Indian Journal of Biochemistry & Biophysics
Vol. 56, December 2019, pp. 460-465

Impact of electroplating industry effluent on the electrophoretic protein pattern of serum in the freshwater fish *Cyprinus carpio*

VJ Florence Borgia[1], AJ Thatheyus[2]* & AG Murugesan[3]

[1]Department of Zoology, J. A. College for Women, Periyakulam- 625 601, Tamil Nadu, India
[2]Department of Zoology, The American College, Tallakkulam, Madurai– 625 002, Tamil Nadu, India
[3]Sri Paramakalyani Centre of Excellence in Environmental Sciences, M.S University, Alwarkurichi- 627 412, Tamil Nadu, India

Received 10 April 2018; *revised 23 May 2019*

The investigation on electrophoretic protein fractions of serum was carried out on the freshwater fish, *Cyprinus carpio* to determine the sublethal toxic effects of electroplating industrial effluent (EIE) using SDS-PAGE. Fish were exposed to 0.004, 0.007, 0.010 and 0.013% of effluent as well as control for twenty eight days. The results revealed the appearance or disappearance of protein fractions in the serum of *C. carpio* compared to control fish after seven, fourteen, twenty one and twenty eight days due to stress caused by metals in the effluent. Thus protein electrophoresis can be a sensitive tool for biomonitoring aquatic pollution.

Keywords: Biomonitoring, *Cyprinus carpio*, Electroplating Industrial Effluent, Serum proteins

Population explosion, use of technological advances, unbridled exploitation of natural resources, rapid industrialization, and anthropogenic activities in agriculture with scant regard for the preservation of ecological balance, have seriously affected the quality of water resources[1]. Among various pollutants, industrial wastes are the major sources of water pollution[2]. Several research works have been carried out to find out the toxic effects of pollutants in organs (such as liver, kidney, brain, and testes) and biomolecules (such as DNA, protein and lipid) of living organisms. The results revealed that most of the pollutants are capable of causing biochemical as well as structural changes in the tissues of living organisms which ultimately affect the entire ecological food chain. These morphological and biochemical changes are used as tools for the clinical diagnosis of diseases[3-10].

Electroplating is regarded as a major polluting industry since it releases toxic substances and heavy metals through effluents, air emissions and solid wastes into the environment[11,12]. Effluent from electroplating industry containing heavy metals put forth direct effects on aquatic health and fish survival. Fish are the most important source of protein and the nutritional value of different tissues of fish depends on their biochemical composition like protein, amino acids, vitamins and mineral contents[13]. Any pollutant in the natural water system would affect the health of aquatic organisms even at molecular level. The toxic effects on proteins of fish may lead to alteration in their structure, and subsequently in their building units of amino acids[14]. The development of electrophoretic techniques made it possible to identify changes in the protein profile of different tissues.

Earlier literature elucidated to the impact of exposure of different pollutants on the electrophoretic protein pattern in the tissues of fishes[15-25]. Hence the present investigation was undertaken to study the sublethal effects of electroplating industrial effluent on the electrophoretic protein pattern of serum in the freshwater fish, *Cyprinus carpio*.

Materials and Methods

Healthy freshwater fish *Cyprinus carpio* (25 ± 5 g) irrespective of sex were collected from a local fish farm and were acclimatized in well aerated, dechlorinated tap water for fifteen days in fibre tanks (150 L capacity). The effluent from electroplating industry was collected and transported immediately to the laboratory and stored in a refrigerator. The physico-chemical characteristics of the effluent and

*Correspondence:
E-mail: jthatheyus@yahoo.co.in

Abbreviations: EIE, Electroplating industrial effluent; KDa, Kilodalton; R_m, Relative mobility; SDS-PAGE, Sodium dodecyl sulphate - Polyacrylamide gel electrophoresis

the median lethal concentration values for 96 h exposure of fish to electroplating industry effluent (EIE) were determined[15]. The sublethal concentrations of 0.004, 0.007, 0.010 and 0.013% of EIE were selected for electrophoretic analysis. After acclimatization, five groups of fish, one for control and others for 0.004, 0.007, 0.010 and 0.013% of EIE were recruited and treated for twenty eight days. Each group housed ten fish (n=10) and the effluent concentrations in the test groups were renewed every day. Feeding was allowed for both experimental and control fish throughout the tenure of experiment. Blood samples were collected from the common cardinal vein situated just below the gills using 1 ml tuberculin syringe fitted with 24 gauge needle after 7, 14, 21, and 28 days of exposure. Blood was allowed to clot overnight at 4°C and was then centrifuged at 400 g for ten min for the serum to be separated. The serum was collected and stored in sterile eppendorf tubes at −20°C until electrophoretic separation. The protein fractions of serum of the fish were separated in SDS-PAGE[16] and were recorded. The molecular weight of the individual subunits of the protein was determined by calculating the relative mobility of the individual subunit using the following formula.

