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Research Article

MORPHOLOGICAL ALTERATIONS AND BIOCHEMICAL CONTENTS OF THE TESTIS OF ADULT MALE FRESHWATER PRAWN *MACROBRACHIUM MALCOLMSONII* (H MILNE EDWARDS) EXPOSSED TO PARAVANAR RIVER POLLUTANTS

***M. Manosathiyadevan, Selvisabhanayakam and Divya velayudhan**
Department of Zoology, Annamalai University, Annamalainagar - 608 002, Tamilnadu, India
**Author for Correspondence*

ABSTRACT
Freshwater bodies are being polluted by a multitude of pollutants from various sources like, industrial effluents, municipal wastes, garbage's, sewage pollutants and agricultural wastes etc. these pollutants contain heavy metals, persistent chemicals and pesticides are carried through food chain, their concentration increasing at each link present a threat to man when we consume the hydrobioants from polluted water. Due to such mixing of pollutants coastal and inland water resources, global fisheries is facing constant decline in prawn stocks. In the Scanning Electron Microscopic Studies showed some remarkable changes in the morphological architecture of the testis and FT-IR spectra revealed significant differences in absorbance intensities between control and polluted testis, reflecting a change in protein and lipid contents in the testis this may probably be attributed, due to the river receiving of effluent sources like untreated industrial, domestic and municipal wastes from the old and new town of Cuddalore, Tamilnadu. The results are discussed in details.

***Key Words**: Pollutants, Testis, Biochemical, FT-IR and Macrobrachium Malcolmsonii*

INDRODUCTION
The river Paravanar contains effluents from different sources affect the aquatic system by depleting and enhancing different physico-chemical parameters thereby affecting the inhabitants. *Macrobrachium malcolmsonii* (Milne Edwards) is the most common freshwater prawn in India. This species is currently much demand in freshwater aquaculture practices. These prawns are widely distributed in tropical, subtropical and temperature zones and are gaining more and more importance as cultivable species.
Industrial effluents contain different toxic substances, affect the aquatic systems, deplete the oxygen content and cause mortality in animals by interfering with their respiratory metabolism (Davis, 1973; Haniffa and Porchelvi, 1985; Jeyachandren and Chockalingam, 1987). Industrial agricultural and domestic wastes pollute the water bodies with heavy metals which reach human tissues through food chain (Kureishy *et al.*, 1979; Saad *et al.*, 1981; Paul and Pillai, 1983 and Ajmal *et al.*, 1985). James *et al.*, (1992) have reported that the indiscriminate discharge of raw and partially treated industrial effluents into aquatic system leads to deterioration of the environment.
Various organic and inorganic wastes in industrial and domestic effluents are responsible for pollution. Non-degradable heavy metals are regarded as hazardous to aquatic ecosystems of their environmental persistence and their tendency for bioaccumulation (Das *et al.*, 2001). As heavy metals are immutable, their biomagnifications has been reported in aquatic ecosystems.
The impact of pollutants on aquatic organisms is highly dependent on the biochemical nature of the organisms. The interaction between the pollutants and the organisms can be understood properly is the various biochemical changes takes place inside the body of the organisms are known. Freshwater prawns are sensitive to contamination of water and pollutants may significantly damage certain physiological and biochemical process when they enter into the organs of these animals. Alteration in the biochemical values in prawns due to environmental pollution provides an indication to understand the mode of action and type of pollutants.

Freshwater prawns constitute one of the major sources of cheap nutrition for the human beings. The nutritional values of prawns depend on their biochemical compositions like proteins, carbohydrates, free amino acids, lipids and mineral contents. It is known that the tissue proteins, carbohydrates and lipids play a major role as energy precursors for prawn exposed to stress conditions. The majority of toxic substances initiate biochemical alterations like, inhibitions of enzyme system, alteration in the level of enzyme and specifically alteration in the permeability of biological membranes.

