Spectral profile index changes as biomarker of toxicity in *Catla catla* (Hamilton, 1822) edible fish studied using FTIR and principle component analysis

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Abstract

The study was carried out to evaluate the toxicity in gill tissue of edible fish *Catla catla* using FTIR spectra. Fourier self-deconvolution obtained by curve fitting was applied in the lipid (3000–2800 cm⁻¹), carbohydrates (1000–1100 cm⁻¹) and in the amide region (1700–1600 cm⁻¹). These spectral changes were used as biochemical parameters to assess the degree of toxicity. A disorder in lipid changes was measured by frequency shift and intensity changes in the CH₂ asymmetric stretching band. This change in the fatty acid composition in fish could be used as biomarkers of toxic effect. Decreases in lactic acid (6–16%) clarify the lipid peroxidation which is the primary mechanism of toxicity. The deconvolution in the amide region shows peaks at 1621 cm⁻¹, 1637 cm⁻¹ due to β sheet; 1652 cm⁻¹ and 1667 cm⁻¹ due to α helix and 1683 cm⁻¹ due to antiparallel β sheet. The results show a decrease by (3–7%) in α helix and increase by (13–40%) in β sheet structure. This shows β sheet formation of protein secondary structure due to toxicity. PCA plots indicate protein and lipids have strong positive loadings. The study shows the spectral variation is considered as an ideal biomarker with a high degree of accuracy of test organism to examine the toxicity of pollutants.

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Graphic abstract

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1 Introduction

Air, water and land are the basic components of life. They are defiled because of increasing population, fast urbanization and industrialization. Extensive industrialization measurably influences the quality of water in lakes, ponds, and rivers especially in India [1]. Natural waters are adulterated by untreated wastes of industry and often contain different metallic compounds. At present, the bioaccumulation of heavy metals in the environment is a remarkable threat to human. Water contamination caused by modern wastage is frequent as they fall into natural water sources and agricultural environment. Leather industry is one of the traditional industries in the world. The demand of leather products led to the emergence of huge commercial tanneries. They meet the huge demands of leather footwear, drums and musical instruments. In India production of leather and the leather industry contributes a major share to the nation's export earnings. The use of a large number of chemicals by tanning industries discharges toxic wastes into the rivers and land. The wastes deliver wide varieties of high strength toxic chemicals. A serious concern to the regulatory bodies and the general public is the safe and long-term management of chromium-containing waste effluents from the tanneries.

Leather industries cause a serious environmental threat due to the discharge of heavy metals, organic and inorganic matter, suspended solids etc. [2, 3]. It is the major source of water pollution with high chemical oxygen demand (COD) and chromium content. The accumulation

of effluents becomes hazardous to the marine organisms and the surrounding human population. Fish is considered an ideal test species in the ecotoxicological studies, to examine the toxicity of pollutants. The wide use of fish is most likely due to their availability and adaptability to laboratory conditions to varying degrees of sensitivity to the toxic substances [4]. Fishes are the most widely distributed aquatic organisms that are susceptible to environmental pollution in the waters. They are also used to evaluate the health of aquatic systems and serve as biomarkers of environmental pollution. They are used in the quality assessment of the marine system due to pollution [5]. The freshwater fish, *Catla catla* are the most common fish consumed due to its high commercial importance worldwide. Therefore, it can be a good model to study the responses to various environmental contaminations. It is the best indicator of aquatic pollution and it is a good choice when compared to other aquatic animals. It is considered for perfect ecotoxicology test, to analyse all probabilities of changing levels of sensitivity to the toxic substance. Biochemical factors such as protein and glucose are highly sensitive and they are used in the detection of stress condition [6]. The alterations of these parameters are mainly used to identify target organs of toxicity. Proteins and lipids are biomarkers commonly used for detecting or diagnosing the pollution level of the environment. These parameters are suitable tools for surveying ecological impacts and stress of aquatic organisms. FTIR is a simple and rapid method to study the cellular changes in biological samples at the molecular level. It is easier to detect

the conformational changes in bimolecular components as well as intermolecular or intermolecular interaction in tissue samples [7, 8]. The change in the spectral alteration can be used in both qualitative and quantitative study of the molecular changes occurring in them [9, 10]. Studies due to the effect of an environmental pollution on the biological sample using FTIR paved the way for better understanding the biomonitoring process for qualitative and quantitative analysis [11, 12]. Hence, the present investigation was made to examine the biochemical changes in gill tissues of *Catla catla* under tannery effluents using the FTIR technique in conjunction with principle component analysis.

