FTIR AND ICP-AES STUDY OF THE EFFECT OF HEAVY METALS NICKEL AND CHROMIUM IN TISSUE PROTEIN OF AN EDIBLE FISH *CIRRHINUS MRIGALA*

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Abstract. The discharge of heavy metal wastes has many obvious impacts on aquatic systems. The objective of this investigation was to determine the effect of heavy metals nickel and chromium at lower and higher sub lethal concentrations of an edible fish *Cirrhinus mrigala* exposed for a period of 28 days. The impact of bioaccumulation of these heavy metals on muscle tissues of the edible fish was related to decrease in protein content. The FT-IR spectra revealed significant differences in the absorption intensities between the control and the treated tissues, pointing to a decrease in the overall protein content on muscle tissues. Also bioaccumulation and elimination of nickel and chromium in the various organs of *Cirrhinus mrigala* have been studied using ICP-AES technique**.** The study indicates that the effect of heavy metal nickel treatment is greater than that of chromium.

Key words: FTIR, heavy metals, ICP-AES, protein.

INTRODUCTION

Proteins are the most important and abundant biochemical constituent present in the animal body, particularly in fish. Protein generally contains a sequence of amino acid residues of the polypeptide chains. Fish, a common source of protein contains a greater quantity of protein than any other living organism, contributing roughly about 75% of the weight of fish. The protein content was experimentally verified for the muscle sample of the catfish, *Claria batrachus*, by Lowery [10] and was found to be 70%. Protein plays a major part in all life systems with a wide variety of structural and functional roles.

Fourier transform infrared spectroscopy is gaining recognition as a promising method in diagnostic medicine and biological studies and is used for studying the molecular structure of protein [4]. The increasing use of FT-IR spectroscopy

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demonstrates that this technique is a valuable tool owing to its high sensitivity in detecting changes in functional groups of tissue components, such as membranes, proteins and nucleic acids, as well as for complex cellular material such as tissues, body fluids or cell cultures [15]. 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. The amide bands of proteins usually appear as strong/sharp bands, the changes and occurring in the relative intensities of their maxima are useful to monitor changes in the protein content quantitatively. Since amide absorption is sensitive to protein confirmation, an increase or decrease in this ratio could be attributed to changes in the composition of the protein pattern [13].

The accumulation of heavy metals in water suggests that fish may serve as a useful indicator for contaminating metals in aquatic systems. Since fishes are often the last link in the aquatic food chain, the metal concentration of many fish species has been analyzed in relation to the metal content of aquatic environment. Bioconcentration of chemicals in aquatic biota is an important factor in the assessment of the potential hazard of chemicals to the environment [6,7]. This parameter can be used to quantify bioconcentration in aquatic biota, and is defined as the ratio of the concentration of the chemicals in the biota (C_B) to that of water (C_W) at equilibrium. The bioconcentration factor (BCF) is usually measured in the laboratory using the test animals and is defined as:

$$BCF = \frac{k_1}{k_2} = \frac{C_B}{C_w} = \frac{\text{Chemical concentration in each part of the fish (µg/g wet weight)}}{\text{Chemical concentration in water (µg/L)}} \quad (1)$$

The excretion rates constant of chemicals from the whole fish body/organ is calculated using the equation:

$$C = C_0 e^{-k_2 t} \quad (2)$$

where C = chemical concentration in the whole fish body /organ (µg/g wet weight) at time t; C_0 = initial chemical concentration in the whole fish body /organ (µg/g wet weight); k_2 = excretion rate constant (h^{-1}), and t = time (h^{-1}).

Knowing the values of k_2 and BCF, k_1 can be calculated using the equation:

$$BCF = \frac{k_1}{k_2} \quad (3)$$

Hence, in the present work the effect of Ni and Cr is shown on the protein contents of muscle tissues and bioaccumulation/elimination of this metal on edible fish *Cirrhinus mrigala* using FT-IR and ICP-AES.

