FTIR study of Nickel and Mercury induced biochemical changes in the muscles tissues of Lates Calcarifer

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ABSTRACT

Nickel and mercury are naturally occurring, highly toxic environmental pollutant. The goal of the present study was investigate the effects of nickel and mercury exposure on the biochemical contents of the muscles tissues of fish Lates calcarifer by using Fourier transform infrared (FT-IR) spectroscopy. FT-IR spectra reveal significant differences in absorbance intensities between the control, nickel and mercury intoxicated tissues, reflecting an alteration on the major biochemical constituents, such as lipids, proteins and nucleic acids of the muscles tissues of Lates calcarifer. Further, it has been observed that mercury has highly altered the biochemical constituents than nickel. Therefore this result concluded that FT-IR spectroscopy can be a successful detection tool in toxicological research.

Keywords: Nickel, Mercury, FT-IR, Lates calcarifer.

1. Introduction

Heavy metal pollution is a major environmental problem in the modern world due to increased human activities. Anthropogenic activities cause an increased discharge of both essential and non-essential metals into natural aquatic ecosystems. The contamination of heavy metal is a serious threat because of their toxicity, long persistence, bioaccumulation and biomagnification. The weathering of rock soil forms, human activities and increased use of metal containing fertilizers in agriculture could lead to continuous rise of the concentration of metal pollutant in water reservoirs, thereby representing the greatest hazard to the human consumer of fish.

Fish are widely consumed in many parts of the world because they are highly nutritious, easily digestible and have high protein content, low saturated fat and also contain omega fatty acids. Nutritional value of fish depends on their biochemical composition which is affected by water pollution because they are constantly exposed to chemicals in polluted and contaminated waters. Fish accumulate metals directly through the water or indirectly through the food chain. Heavy metal bioaccumulation of fish is species-dependent. Feeding habits and life style of species are strongly related to accumulation level. Heavy metals in aquatic environments are transferred through food chain into humans. It is well known that fish muscle is not an active tissue in accumulating heavy metals. But it was informed that heavy metal levels of edible portions (muscle and skin) of some fish in polluted regions exceeded acceptable levels. 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).
From the heavy metals, mercury (Hg) is the most abundant, and is bioaccumulated by aquatic organisms and biomagnified through the food chain (Kojadinovic et al., 2006). The distinction between elemental, inorganic and organic mercury is much more important than oxidation states in determining toxicity as, organic mercury compounds are the most toxic. Nickel introduced into the environment from natural or human sources is circulated through the system by chemical and physical processes and through biological transport mechanisms of living organisms (Sevin, 1980; WHO, 1991). Nickel is essential for the normal growth of many species of microorganisms and plants and several species of vertebrates, including chickens, cows, goats, pigs, rats, and sheep (NAS, 1975; USEPA, 1980; WHO, 1991; USPHS, 1993). These heavy metals accumulated and change the biochemical levels in the fish tissue.

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. The shift in the peak positions, bandwidths and intensities of the bands all give valuable structural and functional information, which may have diagnostic value. With FT-IR spectroscopy, it is possible to monitor changes in the structure and properties of biomolecules such as DNA, RNA, proteins, carbohydrates, lipids in biological tissues and cell simultaneously, Ci et al. (1999). The aim of the present study is to investigate the effects of mercury and nickel exposure on the biochemical contents of the muscle tissues of fish Lates calcarifer by using FT-IR Spectroscopy.

2. Materials and methods

2.1 Test species

Specimens of Lates calcarifer were collected from Rajiv Gandhi Centre for Aquaculture (RGCA), Thirumullaiavasal, Sirkali, Tamil Nadu, India. Specimens were acclimatized to laboratory conditions at Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai for 15 days. Water was changed daily and fish were fed adlibitum with flour pellets and ground dried shrimp twice a day. For experimental studies, fish ranging from 7-10 cm in length and weighing 10-12 g were selected.

2.2. Chemicals

Nickel chloride and mercury chloride were obtained from Merck (Merck Company, Darmstadt, Germany, Glassx India Limited, Bombay, India (No. 17584)) and used without further purification. All chemicals used were of analytical grade.

