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Research Article

Effect of Heavy Metals on Protein Profile of Body Muscles of *Cyprinus carpio*

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ABSTRACT

The study was carried out with commonly cultured C. carpio exposed to two sublethal dose levels of 0.025 and 0.05 ppm of arsenic, mercury, nickel and chromium individually and arsenic in combination with mercury, nickel and chromium. Protein quantity declined in all the heavy metal treatments except chromium, where it showed an increment in protein quantity. Gel electropherograms of muscle protein extracts of C. carpio on their exposure to different heavy metal treatments revealed a definite pattern of variations in their protein profile. It caused the synthesis of some additional protein fractions in almost all the treatments with subsequent deletion of some other protein fractions.

Keywords: *Heavy metal toxicity, SDS – PAGE, stress proteins, body muscles, Cyprinus carpio.*

INTRODUCTION

Natural water is one of the environment's most precious and vital components and is essential to all forms of life. The indiscriminate growth of industries, particularly dealing with the products like textiles, agrochemicals, insecticides, tanneries, paints, dyes, or chloro-alkali metals, discharge heavy loads of effluents in drainages and rivers. Though the water bodies have the potential for self-purification, the dumping of wastes exceeds this limit and alters water's physiochemical and biological characteristics. Development of fisheries in inland water bodies is thus being increasingly impeded due to these environmental constraints, coupled with residual wastes of inorganic fertilizers and pesticides in public health and agricultural sectors (Ghosh et al., 2000).

Today, heavy metals are termed as 'devil in disguise', and they are kept at the top priority list among water pollutants as they are persistent, water-soluble, non-degradable, vigorously oxidizing agents and strongly bind to many biochemical units. They produce cumulative toxicity in small doses over long periods of time and acute toxicity in higher doses.

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Heavy metals adversely affect fish's physiological, histological and biochemical functions (Jain & Sharma, 2003; & Jain & Mittal, 2004). They exert toxic effects on the organisms at the tissue, cellular and molecular levels. Toxicities at the cellular level cause disturbances in reproduction, differentiation and maturation, as exemplified by teratogenesis. They mainly affect the permeability of the cell membrane and disturb energy metabolism, and also decrease the stability of the lysosomal membrane, which disrupts cell functions by releasing various hydrolases at the molecular level. These metals interact with proteins leading to denaturation and precipitation, allosteric effects or enzyme inhibition (Mizrahi & Achituv, 1989). They bind to nucleic acids, leading to irreversible conformational changes. Heavy metals also induce protein synthesis in fish (Ali et al., 2003; & Boone & Vijayan, 2002).

International concern over the increasing hazards of industrial and domestic pollutants has focused particular attention on the toxic effects of heavy metals, exemplified by the effects of organic mercurials on fish, fish predators and human beings. Since then, the effects of lead, chromium, arsenic, cadmium, mercury and copper have been or are currently being reexamined. When the hazard is manifested as an acute lethal episode, the end result is immediately apparent, and investigations for prevention become prompt. When sub-lethal concentrations are present, the "signs and symptoms" become much more subtle; any toxic damage remains undetected for long periods unless specifically sought after, and remedial action may become more difficult or even impossible. The sub-lethal toxicity has received insufficient attention. This study is thus aimed at highlighting the effects of sub-lethal doses of certain commonly available heavy metal pollutants in water, affecting protein profile in locally cultured fish *C.carpio*, which would constitute important biomarkers as clinical tests for determining heavy metal toxicity in fishes.

MATERIALS AND METHODS

Cyprinus carpio weighing 90±15 gm were procured from local freshwater ponds and acclimatized in a tank filled with well-aerated water for seven days, then treated with As, Hg, Ni and Cr individually and As in combinations with others at 0.025 and 0.05 ppm concentrations in plastic tubs of 40 litre capacity. After 45 days of treatment, the fish were dissected, and the body muscles of the control and treated fish were analyzed for total soluble protein by following Bradford's (1976) method.Samples were prepared by crushing 100 mg of tissue in 1 ml chilled phosphate buffer (0.1 M, pH 7.0) along with 50 mg insoluble PVP using pestle and mortal (rinsed with D.D.W. and dried) under cold conditions. The contents were then centrifuged at 10,000 rpm at 4^0C for 15 min in a refrigerated centrifuge. The supernatant containing the proteins was taken in Eppendorf tubes, and pallets were discarded. The supernatant was stored at -20^0C.

Changes in a number of proteins were studied for different treatments by SDS-PAGE, using the discontinuous buffer system of Laemmli (1970). For molecular weight determination, a mixture of the marker proteins was also electrophoresed simultaneously in the same gel in the wells adjacent to the sample wells.