Crespo, (1982) has investigated the morphological changes of dog fish tissues following the treatment of zinc sulphate by Scanning Electron Microscopic (SEM) and reported that characteristic changes have been noticed in the tissues. Pereira, (1988) has observed the morphological changes of heavy metal exposure on Window Pane flounder gills by SEM. Munshi, (1991) have reported that the effect at low on the tissues of *Channa punctatus* using SEM exposed to heavy metal. Kendall and Dale (1979) have studied the tissues of the rainbow trout, *Salmo gairdneri* by SEM and observed changes in the tissues due to heavy metal treatment.

The biochemical changes occurring in the body gives first indication of stress. During the stress an organism needs sufficient energy which is supplied from reserve materials like, protein, lipid and glycogen. If the stress is mild, then only stored glycogen is used as a source of energy, but when the stress is strong, then the energy stored in lipid and protein may be used. The survival and productivity of fresh water prawn in pollutants however depend on their adaptability, which in turn relies on their protein synthetic efficiency.

Protein plays a vital role in the physiology of living organisms. All biological activities are regulated by enzymes and hormones, which are also proteins. Proteins are highly sensitive to heavy metals and are one of the earliest indicators of heavy metals poisoning. The impairment in protein synthesis due to heavy metal stress was reported by many investigators (Jacobs *et al.,* 1977 and Syverson, 1988). Protein synthesis is considered as premier biochemical parameter since it is the most sensitive and earlier indicator for stress. It can be influenced by a large number of exogenous substances, mainly through a reduction of protein synthesizing capacity of the endoplasmic reticulum in the cells.

Lipids are an important constituent of animal tissue and play a prime role in energy metabolism. Lipids are also important in cellular and Sub-cellular membranes.

FT-IR spectroscopy is a highly sensitive analytical technique that can potentially reveal a wealth of quantitative and qualitative information about a given biological sample (Salman *et al.,* 2004). In addition, it makes possible to simultaneously monitor changes in the structure and properties of biomolecules such as proteins, lipids and carbohydrates in biological tissues and cells (Takahashi *et al.,* 1991; Ci *et al.,* 1999 and Wang *et al.,* 2003).

The present study was undertaken to investigate the effect of pollutants on the morphological alterations of the testis by Scanning Electron Microscopic studies and biochemical contents of testis of the freshwater prawn, *Macrobrachium malcolmsonii* by Fourier Transform Infra-red Spectroscopy.

MATERIALS AND METHODS

Description of Study Area

The Paravanar River originating from Virudhachalam Taluk in Cuddalore District, Tamilnadu, India. It is tributary of Gadilum River which originates from the foot of the hills of North eastern part of the Shervarayan hills and runs along for a distance of 250 kms, joins with adjoining Paravanar estuarine otherwise called Uppanar estuary and finally discharging into the Bay of Bengal.

In this river, Station-I is the unpolluted region at the village Alappakkam. The Station-II is the more polluted region at the village Sonaganchavadi and it is received the untreated drainage of municipal and domestic sewage from the cuddalore old and new towns and the wastes from the coconut husk retting grounds are discharged regularly. Agricultural wastes also enter into this area through small drainage channels from the nearby agricultural lands. In addition, effluents from SIPCOT (State Industrial Promotion Corporation of Tamilnadu) industrial complex are discharged into this river which is major

pollutant agents of this river. The distance between Stations-I to Station-II is 4 kms and depth of the river is more than 5 m (Figure 1).

