Pollution Induced Qualitative Variations of Protein in Selected Edible Fish Species of Adayar Estuary, Chennai, Tamil Nadu, India

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***Abstract*— The present study was to evaluate the impact of heavy metal toxicity in estuarine fishes Mugil cephalus and Elops saurus. Much research has used the protein electrophoresis as a valid tool to determine intra and inter-specific variation and health of fish. The control and experimental fishes were dissected out, the protein contents of tissue samples (Muscle, gill, liver, kidney, and brain) were analyzed for alterations in the protein profile using SDS PAGE technique. The protein profile found to be varied much from the control samples.**

Index Terms- SDS PAGE, Heavy metals, toxic effects, protein, muscle, gill, liver, kidney, brain, Mugil cephalus, Elops saurus.

I. INTRODUCTION

Fish constitute approximately half of all the described vertebrates. Fish is a more and more common food and consumption has been increasing at a rate of 3.6% annually since 1961. When regional and national variations are considered into account, per capita consumption has risen from 19.7 kg to 27.7 kg in industrialized countries, where diets generally contain a more diversified range of animal proteins [24].

Proteins are the important biomolecules in a wide spectrum of cellular functions. They interplay between enzymatic and non-enzymatic proteins to govern the metabolic harmony [8]. They are also involved in all the major physiological events to maintain the homeoststis of the cell. Therefore, the estimation of proteins can be considered as a diagnostic tool to regulate the physiological process of cell [10].

The electrophoresis of proteins is a valuable technique for producing systematic data from macromolecules. SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis, is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility. In the past, the identification of fish species was carried out mainly by examining the external morphological characteristics [5].

Electrophoresis of Sarcoplasmic proteins, serum proteins, liver proteins and a number of enzymes has often been used as an aid in the species identification of fish [4], [9]. Soluble proteins of muscle sarcoplasm are amongst the easiest to extract and highly a rich reservoir of species specific and biochemical genetic markers [25], [14], [17], and [1]. The highly water-soluble sarcoplasmic proteins consisting of myoglobin, glycolytic enzymes and other proteins present in intracellular fluid of muscle is frequently used for specific identification [5].

II. MATERIALS AND METHODS

2.1 SAMPLES: Approximately four to five specimens from each species of Mugil cephalus and Elops saurus were collected from Adayar and used as test samples while similar samples from Kovalam estuary (least polluted site) were treated as control [12].

2.2 EXTRACT PREPARATION: The fishes were transported to the laboratory and the tissue samples from collected fish were selected for electrophoresis. Muscle, gill, liver, kidney and brain tissue were cleaned and homogenized in Tris-HCl buffer (pH 6.8). The homogenate was centrifuged for 15 min at +4ºC and 20,000g. The supernatant obtained was used for analysis of proteins by SDS-PAGE.

2.3 ELECTROPHORESIS AND STAINING: SDS-PAGE was performed following the protocol developed by Laemmli and O'Farrell [7] and [13] with slight modifications.

III. RESULTS AND DISCUSSIONS

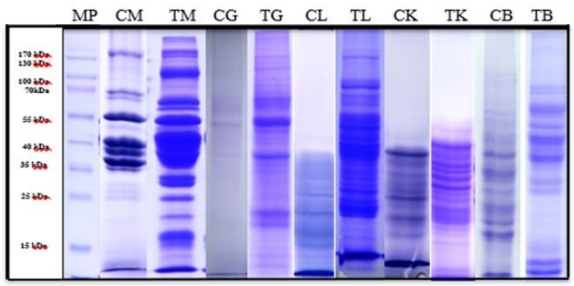
	Band	1	2	3	4	5	6	7	8	9
Marker Protein	RF	0.1	0.14	0.22	0.29	0.39	0.52	0.6	0.76	0.98
	Molecular Weight(kDa)	170	130	100	70	55	40	35	25	15

The fishes collected from Adayar estuary and Kovalam estuary (least polluted site) reveal a definite pattern of variation in protein fractions. (Figures 1 and 7) Muscle, Gill, Liver, Kidney and Brain tissue were dissected out and subjected to SDS – PAGE Electrophoretic analysis. The obtained results were tabulated and protein bands were analysed densitometrically. Densitometric pattern for Muscle, Gill, Liver, Kidney and Brain of Mugil cephalus and Elops saurus reveals a significant quantitative change of molecular weights.