2 Description of the study area

The study area and the effluent discharge points in the river are shown in Fig. 1. The Kaveri river considered for this study travels into various districts of Tamil Nadu. The latitude of Kaveri river is 11.0585435 N and longitude is 79.5787168 E. The total length of this river is 765 km with an average rainfall of 812 mm. The major uses of the river water are agricultural consumption, household consumption and for drinking purpose. But nowadays the quality of the river water is deteriorating because of the discharge effluents from tannery industries and pollution due to sewage wastes, washing, bathing and miscellaneous activities. Kaveri River runs over several km and it reaches the domestic area Ramanathapuram Pudur where it receives effluents discharged from local tannery industries. The effluent samples are measured for the major physical and

chemical parameters and heavy metal analysis. The effluents flowing into the river are collected from the discharge point (S1, S2, S3 and S4) to assess the toxicity due to tannery effluents in the nearby the residential area.

3 Materials and methods

3.1 Measurement of tannery effluent concentration by atomic absorption spectrometer

The collected effluents from sampling points are analysed for heavy metals concentration using Atomic absorption Spectrometer (model AL168 Elico, India) available at ALPHA Lab, Coimbatore, Tamil Nadu. The major concentrations of heavy metals are chromium 6.10 mg/l, Cd 2.93 mg/l, Pb 4.78 mg/l, Cu 0.07 mg/l and Mg 0.89 mg/l. Among the metals, Cu and Mg are below the limit as recommended by the Central pollution control board of India whereas the rest of the metals are found to be above the permissible limit. Further, the chlorides have 2388.36 mg/l and TDS 4080 mg/l. This shows that tannery effluents have higher chromium contents followed by Pb and Cd collected from the sampling points.

3.2 Toxicity study of tannery effluent—determination of LC_{50}

A static bioassay method was used in our laboratory. The experimental fish, *Catla catla* (average length: 7.5 ± 0.5 cm and weight: 6.5 ± 0.5 g) were obtained from Tamil Nadu Fisheries Development Corporation Limited, Aliyar Fish

Fig. 1 Map of sampling station showing a collection of tannery effluents at different sampling point of Kaveri River flowing at Tamilnadu, India

Farm, Tamil Nadu, India. The fish were adapted to laboratory conditions for 15 days in a large tank (1000 l capacity) and fed with groundnut oil cake. Water was renewed daily to avoid accumulation of excretory materials. During acclimatization, the fish were maintained at a natural photoperiod at ambient temperature. The chlorine-free water was used in the present experiment. The physico-chemical parameters of the water were measured following the method of APHA [13]. They are temperature ($29.0 \pm 12^\circ\text{C}$), pH (7.4 ± 0.03), dissolved oxygen ($6.8 \pm 0.5\text{ mg/l}$), total alkalinity ($164 \pm 7.8\text{ mg/l}$), total hardness ($152 \pm 4\text{ mg/l}$) and salinity ($0.04 \pm 0.02\text{ mg/l}$).

Preliminary tests were conducted to find out the median lethal concentration of fish due to tannery effluent for 96 h. For finding LC_{50} , circular plastic container of 20 l capacity was used. Ten fishes were introduced in each container having different concentrations of tannery effluent (1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 and 2.0 mg l^{-1}) for 96 h treatment. The fishes served without the addition of toxicants act as a control. Acute toxicity is determined using a standard static-renewal technique described by the US environmental protection agency for the measurement of toxicity of effluents [14]. The 96 h LC_{50} was calculated by the probit method [15] and found to be 1.9 mg/l . For chronic toxicity study 1/10th of LC_{50} concentration of tannery effluent (0.19 mg/l) was taken and exposed for 10 and 30 days treatments in our study. The samples were grouped into control—group 1, the effluents treated for 10 days and 30 days were grouped as group 2 and 3 respectively. The experiment was carried out with three replicates along with control groups. Upon completion of the stipulated exposure period, the gill tissues selected fish from the control and tannery effluent were taken for further analysis.

