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European Journal of Experimental Biology, 2011, 1 (3):200-208

ISSN: 2248 –9215

FTIR Study of Copper Induced Bimolecular Structural Changes on the Gills and Hepatopancreas of Shrimp, *PenaeusMonodon* (Fabricius, 1798)

Natarajan,E[1]., P. Sivaperumal* and K. Ponnusamy[1]

[1]*CAS in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu (India)*
**Central Institute of Fisheries Education(CIFE), ICAR- Deemed University, Off Yari Road, Versova, Mumbai*

ABSTRACT

Copper is an essential element to the production of hemocyanin and is also involved in diverse life processes, including antioxidant defense, neurotransmitter production, cellular respiration, pigment formation and peptide biosynthesis. It is well documented that copper (Cu) has toxic effects at high concentration. This study was undertaken to present the effect of copper sulfate on gills and hepatpancreas normal control shrimp at molecular level by using Fourier transform infrared (FTIR) spectroscopy. The results mainly reveal that, Cu incubation causes an increase in lipid content and an alteration in protein profile with a decrease in alpha-helix and an increase in beta-sheet structure and random coil in both gills and hepatopancreas which might be reflecting a slight sub toxic effect of copper on normal shrimp at the dose used in the study.

Keywords: Gills; Hepatopancreas; Copper; Lipid content; Protein structure; FTIR spectroscopy.

INTRODUCTION

An essential trace element, copper (Cu), and has fundamental importance to crustaceans. It is essential to the production of hemocyanin and is also involved in diverse life processes, including antioxidant defense, neurotransmitter production, cellular respiration, pigment formation and peptide biosynthesis[1]. However, it has toxic effects at high concentrations. Excess copper exposure has significant toxic implications for proteins that require copper as an integral part of their structure[2]. Such toxicity is reflected at the molecular, cellular and tissue levels in each individual. However, information concerning copper-induced alteration of the biomolecular

constituents of primary organs, gills and hepatopancreas in *Penaeusmonodon*(Fabricius, 1798) has not been widely studied at molecular level.

The gills represent a vital organ in aquatic organisms and play an indispensable role in gaseous exchanges and respiration. Copper may cause damages to gill tissues, thereby reducing oxygen consumption and disrupting the respiratory function of aquatic organisms. Similarly copper may also cause damage to the hepatopancreas. Cells of the hepatopancreas typically consist of digestive and absorbent cells that are believed to mediate digestion, absorption, storage, and excretion and detoxification[3].It was also pointed out that, those copper-induced variations might lead to more important changes in the metabolism by altering the proteins, carbohydrate and lipids[4,5]. Taking into consideration these sparse literatures in this field, it is clearly seen that the effects of copper on major organs such as gills and hepatpancreas have yet to be characterized in detail in normal control *P.monodon*. In the present study, we aimed to study the effect of copper treatment on the gills and hepatopancreas of the *P.monodon* by Fourier transform infrared (FTIR) spectroscopy at molecular level. The biochemical changes in the sub-cellular levels manifest themselves in different optical signatures, which can be detected by IR spectroscopy. Acquisition of IR spectra with high spatial and spectral resolution allows the visualization of the distribution of intrinsic biochemical components such as lipids, proteins, and carbohydrates as well as parameters that reflect a variety of molecular and structural characteristics. This novel method monitors and visualizes the underlying chemistry of the tissue, based on hundreds of vibrational absorption bands that are intrinsic to the sample[6, 7]**.** This technique has been widely used for a rapid and sensitive determination of macromolecular concentration and conformational changes in biological tissues and membranes[7, 8]. Consequently, the spectroscopic study of biological cells and tissues is an active area of research with a primary goal being to elucidate the molecular mechanism of diseases affecting those cells and tissues[7].