3.1 Banding pattern in the tissues of control and Tested Fish Mugil cephalus

The number of protein bands in control muscle of Mugil cephalus muscle was found to be 17 and the molecular weight ranged from 160 kDa to 22 kDa. In the tested muscle the protein bands were 23 and the molecular weights ranged from 216 kDa to 17 kDa (Figure: 2 and Table: 1). In and the gill tissues of control fish the bands were only 7 with the molecular weights between 96 kDa and 21 kDa, but the tested gills showed 20 bands with the molecular weights ranging from 228 kDa to 28 kDa (Figure:3 and Table: 2). The liver of control fish showed 9 bands having the molecular weights were ranging from 59 kDa to 27 kDa and that of tested showed 21 bands ranging from 236 kDa to 16 kDa (Figure: 4 and Table: 3). The kidney tissue of control had 10 bands with molecular weight ranging from 46 kDa to 21 kDa and that of tested had 17 bands with the molecular weight ranging from 54 kDa to 23 kDa (Figure: 5 and Table: 4). The brain tissue of both control and affected showed 15 bands with slight variation in the molecular weight. The molecular weights of control was between 56 kDa and 17 kDa and that of tested ranged between 88 kDa to 17 kDa (Figure: 6 and Table: 5).

Figure: 1 SDS-PAGE analysis of proteins isolated from Mugil cephalus Tissue



MP: Marker Protein, CM: Control Muscle, TM: Tested Muscle, CG: Control Gill, TG: Tested Gill, CL: Control Liver, TL: Tested Liver, CK: Control Kidney, TK: Tested Kidney, CB: Control Brain, TB: Tested Brain.

Figure: 2 Electropherogram and densitometric scan images of protein profile in the muscle tissue of *Mugil cephalus*

Control Tested

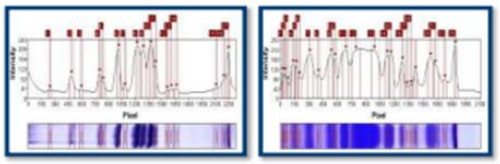


Table 1: Protein banding patterns, relative mobility (Rf) and molecular weights of Muscle tissue of *Mugil cephalus*

Control			Tested		
Band	RF	Molecular weight (kDa)	Band	RF	Molecular weight (kDa)
1	0.105	160	1	0.012	216
2	0.209	103	2	0.025	201
3	0.255	86	3	0.038	187
4	0.344	62	4	0.064	163
5	0.362	58	5	0.08	149
6	0.435	46	6	0.096	137
7	0.465	42	7	0.148	104
8	0.526	36	8	0.191	84
9	0.554	34	9	0.254	63
10	0.59	32	10	0.274	58
11	0.613	31	11	0.327	46
12	0.669	28	12	0.377	38
13	0.689	27	13	0.469	29
14	0.713	26	14	0.533	25
15	0.904	22	15	0.553	24
16	0.942	22	16	0.609	22
17	0.965	22	17	0.632	21
			18	0.652	20
			19	0.674	20
			20	0.749	18
			21	0.774	18
			22	0.821	18
			23	0.873	17

Figure: 3 Electropherogram and densitometric scan images of protein profile in the Gill tissue of Mugil *cephalus*

Control Tested

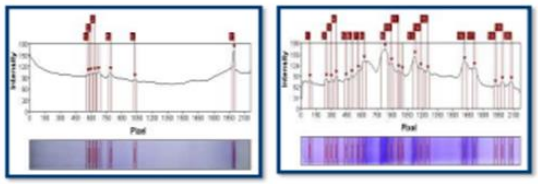


Table: 2 Protein banding patterns, relative mobility (Rf) and molecular weights of Gill tissue of *Mugil cephalus*

Control			Tested		
Band	RF	Molecular weight (kDa)	Band	RF	Molecular weight (kDa)
1	0.266	96	1	0.041	228
2	0.277	93	2	0.115	164
3	0.295	87	3	0.139	148
4	0.312	82	4	0.162	134
5	0.366	68	5	0.205	113
6	0.477	48	6	0.231	102
7	0.925	21	7	0.262	91
			8	0.288	83
			9	0.383	61
			10	0.414	56
			11	0.445	51
			12	0.462	49
			13	0.519	43
			14	0.546	41
			15	0.577	39
			16	0.746	31
			17	0.79	30
			18	0.887	29
			19	0.917	28
			20	0.958	28

Figure: 4 Electropherogram and densitometric scan images of protein profile in the Liver tissue of *Mugil cephalus*

Control Tested

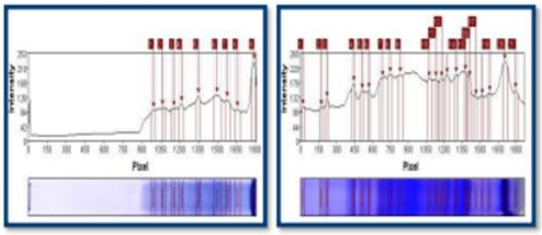


Table: 3 Protein banding patterns, relative mobility (Rf) and molecular weights of Liver tissue of *Mugil cephalus*