3.3 Sample preparation

The gill tissues were lyophilised for 12 h 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. It was 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.

3.4 FTIR analysis

FT-IR spectra were recorded on NEXUS 470 spectrophotometer installed at the Karunya University, Coimbatore, India. A total of 256 scans were taken at a resolution of 4 cm^{-1} and averaged. A blank KBr disk was used as

background. Pellets were scanned at room temperature ($25 \pm 1^\circ\text{C}$) in the $4000\text{--}400\text{ cm}^{-1}$ spectral range. Background spectra were subtracted from the sample automatically. Each sample was scanned under the same conditions with three different pellets. The software OPUS version 6.5 was used to carry out the baseline correction and vector normalization. The spectral deconvolution was obtained using the Origin 8.0 software.

A baseline was subtracted before the curve fitting. To start the curve fitting iteration, peak position, peak height and half bandwidth were chosen. The initial values were taken from second derivatives smoothed by the Savitzky and Golay method with a 9-point window. Gaussian function was used to resolve peak pattern with least goodness-of-fit explained in our earlier study [16].

3.5 Statistical analysis

The results were expressed as $\pm$ standard error of mean (SEM). Gill tissues of *Catla catla* tannery effluent treated group vs control group were analysed using the one-way ANOVA test using SPSS 16.0. *P* values of less than 0.05 were considered as statistically significant.

3.6 Principle component analysis (PCA)

The principle component analysis (PCA) was carried out using SPSS16.0 programming. It is used for data reduction from a larger sample. The PCA was used to our mean-centered, second derivative, and vector normalized spectral data. The results were displayed as score plots. The input value of the samples (both control and treated) is formed as data matrices. The inputs change into scores and loadings which are the characteristics of principal components. It is used for quantitative approaches in discriminating the samples. The scores of the component were plotted to gather data responsible for the variation.

4 Results and discussion

Figure 2 indicates the average spectra of gill tissues of *Catla catla* of control and chronic exposures of tannery effluents for two different periods. The major and minor bands of the infrared spectra of the control groups, tannery effluents treated gill tissues were recorded in Table 1. As observed from Fig. 2 the bands centred at $\sim 3291\text{ cm}^{-1}$ and 3089 cm^{-1} corresponds to amide A and amide B of proteins due to N–H/O–H modes of proteins. The bands rise at $\sim 3013\text{ cm}^{-1}$ which indicates the presence of HC=CH group olefinic molecules. This band is used as a varying measure of degrees of unsaturation of phospholipids [17]. Lipids give rise to a number of absorption in FTIR spectra.

Fig. 2 Average FTIR spectra of *Catla catla* showing control and tannery effluents exposed to different days of exposure in the region of 4000–400 cm^{-1}

The medium band which rises at $\sim 2957 \text{ cm}^{-1}$ is assigned CH_3 asymmetric stretching. The peak at 2927 cm^{-1} and 2855 cm^{-1} can be assigned to asymmetric and symmetric stretching mode of CH_2 modes [18]. This band is mainly screened for the lipids present in the biological system. Strong band $\sim 1645 \text{ cm}^{-1}$ is assigned to amide I and arises due to $\text{C}=\text{O}$ of protein. Amide II bands appear $\sim 1536 \text{ cm}^{-1}$ due to $\text{N}-\text{H}/\text{C}-\text{N}$ mode of vibration [19, 20]. As observed from the Fig. 2 the intensity of amide bands decreases significantly due to the tannery effluents treated for both acute and chronic exposures. Medium intensity bands $\sim 1392 \text{ cm}^{-1}$ arise mainly from COO^- symmetric stretching modes of fatty acids. The band is seen at $\sim 1231 \text{ cm}^{-1}$, and 1083 cm^{-1} were primarily assigned to the asymmetric

and symmetric stretching modes of nucleic acids instead of phospholipids [21]. This band may overlap in the carbohydrates region, as revealed by the deconvolution [22]. There are several bands which appear in the $3000\text{--}2800$ and $1800\text{--}1000 \text{ cm}^{-1}$ region. These bands need extraordinary administration to information investigation since they comprise of several unresolved bands. We used Fourier self deconvolution techniques in the lipid regions ($3000\text{--}2800 \text{ cm}^{-1}$), carbohydrates region ($1000\text{--}1100 \text{ cm}^{-1}$) and amide region ($1700\text{--}1600 \text{ cm}^{-1}$).

