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RESEARCH ARTICLE

STUDIES ON THE IMPACT OF INDUSTRIAL POLLUTANTS ON THE MUSCLE TISSUES OF *LATES CALCARIFER* IN UPPANAR ESTUARY, CUDDALORE, SOUTH COAST OF INDIA, USING FT-IR SPECTROSCOPY

Balakrishnan, K. and *Ronald Ross, P.

Department of Zoology, Faculty of Science, Annamalai University, Annamalai Nagar - 608 002, India

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ABSTRACT

The discharge of industrial effluents having heavy metals throw enormous deleterious impacts on aquatic organisms especially in fish. This potential accumulation of heavy metals in the fish tissues are subsequently transferred to human beings through the food chain. Thus, heavy metal pollution in fish has become an important worldwide concern, not only because of the threat to fish, but also due to the health risks associated with fish consumption. In this context, to investigate the extent of damage to fish tissues, FT-IR Spectroscopy can provide adequate information on the molecular composition of the fish tissues by detecting and analyzing light that is elastically scattered from the tissues following its excitation by monochromatic laser light. The present study aims to analyze the impact of industrial pollutants on the muscle tissues of *Lates calcarifer* inhabiting in Uppanar estuary, Cuddalore, at three different stations with respect to summer season. FT-IR spectra reveal significant differences in absorbance intensities between muscle tissues collected from three stations, reflecting the alterations in the biochemical constituents especially proteins and lipids. The spectral analysis showed variations in composition of bio molecules of the muscle tissue at a wave number region of 3423 to 701 cm^{-1}. The results suggest that FT-IR spectroscopy could be used as a potent tool for the detection of biochemical changes that occur as a result of metal intoxication.

INTRODUCTION

Pollution is a serious problem as 70% of India's surface water resources and ground water reserves have been contaminated by organic, inorganic and biological pollution. Increased industrialization, urbanization, population growth and overall man's greed to overexploit Mother Nature has created a serious threat to all kinds of life in the form of pollution which has now become a global issue. Among all types of pollution, aquatic pollution is of greater concern as each and every kind of the life depends on water and therefore management of aquatic environment in particular has become a major concern in recent years (Deepak Kasherwani *et al.,* 2009). When heavy metals from the industrial effluents reaches the aquatic bodies, they deteriorate the life sustaining quality of water and cause damages to both flora and fauna (Samanta *et al.,* 2005). Several biochemical and physiological responses can occur when aquatic organisms absorb a toxicant, which may be a compensatory response or a toxicity mechanism (Begum, 2004). Fourier transform infrared (FT-IR) spectroscopy is a non-disturbing technique which provides quantitative biochemical information about biological samples. It is a valuable technique due to its high sensitivity in detecting changes in the molecular constituent of tissues, such as lipids, proteins and nucleic acids (Cakmak *et al.,* 2006; Akkas *et al.,* 2007; Venkataramana *et al.,* 2010; Chezhian *et al.,* 2012). The FT-IR spectra of protein are characterized by a set of absorption region known as the amide region and the C–H region. The most widely used modes in protein structure studies in the amide region are amide I, II and III. Since amide absorption is sensitive to protein confirmation, an increase or decrease in this ratio could be attributed to the corresponding changes in the composition of the protein pattern (Palaniappan *et al*., 2009). It is therefore interesting to apply FT-IR spectroscopy in the present investigation to analyze the impact of industrial pollutants on the muscle tissues of *Lates calcarifer* collected from three different stations at Uppanar estuary during summer season.

Corresponding author: Ronald Ross, P.
Department of Zoology, Faculty of Science, Annamalai University, Annamalai Nagar - 608 002, India.

MATERIALS AND METHODS

The present study was carried out in the Uppanar estuary, which runs behind the SIPCOT industrial complex, Cuddalore. Uppanar estuary is a major fishing ground for many kinds of fishes. It receives industrial effluents from SIPCOT industrial complex as well as domestic and municipal sewages from Cuddalore town. Three sampling stations were selected to study the impact of industrial effluents on the muscle tissues of *Lates calcarifer* using FT-IR spectroscopic analysis. The study