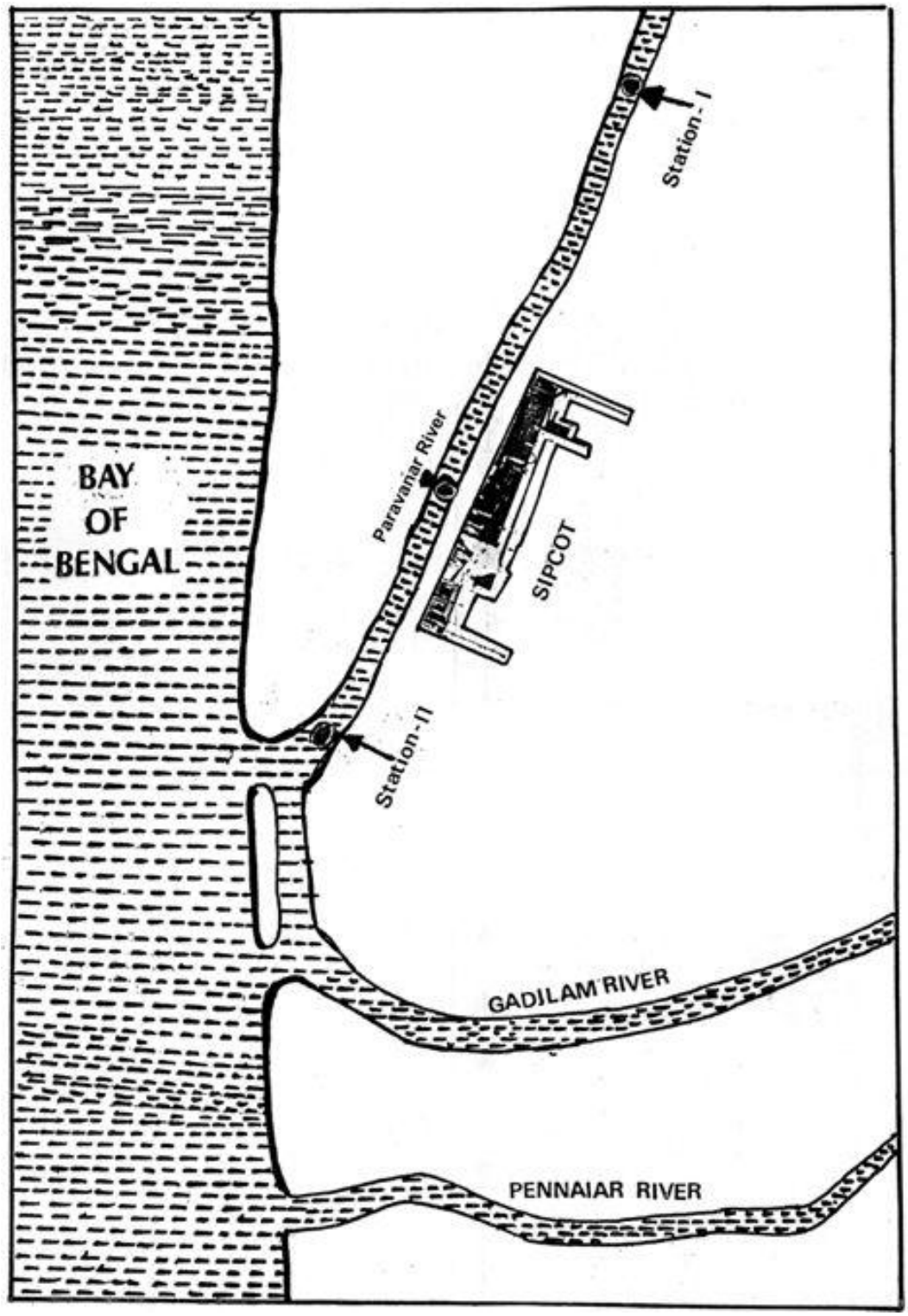

Figure 1: Map showing the study area of the Paravanar River

Physico-chemical parameters of the water collected from Station-I and Station-II of the Paravanar river were studied. Among the various parameters, temperature was high during summer in both stations, at Station-II, the temperature was higher than at Station-I this may be due to the industrial effluents. Statistical analysis revealed that there are significant differences between the two stations. When compare two stations, the pH, dissolved oxygen, salinity, alkalinity, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), total dissolved solids, calcium, nitrite, phosphorus and ammonia content were found to be higher at Station-II. Statistical analysis revealed that there are more significant differences between the three stations.

The accumulation of heavy metals, mercury, cadmium, copper and lead in the sediment were increased at Station-II than at Station-I. The heavy metals (mercury, cadmium, copper and lead) concentration were increased and it is induced several degenerative changes in spermatogenic and interstitial cells, thus inhibiting the function of testis.