2.3. Lethality Studies

The acclimated fish were stocked in glass equipped with continuous air supply. The physico-chemical parameters of water were estimated according to the method described by (APHA, 2005). The maintained parameters: Dissolved oxygen 5.4 ± 0.02 mg/l; pH 8.6 ± 0.2; Water Temperature 39.0 ± 2.0 °C; Salinity 38 ± 0.07 ppt; Total hardness 8.2 ± 2.0 mg/l; Calcium 5.0 ± 0.1mg/l; Magnesium3.0 ± 2.0 and Total alkalinity 16.0 ± 06mg/l. The water was changed every 24 hours throughout the experiment.

For Preliminary studies nickel and mercury was carried out to find the median lethal concentration (LC₅₀) for 96 hrs. For this appropriate amount of nickel chloride and mercury chloride were dissolved in fresh seawater every time to prepare a stock solution of 1000 ppm.
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for each toxicant. For sub-lethal studies, 80 L of water were taken in each 100 L three glass tanks. To the first and second tanks, 1/10 of 96 hrs LC₅₀ concentrations of nickel (4.0 ppm), mercury (3.5 ppm) was added into each fish tanks respectively, while the 3rd tank served as control. Then, 10 fishes were introduced into the each tank and the experiment was maintained for a period of 35 days. Fish were fed ad libitum, and water and toxicants were renewed daily. No mortality was observed throughout the experimental period. After this period, the fish were sacrificed, and muscle tissues were removed and stored at −80°C until sample preparation for FT-IR spectroscopic studies Akkas et al. (2007).

2.4. Sample preparation

The muscle tissues were dried in a lyophilizer (VIRTIS 6KBEL85) for overnight to remove the water content in the samples. The samples were then ground in an agate mortar and pestle in order to obtain muscle powder. The muscle powder was mixed with completely dried KBr at a ratio of 1:100, and then the mixture was subjected to a pressure of 5 tones for 5 min in an evacuated die to produce KBr pellet for use in FT-IR spectrometer.

2.5. Spectroscopic measurement

The measurement of FTIR spectroscopy was performed on a Nicolet-Avatar 300 FTIR spectrometer equipped with a DTGS detector, installed at Centralized Instrumentation and Services Laboratory, Annamalai University. The spectra covered the wave number ranging from 4000 to 400 cm⁻¹. Absorption intensity of the peaks was calculated with base-line method using ORIGIN 8.0 software.

3. Results and discussion

The present study deals with the effect of nickel and mercury on the biochemical contents of Lates calcarifer muscle using FT-IR technique. The average FT-IR Spectra obtained form control, nickel and mercury intoxicated fish tissue samples in the 4000-400 cm⁻¹ wave number region and detailed spectra are presented in Fig 1 and 2. Shifts in peak position changes in intensities and band areas of infrared bands were used to obtain valuable structural and functional information about the system of interest Cakmak et al. (2006). The spectrum consists of several bands arising from the functional groups belonging to proteins, lipids, ester, amide and nucleic acids. The vibrational assignments of FTIR spectra for the control, nickel and mercury intoxicated fish muscle samples are presented in table 1.

From the spectra, there is an overall decrease in the intensity of absorption bands of nickel and mercury intoxicated tissues compared to control sample in the 4000-1800 cm⁻¹ region. In Fig 2 detailed spectral analyses were performed in the 1600-500 cm⁻¹ region and the intensity of nickel intoxicated tissue increase than the control sample. The bands observed at ~ 3435 cm⁻¹ corresponds to Amide A: N–H stretching mainly proteins. In addition, the band is shifted to 3402 cm⁻¹. This large shift might imply a variation in the strength of protein and amide hydrogen bonding due to changes in the plasma chemistries. The band observed at 2957 cm⁻¹ is assigned to CH₂ asymmetric stretching due to lipids. The frequencies of the CH₂ stretching bands of the acyl chains depend on the degree of conformational order/disorder state of lipids Toyran et al. (2008). In the present study there is no large shift in the wave numbers of intoxicated tissue samples. The band observed at 2851 cm⁻¹ is correspond to symmetric stretching vibrations of methylene (–CH₃) groups, mainly monitor lipids.
**Table 1:** FT-IR spectra: Vibrational Assignment of Control, nickel and mercury intoxicated muscle tissues of fish *Lates calcarifer*