4.1 FTIR self deconvolution deduced by curve fitting analysis in the fatty acids region

An interesting observation was made by examining the curve fitting in the region $3050\text{--}2800 \text{ cm}^{-1}$ shown in Fig. 3. The investigation in the region $3050\text{--}2800 \text{ cm}^{-1}$ indicates band $\sim 3013 \text{ cm}^{-1}$ is because of the $\text{HC}=\text{CH}$ stretching modes. As observed from Fig. 3 the band area 3013 cm^{-1} diminishes demonstrating the population of unsaturated lipids. It was observed that the band of olefinic group reduced by 12% and 73% for tannery effluents when compared to control (Table 2). This reduction in the band area and the shift in the frequency value of this band were because of alterations in lipid metabolism induced by tannery treatment. These changes were monitored by the CH_3 asymmetric stretching mode observed in $\sim 2960 \text{ cm}^{-1}$. Considering the control a decrease in the band area of the CH_3 asymmetric stretching vibration by 25% and 56% were observed as the outcome of tannery effluent treatment. This indicates the release of the phospholipids in the gill tissues of *Catla catla*. The shift in frequency and intensity changes in CH_2 asymmetric stretching band $\sim 2927 \text{ cm}^{-1}$ gives information regarding the degree of conformational disorder. In the current work, the shift in peak position to lower values indicates that the lipid disorder increases and

Table 1 Tentative frequency assignment and their functional groups for the Control and tannery effluents treated gill tissues of *Catla catla*

Control	10 Days exposure	30 Days exposure	Frequency assignment
3291 (m)	3298 (m)	3293 (w)	Amide A: mainly $\text{N}-\text{H}$ stretching of proteins
3089 (w)	3081 (w)	3079 (w)	Amide B: $\text{N}-\text{H}$ stretching of proteins
2957 (s)	2953 (m)	2955 (m)	CH_3 asymmetric stretching, mainly lipids
2927 (s)	2928 (m)	2927 (w)	CH_2 asymmetric stretch: mainly lipids
2855 (vw)	2852 (vw)	2858 (vw)	CH_2 symmetric stretch: mainly lipids
1645 (s)	1644 (m)	1642 (m)	Amide I: $\text{C}=\text{O}$ stretching of proteins
1536 (s)	1527 (vw)	1529 (m)	Amide II: $\text{N}-\text{H}$ bending and $\text{C}-\text{N}$ stretching of proteins
1441 (m)	1444 (m)	1446 (vw)	CH_2 bending: mainly lipids
1398 (m)	1395 (vw)	1397 (vw)	COO^- symmetric stretch: fatty acids and amino acids
1231 (m)	1229 (vw)	1226 (vw)	PO_4^{2-} asymmetric stretch
1169 (m)	1164 (m)	1167 (vw)	$\text{C}-\text{O}$ asymmetric stretching of glycogen

s strong, m medium, w weak, vw very weak

Fig. 3 FTIR spectra deduced by curve fitting analysis in the region 3100–2800 cm^{-1} of *Catla catla* showing control and tannery effluents exposed to different days of exposure

acyl chain flexibility decreases [23, 24] (disorder state of lipids). It was observed that the shift in frequency intensity of the band in the lipid region shows adverse in lipid fluidity due to exposure of tannery effluents. In addition, the band area of the CH_2 stretching band was found to be significantly increased at 30 days exposure compared to 10 days exposure. These results suggest an increase in the lipid contents has occurred for 30 days exposure when compared to 10 days. This could be due to the usage of lipid to satisfy the additional energy requirement under stress [25]. Similarly an increase in CH_2 stretching was observed in liver tissues of *rainbow trout* due to estradiol, which was studied using FTIR spectroscopy [26]. Loss of lipids noticed in this study results in lipid synthesis and mobilizing of the stored unsaturated lipid molecules as suggested by Jha [27]. This change in the fatty acid content of fish phospholipid is used as a biomarker indication thus helping in the diagnosis of aquatic pollution [28].