The adult male freshwater prawn *Macrobrachium malcolmsonii* were collected from two stations. And the prawn collected from Station-I as a control prawn were transferred to the water filled plastic pools in the laboratory provided with continuous aeration and transferred to the rectangular fiber glass tanks (100 × 175 cm) of 500 liters capacity in the laboratory containing chlorine free aerated well water. Continuous aeration was maintained using aerators to acclimatize the prawn for a maximum period of 10 days in the laboratory conditions at room temperature (28 $\pm 1^{o}$C). And the prawn collected from polluted region Station-II, the prawns were dissected in the field itself to collect the testis for morphological studies by Scanning Electron Microscopic studies and the biochemical studies by Fourier Transform Infra-Red spectroscopic studies.

Scanning Electron Microscopic Studies

The tissues were removed, cut into 2.3 small pieces and washed with a NaCl 0.9% fixed with chilled glutoraldehyde (3%) prepared in 0.1 ml of phosphate buffer (pH 7.2) for 2 hours in each of the all tissues. These were washed 2.3 times with buffer and then dehydrated in various ascending grades of acetone (30 to 100%) for 30 minutes each Perierra, (1988). The tissues were then subjected to critical point drying followed by mounting on metallic stubs with the help of adhesive tape. The tissues were further trimmed as desired before pasting on 5 to 6 prior to sputtering with gold for 15 min and observations were made with different magnification for making a Scanning Electron Microscopy by using Hitachi Perkin-Elmer D-450 Scanning Electron Microscopy. The testis of the prawn collected from Station-I, and II were dried in vacuum for getting good moisture free specimen was needed. Then the samples were coated gold with full deposition for 3 minutes using polaron Sc 500 sputter coater. Few tungsten line coating was given. This coating has given primarily to prevent charging, since charging destroys samples and clarity of pictures. Then the samples were mounted in stereo scan 440-model electron microscope, UK. The ascertaining voltage given was 20 kw and the beam current used was in between 18.25 P.a. (Pico amperes) notching distance was between 39mm to 17mm. The secondary electron images were taken for all the samples with varied magnifications from 50× to 10,000× (Kotze and Soley, 1990).

FT-IR Analysis

The powered samples were thoroughly mixed with completely dried KBr (100 mg) and subjected to a pressure of 5×10^{6} pa in an evacuated die to produce a clear transparent disc of diameter 13 mm and thickness 1 mm. FT-IR spectra in the region 4000-400 cm^{-1} were recorded at 27 ± 1^{o} on a Nicolet-Avatar 330 FT-IR spectrometer equipped with DTGS (Due-treated Triglycine Sulfate) detector and purged with nitrogen. For each spectrum, 100 interferograms, providing spectral resolution of 4 cm^{-1} were co-added. The frequencies for all sharp bands were accurate to 0.1 cm^{-1}. Each sample was scanned with three different pellets under identical conditions, all of which gave identical spectra. The spectra were analyzed using ORIGIN 8.0 software.

RESULTS AND DISCUSSION

Scanning Electron Microscopic Studies

The scanning electron microscopic photographic studies of the testis of control prawn exhibited lobule covered by numerous myoepithilial cells. Below this, it is found to be covered with folded epithelium. Each testis consists of numerous follicles which were arranged one another and was covered with myoepithelial cells an outer muscular layer and inner apical cells which were folded with numerous secretory vesicles. Scanning electron microscopic observation of tests revealed the occurrence of sperm mass (Figure 2 and 3).

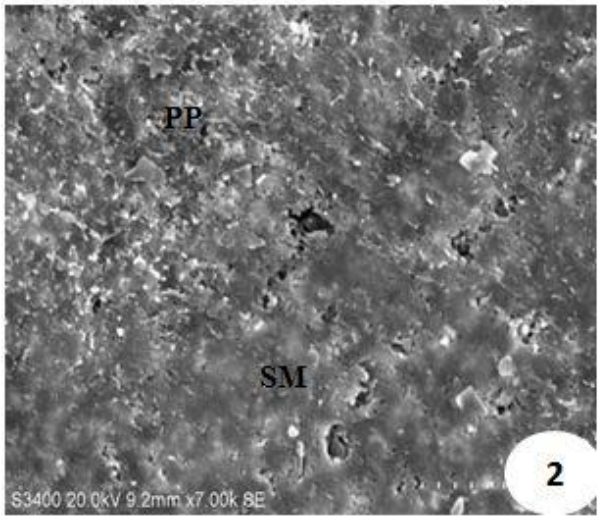

Figure 2: Scanning Electron Microscopic photograph of the testis of control prawn (X 7000k SE);
PP – Pinocytotic Pit; **SM**- Sperm Mass

