-1} showing various bands at ~1741, ~1653, ~1543, ~1456, ~1394, ~1238, ~1163 and ~1088 cm^{-1} corresponding to C=O stretch of triglycerides and cholesterol esters, amide I: C=O stretching of protein α-helix, amide II: N−H bending and C−N stretching of proteins, CH_3 bending of lipids with minor contribution from proteins, COO− symmetric stretching of fatty acids and amino acids, CH_3 and CH_2 stretching of collagen, PO_2− asymmetric stretching of mainly phospholipids and phosphodiester in nucleic acids, C–O asymmetric stretching of glycogen and nucleic acids, and PO_2− symmetric stretch of phospholipids and phosphodiester in nucleic acids respectively. Significant variations in the band areas and absorption frequencies of the selected bands among control and exposed groups can be seen from Table 3 and Fig 3. Comparing to control group, a decrease in the absorption frequency and bands areas of the exposed groups were observed at wavenumber 3364 cm^{-1} and 1741 cm^{-1} to 1543 cm^{-1} in the region of 3600 cm-1 to 3100 cm^{-1} (Fig 3a) and 1800 cm^{-1} to 800 cm^{-1} respectively. While the band areas significantly increased at ~2926 cm^{-1} for Pb^{2+}, at ~1456 cm^{-1} to 1394 cm^{-1} for Cd^{2+} and Cd^{2+}+Pb^{2+}, at ~1308 cm^{-1} for Cd^{2+}+Pb^{2+}, at ~1238 cm^{-1} for all the treated groups and at ~1109 cm^{-1} for Pb^{2+} and Cd^{2+}+Pb^{2+} exposed group.

Discussion

Freshwater contamination with toxic metal is a serious issue in the developing world due to high anthropogenic pressure and industrial expansion (Sun *et al*. 2015). An extensive literature is available on the bioaccumulation of toxic metals in freshwater fish including Cd^{2+} and Pb^{2+} (Hosseini Alhashemi *et al*. 2012, Low *et al*. 2015). However, fewer studies have explained their effects on the structural and compositional changes in various tissues of the exposed organisms (Krishnakumar *et al*. 2012, Palaniappan and Renju 2009). In the present study, an attempt was made to investigate the individual and combined effect of Cd^{2+} and Pb^{2+} on the structural and compositional changes in the liver and muscle of Crucian carp at concentrations closely related to

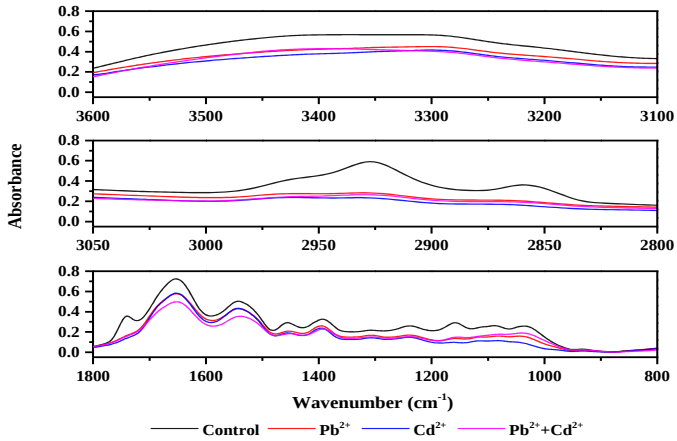

Figure 2. The average FT-IR spectra of Crucian carp's muscle, representing the control, Pb^{2+}, Cd^{2+} and Pb^{2+}+Cd^{2+} exposed groups for 21days in the region of 3600-3100 cm^{-1} (a), 3050-2800 cm^{-1} (b) and 1800-800 cm^{-1} (c).

Table 2: Changes in the selected FT-IR band area values and band area ratios of the selected bands of control, Pb^{2+}, Cd^{2+} and Cd^{2+}+Pb^{2+} exposed Crucian carp's liver tissue.

Band	Control	Pb	Cd	Cd+Pb
3307	21.616 ± 1.012^{c}	16.648 ± 1.796^{b}	15.498 ± 1.018^{b}	13.117 ± 1.637^{a}
2925	4.429 ± 0.564^{c}	3.273 ± 0.411^{b}	3.321 ± 0.392^{b}	2.554 ± 0.369^{a}
1652	8.620 ± 0.828^{c}	5.157 ± 0.256^{b}	5.243 ± 0.195^{b}	2.215 ± 0.212^{a}
1543	3.232 ± 0.688^{c}	4.544 ± 0.366^{d}	2.253 ± 0.245^{b}	1.514 ± 0.138^{a}
1456	2.258 ± 0.283^{a}	2.330 ± 0.285^{a}	2.262 ± 0.413^{a}	3.253 ± 0.303^{b}
1163	0.439 ± 0.036^{c}	0.797 ± 0.109^{d}	0.248 ± 0.035^{b}	0.004 ± 0.002^{a}
1088	1.596 ± 0.169^{ab}	1.376 ± 0.158^{a}	1.771 ± 0.227^{b}	2.596 ± 0.314^{c}
696	0.927 ± 0.024^{d}	0.615 ± 0.014^{b}	0.534 ± 0.033^{a}	0.754 ± 0.081^{c}
A_{1456}/A_{3307}	0.104 ± 0.007^{a}	0.140 ± 0.002^{b}	0.146 ± 0.005^{b}	0.248 ± 0.003^{c}
A_{1653}/A_{2925}	1.946 ± 0.031^{c}	1.576 ± 0.017^{b}	1.579 ± 0.022^{b}	0.867 ± 0.008^{a}