MATERIALS AND METHODS

Brackish water shrimp, *Penaeusmonodon*, approximately 4-months old weighing 23-25 g were purchased from a shrimp farm for the experiment. Shrimp were fed daily ad libitum with chow in holding tanks for approximately 1 week at 25 ° C prior to the experiment. Ten shrimp were randomly selected and killed. Their gills and hepatopancreas were isolated, cleaned several times with pH 8.0 Tris buffer solution (50mM), wiped and then homogenized in the cooled condition. The pooled homogenates were then centrifuged at 1500 X g for 5 min. The supernatant was discarded, and 2 ml of Tris buffer solution was added. The above process was repeated five times, and then the crude homogenates mass was obtained for further use. The total protein concentration in the crude homogenates of shrimp gill and hepatopancreas were determined by the Bio-Rad protein assay kits in the crystalline bovine serum albumin as a standard. The protein contained in this gill and hepatopancreas homogenates were >6 mg/ml. Copper solution (0.5%) was prepared by dissolving Cu powder (company) in a 50 mMTris buffer solution, pH 8.0. Equal volume of homogenate and copper stock solution were mixed (1:1), and then incubated in a 37° C water bath for 3 h.

FT-IR spectroscopic study

FTIR spectra were recorded with a Perkin Elmer–Spectrum RxI Spectrometer equipped with a mullard I–alanine doped triglycine sulfate (DTGS) detector installed at Centralized Instrumentation and Services Laboratory (CISL), Annamalai University. The spectrometer was

continuously purged with dry nitrogen to eliminate atmospheric water vapour and carbon dioxide (CO2). Pellets were scanned at room temperature (25 ± 1 ºC) in the 4000–400 cm–1 spectral range. To improve the signal to noise ratio for each spectrum, 100 interferograms with a spectral resolution of ±4 cm^{-1} were averaged. Background spectra, which were collected under identical conditions, were subtracted from the sample spectra automatically. The frequencies for all sharp bands were accurate to 0.001cm^{-1}. Each sample was scanned under the same conditions with three different pellets.

Peak Fit version (4.1.2) for windows (Cranes Software International Ltd., Bangalore) was used for data acquisition and handling. Second derivative spectra were vector normalized at 2800–3050 cm^{-1}(for the comparisons made in 2800–3030 cm^{-1} region), at 1600–1700 cm^{-1} and at 950–1480 cm^{-1} (for the comparisons made in 1000–1270 cm^{-1} regions). Fourier self-deconvolution and second derivative resolution enhancement were used to narrow the width of infrared bands and increase the separation of the overlapping components with software Peak Fit Version 4.12. The resolution enhancement resulting from self-deconvolution and the second derivative was such that the number and the position of the bands to be fitted were readily determined[9, 10].Curve fitting was accomplished during a curve-fitting process with Peak Fit software (version 4.12) for the amide I band region. Protein secondary structure content was calculated from the area under the individually assigned bands and expressed as a percentage of total area in the amide I region. The band maximum from 1650 to 1658 cm^{-1} was assigned to alpha-helical structure, 1640 to 1610 cm^{-1} to β-sheet, 1700 to 1660 cm^{-1} to β-turn, and 1650 to 1640 cm^{-1} to random coil structure[11].The next step was the calculation of the averages of the individuals belonging to the same groups. The results were presented as ‘‘mean ± standard deviation’’, and the differences between with or without Cu-incubated (0.5%) homogenates of gills and hepatopancreas.

RESULTS AND DISCUSSION

2800–3030 cm^{-1} Region

Average second derivative spectra belonging to the gill and hepatopancreas of the Cu-incubated and without Cu-incubated group are demonstrated in fig.1A-D, respectively. The changes in the percentage area of main functional groups are given in Table 1. As it is seen in Fig. 1 and Table 1; the changes observed between the Cu-incubated and without Cu-incubated groups in this region are quite different for all the investigated regions of the shrimp gill and hepatopancreas. The intensities of the CH_2 asymmetric and the symmetric stretching modes significantly increase in the Cu-treated groups. As it is demonstrated in Fig. 1A-D and Table 1, the intensities of the CH3 symmetric and the asymmetric stretching bands decrease significantly only in the gills of the Cu-treated groups. While the intensity of the CH_3 symmetric stretching band significantly increases in the Cu-treated group of hepatopancreas.

