EFFECT OF HEAVY METALS MIXTURE NICKEL AND CHROMIUM ON TISSUE PROTEINS OF AN EDIBLE FISH *CIRRHINUS MRIGALA* USING FTIR AND ICP-AES STUDY

S. KARTHIKEYAN

Department of Physics, Valliammai Engineering College, Kattankulathur-603203, Tamil Nadu, India,
e-mail: physicskarthik@gmail.com

Abstract. The goal of the study was to investigate the effect of nickel, chromium and its mixtures on biochemical contents of muscle tissues of *Cirrhinus mrigala* fingerlings by Fourier transform infrared spectroscopy. FT-IR spectra revealed significant differences in absorbance intensities between control and metal toxicity in muscle tissues, reflecting a change in protein contents due to heavy metals nickel, chromium and its mixtures. Further the bioaccumulation of heavy metals shows that the influence of one metal on another may exhibit a synergism effect.

Key words: Metal interaction, FTIR, ICP-AES, fish, protein.

INTRODUCTION

Heavy metals constitute a major component of the pollutants of the environment and they accumulate in the biosphere. Assessment of the levels of heavy metals in aquatic system is greatly important, since many of them are toxic even at low concentrations and also persist in the environment for a long period after the source has been removed. The discharge of heavy metal wastes into the receiving waters may result in numerous physical, chemical and biological changes. Heavy metals include a great variety of chemical elements that typically occur in low or trace amounts in the environment, all of which have the potential to provoke toxic effects in organisms [2]. Almost all metals are toxic at higher concentrations and some are lethal even at very low concentrations, although some metals within limits are essential for the life of aquatic organisms, plants as well as human beings to survive and function [14]. The toxicity of a metal depends on the inherent capacity of the metal to affect adversely any biological activity [13]. The response of biota to individual-metal-exposure may differ from its response to that of multiple metals, as mixtures of metals may interact antagonistically,

Received: April 2014;
in final form May 2014.

ROMANIAN J. BIOPHYS., Vol. 24, No. 2, P. 109–116, BUCHAREST, 2014

synergistically or additively. Bioconcentration of chemicals in aquatic biota is an important factor in the assessment of the potential hazard of chemicals to the environment [7-8,10-11]. 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 to that of water at equilibrium.

Fishes are good bioaccumulators and they accumulate metals many times more than that found in the ambient medium. As a secondary consumer, fish in the food chain has equal importance as it forms the major food for the higher tropic levels and is the best biological indicator of pollutant in an aquatic system [15]. Fishes are at the end of the aquatic food chain and may accumulate metals and pass them to human beings through food causing chronic or acute diseases [1]. The fish constitute one of the major sources of cheap nutrition for human beings. The nutritional value of different fish depends on their biochemical content like proteins, carbohydrates, amino acids, lipids and minerals. Protein plays a vital role in the physiology of living organisms. Proteins are highly sensitive to heavy metals and are one of the earliest indicators of heavy metal poisoning. The impairment in protein synthesis due to heavy metal stress has been reported by many investigators [5,7,17].

Spectroscopic techniques are extremely specific and provide a wealth of biochemical information about a given sample. The motivation for using spectroscopic techniques to assess cells and tissues is the fact that these methods detect chemical composition and compo vibrational spectroscopy is very useful. Bands in vibrational spectra are molecule specific and provide direct information about biochemical composition. They are relatively narrow, easy to resolve and sensitive to molecular structure, conformation and environment. Vibrational spectroscopy techniques can thus provide specific biochemical information of cells and tissues caused by variety of conditions and diseases. In particular, X-ray diffraction (XRD), circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy are limited in their application to biological membranes. However infrared spectroscopy has been extensively employed in the study of biological system in order to obtain indication about structural and chemical/physical properties at the molecular level. IR spectroscopic study should be able to provide information concerning the biochemical events underlying the transformation of healthy tissue to a diseased state. Fourier transform infrared (FT-IR) spectroscopy has recently emerged as a novel bio-medical technique; it has the potential to reveal a wealth of qualitative and quantitative information about a given biological sample. The increasing use of FT-IR spectroscopy demonstrates that this technique is a valuable tool because of its ability to monitor simultaneously protein, and lipid components. In the present study *C. mrigala* is chosen since it is the mostly abundant freshwater fish available in Tamil Nadu, India. Moreover, it can withstand to a wide range of experimental conditions and also due to its high value of nutrition. Many reports are available in heavy metals

toxicity but a limited study has been carried out in metal interaction. Keeping this in view an attempt has been made to study the toxic effect of heavy metals nickel and chromium and of their metal mixtures using the FTIR and ICP-AES technique since muscle tissue of *C. mrigala* is consumed by humans due to its high protein contents.