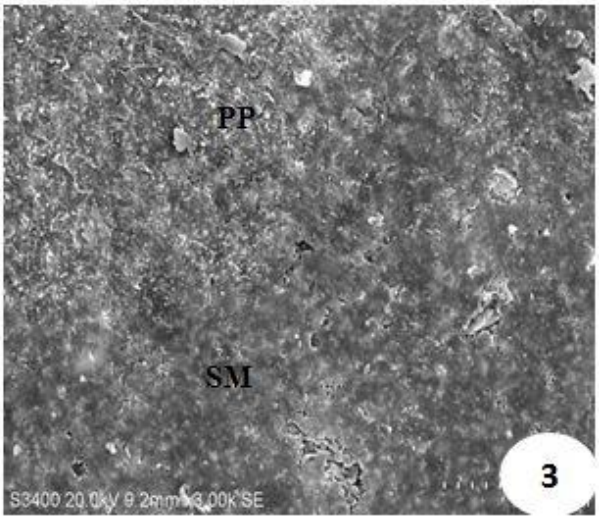

Figure 3: Scanning Electron Microscopic photograph of the testis of control prawn (X 3000k SE);
PP – Pinocytotic Pit; **SM**- Sperm Mass

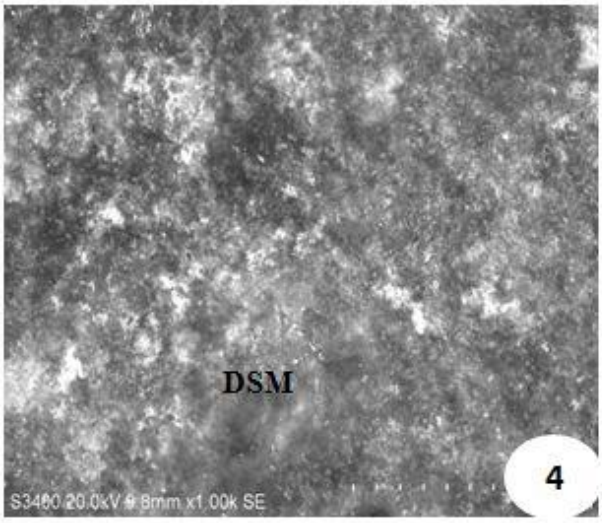

Figure 4: Scanning Electron Microscopic photograph of the testis of the prawn collected from Station –III (X 1000k SE)
DSM - Disintegrated Sperm Mass

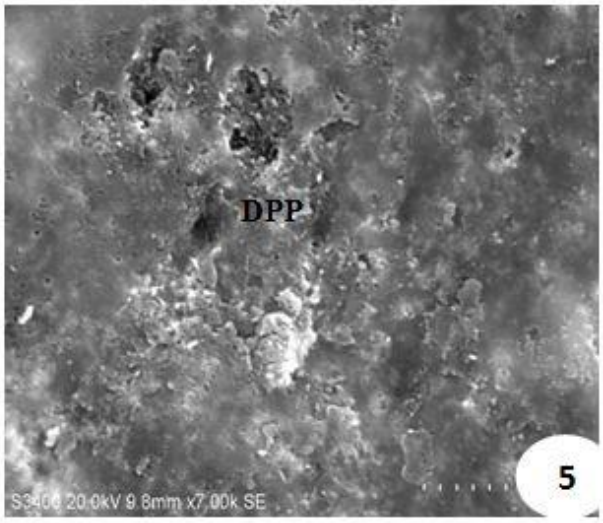

Figure 5: Scanning Electron Microscopic photograph of the testis of the prawn collected from Station –III (X 3000k SE)
DPP - Disintegrated Pinocytotic Pit

The scanning electron microscopic photographs of the testis of prawn collected from Station-II were found to be showed remarkable changes in the architecture of SEM changes such as the tubular testis follicle which was found to be highly pycnotic, comparatively shrunken than control prawn. The testis lobule was externally covered with myoepithelial cells are highly necrotic and pycnotic. The folded cuticle of the prawn collected at Station-II (polluted region) was found to be irregular and highly disintegrated. The apical cells of the testis follicle of the testis of the prawn showed numerous changes like; the sperm mass became disintegrated and disorganized. The spermatids were found to be shrunken with numerous vacuoles were seen in the prawns were perhaps, interfere with the reproductive potentiality of the prawn at Station-II than the Station-I (Figure 4 and 5). These changes may be due to the prawn under stress condition which affect by the pollutants.