EXPERIMENTAL METHODS

Freshly collected animals weighing about 7 ± 1 g body weight and 6 ± 1 cm body length after acclimatization in laboratory for 7 days were divided into four groups. The experiments were carried out at (28±1) °C in an aerated plastic trough. Group 1, consisting of twenty animals, were placed in a plastic trough (45 × 32 × 25) cm and kept in clean water. The group 2 and group 3 fishes were exposed to $1/10^{th}$ and $1/3^{rd}$ of LC_{50} taken as lower (1.08 ppm) and higher (3.61 ppm) concentrations of nickel. The test fishes were exposed to the above-mentioned sub-lethal concentrations separately for a period of 28 days. At the end of the exposure period muscle tissues were isolated and kept in a freezer (–20 °C) prior to analysis. For recovery, a group of fishes were randomly selected from the experimental tank after exposure to toxicity for 28 days and kept in clean water (elimination period) for another period of 28 days and was treated as Group 4. Similarly, the above procedure was adopted for chromium toxicity and the values of $1/10^{th}$ and $1/3^{rd}$ of LC_{50} were 1.82 ppm and 6.06 ppm, respectively. The LC_{50} value was obtained by the probit analysis method [3].

INFRARED ANALYSIS

The samples were lyophilized and transformed into 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 is 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. Each observation was confirmed by taking at least three replicates.

ICP-AES ANALYSIS

For the analysis of metals (nickel and chromium) all glassware were filled with 2N HNO_3 overnight and rinsed several times with double distilled water. The samples were digested by weighing one gram (dry mass) of the sample into a 100 mL Borasil flask and then adding fifteen milliliters of concentrated nitric acid and five milliliters of concentrated perchloric acid. Digestion was performed on a hot plate at 80–90 °C for approximately 120 minutes or until the solutions were cleared. After the digestion was completed, each sample was allowed to cool before being

filtered through a Whatman No. 42 filter paper using a vacuum pump. After filtration, the filtering system was rinsed with distilled water to remove all traces of metal, and each sample was made up to 25 mL with distilled water and stored in acid-washed polyethylene flasks to await the metal concentration analysis. The estimation of nickel and chromium concentrations in the tissue sample of fish was made using an Inductively Complied Plasma Atomic Emission Spectrometer (ISA JOBIN YVON 24 MODEL) and the analytical standard was prepared from the respective stock solution. All the analyses were carried out in four replication and the averages of the values have been reported along with standard deviation.

RESULTS AND DISCUSSION

EFFECT OF NICKEL ON TISSUE PROTEIN IN *CIRRHINUS MRIGALA*

FT-IR spectra of control, lower and higher nickel treated muscles tissues along with their recovery are presented in Fig. 1. 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 control and the treated tissues. From the spectra, it could also be seen that there is an overall decrease in intensity of absorption bands due to nickel treatment and an increase in the intensity of absorption bands in the sample of the recovered animals. Table 1 shows the observed frequencies in the region 4000–400 cm^{-1} along with their vibrational assignment and intensity. The broad band centered at ~3291 cm^{-1} is assigned as the O–H stretching with small contribution from the amide bands of protein. The bands observed at ~2960 cm^{-1} were assigned to the symmetric stretching mode of the methyl end groups of the membrane lipids as well as the methyl side group of the cellular proteins [4]. Also, the sharp bands observed at ~1657 cm^{-1} and at ~1545 cm^{-1} were assigned to the in-plane C=O stretching vibration (amide I) and C–N stretching / N–H bending vibration (amide II) of tissue proteins respectively. The bands observed at ~1446 cm^{-1} and ~1398 cm^{-1} are mainly from the asymmetric and symmetric CH_3 bending modes of the methyl group of proteins respectively [11, 16]. The C–O symmetric stretching mode of carboxylate groups could also have contributed to the latter band. Medium intensity bands observed at ~1240 cm^{-1} are due to the PO_2^- asymmetric stretching modes of phosphodiester indication of phospholipids and amide III [17]. Generally, muscle tissues store energy rich molecule, glycogen which is a glucose polymer and glycogen exhibits the absorption spectrum due to C–O and C–C stretching and C–O–H deformation motions, with peaks at ~1150, ~1080 and ~1045 cm^{-1} [5]. In the present study the bands observed at ~1083 cm^{-1} were assigned as symmetry phosphate stretching [2], glycogen has also made a contribution to the intensity of the band.