$$\text{Relative mobility } R_m \text{ value} = \frac{\text{Distance travelled by individual subunit}}{\text{Distance travelled by the marker dye}}$$

Results

Plate 1 represent the changes in protein banding pattern in the serum of fish exposed to EIE in comparison with control for 7, 14, 21 and 28 days, respectively. In protein electrophoretogram, the marker lane has seven protein bands withthe molecular weights 94, 79, 62, 46, 37, 28, and 13 KDa and with respective relative mobility (R_m) values of 0.35, 0.48, 0.57, 0.70, 0.77, 0.86, and 0.97 cm. On seventh day of treatment, four protein fractions with R_m values of 0.51, 0.59, 0.67 and 0.82 cm nearer to the molecular weight 79, 62, 46 and 28 KDa were identified in untreated control fish serum (Table 1 & Plate 1). The protein bands with the R_m value 0.59 and 0.82 cm and the molecular weight nearer to 62 and 28 KDa completely disappeared in the sublethal concentrations of EIE exposed fish compared to control fish serum.

On fourteenth day of exposure, the serum of control fish exhibited three protein fractions with R_m values 0.52, 0.70, and 0.80 cm nearer to the molecular weight 79, 46 and 37 KDa, respectively (Table 1 & Plate 1). The protein band with the R_m value 0.70 cm

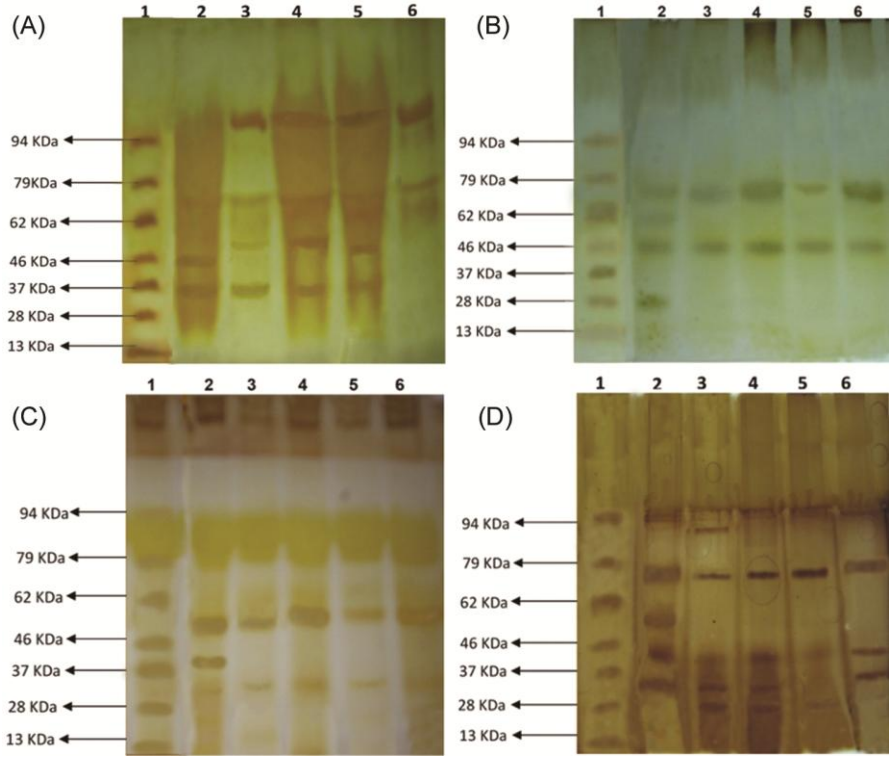

Plate 1 — Effect of EIE on the relative mobility of protein fractions after (A) seven, (B) fourteen, (C) twenty one; and (D) twenty eight days of treatment in the serum of *Cyprinus carpio*. 1- Marker, 2 - Control, 3 - 0.004% of EIE, 4 - 0.007% of EIE, 5 - 0.010% of EIE, 6 - 0.013% of EIE