Control			Tested		
Band	RF	Molecular weight (kDa)	Band	RF	Molecular weight (kDa)
1	0.55	59	1	0.01	236
2	0.589	54	2	0.091	172
3	0.639	48	3	0.118	155
4	0.672	45	4	0.237	99
5	0.746	39	5	0.274	86
6	0.827	34	6	0.305	77
7	0.872	31	7	0.366	63
8	0.918	29	8	0.401	56
9	0.991	27	9	0.446	48
			10	0.573	33
			11	0.604	31
			12	0.63	29
			13	0.654	27
			14	0.695	25
			15	0.737	23
			16	0.755	22
			17	0.783	21
			18	0.811	20
			19	0.844	19
			20	0.912	17
			21	0.961	16

Figure: 5 Electropherogram and densitometric scan images of protein profile in the Kidney tissue of *Mugil cephalus*

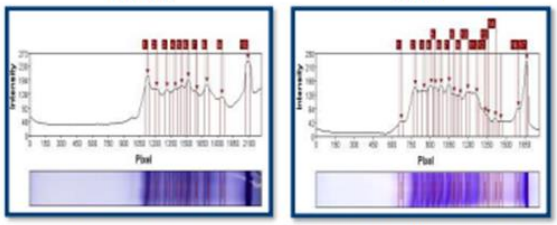


Table: 4 Protein banding patterns, relative mobility (Rf) and molecular weights of Kidney tissue of *Mugil cephalus*

Control			Tested		
Band	RF	Molecular weight (kDa)	Band	RF	Molecular weight (kDa)
1	0.509	46	1	0.382	54
2	0.548	41	2	0.445	44
3	0.592	37	3	0.484	39
4	0.628	34	4	0.515	37
5	0.656	32	5	0.534	35
6	0.684	31	6	0.56	33
7	0.721	28	7	0.596	31
8	0.765	26	8	0.618	30
9	0.83	24	9	0.647	29
10	0.941	21	10	0.68	27
			11	0.719	26
			12	0.759	25
			13	0.772	25
			14	0.803	25
			15	0.828	24
			16	0.907	23
			17	0.943	23

Figure: 6 Electropherogram and densitometric scan images of protein profile in the Brain tissue of *Mugil cephalus*

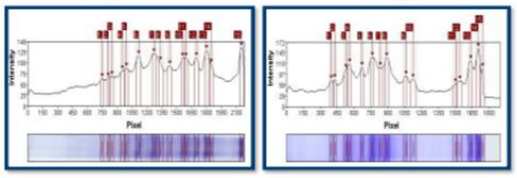


Table: 5 Protein banding patterns, relative mobility (Rf) and molecular weights of Brain tissue of *Mugil cephalus*

Control			Tested		
Band	RF	Molecular weight (kDa)	Band	RF	Molecular weight (kDa)
1	0.344	56	1	0.209	88
2	0.372	51	2	0.227	81
3	0.389	48	3	0.279	65
4	0.438	41	4	0.295	61
5	0.456	39	5	0.353	49
6	0.511	33	6	0.402	41
7	0.583	28	7	0.438	37
8	0.613	26	8	0.469	34
9	0.656	24	9	0.559	27
10	0.716	22	10	0.593	25
11	0.731	21	11	0.791	19
12	0.78	20	12	0.811	18
13	0.827	19	13	0.861	18
14	0.848	19	14	0.897	17
15	0.986	17	15	0.917	17

Figure 7: Boxplot Showing the Molecular Weight of Mugil cephalus Categorized by Tissue

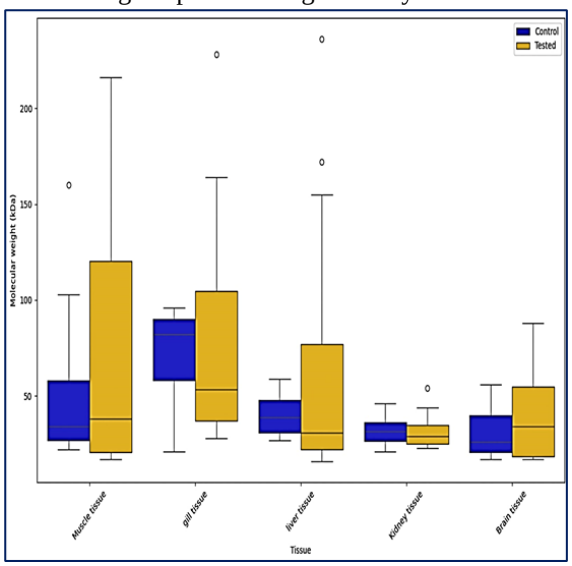
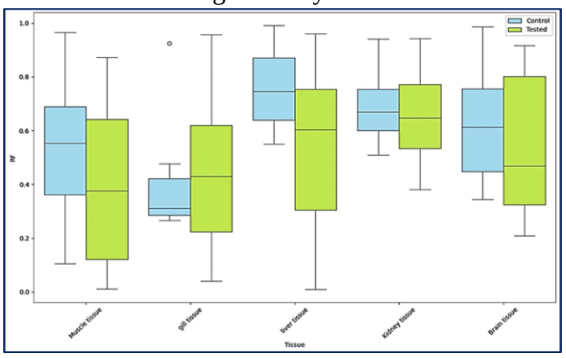