After completion of electrophoresis, staining and background destaining, relative mobilities (Rm values) were calculated for each of the marker proteins and the resolved proteins by the following formula:

$$\text{Rm value} = \frac{\text{Migration distance of protein band (mm)}}{\text{Migration distance of tracking dye (mm)}}$$

The Rm value of marker proteins was plotted against the log of molecular weights of marker proteins using semilogarithmic paper. The molecular weights of different proteins were estimated by matching their Rm values with an appropriate point on the standard curve.

RESULTS AND DISCUSSION

Syversen (1981) reported that heavy metals, in general, interfere with protein synthesis, as concluded from a decrease in protein content of all tested tissues of *O. mossambicus* exposed to mercury. Similarly, Khanee et al. (1991) also reported a gradual decrease in muscle protein content from an initial 182 mg/g to 160 mg/g after 28 days of exposure to sublethal mercury concentration (0.02 ppm) in *O. mossambicus*. In the present study, it is evident that the protein levels in all the heavy metal treatments declined except chromium, which showed an increment of 20.7% (Table-1). A maximum reduction of 21.5% was observed in arsenic (0.05ppm) treatment, followed by mercury and nickel treatments. When heavy metals were in combination, a maximum reduction of 28.3% was observed in arsenic + mercury treatment (0.05 ppm). Kumari (1984) also reported a decrease in the protein content of the liver and muscles by 10.3% and 33.3%, respectively, of *Channa punctatus* when exposed to nickel for 30 days but an increase of 20.1 and 7.7 per cent protein, respectively, when exposed similarly to chromium (2.6 mg/l). The reduction in protein content of fish could be taken to suggest that the fish probably depend more on protein catabolism to meet the energy demands under heavy metal stress. Adaptive utilization of protein to meet the energy demand under stress was reported by Vijayram et al. (1991) and Oikari and Nittyla (1985).

Proteins, the important constituents of animal tissues, play a significant role in spare energy. Proteins are the primary effector 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 of the proteins of the cell is essential due to changes in protein profile during intoxication (Suneetha et al., 2010). A study of gel electropherograms of the muscle protein extracts of *C. carpio* on their exposure to different heavy metal treatments revealed a definite pattern of variations in their protein profile (Table 2, Fig. 1). The control fishes evidenced nine protein fractions and their relative molecular weights ranged in between 40.7 to 154.8 kDa. The protein fraction of 69.1 kDa was maximally expressed; the other fractions for 93.3 and 154.8 kDa were less resolved. Exposure to heavy metal treatments for 45 days caused the synthesis of some additional protein fractions in almost all the treatments, with subsequent deletion of some other protein fractions. Maximum alterations in protein fractions occurred in Cr treatment at both concentration levels, followed by As at 0.05 ppm level. Cr induced the synthesis of three new protein fractions resulting in total twelve fractions, as compared to the control. The protein fraction of 85.1 kDa was relatively less expressed, whereas 154.8 kDa was in higher density over the control at both dose levels. The additional proteins were 28.1, 112.2 and 134.8 kDa. Likewise, As at 0.05 ppm induced the synthesis of two additional protein fractions, resulting in a total of eleven fractions. These fractions were of 112.2 and 134.8 kDa. The protein fractions of 60.2 and 40.7 kDa were less expressed, whereas the fraction of 154.8 kDa was of higher density than the controls. Hg at both concentration levels resulted in the synthesis of a single protein fraction, i.e., 134.8 kDa. Ni at 0.05 concentration also induced synthesis of a protein fraction of 131.8 kDa Mr, with subsequent deletion of a fraction of 93.3 kDa.

Electropherograms of *C. carpio* exposed to different combinations of metals revealed that As in combination with Hg at 0.025 ppm induced synthesis of two new protein fractions of 131.8 and 107.1 kDa , while

at 0.05 ppm, it induced deletion of two fractions of 93.3 and 85.1 kDa . Like As (0.05) concentration of two fractions of 60.2 and 40.7 kDa was reduced, whereas 154.8 kDa was increased. Combination with Cr at the same concentration level also induced the synthesis of three new proteins of 138.0, 134.8 and 131.8 kDa, with concurrent deletion of a fraction of 93.3 kDa. As with Cr, at 0.025 ppm, induced only one additional protein fraction of 112.2 kDa. Two fractions of relative molecular weights of 60.2 kDa and 123.0 kDa were found to be better expressed compared to that in controls. Ni, in combination with As at 0.025 ppm, resulted in the deletion of a fraction of 85.1 kDa, which appeared better resolved in As + Hg (0.025 ppm) treatment. At 0.05 ppm concentration, As + Ni treatment caused no significant change in protein profile over the controls. There has been only better expression of a fraction of 154.8 kDa in both As + Ni and As+ Hg combination treatments at both the test concentrations (Table 3). The present investigations' results demonstrated definite qualitative alterations in protein fractions and their protein intensity profiles. In the earlier studies (Suresh et al., 1991), heavy metal mercury was found to inflict cellular metabolism, thereby leading to impaired protein synthetic machinery in *C. carpio*. The changes in protein profiles recorded after heavy metal treatment were attributed largely to proteotoxic effects of heavy metals. Borgia et al. (2019) also reported the appearance or disappearance of protein fractions in the serum of *C. carpio* compared to control fish due to stress caused by metals in the effluent. When damage to proteins occurs, stress proteins, commonly called hips, are believed to be induced. It has been also known that stress proteins, also known as chaperones, prevent the reactive hydrophobic parts of the damaged proteins from forming non-specific complexes with normal cellular proteins (Weigant et al., 1997). Thus, the alteration in the gel electrophoretic profiles or loss or gain of bands would possibly have some correlations with these stress proteins.