<table>
<thead>
<tr>
<th>Wave Number, cm⁻¹</th>
<th>Control</th>
<th>Nickel Intoxicated</th>
<th>Mercury Intoxicated</th>
<th>Peak assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3435</td>
<td>3402</td>
<td>3402</td>
<td></td>
<td>Amide A: mainly N-H stretching of proteins</td>
</tr>
<tr>
<td>2958</td>
<td>2957</td>
<td>2959</td>
<td></td>
<td>CH₂ asymmetric stretch mainly lipids( fatty acids)</td>
</tr>
<tr>
<td>2851</td>
<td>2850</td>
<td>2857</td>
<td></td>
<td>CH₂ symmetric stretch: mainly lipids</td>
</tr>
<tr>
<td>1641</td>
<td>1643</td>
<td>1641</td>
<td></td>
<td>Amide I, C=O stretching of proteins</td>
</tr>
<tr>
<td>1549</td>
<td>1537</td>
<td>1535</td>
<td></td>
<td>Amide II, N-H bending and C-N stretching of proteins</td>
</tr>
<tr>
<td>1462</td>
<td>1462</td>
<td>1463</td>
<td></td>
<td>CH₃ scissoring: mainly lipids</td>
</tr>
<tr>
<td>1402</td>
<td>1413</td>
<td>1407</td>
<td></td>
<td>COO⁻ symmetric stretching: mainly fatty acids</td>
</tr>
<tr>
<td>1215</td>
<td>1235</td>
<td>1237</td>
<td></td>
<td>PO-2 asymmetric stretch: mainly nucleic acids with the little contribution from phospholipids</td>
</tr>
<tr>
<td>1173</td>
<td>1171</td>
<td>1172</td>
<td></td>
<td>C–O asymmetric stretching: glycogen</td>
</tr>
<tr>
<td>1048</td>
<td>1047</td>
<td>1051</td>
<td></td>
<td>C–O stretching of carbohydrate</td>
</tr>
</tbody>
</table>

**Figure 1:** The average FTIR spectra of the control, nickel and mercury intoxicated muscle tissues of *Lates calcarifer* in the 4000–400 cm⁻¹ regions.
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Figure 2: The average FTIR spectra of the control, nickel-and mercury intoxicated muscle tissues of Lates calcarifer in the 1700-500 cm\(^{-1}\) regions.

From the spectra, there is an overall decrease in the intensity of absorption bands of nickel and mercury intoxicated tissues compared to control sample in the 4000-1800 cm\(^{-1}\) region. In Fig 2 detailed spectral analyses were performed in the 1600-500 cm\(^{-1}\) region and the intensity of nickel intoxicated tissue increase than the control sample. The bands observed at ~ 3435 cm\(^{-1}\) corresponds to Amide A: N–H stretching mainly proteins. In addition, the band is shifted to 3402 cm\(^{-1}\). This large shift might imply a variation in the strength of protein and amide hydrogen bonding due to changes in the plasma chemistries. 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). In the present study there is no large shift in the wave numbers of intoxicated tissue samples. The band observed at 2851 cm\(^{-1}\) is correspond to symmetric stretching vibrations of methylene (–CH\(_2\)) groups, mainly monitor lipids.

The band observed at ~1641 and ~1549 cm\(^{-1}\) correspond to amide I and amide II vibrations of structural proteins, respectively. The changes in peak position of amide bands may quantitatively reflect alterations in the composition of protein secondary structure (Susi and Byler, 1983). These vibrations are influenced by secondary structure of the protein, since this involves protein folding with hydrogen boning between peptide bonds. Characteristic infrared absorption bonds of peptide linkage at these amide I and amide II regions correspond to the alpha-helix protein structure (Rice-Evans, 1991; Wong, 1995). The conformational changes of proteins may be caused by the binding of metals ions to some amino acids of polypeptide chain, which affect the lipid-protein interactions within the plasma membrane (Akahori et al., 1999).