Figure 8: Boxplot Showing the RF of Mugil cephalus Categorized by Tissue

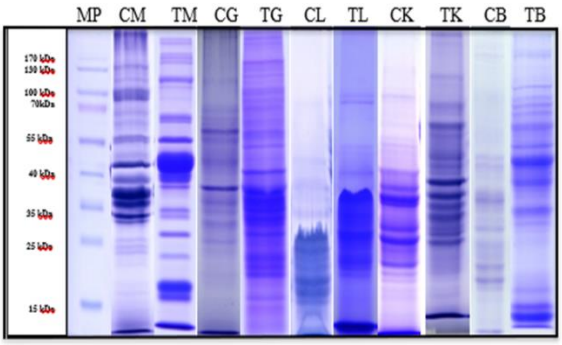


3.2 Banding pattern in the tissues of control and Tested Fish Elops saurus

The muscle tissue of control Elops saurus showed similar number of protein bands of 22 but the molecular weight of control ranged from 232 kDa to 22 kDa and that of tested ranged from 216 kDa to 17 kDa (Figure: 10 and Table: 6). The gill of control had 14 bands with molecular weights ranging from 262 kDa to 21 kDa and that of tested had 24 bands having molecular weights in range 262 kDa to 28kDa (Figure: 11 and Table: 7). The liver of control had 10 bands with molecular weight 138 kDa to 31 kDa and that of tested had 13 protein bands with molecular weights 108 kDa to 16 kDa (Figure: 12 and Table: 8). The kidney tissue of control had 15 bands with molecular weight ranging between 108 kDa to 23 kDa and that of tested had 19 bands with the molecular weight 235

kDa to 21 kDa (Figure: 13 and Table: 9). The brain tissue of control had 11 bands with molecular weight 160 kDa to 17 kDa and tested brain showed 15 bands with molecular weight ranging between 166 kDa to 17 kDa (Figure: 14 and Table: 10).

Figure: 9 Banding pattern of Elops saurus Tissue



MP: Marker Protein, CM: Control Muscle, TM: Tested Muscle, CG: Control Gill, TG: Tested Gill, CL: Control Liver, TL: Tested Liver, CK: Control Kidney, TK: Tested Kidney, CB: Control Brain, TB: Tested Brain.

Figure: 10 Electropherogram and densitometric scan images of protein profile in the muscle tissue of *Elops saurus*

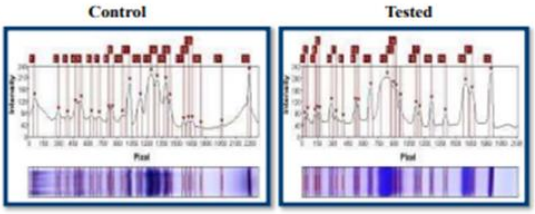


Table: 6 Protein banding patterns, relative mobility (Rf) and Molecular weights of Muscle tissue of *Elops saurus*

Control			Tested		
Band	RF	Molecular weight (kDa)	Band	RF	Molecular weight (kDa)
1	0.024	232	1	0.012	216
2	0.128	145	2	0.026	200
3	0.169	121	3	0.059	167
4	0.204	105	4	0.07	157
5	0.228	95	5	0.082	148
6	0.272	80	6	0.136	111
7	0.306	71	7	0.157	100
8	0.347	61	8	0.191	84
9	0.365	57	9	0.247	65
10	0.408	50	10	0.261	61
11	0.439	46	11	0.319	48
12	0.486	40	12	0.394	36
13	0.533	36	13	0.425	33
14	0.559	34	14	0.435	32
15	0.597	31	15	0.456	30
16	0.615	30	16	0.527	25
17	0.674	28	17	0.544	24
18	0.695	27	18	0.6	22
19	0.712	26	19	0.664	20
20	0.75	25	20	0.758	18
21	0.842	23	21	0.787	18
22	0.961	22	22	0.876	17