4.2 FTIR self deconvolution deduced by Curve fitting analysis in the carbohydrates region

The results of deconvolution in the region 1200–900 cm^{-1} shows the existence of glycogen $\sim 1013 \text{ cm}^{-1}$, glucose $\sim 1038 \text{ cm}^{-1}$ and lactic acid $\sim 1126 \text{ cm}^{-1}$ respectively. From Fig. 4a remarkable decrease in glycogen level was perceived due to tannery effluent toxicity. The band area decreases by 5–15% due to effluent treatment (Table 3). This decrease in glycogen has additionally been recommended by Shaffi [29], to clarify consumption in glycogen. Comparable consumption in glycogen content in the current study might be credited to the usage of glycogenolysis due to effluents stress [30]. From Fig. 4 a 5% reduction in glucose was observed due to tannery effluent exposed for a period of 10 days. But there is a slight increment (18%) in the glucose concentration noted at 30 days exposure. This mobilization of glucose is due to its availability for utilization by the tissues in the normal metabolic process which is inevitable when exposed to the toxic medium.

Fig. 4 FTIR spectra deduced by curve fitting analysis in the region 1200–900 cm^{-1} of *Catla catla* showing control and tannery effluents exposed to different days of exposure

Table 2 Results of curve fitting analysis for the Control and tannery effluents treated gill tissues of *Catla catla* in the fatty acids region (3050–2800 cm^{-1}) and their band assignments

Control		10 Days exposure		30 Days exposure		Assignments
Wave number (cm^{-1})	% Area	Wave number (cm^{-1})	% Area	Wave number (cm^{-1})	% Area	
2927	37.49 ± 0.79	2921	51.48 ± 0.94	2925	72.88 ± 1.25	CH_2 asymmetric stretching lipids
2960	52.59 ± 0.64	2954	39.48 ± 1.74	2963	23.18 ± 0.96	CH_3 asymmetric stretching Lipids
3013	11.24 ± 0.56	3015	9.93 ± 0.46	3018	3.03 ± 0.24	Olefinic $\text{HC}=\text{CH}$ lipids

The values are the mean $\pm$ SE for each group ($n=3$). The degree of significance was $P < 0.05$

Table 3 Results of curve fitting analysis for the control and tannery effluents treated gill tissues of *Catla catla* in the carbohydrates moiety region (1000–1150 cm^{-1}) and their band assignments

Control		10 Days exposure		30 Days exposure		Assignments
Wavenumber (cm^{-1})	% Area	Wave number (cm^{-1})	% Area	Wave number (cm^{-1})	% Area	
1013	50.77 $\pm$ 0.85	1014	48.12 $\pm$ 1.04	1013	42.95 $\pm$ 1.47	Glycogen
1038	37.73 $\pm$ 0.46	1037	35.96 $\pm$ 0.69	1035	44.36 $\pm$ 0.58	Glucose
1126	14.58 $\pm$ 0.64	1125	12.26 $\pm$ 0.38	1127	13.75 $\pm$ 0.26	Lactic acid

The values are the mean $\pm$ SE for each group ($n = 3$). The degree of significance was $p < 0.05$