close to the molecular weight 46 KDa was absent in all the effluent treated fish. Compared to control the new protein fraction with the R_m value 0.30 cm near to the molecular weight 94 KDa appeared in fish serum treated with 0.004, 0.007, 0.010 and 0.013% of EIE and the band with the R_m value 0.65 cm in between the molecular weight 62 and 46 KDa was identified in fish exposed to 0.004, 0.007, and 0.010% of EIE compared to control.

After exposure to EIE for twenty one days, in control fish serum five protein fractions were identified with the R_m values of 0.35, 0.51, 0.63, 0.73 cm and 0.82 cm near to the molecular weight 94, 79, 62, 46, and 28 KDa, respectively (Table 1 &

Table 1 — Changes in Relative Mobility (cm) of the Electrophoretic protein profile in serum due to chronic exposure to sublethal concentrations of electroplating industrial effluent for day seven,fourteen, twenty one and twenty eight post treatment in *Cyprinus carpio*.

Treatment period (Days)	Marker	Concentrations of electroplating industrial effluent (%)				
		0 (Control)	0.004	0.007	0.010	0.013
	0.35	-	-	-	-	-
7	0.48	-	-	-	-	-
	-	0.51	0.51	0.51	0.51	0.51
	0.57	-	-	-	-	-
	-	0.59	-	-	-	-
	-	0.67	0.67	0.67	0.67	0.67
	0.70	-	-	-	-	-
	0.77	-	-	-	-	-
	-	0.82	-	-	-	-
	0.86	-	-	-	-	-
	0.97	-	-	-	-	-
	-	-	0.30	0.30	0.30	0.30
14	0.35	-	-	-	-	-
	0.48	-	-	-	-	-
	-	0.52	0.52	0.52	0.52	0.52
	0.57	-	-	-	-	-
	-	-	0.65	0.65	0.65	-
	0.70	0.70	-	-	-	-
	0.77	-	-	-	-	-
	-	0.80	0.80	0.80	0.80	-
	0.86	-	-	-	-	-
	0.97	-	-	-	-	-
	0.35	0.35	-	-	-	-
21	-	-	0.38	-	-	-
	0.48	-	-	-	-	0.48
	-	0.51	0.51	0.51	0.51	-
	0.57	-	-	-	-	-
	-	0.63	-	-	-	-
	0.70	-	-	-	-	-
	-	0.73	0.73	0.73	0.73	0.73
	0.77	-	-	-	-	-
	-	-	-	-	-	0.80
	-	0.82	0.82	0.82	-	-
	0.86	-	0.86	0.86	0.86	-
	0.97	-	-	-	-	-
	0.35	-	-	-	-	-
28	0.48	-	-	-	-	-
	0.57	-	-	-	-	-
	-	-	-	0.62	0.62	0.62
	-	0.65	0.65	-	-	-
	0.70	-	-	-	-	-
	-	0.73	-	-	-	-
	0.77	-	-	-	-	-
	-	0.81	0.81	0.81	0.81	0.81
	0.86	-	-	-	-	0.86
	-	0.89	-	-	-	-
	0.97	-	-	-	-	-

Plate 1). The protein with the R_m value of 0.38 cm nearer to the molecular weight 94 KDa in fish exposed to 0.004% of EIE and the fractions with the R_m values 0.48 and 0.80 cm and the molecular weight 79 and 37 KDa in fish subjected to 0.013% of EIE were not matched with that of the control. The fraction with the R_m values 0.86 cm and the molecular weight 28 KDa was seen in fish exposed to 0.004, 0.007, and 0.010% of EIE which also not matched with that of the control. The bands with the R_m values 0.35 and 0.63 cm and the molecular weight nearer to 94 and 62 KDa were completely disappeared in the fish composed & sublethal concentrations compared to control fish.