MATERIALS AND METHODS

EXPERIMENTAL STUDY

The test animals were divided into four groups. 20 each of the test animals were exposed to the respective concentrations such as Group 1 serves as controls, Groups 2 and 3 were exposed to 1/3rd of LC_{50} (2.87 ppm) of nickel and chromium separately. Group 4 were exposed to metal mixtures (nickel and chromium (1:1)) of 2.87 ppm for 7 days. The median lethal concentration 96h LC_{50} was found by the Probit method [3]. At the end of each exposure periods (seven days) muscle tissues was dissected and preserved at –20 °C prior to analysis.

INFRARED ANALYSIS

FT-IR spectra were recorded at room temperature (25±1 °C) on a Perkin Elmer-Spectrum RxI Spectrometer equipped with a mullard Ialanine doped deuterated triglycine sulphate (DTGS) detector installed at Centralised Instrumentation and Services Laboratory, Annamalai University. Pellets were scanned at room temperature in the 4000–400 cm^{-1} spectral ranges. 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 frequencies for all sharp bands were accurate to 0.001 cm^{-1}. Absorption intensity of the peaks was calculated by baseline method. Special care was taken to prepare the pellets at the same thickness by taking the same amount of sample and applying the same pressure. Therefore, in the present study it is possible to directly relate the intensities of the absorption bands to the concentration of the corresponding functional groups.

ESTIMATION OF NICKEL AND CHROMIUM

Muscle tissue samples were digested with concentrated nitric acid and perchloric acid by the standard digestion method [18]. The concentrations of Ni and Cr present in the tissues were estimated by ICP-AES available in the CAS in Marine biology, Annamalai University, Annamalai Nagar.

RESULTS AND DISCUSSION

EFFECT OF METAL MIXTURE ON TISSUE PROTEINS

FT-IR spectra of muscle tissues exposed to nickel, chromium and its mixture (1:1) along with control tissue have been presented in Fig. 1. The observed frequencies in the region 4000–400 cm^{-1} have also been given in Table 1 along with vibrational assignments and intensities. The infrared spectra of protein are characterized by a set of absorption regions 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, amide II and amide III. The amide I band arises principally from the C=0 stretching vibration of the peptide group. The amide II band is primarily N–H bending with a contribution from C–N stretching vibrations. The amide III absorption is normally weak, and arises primarily from N–H bending and C–N stretching vibrations. In the present study, the band observed at ~ 3293 cm^{-1} is due to OH/NH stretching vibration of water/primary amine. The spectral region between 2800 and 3000 cm^{-1} is due to CH stretching region of saturated hydrocarbons largely representing lipids and proteins. In these regions, the bands observed are: the CH_3 symmetric stretching (~2960 cm^{-1}), CH_2 asymmetric stretching (~2927 cm^{-1}) and the CH_2 symmetric stretching (~2874 cm^{-1}). The band observed at 1449 cm^{-1} is due to CH_3 asymmetric proteins. It is that bands observed at 1652 cm^{-1}, 1541 cm^{-1} and 1249 cm^{-1} are mainly due to amide I, amide II and amide III bands of proteins [4]. Also the weak band at ~1082 cm^{-1} is assigned to PO_2^- symmetric stretching of glycogen. The amide absorptions are considered sensitive to protein conformation; hence an increase or a decrease in the ratio of the intensities of the bands at ~1540 cm^{-1} (amide II) and ~1650 cm^{-1} (amide I) could be attributed a change in the composition of the whole protein [15].

In the present study the ratio of intensities between I_{1652} and I_{1541} (amide II/amide I) decreases from 0.58 to 0.50 for nickel treated samples, to 0.54 for chromium treated samples and to 0.48 for metal mixture (nickel and chromium), which reflects the change of about 13%, 7% and 17% for nickel, chromium and metal mixtures respectively (Table 2). Also the peak intensity ratio (I_{1541}/I_{3291}) due to nickel, chromium and its mixture decreases from 0.69 to 0.59, 0.61 and 0.57 respectively, which corresponds to a change of 14%, 11% and 17% respectively.

The ratio of the intensity of absorption of the 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 0.8 to 0.4, 0.2 & 0.33 for metals (nickel and chromium) and their mixtures. It is observed from these absorption bands a decrease of 50%, 75% and 59% of methyl group of proteins due to metals individually and in binary solution. Also, there is a reduction in the intensity of the absorption band in 1200–1000 cm^{-1} due to metal treatments. Also, the C=O stretching band (amide I) shifted from 1652 cm^{-1} to 1664 cm^{-1} for nickel treatment indicating either a structural rearrangement of the existing proteins or the expression of a new set of proteins.