Rice-Evans et al. (1991) suggest that all the constituent amino acid side chains in proteins are susceptible to free radicals, but some are more vulnerable than others. Thus, exposure of proteins to free radical-generating systems may induce secondary structural changes, since secondary structure is stabilized by hydrogen bonding of peptide backbone, and interference with the functional groups of the peptide bonds may cause secondary structural modifications. They have postulated that the amide I and II region shifts corresponding to the alpha -helix protein conformational change. In the present study, amide II shift to lower value in both
nickel and mercury intoxicated tissue samples. This indicates the altered protein secondary structure due to effect of nickel and mercury intoxication. The bands at ~1462 and ~ 1402 cm\(^{-1}\) are due to CH\(_3\) scissoring, mainly lipids and COO\(–\) symmetric stretching of fatty acids respectively. The band at ~1215 and ~1173 cm\(^{-1}\) are due to PO\(_2\)- asymmetric stretching mainly nucleic acids with little Contribution from phospholipids and C–O asymmetric stretching of glycogen respectively, originate mainly from the phosphodiester backbone of cellular nucleic acids Wang et al. (1997). The increase in the intensity of the ~1237 cm\(^{-1}\) band implies an increase in the relative content of the nucleic acids in the mercury intoxicated tissues Ci et al. (1999). In these regions nickel intoxication only acutely affect the muscle tissue. The band at 1051 cm\(^{-1}\) in the mercury intoxicated tissue is due to C–O stretching of carbohydrate. In addition the intensity decrease in the band from control and this reflects the changes in the quantity of glycoprotein content due to mercury intoxication. Our obtained results were correlated with previous studies. Filipe et al. (1995) have found that zinc has an inhibiting effect on the spontaneous lipid peroxidation in rat brain. Their results support the effect of zinc in antioxidant properties. Liver and gill tissues showed higher metal concentrations than muscles tissue. Yilmaz et al. (2007) reported that in Leuciscus cephalus and Leporinus gibbosus, cadmium, cobalt and copper accumulations in the liver and gills were maximum, while these accumulations were least in the fish muscle. The higher levels of trace elements such as lead and chromium in liver relative to other tissues may be attributed to the affinity or strong coordination of metallothionein protein with these elements (Ikem et al., 2003). Similar results were reported by George, (1989) on dab, and also by Stegeman and Hahn, (1994); the EROD activity was strongly decreased after Cd\(^{2+}\) treatment; however, this does not reflect inhibition of the enzyme activity, but a decreased de novo synthesis of protein. George and Young, (1986), Fair, (1986), and Forlin et al. (1986) observed similar results: the administration of CdCl\(_2\) i.p. at doses lower than 2 mg kg\(^{-1}\) decreased the liver microsomal EROD activity in plaice, bass, and trout, respectively. Samuel et al. (2005) have also reported decreased sulfhydryl proteins in the rat brain regions due to arsenic treatment. Such like heavy metals pollutant are changes the nature of the biochemical level and properties of the body tissues that were clearly indicated by the FTIR spectroscopy technique.

4. Conclusion

Nickel and mercury intoxication induced changes in Lates calcarifer were not similar in both frequency alterations and intensity. Intoxication stress highly altered the metabolism of the muscle of Lates calcarifer. The molecular changes may retard the growth of species. FT-IR spectra reveals significant differences in absorbance intensities between the control, nickel and mercury intoxicated muscle tissue, thus reflecting an alteration on the major biochemical constituents, such as lipids, proteins and nucleic acids of the muscle tissue of Lates calcarifer. Further, it is observed that sublethal nickel and mercury exposure causes some alteration in protein profile with a decrease in \(\alpha\)-helix and an increase in random coil structure. Finally the present study show that the FT-IR spectroscopy is a very informative technique to differentiate mercury and nickel intoxicated tissue from normal ones at the molecular level.

5. Acknowledgements

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6. References