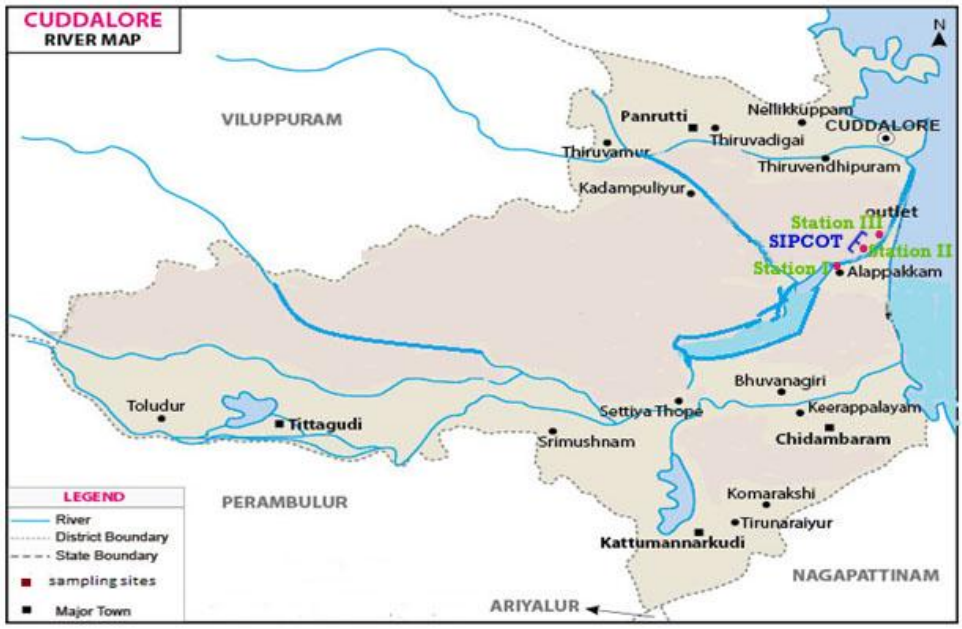

Fig. 1. Location map of study area

was undertaken from January 2012 to December 2012 during summer (April to June) season. The station I is 10 Km upstream, station II is purely industrial zone and station III is 2 Km away from station II (Fig.1).

Live *Lates calcarifer* were collected from the Uppanar estuary at stations I, II and III during summer season with the help of local fisher folks. In the field itself, the fishes were dissected and the muscle tissue was quickly removed and stored at –80 ºC until spectroscopic studies were carried out. The samples were lyophilized and made into a fine powder. The tissue powder samples and KBr (all solid dry state) were again lyophilized in order to remove most bound water which might interfere with the measurement of the amide I band. Approximately 5 mg of the sample was mixed with 100 mg of dried KBr and then pressed into a clear pellet of 13 mm diameter and 1 mm thickness. Absorbance spectra were recorded using Nicolet Avatar-360 FT-IR spectrometer equipped with a KBr beam splitter and a DTGS detector installed at the Centralized Instrumentation and Services Laboratory, Annamalai University. For each spectrum 100 scans were co-added, at a spectral resolution of 4 cm^{-1}. The spectrometer was continuously purged with dry nitrogen. The absorption intensity of the peak was calculated using the base line method.

RESULTS AND DISCUSSION

The broad band that appeared in the muscles of *Lates calcarifer* collected from station I at 3421cm^{-1} indicated the presence of OH with alcohols and phenols. The peaks that appeared in the region of 2956cm^{-1} showed the presence of aliphatic components. The regions of 2853cm^{-1} and 2923cm^{-1} indicated the presence of lipids and proteins respectively. The peak that appeared in the region of 1653cm^{-1} was assigned as primary amides and 1541cm^{-1} was assigned to the secondary amides of NH. Aliphatic compounds, carboxylic acids, sulfomes and alcohols were assigned in the regions between 1457cm^{-1} and 1075cm^{-1} (Table 1 and Fig. 2).

Table 1. Station I: Muscle

Wave number	Assignments	Bond
3421.28	OH in alcohols and phenols	OH
2956.43	CH_3 and -CH_2- aliphatic compounds	CH
2923.67	CH_3 and -CH_2- aliphatic compounds	CH
2853.07	CH_3 and -CH_2- aliphatic compounds	CH
1653.96	C=O- primary amides	C=O
1541.3	NH secondary amides	NH
1457.8	CH_3 aliphatic compounds	CH_3
1395.42	Carboxylic acids	COO-
1304.6	SO_2 in sulfomes	SO_2
1238.27	C-N aromatic amides	-C-N
1159.41	C-OH in alcohols	C-O
1075.59	C-N_2 in primary alcohols	C-N

Table 2. Station II : Muscle

Wave number	Assignments	Bond
3423.25	OH in alcohols and phenols	OH
2956.43	CH_3 and -CH_2- aliphatic compounds	CH
2924.37	CH_3 and -CH_2- aliphatic compounds	CH
2849.59	CH_3 and -CH_2- aliphatic compounds	CH
1637.15	C=O in primary amides	NH
1545.66	NH secondary amides	CH_3
1458	COO- group in carboxylic acid	fill
1395	COO- group in carboxylic acid	fill
1301.86	SO_2 in sulfomes	SO_2
1236.11	C-N in aromatic amides	C-O
1173.11	C-N_2 in primary alcohols	C-N
1118.32	Aromatic C-H plane bend	C-H
934.79	Vinyl C-H out off plane bend	C-H
899.17	Vinyl C-H out off plane bend	C-H