Figure: 11 Electropherogram and densitometric scan images of protein profile in the Gill tissue of *Elops saurus*

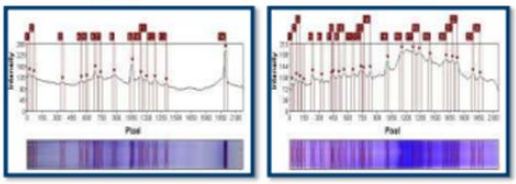


Table: 7 Protein banding patterns, relative mobility (Rf) and molecular weights of Gill tissue of *Elops saurus*

Control			Tested		
Band	RF	Molecular weight (kDa)	Band	RF	Molecular weight (kDa)
1	0.011	262	1	0.011	262
2	0.032	241	2	0.031	239
3	0.164	142	3	0.049	220
4	0.253	101	4	0.067	203
5	0.277	93	5	0.11	168
6	0.315	81	6	0.154	139
7	0.34	74	7	0.2	115
8	0.403	60	8	0.21	111
9	0.487	47	9	0.236	100
10	0.528	42	10	0.26	91
11	0.554	39	11	0.302	79
12	0.593	35	12	0.341	69
13	0.645	31	13	0.353	67
14	0.92	21	14	0.385	60
			15	0.47	48
			16	0.536	42
			17	0.591	38
			18	0.621	36
			19	0.672	34
			20	0.749	31
			21	0.779	31
			22	0.802	30
			23	0.879	29
			24	0.912	28

Figure 12: Electropherogram and densitometric scan images of protein profile in the Liver tissue of *Elops saurus*

Control Tested

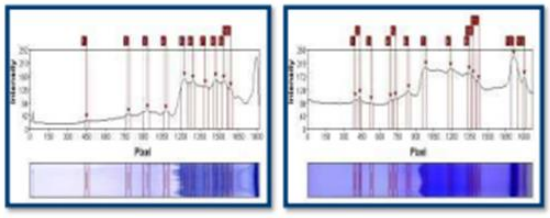


Table: 8 Protein banding patterns, relative mobility (Rf) and molecular weights of Liver tissue of *Elops saurus*

Control			Tested		
Band	RF	Molecular weight (kDa)	Band	RF	Molecular weight (kDa)
1	0.246	138	1	0.212	108
2	0.431	80	2	0.232	101
3	0.512	65	3	0.282	84
4	0.593	53	4	0.368	62
5	0.673	45	5	0.391	58
6	0.71	41	6	0.448	48
7	0.764	37	7	0.525	38
8	0.81	34	8	0.636	28
9	0.845	33	9	0.718	24
10	0.875	31	10	0.736	23
			11	0.761	22
			12	0.916	17
			13	0.964	16

Figure 13: Electropherogram and densitometric scan images of protein profile in the Kidney tissue of *Elops saurus*

Control Tested

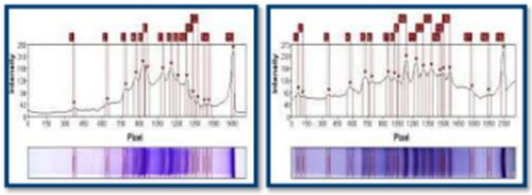


Table: 9 Protein banding patterns, relative mobility (Rf) and molecular weights of Kidney tissue of *Elops saurus*

Control			Tested		
Band	RF	Molecular weight (kDa)	Band	RF	Molecular weight (kDa)
1	0.212	108	1	0.03	235
2	0.364	57	2	0.051	216
3	0.449	43	3	0.166	140
4	0.495	38	4	0.261	100
5	0.527	36	5	0.328	79
6	0.547	34	6	0.359	72
7	0.619	30	7	0.432	57
8	0.658	28	8	0.46	53
9	0.688	27	9	0.479	50
10	0.729	26	10	0.513	45
11	0.758	25	11	0.557	40
12	0.779	25	12	0.589	37
13	0.81	24	13	0.625	34
14	0.829	24	14	0.663	32
15	0.942	23	15	0.678	31
			16	0.707	29
			17	0.806	25
			18	0.881	22
			19	0.948	21

Figure: 14 Electropherogram and densitometric scan images of protein profile in the Brain tissue of *Elops saurus*

Control Tested

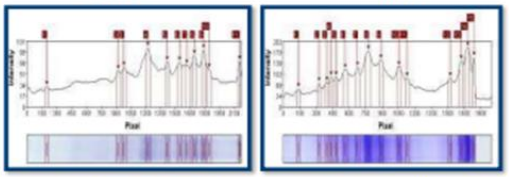


Table: 10 Protein banding patterns, relative mobility (Rf) and molecular weights of Brain tissue of *Elops saurus*