Table 1

FT-IR frequency assignment of control, nickel, chromium and their metal mixtures of muscle tissues of *Cirrhinus mrigala*

Control	Interaction			Frequency assignment
	Nickel treatment	Chromium treatment	Ni+Cr treatment	
3293 (s)	3288 (m)	3301 (m)	3281 (vw)	O–H stretching / N-H stretching
2960 (m)	2960 (m)	2961 (w)	2960 (vw)	CH_3 symmetric stretching; lipid, protein
2927 (m)	2927 (w)	2930 (m)	2926 (vw)	CH_2 symmetric stretching; mainly lipid, protein
2874 (w)	2875 (vw)	2874 (vw)	2870 (vw)	CH_2 symmetric stretching; lipid, protein
1652 (vs)	1664 (m)	1653 (m)	1652 (w)	Inplane C=O stretching (Amide I)
1541 (s)	1534 (w)	1539 (m)	1539 (vw)	C–N stretching / N–H bending (Amide II)
1449 (m)	1448 (vw)	1449 (m)	1455 (vw)	CH_3 asymmetric bending; protein
1395 (m)	1395 (vw)	1393 (m)	1394 (vw)	CH_3 symmetric bending; protein
1240 (w)	1237 (vw)	1238 (vw)	1239 (vw)	PO_2^- asymmetric stretching /Amide III
1082 (w)	1084 (vw)	1081 (vw)	1080 (vw)	PO_2^- symmetric stretching (glycogen)

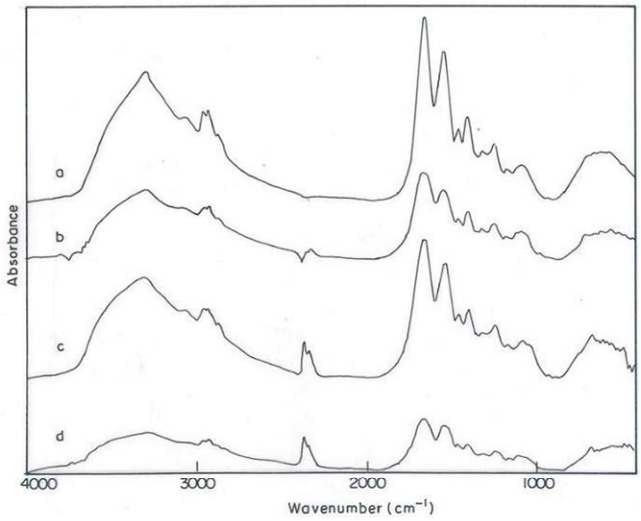

Fig. 1. FT-IR spectra of muscle tissue of *Cirrhinus mrigala* (a) control; (b) nickel treated; (c) chromium treated; (d) nickel+chromium treated.

BIOACCUMULATION OF METAL MIXTURES

It could be seen from the study of Table 3 that nickel levels as high as 50 times the normal nickel concentration in water were found in fishes exposed to 2.35 ppm of nickel whereas it was only 18 times that of the chromium concentration in water in fish exposed to 2.35 ppm of chromium.

Table 2

FT-IR absorption intensity ratios of selected bands in the muscle tissues of *Cirrhinus mrigala*

Treatment		I_{1541}/I_{1652}	I_{1541}/I_{3293}	I_{2960}/I_{2873}
Control		0.58	0.69	0.80
Interaction	Nickel treatment	0.50	0.59	0.40
	Chromium treatment	0.54	0.55	0.20
	Nickel+chromium treatment	0.48	0.61	0.33

It could also be seen that there is a slightly higher nickel content in fish from the chromium treated groups compared to the those exposed to nickel alone, leading to the conclusion that the presence of chromium promoted the retention of nickel by 51%. Similarly an increase of 43% chromium was observed in the presence of nickel. It could also be seen from the table that the combined effect of the metal mixtures is greater than that of individual exposure which exhibits the synergistic tendency of the metal. The possible explanation for enhanced accumulation is that the presence of one metal inhibits the elimination of another. Also, it has been observed that a considerable level of concentration of nickel and chromium was observed in control animals and it could be due to the presence of nickel (0.21±0.02 ppm) and chromium (0.14±0.07 ppm) in the water used in the present study which was drawn from the general source.

Table 3

Accumulation of nickel, chromium and their metal mixture in muscle tissues of *Cirrhinus mrigala* exposed to 7 days

Metal	Control	Ni	Cr	Ni+Cr
Ni treatment	3.652±0.226	17.462±3.456	–	36.283±1.626
Cr treatment	6.614±1.263	–	11.677±1.471	20.713±1.860

The results of the present study a low level of chromium and this may possibly explain the reduced bioavailability of chromium when compared to that of nickel. Also when the animals are exposed to nickel and chromium simultaneously

(2.35 mg Ni and 2.35 mg Cr) an increased absorption of nickel and chromium was observed in the muscle tissues. This increased bioavailability of nickel and chromium compared to that of the individual metal shows the synergetic effect among these metals. This confirms the combined exposure signifying the greater toxicity of the metal mixture compared to individual metals.