1600-1700 cm^{-1}Region: The average spectra belonging to the gill and hepatopancreas of the Cu-incubated and without Cu-incubated groups in the 1600-1700 cm^{-1}region are shown in Fig. 2A–D, respectively. The changes in the percentage area of the main functional groups are given in Table 3. Since the peak maximum of the Amide I band occurs at different frequencies for various types of hydrogen- bonded secondary structures, the discrete types of secondary structure in proteins can be identified by the frequencies of their maxima in the FTIR spectra. As it is seen in Fig. 2 and Table 2, Cu-treatment causes a significant decrease in the intensities of alpha-helix

and Beta-turn of Amide I band of the gill and the hepatopancreas. The intensity of beta-sheet of Amide I band significantly increases in all the investigated regions of the Cu-treated group. The figure and Table reveal that the intensities of the random coil increased significantly only in the gill homogenate of the Cu-incubated group with respect to the without Cu-incubation. However, the intensity of random coil did change significantly in the hepatopancreas.

1000–1270cm^{-1} Region: The average spectra belonging to the gill and hepatopancreas of the Cu-incubated and without Cu-incubated groups in the 1000–1270 cm^{-1}region are demonstrated in Fig. 3A–D, respectively. The main changes in the percentage area of the investigated functional groups are given in Table 4. The band located at around 1230 cm^{-1} is a PO_2^- asymmetric stretching band which arises from the phospholipids and nucleic acids. As it is shown in Fig. 3 and Table 3, the intensity of this band decreases significantly in the gill and hepatopancreas homogenates of the Cu-incubated group. The band at 1151 cm^{-1} is a C–O stretching band arising due to the presence of glycogen and nucleic acid in the system and the band giving absorption at 1171 cm_1 is due to CO–O–C asymmetric stretching arising from ester bonds in cholesterol esters and phospholipids. The other band in the spectrum of gill and hepatopancreas of Cu-treated group in which we see decrease in the intensity value gives an absorption peak at 1083 cm^{-1}. This band can be assigned as the PO_2^- symmetric stretching band arising from phospholipids and nucleic acids or due to the C–O stretching band which mainly arises from glycogen, oligosaccharides and glycolipids present in the system.

In the present study, we have analyzed the spectral changes in 1000–3030 cm^{-1} region in detail by FTIR spectroscopy. The dominant lipid bands in the 2800–3030 cm^{-1} region originate from the C–H stretching vibrations of the fatty acyl chains of membrane lipids[7]. The increase in lipid content might be due to the disturbance of lipid metabolism in Cu-treated groups. Accumulation of fatty acids and their metabolites, such as long chain acyl-CoA and long chain acyl-carnitine, has been associated with cell damage. Alterations in the composition of membrane phospholipids are also considered to change the activities of various membrane bound enzymes and subsequently gills and hepatopancreas function under different pathophysiological conditions[12]. However, we observed a significant decrease in the content of the CH3 symmetric and asymmetric stretching group in gills homogenate and the CH3 symmetric stretching group in heptopancreas of the Cu-treated shrimp. These changes might be implying an alteration in lipid and protein profiles leading to modifications in membrane composition[9].