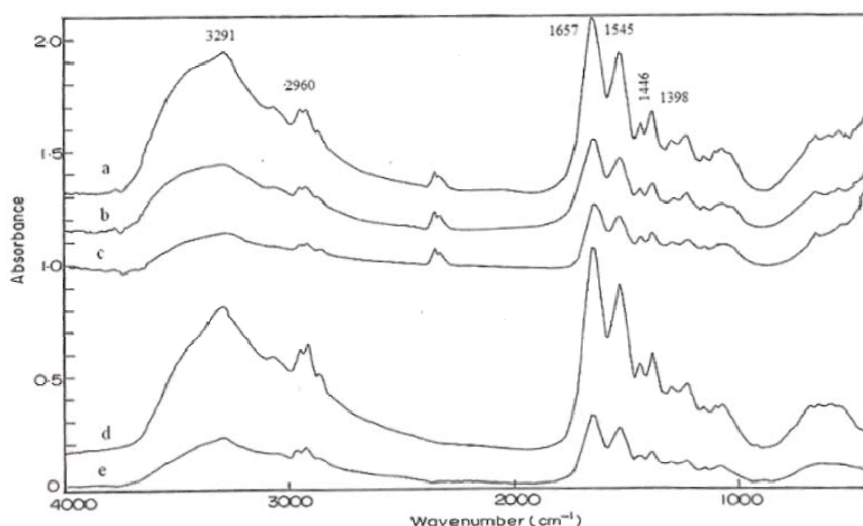

Fig. 1. FTIR spectra of tissues protein of edible fish *Cirrhinus mrigala*; (a) control; (b) lower nickel treated; (c) higher nickel treated; (d) lower recovery; (e) higher recovery.

According to Edordo Benedetti *et al.* [1] increase or decrease in the ratio of the intensities of the bands of 1540 cm^{-1} and 1650 cm^{-1} could be attributed to change in the composition of the whole protein pattern. The measured intensity ratios of the selected bands I_{1545}/I_{1657}, I_{1545}/I_{3291} and I_{2960}/I_{2873} are given in Table 2. It has been observed from the table that the ratio of amide II/amide I decreased from 0.82 to 0.63 for lower nickel treatment and to 0.52 for higher nickel treatment, which corresponds to a decrease of 23% and 36% respectively.

These decreased ratios reflect an alteration in the level of total proteins due to nickel treatment. During the period of recovery this ratio increases from 0.63 to 0.65 and from 0.52 to 0.60, which corresponds to 3% and 15% for lower and higher treatment respectively. The ratio of the peak intensities of the bands ~1545 cm^{-1} and ~3291 cm^{-1} (I_{1545}/I_{3291}) may be used as an indicator of the relative concentration of proteins to water included near the surface of biological tissues. In the present study the ratio I_{1545}/I_{3291} decreases from 0.72 to 0.60 and 0.55 for a nickel treated sample which corresponds to 16% and 23% decrease in the ratio. Also, during the period of recovery there is gain in intensity from 0.60 to 0.63 and 0.55 to 0.69, which corresponds to 5% and 25% gain in the ratio. These results suggest that the relative concentration of protein to water in the tissue membrane is considerably lower in nickel treated tissues compared with that of control. Also the ratio of the intensity of absorption of bands between $I_{2960}/\ I_{2873}$ could be used as the main contribution of the number of methyl groups in protein fibers. In the present study the ratio of the methyl band and methylene band decreased from 0.80 to 0.20 for lower nickel treatment and to 0.10 for higher nickel treatment which corresponds to

a decrease of 75% and 88% respectively. This decreased ratio indicates a decrease in number of methyl groups compared to methylene groups in nickel intoxicated tissues. This decrease ratio indicates the main contribution for the low number of protein fibers. During the period of recovery this ratio increases from 0.20 to 0.33 and from 0.10 to 0.14, which corresponds to 65 % and 40 % gain in the ratio as supported by increased excretion rate constant (k_2) from the bioaccumulation study reported in Table 3.