Control			Tested		
Band	RF	Molecular weight (kDa)	Band	RF	Molecular weight (kDa)
1	0.091	160	1	0.073	166
2	0.425	43	2	0.172	104
3	0.452	39	3	0.209	88
4	0.564	29	4	0.23	80
5	0.655	24	5	0.254	73
6	0.711	22	6	0.297	61
7	0.745	21	7	0.357	48
8	0.781	20	8	0.409	40
9	0.825	19	9	0.468	34
10	0.851	19	10	0.555	27
11	0.992	17	11	0.594	25
			12	0.802	18
			13	0.855	18
			14	0.886	17
			15	0.915	17

Figure 15: Boxplot Showing the Molecular Weight of Elops saurus Categorized by Tissue

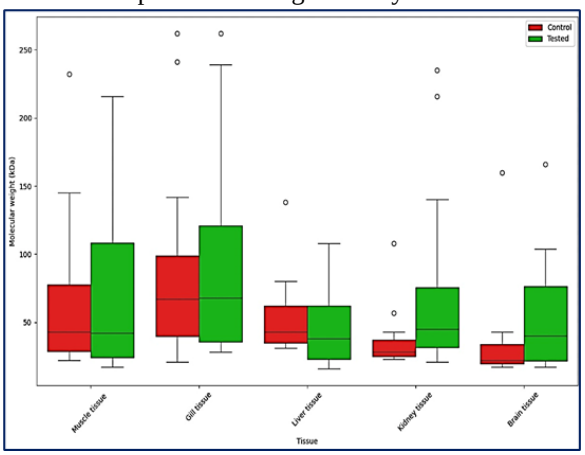


Figure 16: Boxplot Showing the RF of Elops saurus Categorized by Tissue

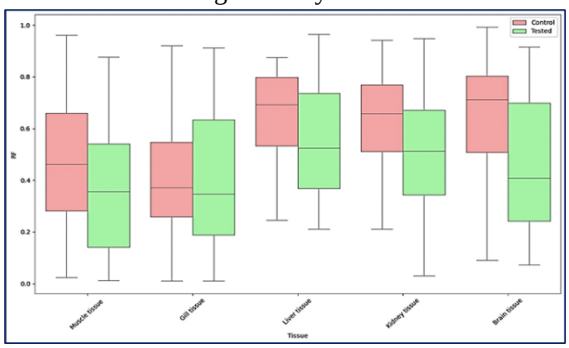


Figure 7 and 15 are the box plot showing the molecular weight of Mugil cephalus and Elops saurus categorized by tissue. Figure 8 and 16 refers the RF values. These boxplots provide a concise summary of the data and provide a robust view of the data's central tendency and distribution.

Proteins are among the most abundant biological molecules present in living tissue and have varied functions in various biochemical and physiological processes. Protein expression profile is a good approach to visualize the pattern of proteins expressed under stress [27] and [20]. SDS PAGE is used in identification of protein expression, apart from this the protein profiles may present useful tool for the understanding of response mechanisms triggered by changes in the environment [16]. Protein profiling study is very useful in assessing the fishes at various climatic and diseased conditions as it is highly useful in exploiting health status of fishes. Similar observations were observed by Vidya Sagar reddy and Vijaya, [26]. Agarose gel electrophoresis is performed routinely by molecular biologists as both an analytical and a preparative method for characterization of nucleic acids [3].

In the present study the tissues of the control fish species showed less number of protein bands except the brain tissue of control and tested Mugil cephalus and muscle tissue of control and tested Elops saurus which has same number of protein bands with slight variations in the molecular weights. The tissues of the tested fish species showed increase in number of protein bands with much variation in their molecular weights. This is in accordance with the study by Depasree et al., [2] in Channa punctatus exposed to fungicide. Occurrence of many new proteins in the tissues of the fish species of Adyar estuary could be the stress proteins to overcome the toxic effect of heavy metals. Similar findings were reported by Muthukumaravel et al., [11] in the fish Oreochromis mossambicus exposed to cadmium, by Salahuddin and Khola [18] in the tissues of fresh water fish Channa gachua and by Shalaka Sadekarpawar in oreochromis mossambicus and Labeo rohita exposed to plant nutrient [19].

Proteins play a very important role in the physiology of living organisms. All physiological activities are regulated by enzyme and hormones, which are also proteins. If any alteration takes place in the proteins, it may have an antagonistic impression on their vital and complex groups of biological materials, proteins comprise the nitrogenous constituents of the body, thus they perform different biological functions. Similar observations were also made by Prashanth [15] and Sheik Mohamed Salahudeen et al., [21].