The by-product lactic acid formed is put to use for cell functions. This clarifies the possible synthesis of carbohydrates to overcome the glucose level under stress conditions. The decrease in glycogen results in higher demand for carbohydrate to meet energy demands during stress conditions. The reduction in glycogen level is considered as the result of greater stress the organs experience during the detoxification process. An unusual lactic acid is found to diminish radically (6–16%) for both treatments which could clarify the lipid peroxidation which is the primary mechanism of toxicity. It is seen at chronic exposure concentration lactic acid raises, proposing oxidative stress resulting in altered levels of lipid metabolism due to toxicants. A similar effect was studied by Jerome et al. [31], due to the effect of industrial effluent on gill tissues of *Callinectes amnicola* resulting in a decrease lipid peroxidation. This brings about the disruption of enzymes associated with carbohydrate metabolism. The consumption of glycogen level by fish may because of the utilization of energy due to toxic stress. Similar result in a reduction in carbohydrate metabolism was reported by Tilak et al. [32] in *Channa punctatus* due to exposure of thermal power plant effluent. The decrease in the glycogen concentration is increased by the utilization of energy resource to overcome the toxic stress which normally enhances glycogen utilization [33]. In addition, the decline in glycogen may be due to use in the formation of glycoproteins and glycolipids of various cells and other membranes. Similar results in carbohydrates reduction were observed by Muley et al. [34], due to the toxic effect of industrial effluents in *Labeo rohita*. Sobha Rani et al. [35], observed a huge exhaustion in carbohydrate metabolism in different tissues of freshwater fish *O. mossambicus* under toxic conditions. Perceptions of the present review demonstrated that effluents to sub-lethal concentrations causes a decrease in the biochemical composition (glycogen, protein and lipid). Umminger [36] hypothesized that glycogen is utilized as the immediate energy source by the fish under stress conditions. Our FTIR study supports the decline in the glycogen level in fish tissues exposed to sub-lethal concentrations when compared to control. This may be due to the reduction of Cr(VI) to Cr(III) which is a major component in tannery effluents. Further, this Cr(III) ions can bring about the production of

reactive oxygen species (ROS) leading to oxidative stress in the exposed animals [37, 38]. Chen et al. [39], studied the effect of chromium on tissues, organs of *Oryzias latipes* with respect to biochemical changes leading to an increase in lipid peroxidation and histopathological alteration. We can get from the eventual outcomes of the present study that the dynamic gathering of tannery effluents in gill target tissues influenced the decline in the glycogen level. The present work shows that tannery effluents brought about changes in the carbohydrate metabolism in *Catla catla*, resulting in a further decrease in glycogen values. Subsequently an increase in glucose content resulting in a decrease in lactic acid which are more pronounced due to anaerobic metabolism to make a by-product during ATP synthesis.

4.3 FTIR self deconvolution deduced by curve fitting analysis in the amide I region

Understanding of the protein components of the cell seems to be distinctly vital in studying fundamental changes in the secondary structure of protein among tannery intoxication. The synthesis of protein and degradation are sensitive due to varying physical and chemical modulators. Deconvolution made in the amide I region of 1720–1600 cm^{-1} are used to study the secondary structural changes in proteins. The Fig. 5 shows the presence of five peaks 1683, 1667, 1652, 1637 and 1621 cm^{-1} obtained after the results of the curve fitting analysis for control and treated samples. The peak due of 1621 cm^{-1} , 1637 cm^{-1} assigns to β sheet; 1652 and 1667 cm^{-1} assigns to α helix and 1683 cm^{-1} due to antiparallel β sheet [16, 40]. It is observed from Table 4 that the percentage area of α helix declines by 3–7% and an increase in β sheet structure by 13–40% was noticed. This may be because of the changes or synthesis of proteins which were consistent with the mechanisms of β sheet formation. The β -sheet structure in the effluents treated gill tissues was formed because of the intermolecular hydrogen-bond interaction that modifies the secondary structure of proteins in the gill tissues [41, 42]. The further antiparallel β sheet was formed and an increase in band area 9–12% was observed with respect to control. This change in the secondary structure

Fig. 5 Secondary structure of protein deduced by curve fitting analysis in the region 1720–1600 cm^{-1} of *Catla catla* showing control and tannery effluents exposed to different days of exposure

is in agreement with our earlier studies using FTIR on fish tissues [17]. Similar results of a decrease in the protein profile exposed to effluent toxicity of *Channa punctatus* (Bloch) were reported by Maruthi and Subba Rao [43]. Proteins possess a remarkable position in the metabolism of cells and enzymes, which intervene at different metabolic pathways. These results suggest that the structural changes in the protein of the gill tissues were significantly affected by tannery effluents. In the present study, protein compositions were altogether decreased in the tannery treated group when contrasted with the control group proposing solid markers of stress impact of the tannery treatment. Khan et al. [44], studied the toxic impact of Cd and Pb resulting in changes in lipids, proteins on *Cru-cian carp* (*Carassius auratus gibelio*) using FTIR analysis. The outcomes revealed that toxicity due to tannery influences the intermediary metabolism of *Catla catla* utilizing FTIR can fill in as incredible biomarkers of tannery effluent contamination.