The station II fish muscle tissue had showed significant changes in FTIR spectroscopic analysis with regard to functional groups and vibrations. The spectra ranged from

3423cm^{-1} to 899cm^{-1}. The broad peak 3423cm^{-1} indicated the functional groups such as OH in alcohols and phenols. The peaks 2956cm-1, 2924cm^{-1} and 2853cm^{-1} coded with the functional groups as aliphatic compounds and may perhaps be present in lipids. The peaks from 1637cm^{-1} to 899cm^{-1} imitate the same functional groups in their organic compounds such as phenolic, aliphatic groups, ureas, aromatic nitro compounds, carboxylic acid salts and aromatic amine groups (Table 2 and Fig. 3). The station III had significant changes from station II and I in peak ranges. The spectra ranged between the 3425cm^{-1} and 701cm^{-1}. The typical peak observed in 1044cm^{-1} intimated the existence of organophosphorus. Other peaks were similar to that of station II and it had the same functional groups (Table 3 and Fig.4).

Table 3. Station III : Muscle

Wave number	Assignments	Bond
3425.71	OH in alcohols and phenols	OH
2953.69	CH_3 and -CH_2- aliphatic compounds	CH
2924.12	CH_3 and -CH_2- aliphatic compounds	CH
2852.33	CH_3 and -CH_2- aliphatic compounds	CH
1657.62	C=O in primary amides	NH
1640.58	C=O in secondary amides	NH
1547.3	NH secondary amides	CH_4
1529.17	NH_3+ in amino acids or hydrochlorides	NH_3+
1378.56	COO- group in carboxylic acid	fill
1227.9	C-N in aromatic amides	C-O
1167.63	C-N_2 in primary alcohols	C-N
1044.36	Organophosphorus	P-C-O
701.94	Aliphatic chloro compounds	C-Cl

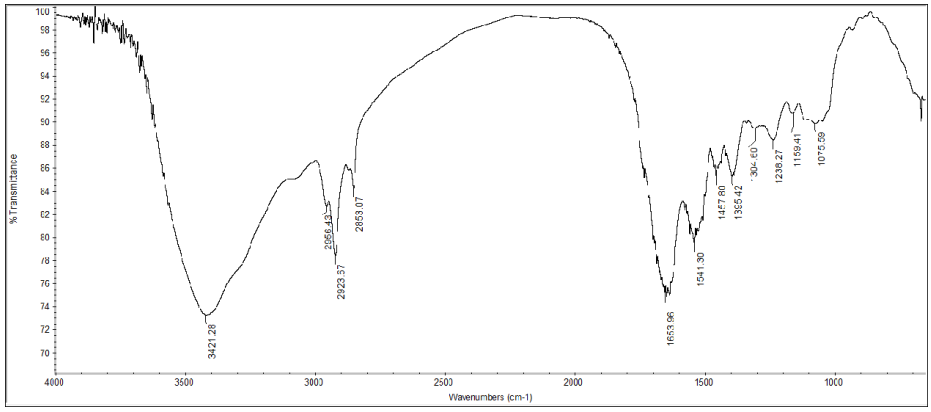

Fig.2.

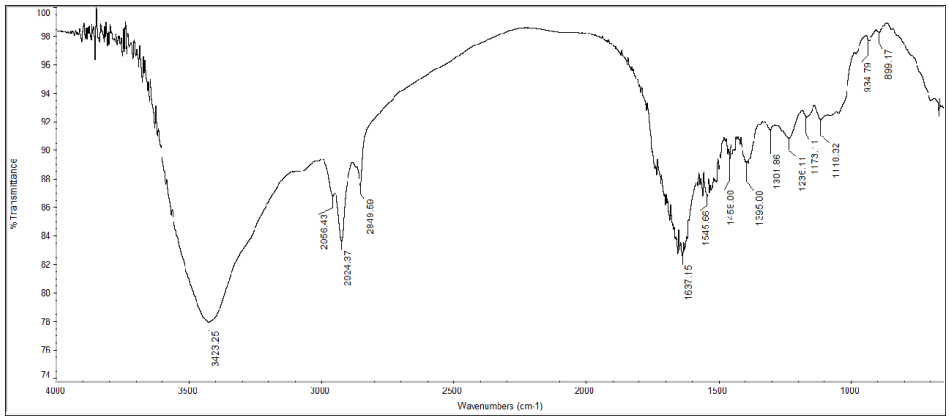

Fig. 3.