FT-IR spectroscopic studies

The representative FT-IR spectra of the testis of the control prawn and the testis of prawn collected from polluted region in the 4000-400 cm-1 wave number region are presented in figures 6 and 7. The main bands are labeled in the figure. The vibrational assignment of FT-IR spectra of control testis and the test is collected from polluted region is presented in Table 1. It was based on the probable frequencies of vibrations of control testis and the testis of prawn collected from polluted region frequencies as reported in the references and their relative intensity in the FT-IR spectra. As can be seen in the figure, the FT-IR spectrum of the testis of *Macrobrachium malcolmsonii* is quite complex and contains several bands belonging to proteins, lipids and nucleic acids.

Figure 6 shows the spectra of the control testis were normalized with respect to the band at 3327cm^{-1} (Amide A). In the present study, the broad band centered at ~3327 cm^{-1} was assigned as the N-H stretching bands of proteins (Akkas *et al.,* 2007). In figure 7 the N-H stretching bands of proteins band shift at 3315cm^{-1} (Amide A: N-H stretching bands of proteins) (Akkas *et al.,* 2007; Palaniappan *et al.,* 2008). It indicates the N-H stretching bands of proteins decreased in the testis of the prawn collected from polluted region due to the pollutants of the Paravnar River.

The obsorption peak at ~2927 cm^{-1} (Figure 6) was assigned to the asymmetric and symmetric stretching modes of methylene chain membrane lipids (Akkas *et al.,* 2007; Palaniappan *et al.,* 2008). In figure 7 the asymmetric and symmetric stretching modes of methylene chain membrane lipids band was assigned at ~2924 cm^{-1} (Figure 7). It indicate the asymmetric and symmetric stretching modes of methylene chain membrane lipids was shifted in the testis of the prawn collected from polluted regions due to the pollutants of the Paravnar river.

The obsorption peak at ~2852 cm^{-1} (Figure 6) was assigned CH2- symmetric stretch: mainly lipids (Akkas *et al.,* 2007; Palaniappan *et al.,* 2008). In figure 2 CH_2- symmetric stretch: mainly lipids band was assigned at same ~2853 cm^{-1} (Figure 7).

The sharp bands observed at ~1655 cm^{-1}(Figure 6) correspond to amide I and II vibration of structural proteins, respectively. The amide I absorption is mainly associated with the C=O stretching vibration of the protein amide (Palaniappan *et al.,* 2008). The sharp bands observed at ~1654 cm^{-1}(Figure 7) the amide I absorption is mainly associated with the C=O stretching vibration of the protein amide is shifted due to the presence of pollutants.

The sharp bands observed at ~1534 cm^{-1}(Figure 6) correspond to amide I and II vibration of structural proteins, respectively. The amide I absorption is mainly associated with the C=O stretching vibration of the protein amide (Palaniappan *et al.,* 2008). The sharp bands observed at ~1541 cm^{-1}(Fig. 7) the amide I absorption is mainly associated with the C=O stretching vibration of the protein amide is shifted due to the presence of pollutants.

The obsorption peak at ~1236 cm^{-1}(Figure 6 and 7) was due to PO^{-}_2 asymmetric stretching modes of phosphodiester group in nucleic acids with little contribution from phospholipids (Cakmak *et al.,* 2006).

The band observed at ~1163 cm^{-1}(Figure 6) was assigned to CO-O-C asymmetric stretching arising from ester bonds in cholesterol esters and phospholipids (Toyran *et al.,* 2007).