Cu-incubation causes a decrease in the content of Amide I alpha-helical structure of the gills and hepatopancreas (Fig. 2A-D and Table 2). Our results also reveal that Cu-treatment causes an increase in the concentration of beta-sheet structure in both gill and hepatopancreas (Fig. 2A-D and Table 2). Thus, we can deduce that Cu-treatment might be causing some important structural alteration in the protein secondary structure of the shrimp gill and hepatopancreas. The epithelial membrane covering the outside of gill and hepatopancreas tissue may first be exposed to Cu in solution, resulting in changes in structural conformation of bimolecular components of gill hepatopancreas tissues and progressively lose its function. The shrimp gills and hepatopancreas homogenates shows the maximum peaks of Amide I bands at 1650-1658 cm^{-1}, suggesting that the predominant proportion of protein is in the alpha-helical conformation. Once the Cu was incubated in the gill and hepatopancreas, the Amide I peak shifted gradually from 1650-58 cm^{-1} to 1640 and finally to 1640 cm^{-1}. This shift is assignment with a random coil and beta-sheet

structure. As it is shown in fig 2A-D and Table 2 with Cu-incubation, corresponding to alterations of the protein conformation from alpha-helix to random coil and beta-sheet conformation. The changes in peak position and height of second derivative Amide I band may quantitatively reflect alterations in the composition of protein secondary structures [13]. The random coil and beta-sheet structures were more pronounced in protein secondary conformations of the gill and hepatopancreas homogenates after treatment with Cu. This finding was consistent with the mechanism of beta-sheet formation by converting alpha-helical structure into beta-sheet conformation [14, 15,16]. In the present study, the decrease in alpha-helix structure of the shrimp gill and hepatopancreas homogenates might be responsible for the increase in beta sheet and random coil structures; which were consistent with the mechanism of beta-sheet formation[15] . The beta-type structure in the gill homogenate induced by Cu suggests that the increase of the intermolecular hydrogen-bond interactions forms aggregates of higher molecular weight, and then to modifies the secondary structures of protein in the gill homogenate[16].

The decrease in the intensity of the PO_2^- asymmetric stretching band in the gill and hepatopancreas of the shrimp due to Cu-treatment implies decrease in the phospholipid content (Fig. 3 and Table 3). The decrease in the intensity of the band at 1151 cm^{-1}in the Cu-treated group of gill and hepatopancreas might be resulting from decrease in the content of glycogen, which might be due to the disturbance of carbohydrate and lipid metabolism. Furthermore, our results also show that, in the Cu-incubated groups of the gill and hepatopancreas, the intensity of the band at 1151 cm^{-1} decreases and the intensity of the band at 1171 cm^{-1}increased with respect to the control. In both gill and hepatopancreas of the Cu-treated groups, there is decrease in the intensity of the band located at around 1083 cm^{-1}. This decrease might be due to decrease in the lipid content and/or glycolipid content and/or glycogen content.

By considering the results of both gill and the hepatopancreas of the shrimp, an apparent increase in the content of lipids was observed. The findings of our study are important in this aspect since they might be revealing that lipid metabolism is altered due to Cu-treatment. Our results are in agreement with a previous study which has shown that the fatty acid content of hepatopancreas, gills and muscles increases due to Cu-treatment in Crab. However, when exposed to copper, the ultrastructure and mcrostructure[17] of the gills and hepatopancreas would be damaged in *Macrobrachumrozenbergii* , consequently the indispensable cytological energy consumption was diminished, further decreasing the respiratory ability of gills and more significantly decreasing the cell respiratory-related physical activities. Similarly copper may also cause damage to the hepatopancreas. Cells of the hepatopancreas typically consist of digestive and absorbent cells that are believed to mediate digestion, absorption, storage and excretion and detoxification [3]**.** Moreover, copper may interact with nuclear proteins, altering the complex structure of chromatin or catalytic activities of the enzymes involved in DNA and RNA metabolism. Copper reduces the rate of protein synthesis by reducing the rate of RNA synthesis[18]. The findings of the current study related to the changes in the lipid and protein content due to Cu are also important since it is known that ion channels are affected by lipid bilayer composition [10, 11]**.** Therefore, the structural damage to the gills and hepatopancres homogenate due to Cu-treatment observed in the present study would impair the physiological functioning of the gill and hepatopancreas.