Values are means ± SD for five fish in each group
Values with different superscript letter are significantly different at P<0.05

Table 3. Changes in the selected FT-IR band area values and band area ratios of the selected bands of control, Pb^{2+}, Cd^{2+} and Cd^{2+}+Pb^{2+} exposed Crucian carp's muscle tissue

Band	Control	Pb	Cd	Cd+Pb
3364	41.258±2.067^{d}	29.92±2.132^{c}	21.757±2.224^{a}	25.792±2.738^{b}
2926	2.603±0413^{c}	2.492±0.459^{d}	1.731±0.145^{a}	1.960±0.290^{a}
1741	4.975±0.545^{c}	1.366±0.228^{ab}	0.652±0.082^{a}	2.073±0.341^{b}
1653	3.356±0.531^{a}	2.283±0.781^{a}	2.591±0.210^{a}	2.1974±0.482^{b}
1543	17.803±0.496^{a}	17.531±0.472^{a}	10.533±0.774^{c}	7.196±0.789^{b}
1456	1.617±0.749^{a}	1.587±0.381^{a}	5.265±0.854^{b}	4.178±0.648^{b}
1394	0.735±0.235^{b}	0.343±0.205^{a}	2.434±0.459^{d}	1.541±0.327^{c}
1308	2.388±0.852^{a}	1.403±0.161^{a}	2.169±0.130^{a}	5.266±0.542^{b}
1238	1.434±0.830^{a}	1.762±0.526^{b}	2.958±0.748^{a}	1.559±0.414^{a}
1163	2.127±0.462^{b}	0.928±0.097^{a}	0.504±0.407^{a}	0.939±0.376^{a}
1109	23.301±1.847^{b}	24.573±1.134^{a}	23.317±1.152^{b}	24.100±2.081^{c}
1088	0.853±0.421^{a}	0.854±0.210^{a}	0.301±0.083^{c}	0.725±0.056^{b}
A_{1741}/A_{3364}	0.121±0.004^{b}	0.045±0.002^{a}	0.030±0.007^{a}	0.080±0.005^{c}
A_{1543}/A_{2926}	6.839±0.081^{c}	7.035±0.023^{c}	6.085±0.031^{b}	3.671±0.067^{a}

contaminated environment by FT-IR spectroscopy (Bing *et al*. 2013, Khan *et al*. 2014, Wang *et al*. 2013, Zhou *et al*. 2007).

The FT-IR spectra of the two tissues for control and exposed groups of Crucian carp revealed significant differences in terms of band intensities and band areas (Fig 1). The band at ~3364 cm^{-1}in the spectra of muscle tissue of Crucian carp, which mainly correspond to amide A and amide B: N− H stretching of protein revealed significant reduction in band areas of intoxicated groups (Fig3a). The absorption band at ~3307 cm^{-1} in the liver tissue originates from (amide A) N− H stretching and O− H stretching modes in water, since water was removed during sample preparation, thus the band can only be considered due to protein and polysaccharide in the sample. As seen from Fig 2a, a significant reduction in the band areas of the three exposed groups suggests a proportional decline or deterioration in the content of protein and polysaccharides. One of the possible mechanisms responsible for protein modification and misfolding is oxidative stress, because toxic metals such as Cd^{2+} and Pb^{2+} are the potential producer of oxidative stress (Ashry *et al*. 2010, Pathak and Khandelwal 2006).

The band at ~2925 cm^{-1} arising from the olefinic region of CH_2 asymmetric stretching mainly monitors lipids (Bogomolny *et al*. 2008, Bozkurt *et al*. 2010). Reduction in the corresponding band intensities (Table 2 & 3) in the exposed groups revealed a decrease in the proportion of unsaturation in acyl chain of lipid, which indicates increase in lipid peroxidation (Garip and Severcan 2010) with highest lipid peroxidation being caused by the combined exposure of Cd^{2+}+Pb^{2+}. This can also be deduced from the selected band area ratios at $A_{1456/3307}$ and $A_{1741/3364}$ in table 2 and 3 respectively. Increase in the content of lipid was thought to be important for regulation of membrane functions in a cell (Ibarguren *et al*. 2014). However, in our study, disturbed metabolism of lipids might be the possible reason for increase in lipid peroxidation in Cd^{2+} and Cd^{2+}+Pb^{2+} intoxicated liver and muscle tissue.