Table 1

FTIR frequency assignment for muscle tissues of control, nickel, chromium treated and their recovery of *Cirrhinus mrigala*

Wave number (cm^{-1})									**Frequency Assignment**
Control	**Nickel treatment**		**Recovery**		**Chromium treatment**		**Recovery**		
	Lower	**Higher**	**Lower**	**Higher**	**Lower**	**Higher**	**Lower**	**Higher**	
3291 (s)	3296 (w)	3285 (w)	3294 (m)	3292 (w)	3295 (m)	3290 (m)	3294 (m)	3287 (w)	O–H stretching /N–H stretching
2960 (s)	2960 (w)	2960 (w)	2960 (m)	2960 (w)	2961 (m)	2960 (m)	2961 (m)	2959 (w)	CH_3 symmetric stretching; lipid, protein
2926 (s)	2924 (m)	2926 (vw)	2927 (m)	2927 (w)	2928 (m)	2929 (m)	2928 (m)	2926 (w)	CH_2 asymmetric stretching; mainly lipid, protein
2873 (m)	2872 (vw)	2872 (vw)	2874 (m)	2874 (vw)	2874 (m)	2875 (w)	2874 (vw)	2872 (vw)	CH_2 symmetric stretching; lipid, protein
1657 (s)	1659 (s)	1657 (w)	1653 (s)	1653 (m)	1655 (s)	1654 (w)	1651 (s)	1655 (m)	inplane C=O stretching(Amide I)
1545 (s)	1546 (m)	1545 (w)	1542 (s)	1541 (m)	1544 (s)	1542 (m)	1538 (m)	1538 (w)	C–N stretching / N–H bending(Amide II)
1446 (m)	1445 (m)	1444 (vw)	1450 (m)	1448 (vw)	1448 (w)	1452 (vw)	1449 (w)	1449 (vw)	CH_3 asymmetric bending; protein
1398 (s)	1397 (w)	1397 (w)	1396 (s)	1395 (w)	1396 (m)	1396 (w)	1393 (m)	1393 (w)	CH_3 symmetric bending; protein
1240 (m)	1238 (w)	1238 (vw)	1240 (m)	1238 (vw)	1239 (w)	1237 (w)	1236 (m)	1237 (vw)	PO_2^- asymmetric stretching /Amide III
1083 (m)	1081 (w)	1083 (vw)	1083 (w)	1082 (vw)	1081 (vw)	1082 (vw)	1082 (w)	1084 (vw)	PO_2^- symmetric stretching (glycogen)

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

EFFECT OF CHROMIUM ON TISSUE PROTEIN IN *CIRRHINUS MRIGALA*

Fig. 2 shows the absorption spectra of chromium treated tissues along with the respective recovery and control tissues. The absorption bands are consistent with the general features. The tentative frequency assignments for the absorption have been reported in Table 1 and the measured intensity ratios are given in Table 2. It could be observed from the spectra that the ratio of absorption intensity of the band I_{1545}/I_{1657} decreases from 0.82 to 0.69 and to 0.56 for lower and higher treatment which corresponds to 16% and 32% decrease in the ratio. Also during the period of recovery the ratio decreases further from 0.69 to 0.45 and 0.56 to 0.46 which corresponds to 34% and 17% by value. This decreased ratio indicates that even after the chromium treatment was withdrawn, the level of protein continued to decrease during the period of recovery. Also the ratio of I_{1545}/I_{3291} decreases from 0.72 to 0.70 and to 0.62 which corresponds to the change of 3% and 14% for lower and higher chromium treatment respectively. Further, this ratio decreases from 0.70 to 0.58 and 0.62 to 0.59 during the recovery period respectively. Also there is a considerable decrease in the intensity of the absorption bands in the region 1200–1000 cm^{-1}. The ratio of methyl band and methylene band decreases from 0.80 to 0.66 for lower chromium treatment and 0.50 higher treatments which corresponds to decreases of protein fibers by 18 % and 38% respectively. Further the ratio of the methyl band and methylene band decreased from 0.66 to 0.53 for lower and 0.50 to 0.30 for higher chromium treated recovery which corresponds to a decrease of 20% and 40% respectively. This decreased ratio indicates a decrease in number of methyl groups compared to methylene groups in chromium intoxicated tissues which corresponds to the decrease in the contents of protein fibers even during the period of recovery. It was supported from decreased excretion rate constant $k_2 = 0.005\pm0.001$ h^{-1} than that of nickel (Table 3).