Table 1: Changes in the percentage area of the main functional groups in the region 2800-3030 cm^{-1} region with or without incubation with Cu solution (0.5%)

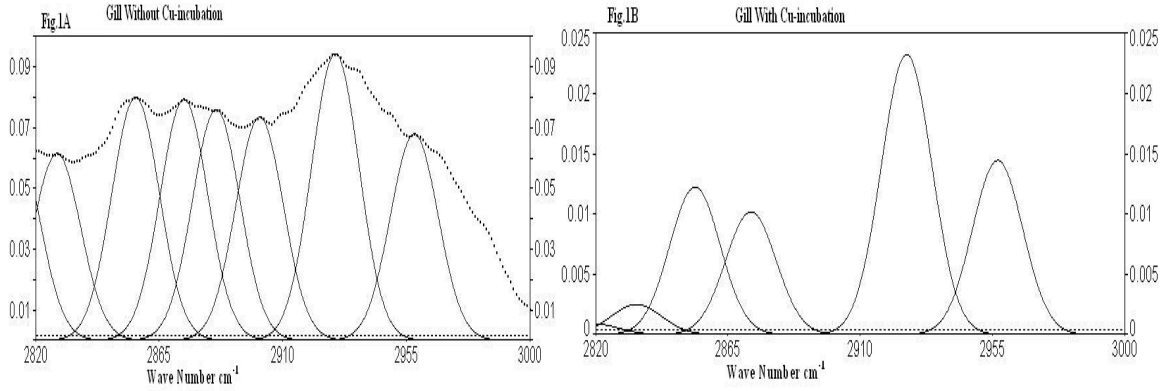
Functional groups	Gill homogenate		Hepatopancrease	
	Without Cu	With Cu	Without Cu	With Cu
CH_3asym.str.(at 2953 cm^{-1})	8.20± 0.50	6.09± 0.45	7.80± 0.68	6.19± 0.58
CH_2asym.str.(at 2925 cm^{-1})	5.08±0.48	6.53±0.56	8.33±2.89	5.09±1.97
CH_3sym.str.(at 2873 cm^{-1})	14.28±0.15	13.58±0.36	12.48±1.00	14.47±0.64
CH_2sym.str.(at 2854 cm^{-1})	7.38±0.94	11.12±2.71	9.71±3.28	8.60±3.30

Table 2: Changes in percentage area of the different secondary structures in the Amide I: 1600-1700 cm^{-1} region with or without incubation with Cu-solution (0.5%)

Functional groups	Gill homogenate		Hepatopancrease	
	Without Cu	With Cu	Without Cu	With Cu
α-helical.(1650-1658 cm^{-1})	23.50± 0.44	21.54± 0.89	23.20± 0.28	18.20± 0.34
β-sheet (1640-1610 cm^{-1})	20.39±0.79	26.45±0.77	22.06±0.29	35.68±0.69
Random coil (1640-1650 cm^{-1})	18.48±0.28	23.38±0.88	19.07±0.47	18.39±0.20
β-turn (1660-1700 cm^{-1})	37.56±0.29	28.63± 0.34	35.62±0.29	27.68±0.29

Table 3: Changes in the percentage area of the main functional groups in the region 1000-1270 cm^{-1} region with or without incubation with Cu-solution (0.5%)

Functional groups	Gill homogenate		Hepatopancrease	
	Without Cu	With Cu	Without Cu	With Cu
Po_2^- asym.str.(at 1230 cm^{-1})	6.68± 2.81	4.44± 1.79	3.94± 0.70	1.60± 0.57
C-O-O-C asym.str.(at 1171cm^{-1})	5.45±2.38	4.34±0.40	5.75±0.74	5.04±0.19
C-O str.(at 1151 cm^{-1})	7.40±1.15	5.25±0.37	4.45±1.55	2.41±0.64
PO_2^- sym.str.(at 1083 cm^{-1})	5.60±0.52	3.14±0.75	2.19±0.15	1.27±0.16