Apart from their protein-damaging action, heavy metals have been reported to cause chromosomal damage (Bartoli et al., 1991), increased DNAase activity (Joshi & Desai, 1988), and decrease in DNA and RNA levels (Chaudhary, 2004). Therefore, the change of gel electrophoretic band profile and total protein contents might actually reflect damage in DNA or protein synthesizing system by heavy metal treatments.

Various authors (Boone & Vijayan, 2002; Tabche, 2002; Ali et al., 2003; & Chaudhary, 2004) reported the induction of stress proteins by heavy metals. This study observed that metals are effective inducers of stress proteins, although the specific stress proteins induced could vary considerably. The type and dose of metal administered influenced this. This statement is exactly supported by Goering and Fisher, 1995 and Sanders et al. 1996. This specificity in response makes it difficult to offer generalizations regarding the induction of specific stress proteins by metals. These differences in protein induction might also reflect differences in the mechanisms of action by which specific metals elicit toxicity.

Based on the synthesis of stress proteins on exposure to heavy metals, a protein profile study could constitute an important biomarker as a clinical test for determining heavy metal toxicity in fish. Osman et al. (2010) also inferred protein electrophoresis is a sensitive tool for biomonitoring aquatic pollution. 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 practised to avoid the entry of toxic pollutants into the environment. Hence appropriate removal of heavy metals from industrial effluent is necessary before discharge into aquatic systems to improve aquatic health.

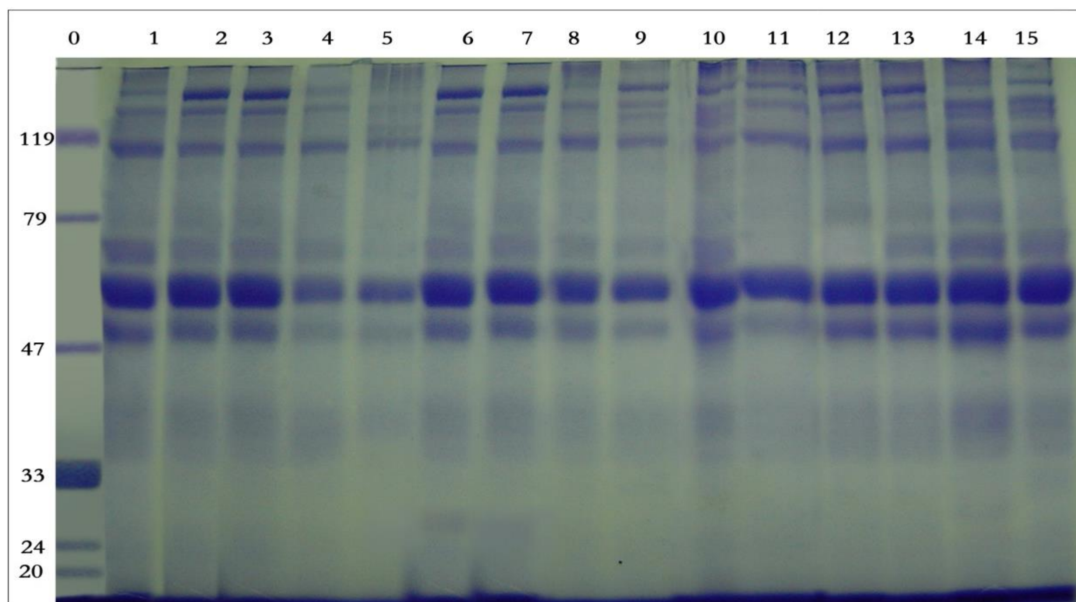

Fig. 1: Gel electropheregram of body muscles of *C. carpio* exposed to different heavy metal treatments

Lane 0- Molecular weight marker (molecular weight (kDa) of each band is given at side).