Table 2

FTIR absorption intensity ratios of selected bands in the muscle tissues of *Cirrhinus mrigala*

<table>
<tr><th colspan="2">Treatment</th><th>I_{1545}/I_{1657}</th><th>I_{1541} / I_{3293}</th><th>I_{2960}/I_{2873}</th></tr>
<tr><td></td><td>Control</td><td>0.82</td><td>0.72</td><td>0.80</td></tr>
<tr><td rowspan="2">Nickel treatment</td><td>Lower sub-lethal concentration</td><td>0.63</td><td>0.60</td><td>0.20</td></tr>
<tr><td>Higher sub-lethal concentration</td><td>0.52</td><td>0.55</td><td>0.10</td></tr>
<tr><td rowspan="2">Nickel recovery</td><td>Lower sub-lethal concentration</td><td>0.65</td><td>0.63</td><td>0.33</td></tr>
<tr><td>Higher sub-lethal concentration</td><td>0.60</td><td>0.69</td><td>0.14</td></tr>
</table>

Table 2 (continued)

Chromium treatment	Lower sub-lethal concentration	0.69	0.70	0.66
	Higher sub-lethal concentration	0.56	0.62	0.50
Chromium recovery	Lower sub-lethal concentration	0.45	0.58	0.53
	Higher sub-lethal concentration	0.46	0.59	0.30

Table 3

Bioconcentration factor (*BCF*), uptake rate (k_1) and elimination rate (k_2) constants in muscle tissues exposed to nickel and chromium for 28 days

Organs	**Control**	**Accumulation**	**Lower sub-lethal concentration**			**Higher sub-lethal concentration**		
			BCF	k_1 (h^{-1})	k_2 (h^{-1})	***BCF***	k_1 (h^{-1})	k_2 (h^{-1})
Nickel	8.72 ± 0.84	22.95 ± 3.14	21.20 ± 1.26	0.059 ± 0.03	0.003 ± .001	86.35 ±5.63	23.91 ± 1.82	0.112 ±0.02
Chromium	20.43 ±1.32	48.63 ±3.78	26.70 ±1.80	0.07 ±0.3	0.03 ±0.2	217.6 ±0.43	91.2 ±5.26	0.09 ±0.002

The differences between controls and exposures are statistically significant ($P < 0.005$). ($n = 4$) k_1 was calculated by $k_2 \times BCF$ ($n = 4$).

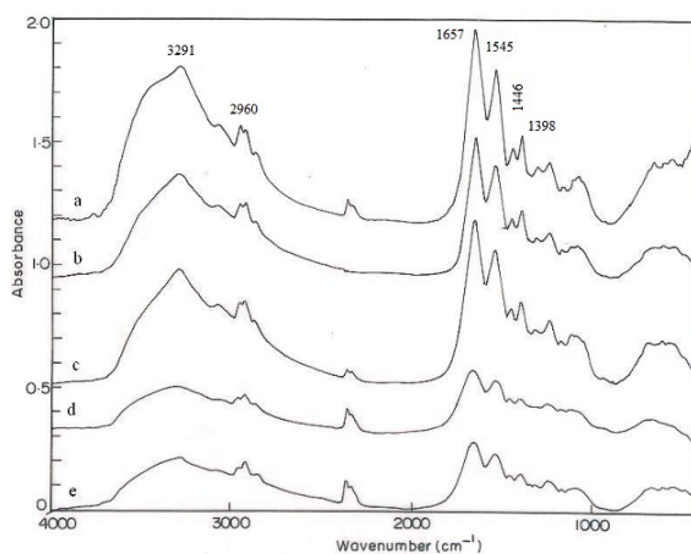

Fig. 2. FTIR spectra of tissues protein of edible fish *Cirrhinus mrigala*; (a) control; (b) lower chromium treated; (c) higher chromium treated; (d) lower recovery; (e) higher recovery.