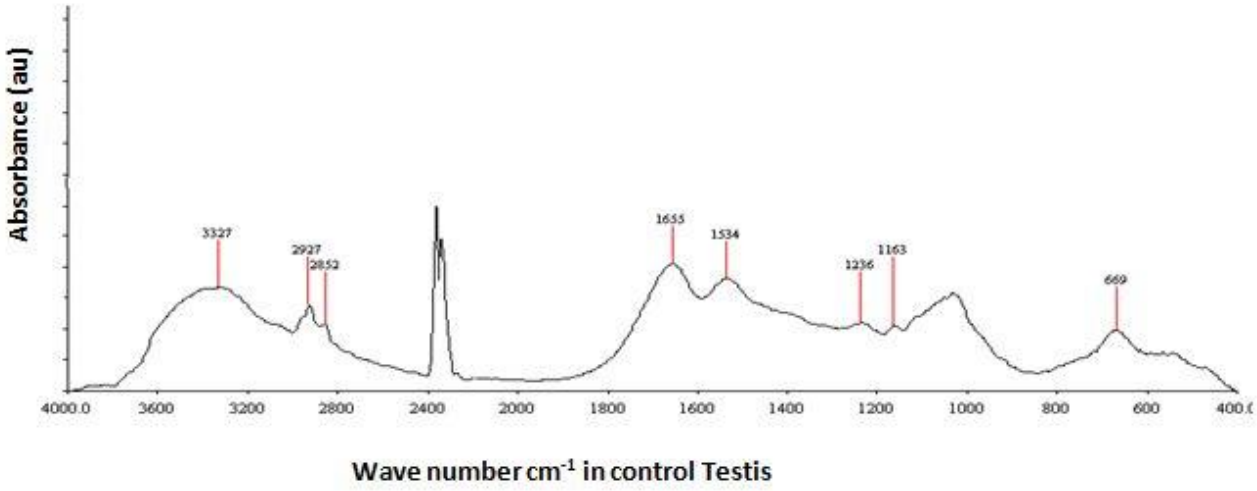

Figure 6: Representative FT-IR spectra of Control Testis of freshwater prawn *Macrobrachium malcolmsonii* in the 4000-400 cm^{-1} region

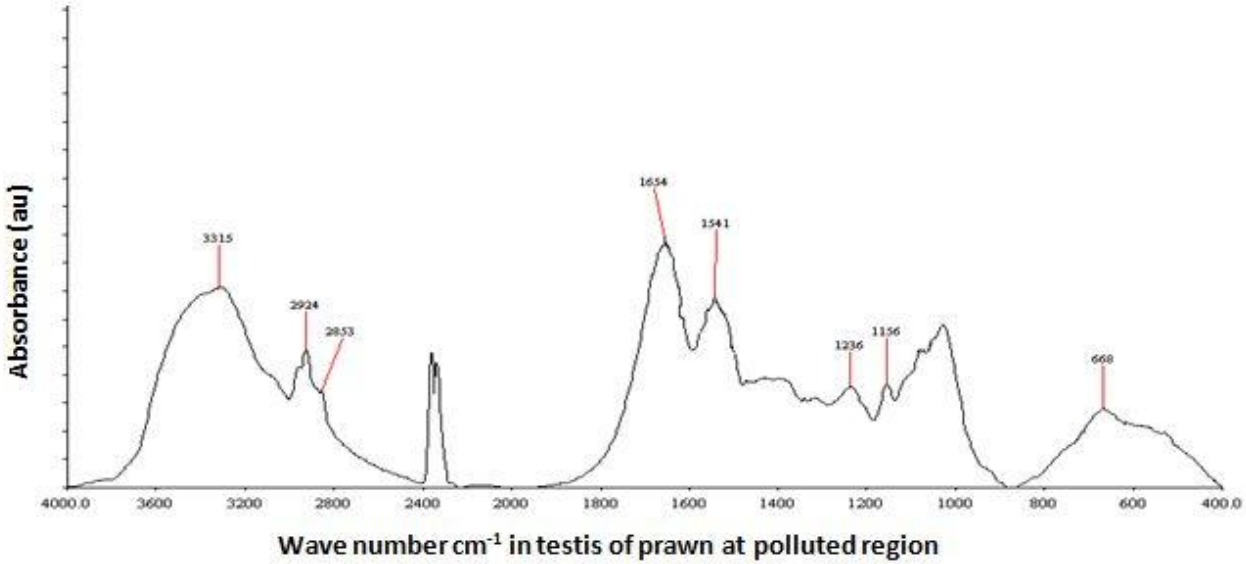

Figure 7: Representative FT-IR spectra of the Testis of freshwater prawn *Macrobrachium malcolmsonii* collected from polluted region in the 4000-400 cm^{-1} region