Biomarkers refer typically to physiological or biochemical responses that serve as sensitive indicators of exposure to contaminants and or sublethal stress. Whereas the reaction to environmental perturbations starts at cellular level, the use of stress proteins as markers provides an early warning to prevent damage to higher organizational levels. The relationship between exposure to stressors such as heavy metals or environmental toxins and synthesis of stress proteins has the potential to act as significant indicator of environmental stress.

According to Tahmina Hoq and Asha Rani Das [23] the use of stress proteins as biomarkers may serve as an indicator of environmental perturbation in situations where the response to stress in chronic, sublethal exposures and it is still possible to prevent the biological consequences of exposures which affect organismal or higher organizational levels and according to them the perseverance of the stress response may correlate with the intensity of the stressor. If there is continuous exposure to severe environmental stress may lead to a physiological state from which the animal can no longer maintain the stress protein response and recover and ultimately lead to death of the organism and this may also be related to the present study where the fishes were taken from the natural environment.

According to Tahmina Hoq and Asha Rani Das [23]. A dominant band of 70 kDa is considered stress protein for biomarker is easy to use and sensitive for assessing water quality. Stress disrupts the regular conformation of proteins demanding the aid of HSP gene to regularize the effect. In fish, stress might be due to any minor alteration in the environment. Fish are exposed to numerous kinds of stressors such as microbial infection, toxic exposure, traumatic damage, radiation, or nutritional deficiency. Therefore, the modulation of HSP genes is more apparent in fish in producing 70 KDa protein and can be studied as molecular biomarkers of stress [6]. because under adverse physiological conditions, there is a notable elevation of these proteins [22]. Comparing this with the present study it can be concluded that even though there were expression of many genes and increased the production of protein bands the fish species did not produce the stress protein of 70 KDa. This indicates that the fish species exposed to similar concentration of heavy metals for long term and this might have helped in over coming the stress and adopting for the natural environment.

CONCLUSION

The present study points out only the initial but relevant information on aspects of the response of fish to environmental stresses. Continuous exposure to environmental stress which changed according to the seasons may lead to a physiological state from which the animal was able to maintain and overcome the stress and recover. The results of the polyacrylamide gel electrophoresis (SDS-PAGE) and densitometric analysis demonstrated that the number of protein bands in the test samples were more comparing with the control samples. The increase in number of protein bands may be due to the expression of many genes. Even though there were expression of many genes from the study it is clear that the stress protein of 70 KDa was not produced. This shows the fish species were able to cope up with the pollutants in its natural ecosystem.

REFERENCES

[1] Buth, D.J. & Murphy, R.W. (1999). The use of isozyme characters in systematic studies. Bio Syst Ecol, 27, 117-129.

[2] Deepasree, M.I. & Rajendran Nair, M.S. (2017). Histological and Protein profile alterations of liver of freshwater fish Channa punctatus (Bloch), on exposure to fungicide, Fytran. International Journal of Science and Research, 6(6), 1370-1374.

[3] Drew S. Sowersby, L. Kevin Lewis. (2024). SURE gel electrophoresis: A method for improved detection and purification of dilute nucleic acid samples, Analytical Biochemistry, Analytical Biochemistry, vol.684 ISSN 0003-2697, https://doi.org/10.1016/j.ab.2023.115373.

[4] Focant ,B., Jacob, M.F. & Huriaux, F. (1981) Electrophoretic comparison of the proteins of some perch (Perca fluviatilis L.) head muscles. J. Muscle Res. Cell Motil. 2(3), 295-305.

[5] George Jesslin, Arumugam Sabaridasan, Ponnu Edwinthangam, Radhakrishanan Palanikani & Ramaiah Soranam. (2013). Electrophoretic Studies on the Muscle Proteins of Three Species of Genus Puntius (Osteichthyes - Cyprinidae) International Journal of Research in Fisheries and Aquaculture, 3(2): 16-20

[6] Kayhan, F. E., & Susleyici Duman, B. (2010). Heat Shock Protein Genes in Fish.Turkish Journal of Fisheries and Aquatic Sciences, 10(2), 287–293. http://doi.org/10.4194/trjfas.2010.0218.

[7] Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the Head of Bacteriophage T4, Nature, 227 (5259), 680-685

[8] Lehninger, A. (1984). Principles of Biochemistry, Published by Worth Publishers Inc.,U.S. ISBN 10: 087901136X ISBN 13: 9780879011369. Worth Publishers

[9] Miyazaki, J.I., Hirabayashi, T., Hosoya, K. & Iwami, T.A. (1998) Study of the systematics of cyprinid fishes by two dimensional gel electrophoresis. Environ. Biol. Fish. 52, 173-179.