BIOACCUMULATION AND ELIMINATION OF HEAVY METAL NICKEL AND CHROMIUM

The accumulation of nickel in the muscle tissues and the Bioconcentration factor (*BCF*), uptake rate constant (k_1) and excretion rate constant (k_2) were also computed [6, 7] and they have been reported in Table 4. The muscle accumulated the least concentration of nickel *BCF* = 21.20±1.26 and 23.91±1.82) when compared to that of chromium. The uptake and excretion rates were also lower ($k_1 = 0.059\pm0.038$, 0.112 ± 0.021 and $k_2 = 0.003\pm0.001$, 0.005 ± 0.001) for both levels of concentration. The chromium concentration was found to be *BCF* = 15.05 ± 1.20. Further the uptake and excretion rate constants were found to be $k_1 = 0.059 \pm 0.002$ h^{-1} and $k_2 = 0.004 \pm 0.001$ h^{-1}. It can be observed from the results presented above that in the case of nickel treatment there is a decrease in the protein content as could be seen from the decrease in the ratios of $I_{1545}/I_{1657,}$ $I_{1545}/$ I_{3291} and I_{2960}/I_{2873} for both lower and higher nickel treatment. Also there is a gain in the ratio of these bands, during the period of recovery, which indicates an increase in the protein content. The gain in the total protein content could be attributed to reduced proteolysis due to the elimination of nickel after the animals have been transferred to normal water. Also, it could be observed that due to chromium treatment, there is depletion in the protein content as evidenced by a decrease in the intensity ratios. Interestingly it can also be observed that even during the period of recovery there is a decline in the protein content, which may be due to the increased proteolysis and possible utilization of the products of their degradation for metabolic purpose. This could have been fed into TCA cycle through aminotransferase system to cope with the excess demand of energy during the elimination of the toxicant from the body [9]. Palaniappan *et al.* [12] has studied the effect of zinc on tissue protein of *Labeo rohita* using FTIR. Ragothaman [14] has also observed a decline in the protein level of *Cirrhinus mrigala* exposed to cadmium. Karthikeyan [8] also studied the effect of heavy metals nickel and chromium on bone minerals of *Cirrhinus mrigala*. Hence the present finding is in good agreement with the earlier reports, i.e. due to the treatment of heavy metals nickel and chromium, total protein content of muscle tissue decreases and that the former is more toxic. The main features in the spectra of all the tissues is that the amide I absorption band at ~1650 cm^{-1} arises from the stretching vibration of the C=O group of amide band in proteins. In isolated proteins and lipid complex the position of the amide I maxima is used to estimate the secondary structure of protein. However, as amide I absorption of all proteins present in tissues are observed simultaneously and it is not usually possible to assign particular amide I absorption to particular secondary structures within any position in IR spectra of tissues.

Further, the structure of proteins solution and in the membrane environment is determined predominantly by the amide I maxima in FT-IR spectroscopy. All proteins and almost all peptides are composed of mixtures of different structural elements and each of these has a characteristic hydrogen bonding pattern including

amide C=O and N–H group; therefore the amide C=O groups associated with each structural element may be expected to have a characteristic electron density. This in turn will produce a characteristic amide I absorption frequency. The observed shift towards high wave number and changes in band intensity due to nickel treatment as well as to chromium indicate either a structural rearrangement of the existing proteins or the expression of a new set of proteins.

CONCLUSION

The effect of nickel and chromium on muscle tissue proteins was studied using FTIR technique. The variations in the intensity ratio of I_{1540}/I_{1650}, I_{1545}/I_{3291}, and I_{2960}/I_{2873} were used to monitor changes in the total protein content of the muscle tissues. The highest accumulation of nickel on muscle tissues shows that the toxicity is more pronounced in nickel than in chromium and this was supported by decrease in protein content in muscle tissue by FTIR spectroscopy. Further Bioaccumulation of heavy metals was higher in the case of nickel than that of chromium and this supports decrease in protein contents as evidenced by FTIR study.