On day twenty eight, the control fish serum exhibited four protein bands with the R_m values of 0.65, 0.73, 0.81 and 0.89 cm near to the molecular weight 46, 46, 37, and 28 KDa, respectively (Table 1 & Plate 1). The band with the R_m value 0.62 cm in between the molecular weight 62 and 46 KDa in fish exposed to 0.007, 0.010, and 0.013% of EIE and the band with the R_m value 0.86 cm and molecular weight 28 KDa in fish subjected to 0.013% of EIE were identified. The band with the R_m value of 0.65 cm nearer to the molecular weight 46 KDa disappeared in fish exposed to 0.007, 0.010 and 0.013% of EIE and the fractions with the R_m values of 0.73 and 0.89 cm near to the molecular weight 46 and 28 KDa noticed in control fish serum completely disappeared in all the experimental effluent treated fish.

Discussion

Proteins, the important constituents of animal tissues play a significant role in spare energy. Proteins are the primary effectors molecules of all living systems and any adaptive responses to environmental, physiological or pathological conditions will be reflected by alterations in protein activity or content. Hence, the study on the proteins of the cell is essential due to changes in protein profile during intoxication[26]. In the current work of protein electrophoretic analysis, several changes were noticed in protein banding patterns of serum of electroplating industrial effluent exposed *C. carpio*. The influence of physiology by electroplating industrial effluent is due to the interaction between the animal and the effluent. The appearance or disappearance of protein fractions in the serum of *C. carpio* may be because of the electroplating industrial effluent. Munshi *et al.*[27] observed the appearance and disappearance of some protein fractions in the serum of *Heteropneustes fossilis* after exposure to malathion. Badawy *et al.*[28] reported the electrophoretic serum proteinograms of *Clarias gariepinus*. Bano and Hasan[29] stated that the decline in serum albumin could be attributed to its disturbed synthesis due to functional effects on liver. Yilmaz *et al.*[19] detected overexpression of proteins and inhibition of proteins in the serum of *Capoeta capoeta capoeta* exposed to cobalt parahydroxy-benzoate. Jyothirmayee *et al.*[30] studied the alterations in the serum protein electrophoretic profile of the edible fish, *Anabas testudineus* and *Clarias batrachus* due to chromium and endosulfan, and revealed that these toxicants were carried by serum proteins, and stored in gill, liver and kidney before being excreted. Osman *et al.*[31] found variations in serum proteins of *Oreochromis niloticus* after exposure to different doses (1/5, 1/10 and 1/20 of LC_{50}) of copper sulphate and lead acetate for a period of 2, 4, and 6 weeks. *Clarias gariepinus* exposed to deltamethrin exhibited loss of some fractions in its serum proteinograms[32]. Sharf-Eldeen and Abdel-Hamid[33] also reported that copper induced disappearance of protein fractions in fish serum.

In the present investigation, the number of protein bands compared to control decreased from four to two, three to two, five to three and four to three after seven, fourteen, twenty one and twenty eight days of exposure to 0.013% of electroplating industrial effluent, respectively and the disappearance of bands was due to the interference of metals in protein synthesis. The reduction in protein bands may be because of the effect on protein synthesis or the depletion of reserve proteins for meeting the stress caused by metals. Palaniappan and Muthulingum[34] reported that the depletion of protein fraction may have been due to their degradation and possible utilization for metabolic purposes. In the current study new protein bands also appeared in the tested fish. New protein fractions may be attributed to toxicant induced cell damages. Damage of the tissue would have resulted in leakage of cellular proteins[35]. It can be inferred that these protein bands could be stress proteins due to metal exposure. Proteins are responsible for cellular functions based on physical stimuli. Based on the results, it can be inferred that protein electrophoresis is a sensitive tool for biomonitoring aquatic pollution[36].

In view of the importance of fish in the diet of human beings, it is necessary that biological monitoring of water and fish meat for consumption has to be undertaken regularly to ensure continuous

safety of food. Safe disposal of industrial effluents must be practiced to avoid the entry of toxic pollutants into the environment. Hence appropriate removal of heavy metals from electroplating industrial effluent is necessary before discharge into aquatic systems to improve aquatic health.

References

1 Gao L, Wang Z, Shan J, Chen J, Tang C & Yi M, Aquatic environmental changes and anthropogenic activities reflected by the sedimentary records of the Shima River, Southern China. *Environ poll*, 224 (2017) 70.