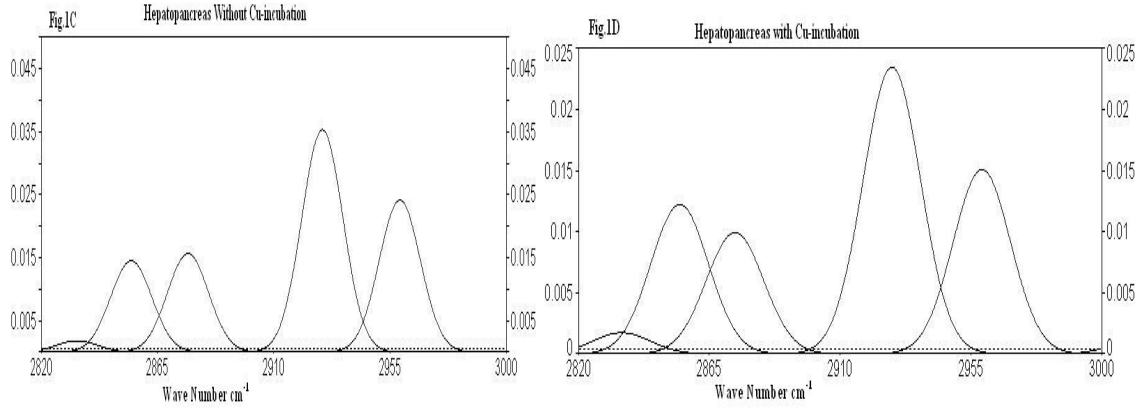


Fig.1A-D.The average spectra belonging to the gill and hepatopancreas of the Cu-incubated and without Cu-incubated groups in the 2800-3030 cm^{-1}region.Second derivative average spectrum belonging to the gill without Cu-incubation (A), with Cu-incubation (B), and hepatopancreas without Cu-incubation (C), and with Cu-incubation (D).