REFERENCES

1. BENEDETTI, E., E. BRAMANTI, R. PAPIMESCHI, I. ROSSI, E. BENEDETTI, Determination of the relative amount of nucleic acids and proteins in leukemic and normal lymphocytes by means of Fourier transform infrared, *Appl. Spect.*, 1997, **51**, 792–797.
2. DIEM, M., S. BODYSTON WHITE, L. CHRIBOGA, Infrared spectroscopy of cells and tissues: Shining light onto a novel subject, *Appl. Spect.*, 1999, **53**(4), 148A–161A.
3. FINNEY, D.J., *Probit Analysis,* Cambridge University Press, Cambridge. 1971.
4. GRDADOLNIK, J., A FTIR investigation of protein conformation, *Bull. Chem. Technol.,* 2002 **21**(1), 23–34.
5. JACKSON, M., L.P.CHOO, P.H. WATSON, W.C. HALLIDAY, H.H. MANTSCH, Beware of connective tissue proteins: Assignment and implications of collagen absorptions in infrared spectra of human tissues, *Biochem. Biophys. Acta*, 1995, **1270**(1), 1–6.
6. KARTHIKEYAN, S., PL.RM. PALANIAPPAN, S. SABHANAYAGAM, Bioaccumulation of nickel in various organs of fresh water fish *Cirrhinus mrigala* exposed to sub-lethal concentrations, *Indian J. Environ. Prot*., 2005, **25**(7), 629–634.
7. KARTHIKEYAN, S., PL.RM. PALANIAPPAN, SELVI SABHANAYAGAM, Influence of pH and water hardness upon nickel accumulation in edible fish *Cirrhinus mrigala*, *J. Environ. Biol.,* 2007, **28**(2), 489–492.
8. KARTHIKEYAN, S., X ray diffraction and fourier transform study of toxic effect of heavy metals nickel and chromium on bone tissues of an edible fish *Cirrhinus mrigala*, *Acta Phy., Pol.,* 2012, **122**(1), 236–239.
9. KLASSAN, C.D., Heavy metal and antagonists, In: *Pharmacological Basis of Therapeutics*, A.G. Gilman, L.S Goodman, and G. Gilman, eds., London, 1980.
10. LOWERY, O.H., N.J. ROSEBROUGH, FARRAL, R.J. RANDAL, Protein measurement with the folin phenol reagent, *J. Biol. Chem*., 1951, **193**, 265–275.

11. NAUMANN, D., D. HELM, H. LABISHINSKI, P. GIRBRECHT, The characterisation of microorganisms by Fourier transform infrared spectroscopy, In: *Modern Techniques for Rapid Microbiological Analysis*, W.H. Nelson, ed., VCH Verlag Chemie, New York. 1992.
12. PALANIAPPAN, PL.RM, K.S. PRAMOD, K. VIJAYASUNDARAM, FTIR study of zinc-induced biochemical changes in the liver of Indian carp *Labeo rohita*, *J. Appl. Spectro.*, 2008, **75**(5), 752–758.
13. PALANIAPPAN, PL.RM, R. VIJAYASUNDARAM, The effect of arsenic exposure and the Efficacy of DMSA on the proteins and lipids of the gill tissues of *Labeo rohita*, *Food and Chemical Toxicology*, 2009, **47,** 1752–1759.
14. RAGOTHAMAN, G., *Effect of cadmium as a pollutant on certain metabolite content on Cirrhinus mrigala (Hamilton) fingerlings*, Ind. Nat. Symp. on Fish and their Environ, Dept. of Aquaculture, Biol. Fish, University of Kerela, 1988.
15. VENKATARAMANA, G.V., J. KOMAL KUMAR, A.G. DEVI PRASAD, P. KARIMI, Fourier transform infrared spectroscopic study on liver of freshwater fish *Oreochromis mossambicus*, *Romanian J. Biophys*., 2010, **20,** 315–322.
16. WONG, P.T.T., E.D. PAPAVASSILIOU, B. RIGAS, Phosphodiester stretching bands in the infrared spectra of human tissues and cultured cells, *Appl. Spect.* 1991, **45**(9), 1563–1567.
17. ZEROUAL, W., C. CHOISY, S.M. DOGLIA, H. BOBICHON, Monitoring of bacterial growth and structural analysis as probed by FT-IR Spectroscopy, *Biochemica et Biophysica Acta.*, 1994, **1222**(2), 171–178.