2 Hounslow A, *Water quality data: analysis and interpretation.* (CRC press) 2018, 12.

3 Nagpura NS, Srivastava R, Kumar R, Dabas A, Kushwaha B & Kumar P, Assessment of pollution river ganges by tannery effluents using genotoxicity biomarkers in murrel fish, *Channa punctatus* (Bloch). *Indian J Exp Biol*, 53 (2015) 476.

4 Toteja R, Seema M, Sripoorna S, Abraham JS & Gupta R, Influence of copper and cadmium toxicity on antioxidant enzyme activity in fresh water ciliates. *Indian J Exp Biol*, 55 (2017) 694.

5 Bhutia RD, Khandelwal B, Sherpa ML & Singh TA, Oxidation products of DNA, lipid and protein among the individuals progressing towards metabolic syndrome. *Indian J Biochem Biophys*, 56 (2019) 155.

6 Li Y, Ma X, Huang K & Bai Z, Applications of serum amino acid levels in identifications of cancer. *Indian J Biochem Biophys*, 56 (2019) 53.

7 Jaiswal A & Srivastava JP, Changes in reactive oxygen scavenging systems and protein profiles in maize roots in response to nitric oxide under waterlogging stress. *Indian J Biochem Biophys*, 55 (2018) 26.

8 Kumari K, Krishnan G, Pathakota, GB, Annam PK & Kumar S, Acute toxicity of copper sulphate on *Catla catla* larvae and its effect on expression of three commonly used housekeeping genes, *Indian J Biochem Biophys*, 55 (2018) 12.

9 Kumar P & Singh P, *Tribulus terrestris* ameliorates aluminium chloride-induced alterations in oxidative status and functional markers in the liver, kidney, brain and testis of the laboratory mouse. *Indian J Biochem Biophys*, 53 (2016) 179.

10 Zaidi S, Bhatnagar V, Patel AB, Sinha S, Choudhari R & Shivgotra V, neurobehavioural, biochemical and immunological manisfestations in workers exposed to organophosphate insecticides. *Indian J Biochem Biophys*, 52 (2015) 305.

11 Jiawei T, Yizhen Z, Jiajun S, Xuelu S, Chao S & Chunhui, Z, Occurrence and characteristics of perfluoroalkyl substances (PFASs) in electroplating industrial wastewater. *Water Sci Technol*, 79 (4) (2019) 731.

12 Kul M & Oskay KO, Separation and recovery of valuable metals from real mix electroplating wastewater by solvent extraction. *Hydrometallurgy*, 155 (2015) 153.

13 Muthukumaravel K, Kumarasamy P, Amsath A & Paulraj MG, Toxic effects of cadmium on the electrophoretic protein patterns of gill and muscle of *Oreochromis mossambicus*. *E J Chem*, 4 (2007), 284.

14 Olfat MW, Shyma MS & Ashraf YD, Effects of pulp and paper industrial effluent on some blood parameters, gonads and flesh proteins in experimentally exposed striped seabream *Lithognathus mormyrus*. *Egypt J Aquat Res*, 30 (2004) 25.

15 Sornaraj R, Thanalashmi S & Barskara P, Influence of heavy metals on biochemical responses of the freshwater air breathing fish *Channa punctatus. J Ecotoxicol Environ Monit*, 5 (1995) 19.

16 Kumari SA, Effect of water pollution on esterases in *Channa punctatus* (Bloch) from Hussainsagar Lake, Hyderabad. *Indian J Fish*, 50 (2003) 135.

17 Hamdy MA, Gene expression of heat shock protein (HsP70) in some body organs of the fish *Tilapia zillii* after exposure to the neem biopesticide triology. *J Egypt Ger Soc Zool*, 44 (2004) 1.

18 Rizkalla EH, Ramadan AA, Shalaby AM & Rashed MA, Electrophoretical variability in plasma protein of common carp (*Cyprinus carpio*) as a result of experimental exposure to different concentrations of copper, cadmium or zinc individually/ combined. *Third Conference of Role of Biochemistry in Environment and Agriculture, Cairo University, Egypt*, (2006) 27.

19 Yilmaz M, Ersan Y, Karaman M, Ozen H, Koc E & Necefoglu H, Toxic effects of cobalt parahydroxybenzonate on tissue histopathology and serum proteins in *Capoeta capoeta capoeta*. *Fresen Environ Bull*, 17 (2008) 1322.