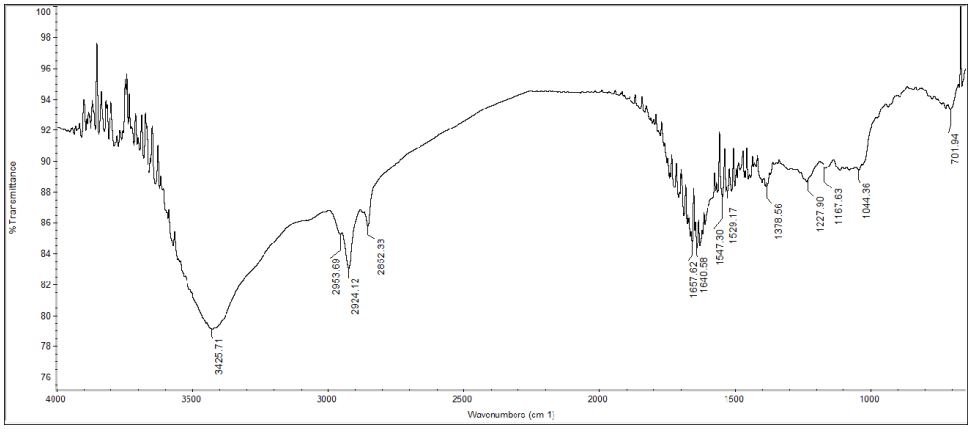

Fig. 4

Raman spectroscopy is a non-destructive technique and can provide quantitative chemical composition and identify tissue constituents. The nutritional value of the different organisms depends on their biochemical constituents like proteins, carbohydrates, lipids, amino acids and minerals (Jagadeesasn *et al*., 2005). The FT-IR spectra of tissues are consistent with the general features and are characterized by a set of absorption region known as the amide region and C–H region. The spectra revealed difference in bandwidth, signal intensity values and signal intensity ratios between the muscle tissues in the three stations. From the spectra, it could also be understood that there is an overall decrease and increase in the intensity of absorption bands due to the effect of toxic pollutants. The broad band spectra of muscle tissues in all the three stations at a peak of ~3421 cm^{-1}, 3423 cm^{-1} and 3425 cm^{-1} were assigned as the O–H stretching with small contribution from the amide bands of protein. The bands observed at ~2956 cm^{-1}, ~2923cm^{-1}, ~2853 cm^{-1} were assigned to the symmetric stretching mode of the methyl end groups of the membrane lipids as well as the methyl side groups of the cellular proteins. Similar peaks were also observed by Karthikeyan (2012) in the muscle tissues intoxicated with nickel and chromium in *Cirrhinus mrigala.* The band observed at 2957 cm^{-1} is assigned to CH_2 asymmetric stretching due to lipids. The frequencies of the CH_2 stretching bands of the acyl chains depend on the degree of conformational order/disorder state of lipids (Toyran *et al.,* 2008).

The band observed at ~1653 and ~1541 cm^{-1} correspond to amide I and amide II vibrations of structural proteins, respectively. These vibrations are influenced by secondary structure of the protein, since this involves protein folding with hydrogen bonding between peptide bonds (Wong, 1995). The bands at ~1458 and ~ 1395 cm^{-1} are due to CH_3 scissoring, mainly lipids and COO^- symmetric stretching of fatty acids respectively. Similar peaks were also observed by Chezhien *et al*. (2012) in the muscle tissues of *Lates calcarifer* intoxicated with nickel and mercury. These changes in absorption of specific vibrational bands suggest changes in the relative concentrations of proteins and lipids in the muscle tissues due to the toxic effects of pollutants. The band at 1458 cm^{-1} and 1395 cm^{-1} is mainly due to the COO^- symmetric stretching vibration in amino acids and fatty acids (Cakmak *et al*., 2003). The asymmetric and symmetric phosphate stretching bands at 1236 and 1080 cm^{-1} respectively, originated mainly due to the phosphodiester backbone of cellular nucleic acids (Wang *et al*., 1997).

Conclusion

The heavy metal stress highly altered the metabolism of the muscle tissues of *Lates calcarifer.* FT-IR spectra reveal significant differences in the absorbance intensities between the muscle tissues in the three stations, thus reflecting an alteration on the major biochemical constituents, such as proteins and lipids of the muscle tissues of *Lates calcarifer.* The band areas and intensities of amide bands in tissues indicate the protein quantity of the system in the muscle. To conclude, the results suggest that FT-IR Spectroscopy could be used as a potential and viable tool for the discrimination between normal and metal intoxicated tissues and to detect the extent of biochemical changes that could have occured as a result of metal intoxication.

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