[10] Mushigeri, (2003). Effects of fenvalerate on the metabolism ofIndian major carp Cirrhinus mrigala, Ph.D. thesis, Karnataka University, Dharwad, Karnataka, India.

[11] Muthukumaravel, K., Kumarasamy, P., Amsath, A., & Paulraj, M. G. (2007). Toxic Effect of Cadmium on the Electrophoretic Protein Patterns of Gill and Muscle of Oreochromis mossambicus. E-Journal of Chemistry, 4(2), 284–286. http://doi.org/10.1155/2007/834538.

[12] Nandini S and M. C. John Milton (2018) Assessment On Heavy Metals In Water And Sediments Of Adayar Estuary, Tamil Nadu, India. IJRAR September 2018, Volume 5, Issue 3 www.ijrar.org (E-ISSN 2348-1269, P- ISSN 2349-5138).

[13] O' Farrell, P.H., High resolution two-dimensional electrophoresis of proteins, J Biol Chem.1975, 25; 250 (10): 4007-21.

[14] O'Rourke, F.J. (1974). Fish In: Biochemical & Immunological Taxonomy of Animals. (Ed. C.A. Wright) 271-282 Academic Press, London.

[15] Prashanth, M .S.(2007). Impact of free cyanide on protein level in the Indian major carp, Catla catla. J. Ecotoxicol. Environ. Monit.,17(2): 159-166.

[16] Radoslav Dekic, Jassna Friscic, Aleksandar Ivanc & Biljana Kukavica, (2016). Characterization of proteins from Popovo minnow (Delminichthys ghetaldii Steindachner, 1882) muscle.Turkish Journal of Fisheries and Aquatic Sciences, 16(3), 637-642. http://doi.org/10.4194/1303-2712-v16_3_17

[17] Ryman, N. & Utter, F.M. (1987). Manual for starch gel electrophoresis: A method for detection of genetic variation. NOAA Technical Report, NMFS, 1- 61.

[18] Salahuddin R. & Khola K. (2013) The effect of chromium on glycogen content of certain tissues of fresh water fish Channa gachua. Global Journal of Science Frontier Research, 13(7), 27-30.

[19] Shalaka Sadekarpawar, Parth Pandya & Pragna Parikh, (2015). Proteotoxicity studiesin liver, muscle, kidney and gills of Oreochromis mossambicus and Labeo rohita exposed to plant nutrient (Librel), International Journal of Research Studies in Biosciences, 3(6), 29-36.

[20] Shamshad Begam, S. Suvarchala, G. & Philip, G.H. (2016). Protein profile in tissues of Zebrafish exposed to Deltamethrin. J.Bio.Innov, 5 (6), 914-922.

[21] Sheik Mohamed Salahudeen, B., Muthukamaravel, K. & Kumarasamy, P. (2014). Impact of nitrate fertilizer urea on the electrophoretic protein pattern of different organs in the freshwater fish Oreochromis mossambicus. International Journal of Recent Scientific Research, 5(1), 133-135.

[22] Sung, Y.Y., MacRae, T.H., (2011). Heat Shock Proteins and Disease Control in Aquatic Organisms. J Aquac Res Development S2:006. http://doi.org/10.4172/2155-9546. S2-006.

[23] Tahmina Hoq & Asha Rani Das, (2017). Fish Stress Protein: An Approach for Biomonitoring of Water Quality in Bangladesh. American Journal of Zoological Research, 5(2), 24-28. http://doi.org/ 10.12691/ajzr-5-2-1.

[24] Tomm, J.M., van Do, T., Jende, C., Simon, J.C., Treudler, R., von Bergen, M. &Averbeck, M. (2013). Identification of New Potential Allergens from Nile Perch (Lates niloticus) and Cod (Gadus morhua). J Investig Allergol Clin Immunol, 23(3), 159-167

[25] Tsuyuki, H., Roberts, E., & Vanstone, W. E. (1965). Comparative Zone Electropherograms of Muscle Myogens and Blood Hemoglobins of Marine and Freshwater Vertebrates and Their Application to Biochemical Systematics. Journal

of the Fisheries Research Board of Canada, 22(1), 203–213. http://doi.org/10.1139/f65-017.

[26] Vidya Sagar Reddy, G. & Ch. Vijaya. (2014). Protein profiles of selected commercial freshwater fishes of Nellore, India. International journal of scientific research, 3(4), 1-3.

[27] Wilson, E.D., Fisher, K.H. and Flugua, M.E (1975). Principles of nutrition. John Wiley and Sons Inc. New York. pp., 49-95.