Lane 1- Control; Lane 2 - Hg (0.025); Lane 3 - Hg (0.05)
Lane 4- Ni (0.025); Lane 5 - Ni (0.05); Lane 6 - Cr (0.025)
Lane 7- Cr (0.05); Lane 8 - As (0.025); Lane 9- As (0.05)
Lane 10 - As + Hg (0.025)' Lane 11 - As + Hg (0.05)
Lane 12 - As + Ni (0.025); Lane 13 - As + Ni (0.05)
Lane 14 - As + Cr (0.025); Lane 15 - As + Cr (0.05)

Table 1: Alterations in protein quantity (mg/g) in body muscles of *C. carpio* exposed to different heavy metals

Treatment / concentration (ppm)	Control	Mercury 0.025 0.05	Nickel 0.025 0.05	Chromium 0.025 0.05	Arsenic 0.025 0.05	AS+Hg 0.025 0.05	AS+Ni 0.025 0.05	AS+Cr 0.025 0.05
Mean±S.E.	208.17±1.98	181.73±1.32 174.03±1.30	201.09±0.92 196.09±0.61	232.94±1.94 251.26±1.61	174.44±1.46 163.41±1.34	166.11±1.72 149.25±1.57	173.40±0.56 159.87±1.45	198.80±0.23 191.10±0.60
% Alteration*		-12.7 -16.8	-3.4 -5.8	+11.9 +20.7	-16.2 -21.5	-20.2 -28.3	-16.7 -23.2	-4.5 -8.2

Note:* Indicate increase (+) or decrease (-) in protein quantity over the control

Table 2: Protein profile of muscles of *Cyprinus carpio* exposed to different heavy metals

Sr. No.	Rm value	M.W. (k.Da.)	Control	Hg 0.025	Hg 0.05	Ni 0.025	Ni 0.05	Cr 0.025	Cr 0.05	As 0.025	As 0.05
1	0.041	154.8	+	++	++	+	+	++	++	+	++
2	0.083	141.2	++	++	++	+	+	++	++	++	++
3	0.10	134.8	-	+	+	-	-	+	+	-	++
4	0.11	131.8	-	-	-	-	+	-	-	-	-
5	0.13	125.8	++	++	++	++	++	++	++	+	++
6	0.14	123.0	++	++	++	++	++	++	++	++	++
7	0.18	112.2	-	-	-	-	-	+	+	-	+
8	0.26	93.3	+	+	+	-	-	+	+	+	+
9	0.30	85.1	++	+	+	+	+	+	+	+	+
10	0.39	69.1	+++	+++	+++	+++	+++	+++	+++	+++	+++
11	0.45	60.2	++	++	++	+	+	++	++	++	+
12	0.62	40.7	++	++	++	++	+	++	++	++	+
13	0.79	28.1	-	-	-	-	-	+	+	-	-
Total number of proteins			**9**	**10**	**10**	**8**	**9**	**12**	**12**	**9**	**11**

Note - +: Least band intensity
++: Medium band intensity
+++ Highest band intensity

Table 3: Protein profile of muscles of *Cyprinus carpio* exposed to different combinations of heavy metals

Sr. No.	Rm value	M.W. (k.Da.)	Control	AS+Hg 0.025	AS+Hg 0.05	AS+Ni 0.025	AS+Ni 0.05	AS+Cr 0.025	AS+Cr 0.05
1	0.041	154.8	+	++	++	++	++	+	+
2	0.083	141.2	++	++	++	++	++	++	++
3	0.091	138.0	-	-	-	-	-	-	+
4	0.100	134.8	-	-	-	-	-	-	+
5	0.108	131.8	-	+	-	-	-	-	+
6	0.13	125.8	++	++	++	++	++	++	++
7	0.14	123.0	++	++	++	++	++	+++	+++
8	0.18	112.2	-	-	-	-	-	+	-
9	0.20	107.1	-	+	-	-	-	-	-
10	0.26	93.3	+	+	-	+	+	+	-
11	0.30	85.1	++	++	-	-	++	++	++
12	0.39	69.1	+++	+++	+++	+++	+++	+++	+++
13	0.45	60.2	++	++	+	++	++	+++	+++
14	0.62	40.7	++	++	+	++	++	++	++
Total no. of proteins			**9**	**11**	**7**	**8**	**9**	**10**	**11**

Note - +: Least band intensity
++: Medium band intensity
+++: Highest band intensity

CONCLUSION

It is concluded that heavy metals induced the synthesis of some new proteins called stress proteins over the control with subsequent deletion of a few proteins. It could act as an important biomarker for determining heavy metal toxicity. In general, protein quantity declined on exposure to heavy metals. Decreased levels of protein might be related to proteolysis to meet increased energy demands by stress situations.

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