The band observed at ~1156 cm^{-1}(Figure 7) was assigned to CO-O-C asymmetric stretching arising from ester bonds in cholesterol esters and phospholipids are shifted due to the pollutants of the Paravanar river. Obsorption peak at ~669 and ~668 cm^{-1}(Figure 6 and 7) was assigned which are respectively due to the ring breathing modes of the DNA bases (Adinine, Guanine) and ring breathing modes of the DNA bases (Guanine, Thymine) (Chen *et al.,* 2006).

According to Benedetti *et al.,* (1997) an increase or a decrease in the ratio of the intensities of the amide bands at 1540 cm^{-1} and 1650 cm^{-1} could be attributed to a change in the composition of the whole protein pattern the ratio of the peak intensities of the bands at ~ 1080 and ~ 1540 cm^{-1} was used by Ozaki *et al.,* (1989) to indicate the relative concentration of the glycoprotein content.

Ram and Sathyanesan (1984) have noticed a fall in the level of total protein content in the kidney and liver tissues of *Channa punctatus* exposed to mercury. Similar results were observed in *Cyprinus Caipio* when exposed to nickel (Sreedevi *et al.,* 1992).

The decreased quantity of protein in the liver and brain tissues might be due to its conversion to amino acid residue in order to increase amino acid pool. The decreased protein content in the tissues was due to non-selective blocking of phosphorylation process in the tissues and also it can be influenced by large number of exogeneous substances, mainly through a reduction of protein synthesizing capacity of the endoplasmic reticulum in the cells. Meenakshi and Lomte, (1998) also observed a reduction in lipid content which could be due to the reduced synthesis of lipid or increased activity of lipase involved in oxidation of lipid.

According to Ramana Rao and Ramamurthi, (1982) this may be due to the increase in activity of enzymes lipase in increasing lypolytic activity to meet the increased demand of energy during stress.

Shivaprasad Rao *et al.,* (1982) also reported a decrease in total lipids its might be due to drastic decrease in glycogen content in the same tissue which is an immediate source of energy during toxic stress conditions. After glycogen, lipid content may be used for energy production to overcome toxic stress.

Many of the investigators to study the prawn tissues by using Fourier Transform Infrared spectroscopy Icononmidou *et al.,* (2000) and Pornat *et al.,* (2007). Structural, compositional and functional changes in rainbow trout by using Fourier Transform Infrared spectroscopy spectroscopy reported by Gulgun *et al.,* (2006).

FT-IR spectroscopy monitors the vibrational modes of functional groups within biomolecules and enables a correlation between chemical information and histological structures (Jackson and Mantsch, 1993; Melin *et al,* 2000; Li *et al.,* 2002; Toyran *et al,* 2004, Severcan *et al.,* 2005a; Severcan and Haris, 2003). Shifts in peak positions, changes in bandwidths, intensities, and band area values of the infrared bands are used to obtain valuable structural and functional information about the system of interest Takahashi *et al.,* (1991); Liu *et al.,* (1996); Yano *et al,* (1996); Fung *et al.,* (1997); Ci *et al,* (1999); Kidder *et al.,* (1999); Melin *et al.,* (2001); Toyran and Severcan, (2003).

In the present FT-IR study, it has been observed that in the testis, the amount of protein contents were found to be decreased whereas the amount of lipid content were increased in the testis of the prawn collected from polluted region than the control prawn, may be suggested due to the presence of more pollutants at polluted region caused drastic changes due to less proteolytic and more lipolytic activity. Similar results were reported by Dogan *et al.,* (2007); Akkas *et al.,* (2007) and Palaniappan *et al.,* (2008).

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