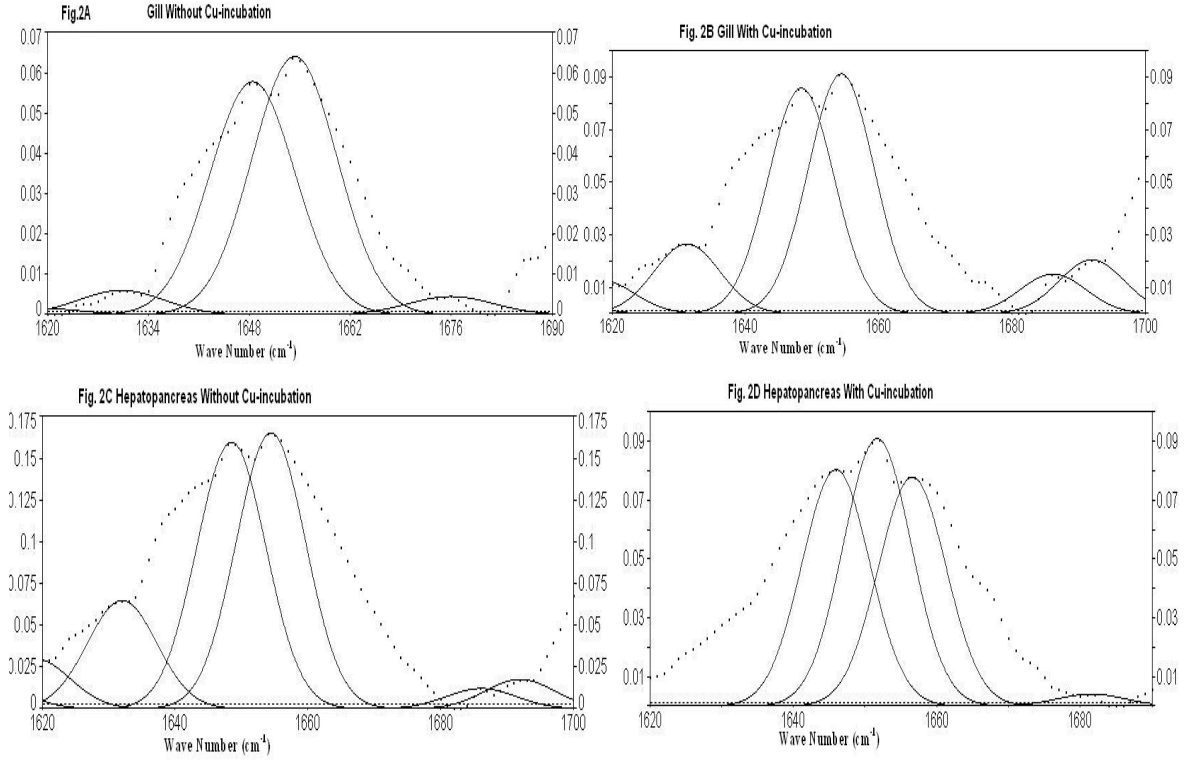


Fig.2A-D.The average spectra belonging to the gill and hepatopancreas of the Cu-incubated and without Cu-incubated groups in the 1600-1700 cm^{-1}region.Second derivative average spectrum belonging to the gill without Cu-incubation (A), with Cu-incubation (B), and hepatopancreas without Cu-incubation (C), and with Cu-incubation (D).