20 Chaudhry AS & Jabeen F, Assessing metal, protein and DNA profiles in *Labeo rohita* from the Indus River in Mianwali, Pakistan. *Environ Monit Assess*, 174 (2011) 665.

21 Veeraiah K, Raju KJ, Padmavathi P, Rani AS & Vivek CH, Cadmium chloride induced changes in protein molecules of the freshwater fish *Cirrhinus mrigala* (Hamilton). *Innov J Life Sci*, 1 (2013) 18.

22 Veeraiah K, Padmavathi P, Rao ST & Vivek CH, Methyl parathion (50% EC) induced changes in protein and DNA banding patterns in the fish *Channa punctatus* (Bloch). *Int J Bioassays*, 4 (2014) 3632.

23 Athisuyambulingam M, Rao RS & Manambrakat V, Effect of copper on the characterization of proteins in the spiny lobster, *Panulirus homarus homarus* (Linnaeus, 1758). *Int J Aquat Biol*, 2 (2014) 129.

24 Sripriya L, Vijayalakshmi M, Sumathy R & Sharmila J, The impact of textile dyes on the biochemistry and histology of liver, a freshwater fish, tilapia, *Oreochromis mossambicus* (Peters). *Int J Pharma Bio Sci*, 5 (2014) 271.

25 Arivu I, Muniyan M, Muthulingam M, Parthiban P, Ambedkar G, Kamalkanth S & Anbarasan R, Effect of 2, 4-dichlorophenoxy acetic acid on protein changes of freshwater fingerlings *Labeo rohita* (Hamilton) under SDS-PAGE gel separation. *Int J Toxicol Appl Pharmacol*, 5 (2015) 7.

26 Suneetha K, Kumar KG & Veeraiah K. Changes in protein subunits induced by endosulfan and fenvalerate in freshwater fish *Labeo rohita* through SDS-PAGE. *J Environ Biol*, 31 (2010) 759.

27 Munshi JD, Dutta HM, Singh NK, Roy PK, Adhikari S, Dogra JV & Ali MM, Effect of malathion, an organophosphorus pesticide, on the serum proteins of *Heteropneustes fossilis* (Bloch). *J Environ Pathol Toxicol Oncol*, 18 (1999) 79.

28 Badawy EA, El-Serafy SS & Menofiya J, Comparative biochemical genetic studies on *Clarias gariepinus* from different polluted localities. *Agric Res*, 23 (1998) 1705.

29 Bano Y & Hasan M, Histopathological lesions in the body organs of catfish (*Heteropneustes fossilis*) following mercury intoxication. *J Environ Sci Health*, 25 (1990) 67.

30 Jyothirmayee S, Reddy V, Theophilus J, Nagaraju T, Balaravi P & Reddy PU, Effect of chromium and endosulfan on serum proteins of *Anabas testudineus* and *Clarias batrachus* – A comparative study. *Indian J Comp Anim Physiol*, 24 (2006) 72.

31 Osman MM, El-Fiky SA, Soheir YM & Abeer AI, Impact of water pollution on histopathological and electrophoretic characters of *Oreochromis niloticus* fish. *Res J Environ Toxicol*, 3 (2009) 9.

32 Salama MAM, *Experimental studies on the cytogenetics of a certain freshwater fish*, Ph.D. Thesis, Zagazig University, Sharkia, Egypt, (2001) 162.

33 Sharaf-Eldeen K & Abdel-Hamid NH, Sublethal effects of copper sulfate, malathion and paraquat on protein pattern of *Oreochromis niloticus*. *Egypt J Aqua Biol Fish*, 6 (2002) 167.

34 Palaniappan R & Muthulingam M, Effect of Heavy Metal, Chromium on Protein and Amino Acid Contents in Gill, Liver and Kidney of Freshwater Fish, *Channa striatus* (Bloch). *Int J Curr Microbiol App Sci*, 5 (2016) 372.

35 Benninghoff AD, Toxicoproteomics – The next step in the evolution of biological monitoring. *Biochem Phys*, 86 (2007) 233.

36 Osman AGM, Al-Awadhi RM, Harabawy ASA & Mahmoud UM, Evaluation of the use of protein electrophoresis of the African catfish *Clarias gariepinus* (Burchell, 1822) for biomonitoring aquatic pollution. *Environ Res J*, 4 (2010) 235.