The band at ~1652 cm^{-1} and ~1543 cm^{-1} (Fig 2b & Fig 3c) corresponds to amide I and amid II, investigating the structural proteins, respectively. Intoxication of Cd^{2+} and Pb^{2+} shows decrease in band areas with maximum decline in the band area due to combined exposure of the two metals. Contrary to the Cd^{2+} and Cd^{2+}+Pb^{2+} exposed groups, exposure to Pb^{2+} caused elevation of band area (4.544±0.366) at ~1543 cm^{-1} in the liver tissue (Table 2) which further revealed that intoxication of Cd^{2+} and Cd^{2+} +Pb^{2+} might decrease the α-helical structure of protein in the studied tissues. In another study, intoxication of Zn also decreased the α-helical structure of protein in the muscle of *Labeo rohita* (Palaniappan and Renju 2009). The band area at ~1394 cm^{-1} (Table 3) attributed to symmetric stretch of carboxylate, increased from 0.735±0.235 to 2.434±0.459 and 1.541±0.327 in Cd^{2+} and Cd^{2+}+Pb^{2+} exposed group respectively but decreased in the Pb^{2+} exposed group (0.343±0.205). This might confirm the partial oxidation of protein and lipid at ~1652 cm^{-1} and ~2925 cm^{-1} position respectively, which resulted in high content of fatty acid and amino acid at ~1394 cm^{-1} (Palaniappan and Pramod 2011). A significant increase in the band area was noticed at ~1308 cm^{-1} for Cd^{2+}+Pb^{2+} exposed group (Table 3), which mainly explain changes in collagen, a common fibrous protein, with many important functions and an indicator of several pathological conditions (Sivakumar *et al*. 2014). Similarly, changes in the intensity of phospodiester band at ~1088 cm^{-1} showed an increase in the content of nucleic acids of liver but decrease in muscle. Previously a decrease in the nucleic acid content was also observed in the arsenic intoxicated brain tissue of *Labeo rohita* (Palaniappan and Vijayasundaram 2008). Significant changes in the band areas at 3307, 1543, 1163, 696 of liver tissue and 3364, 2926, 1741, 1653, 1543, 1456, 1394, 1163 of muscles tissue further suggested that Cd had more drastic effect on the tissue architecture as compared to Pb. In a previous study, Tatrai *et al*. (2001) observed

that Cd caused more severe oxidative stress, membrane damage and inhibition of protein synthesis than Pb during exposure of type II pneumocytes to Cd and Pb.

The pronounced mechanism of Cd^{2+} and Pb^{2+} toxicity is the production of reactive oxygen species which further aggravate the normal homeostasis of the cells thereby affecting the biochemical integrity (Dewanjee *et al*. 2013, Souid *et al*. 2013). However, cells have marked defense mechanism to overcome the production of oxidatively modified protein with an increased proteolysis (Chondrogianni *et al*. 2014). This might be the possible reason for altered protein structure and concentration in the present study coinciding with earlier observations about Cd intoxicated liver tissue of rate by FT-IR analysis (Krishnakumar *et al*. 2012). Lipid peroxidation is another offshoot of oxidative damage caused by Cd and Pb due to indirect generation of free radicals (Sun *et al*. 2011). These free radicals attack cell membranes and cause disintegration of their vital cellular component such as protein and lipids resulting in lipid peroxides production (Krishnakumar *et al*. 2012, Brucka-Jastrzębska 2010). However, each toxic metal has unique toxicity mechanism, for example Pb^{2+} has the ability to compete for diverse polyvalent cations (Ca, Zn, Mg) in their binding sites (Garza *et al*. 2006). This evolutionary characteristic of Pb^{2+} may affect several physiological functions with the onset of structural and compositional change in the cells and tissues, whereas Cd has the ability to reduce the activity of glutathione reductase, which is a pre requisite for membrane integrity (Tatrai *et al*. 2001). Thus, it is imperative to further investigate the toxicity mechanism of these metals and their interactions in details.

The findings of the present stuady suggest that exposure to Cd^{2+} and Pb^{2+} alone or in combination at relatively low concentrations can significantly change the biochemical constituents of liver and muscle in Crucian carp. In general, a decrease in the protein and increase in lipid contents were observed from the spectra of the two tissues with marked changes caused by the combined exposure of the two metals. Alterations in the intensity and band areas at amide I responded differently to the exposed groups, revealing a differential response of the structural protein to Cd^{2+} and Pb^{2+} exposure in the liver. Also, exposure to Cd^{2+} and Pb^{2+} caused conformational changes in protein structure with depleted α-helix and alterations in the nucleic acids content. Moreover, FT-IR spectroscopy was found an effective and rapid technique in monitoring the lucid effect of environmental contaminants on biochemical integrity.

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