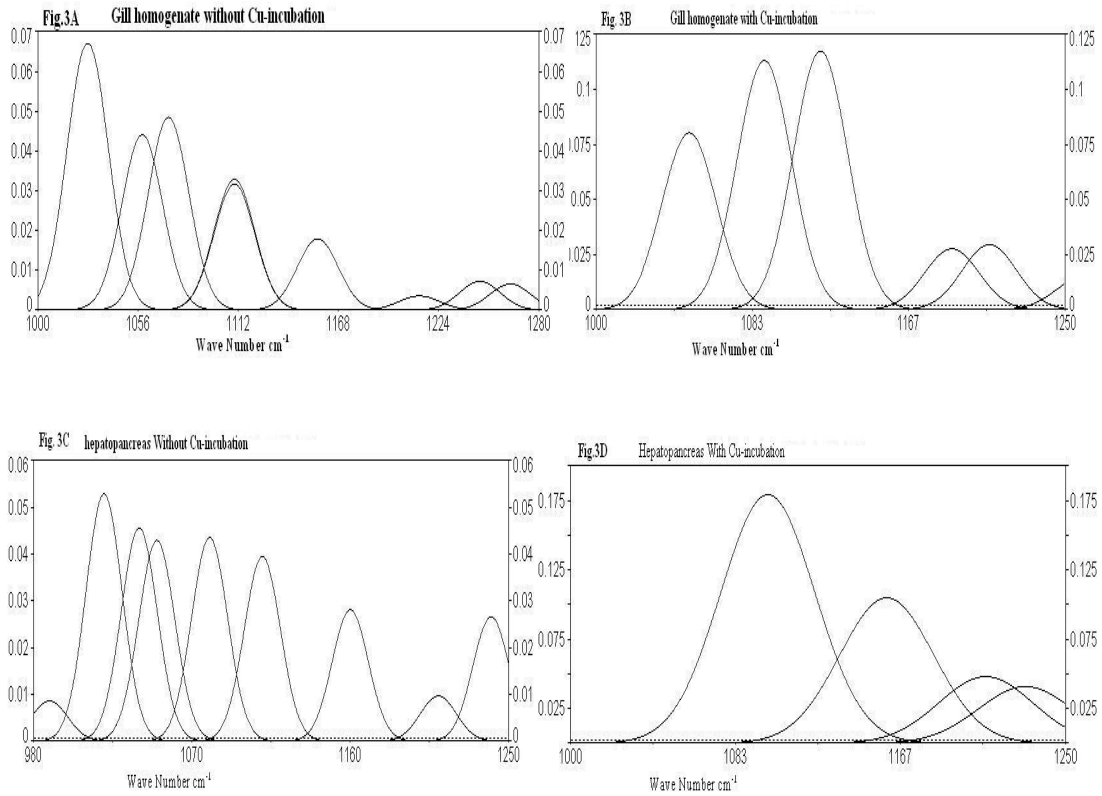


Fig.3A-D.The average spectra belonging to the gill and hepatopancreas of the Cu-incubated and without Cu-incubated groups in the 1000-1250 cm^{-1}region.Second derivative average spectrum belonging to the gill without Cu-incubation (A), with Cu-incubation (B), and hepatopancreas without Cu-incubation (C), and with Cu-incubation (D).

CONCLUSION

In the current work, we showed that Cu-incubated at the dose (0.5%) used in this study increases lipid content and causes some alterations in protein profile with a decrease in alpha-helix and increase in beta-sheet structures in the shrimp gill and hepatopancreas. The present study elucidated the relationship between biomolecules would provide more practical applications for chemical contaminants analysis.

Acknowledgements
The author is grateful to the Dean, CAS in Marine Biology, Faculty of Marine Science,Parangipettai (AnnamalaiUniversity,Tamil Nadu) for their kind permission, support, and motivation for the work.

REFERENCES

[1] Danks, D.M., **1988**. *Annual Review Nutrition* 8, 235–57.
[2] Heath, A.G., **1995**.CRC Press, Boca Raton.
[3] Vogt, G., **1990**. *Aquatic Organism* 9, 45–61.

[4] Nagabhushanam, R., Deshpande J. and Sarojini, R.**1972**. *Proc. Nat. Symb. Ecotoxi*., 73-84.
[5] Patil, P.N., **1986**. Ph.D.Thesis, Marathwada University, Aurangabad.
[6] Lester,D.S., Kidder L.H., Levin I.W., Lewis E.N., **1998.** *Cell. Mol. Biol.* (Noisy-le-grand) 44, 29–38.
[7] Toyran,N., Lasch P., Naumann D., Turan B., Severcan F.,**2006**. J. 397, 427–436.
[8] Dogan,A., Siyakus G., Severcan F., **2007**. *Food Chem*. 100, 1106–1114.
[9] Gaigneaux,A., Ruysschaert J.M., Goormaghtigh E.,**2002**. *Eur. J. Biochem*.269, 1968–1973.
[10] Goforth,R.L., Chi A.K., Greathouse D.V., Providence L.L., Koeppe R.E., Andersen O.S. ,**2003**. *J. Gen. Physiol*. 121, 477–493.
[11] Lee, A.G., **2003**. *Biochim. Biophys. Acta* 1612, 1–40.
[12] Dhalla, N.S., Elimban V., Rupp H., **1992**. *Mol. Cell. Biochem*. 116, 3–9.
[13] Susi, H., Byler, D.M., **1983**. *Biochem.Biophys.Res.Commun*. 115, 391-397.
[14] Clark, A.H., Saunderson, D.H.P., Suggett, A., **1981**. *Int. J. Peptide Protein Res*. 17, 353-364.
[15] Creighton, T.E., **1984**. *Adv.Biophys,* 18, 1-20.
[16] Kim, Y., Rose, C.A., Liu, Y., Datta, G., Tu, A.T., **1994**. *J.Phar. Sci*. 83, 1175-1180.
[17] Li, N., Zhao, Y.L., Yang, J., **2007**. *Archives of Environmental Contamination and Toxicology*, 3-45.
[18] Viarengo, A., **1985**. *Bulletin of Pollution* 16, 153–8.