CONCLUSION

The results of the present study show that accumulation of nickel in the tissues of *Cirrhinus mrigala* is higher than that of chromium. It suggests that cell membranes are more permeable to nickel than to chromium which no doubt accounts for the greater toxicity of the former element to these organisms. The toxicity of nickel may be due to nickel being in contact with the skin (body surface), penetrating the epidermis and combining with body proteins. But, in the case of chromium Cr(VI) after entering the cell it is readily reduced to Cr(III). This intracellular reduction of Cr(VI) to Cr(III) helps maintain a low level of chromium and this may possibly explain the reduced bioavailability of chromium when compared to that of nickel.

The interaction of heavy metals nickel and chromium shows that nickel is more toxic than chromium and exhibits a synergism effect. 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. Thus it could be inferred that the secondary structure of an element is present with the protein from the position of the amide I band. The amide I band observed at ~1650 cm^{-1} indicates that the protein is predominantly in an α-helical structure. The observed shift towards high wave number and changes in band intensity due to metal mixture indicate either a structural rearrangement of the existing proteins or the expression of a new set of proteins.

REFERENCES

1. AL-YOUSUF, M.H., M.S. EL-SHAHAWI, S.M. AL-GHAIS, Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex, *Sci. Total Env.,* 2000, **256,** 87–94.
2. ANTÓN, A., T. SERRANO, E. ANGULO, G. FERRERO, A. RALLO, The use of two species of crayfish as environmental quality sentinels: the relationship between heavy metal content, cell and tissue biomarkers and physico-chemical characteristics of the environment, *Sci. Total Environ.*, 2000, **247**, 239–251.
3. FINNEY, D.J., *Probit Analysis,* Cambridge University Press, Cambridge, 1971.

4. HAALAND, D.M., H.D.T. JONES, E.V. THOMAS, Multivariate classification of the infrared spectra of cell and tissue samples, *Appl. Spect*., 1997, **51**, 340–345.
5. JACOBS, J.M., N. CARMICHAEL, J.B. CAVANAGH, Ultrastructural changes in the nervous system of rabbits poisoned with methyl mercury, *Tox. App. Pharmacol*., 1997, **39,** 249–261.
6. KARTHIKEYAN, S., C. KARTHIK, SELVI SABHANAYAGAM, Bioaccumulation and elimination of chromium in an edible fingerlings of *Cirrhinus mrigala* exposed to sub-lethal concentrations, *Biosci. Biotech. Res. Asia,* 2007, **4**, 589–594.
7. KARTHIKEYAN, S., FTIR and ICP-AES study of the effect of heavy metals nickel and chromium in tissue protein of an edible fish *Cirrhinus mrigala*, *Romanian J. Biophys.*, 2012, **22**, 95–105.
8. 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**, 489–492.
9. 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.
10. KARTHIKEYAN, S., P. MANI, Effect of heavy metals on tissue protein of an edible fish *Cirrhinus mrigala* as dependent on pH and water hardness, *Biophysics*, 2014, **59**, 321-325.
11. KARTHIKEYAN, S., PL.RM. PALANIAPPAN, SELVI SABHANAYAGAM, Bioaccumulation of nickel in various organs of fresh water fish *Cirrhinus mrigala* exposed to sub-lethal concentrations, *Ind. J. Environ. Prot.*, 2005, **25**, 629–634.
12. LOWERY, O.H., ROSEBROUGH, N.J., A.L. FARR, R.J. RANDALL, Protein measurement with the folin phenol reagent, *J. Biol. Chem.,* 1951, **193**, 265–275.
13. MEMMERT, U., Bioaccumulation of zinc in two freshwater organisms (*Daphnia magna*, crustacea and *Brachydanio rerio*, pisces), *Wat. Res.*, 1987, **21**, 99–106.
14. MWACHIRO, E.C., V.S. DURVE, Heavy metal status of the reservoir Bari near Udaipur (Rajasthan) and the accumulation of the metals in fish organs, *Poll. Res*., 1997, **16**, 67–74.
15. OZAKI, Y, F. KANEUCHI, Nondestructive analysis of biological materials by ATR/FT-IR spectroscopy. Part II: Potential of the ATR method in clinical studies of the internal organs, *Appl. Spect.,* 1989, **43**, 723–725.
16. SEBASTIN, C.D, *Freshwater Fishes, Handbook on Aquafarming*, Marine Products Export Development Authority, Kochi, India, 1995.
17. SYVERSEN, T.L.M, Effects of methyl mercury on protein synthesis *in vitro*, *Acta*. *Pharmacol*. *Ed. Toxicol*., 1981, **49**, 422–426.
18. TOPPING, G., Heavy metals in shellfish from Scottish water, *Aquaculture*, 1973, **1**, 379–384.