4.4 Principle components analysis of the gill tissue of *Catla catla* exposed to tannery effluents

PCA enables quantitative changes among the spectra in terms of principle components, based on the criteria of uncorrelated difference. The PCA obtained from infrared spectra are better reflected from higher eigenvalues and % of the variance as shown in Fig. 6a. The plots indicate that the control and tannery treated samples are successfully well discriminated with each other. Control and tannery treated samples have a strong separation in the positive components of the score plots. The highest absolute eigenvalue corresponds to components 1 and 2. The first principal components describe over 97% of all spectral features. The component 2 accounts for 3% difference of the spectral values. The third component is neglected due to lower eigenvalues. The results of the PCA are best displayed graphically, as loading plots versus the wavenumber showing significant variation in the biochemical composition (Fig. 6b). By reviewing the loading plots, it is clear that protein and fatty acids have a positive loading showing highest variation obtained from our sample. A distinct variation was evident from the infrared spectra of the samples studied. The glycogen, glucose and lactic acid of the carbohydrate moieties are having the least variation of loading values due to effluent treatment.

5 Conclusion

Spectral bands obtained by deconvolution technique were used for a quantitative measure of toxicity on gill tissues of *Catla catla*. The decrease in the frequency and intensity changes of asymmetric CH_2 stretching showed a reduction in lipid contents due to tannery effluents. This reduction is more pronounced with exposure periods. Changes in carbohydrate metabolism bring about an increase in glucose level in both the types of treatment of gill tissues of *Catla catla*. The decrease in lipid and protein contents is

Table 4 Results of curve fitting analysis expressed as a function of percentage areas of protein secondary structures for the control and tannery effluents treated gill tissues of *Catla catla* in the Amide I region and their band assignments

Control		10 Days exposure		30 Days exposure		Assignments
Wave number (cm^{-1})	% Area	Wave number (cm^{-1})	% Area	Wave number (cm^{-1})	% Area	
1621	8.89 ± 0.46	1619	11.05 ± 0.73	1618	14.38 ± 0.64	β Sheet
1637	20.34 ± 0.61	1636	22.09 ± 0.65	1634	26.77 ± 0.68	β Sheet
1652	28.58 ± 0.71	1651	26.72 ± 0.32	1656	26.95 ± 0.14	α Helix
1667	26.03 ± 0.56	1666	25.26 ± 0.27	1665	24.64 ± 0.39	α Helix
1683	14.81 ± 0.48	1682	16.18 ± 0.52	1683	16.58 ± 0.63	β Sheet antiparallel structure

The values are the mean $\pm$ SE for each group ($n = 3$). The degree of significance was $P < 0.05$

Fig. 6 **a** PCA plots showing distinct variation in the biochemical composition of gill tissues of *Catla catla* showing Control and tannery effluents exposed to different days of exposure. **b** Variation of

the factor loading obtained from the PCA method with the corresponding wavenumber

because of the repair mechanism of the gill tissues with the creation of lipoproteins, key constituents of cell membranes. Further, the depletion of tissue proteins is due to lower protein synthesis because of metallic stress with the secretions of mucoproteins, which are eliminated in the form of mucus. PCA study shows the distinct variation among the samples studied when compared with control. It shows that the major component responsible for variation is proteins due to β sheet formation, followed fatty acids and carbohydrates. The results conclude that the use of infrared spectroscopy with self deconvolution and PCA techniques serve to assess the toxicity of the effluent in aquatic organisms. Further, the selected spectral bands are used as a potential tool in assessing the toxicity in the marine system with better reliability and accuracy.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

Ethical approval Ethical approval is not required for carrying out experiments with fishes in India.

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