



## Investigation of Protein Patterns by Using Sds Page in Kidney, Liver and Brain of Cyprinus Carpio

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Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 02 Nov 2023	<p>Western transfer and dodecyl sodium sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) are two of the most widely used and beloved techniques in cancer research to identify proteins and glycoproteins. the protein fraction expression on the protein profile is significantly lower on the seventh day than it is on the eighth, the protein fraction expression is directly inversely related to the number of days. In order to determine the number of protein bands expressed in different tissues of Cyprinus carpio exposed to mercury and lead as the number of days rose, electrophoretic research was conducted. The difference between the 27 bands for lead and 36 bands for mercury on the seventh day and the 29 bands for lead and 40 bands on the thirty-first day suggests that as the number of exposure days to lead and mercury rises, so do the number of protein bands that are expressed. Using gel electrophoresis, a mixture of proteins is categorized by kind and molecular weight in the Western Blotting method. The results are then put into a membrane, which results in the formation of a band for each protein. Two HSP70 gene bands were found in Cyprinus carpio tissues after exposure to lead and mercury.</p> <p><b>Keywords:</b> Cyprinus carpio, Mercury, Lead, HSP70, Western Blotting, SDS, Gene expression.</p>
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### 1. Introduction

One of the most widely used methods in many scientific fields, including molecular biology, biochemistry, forensic sciences, etc., is sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), which can separate proteins on a gel based on the length of their polypeptide chains. As a result, SDS-PAGE is a frequently used technique in many disciplines to categorise proteins according to their electrophoretic mobility. SDS-PAGE analysis is a crucial indicator for toxicological investigations on fish, according to Muhammad (2018). The significance of those parameters, their value as biomarkers in fish toxicology, and our new findings were presented in this paper. Fish toxicity studies frequently used hemato-biochemical parameters. Protein electrophoresis has been successfully employed in numerous studies to identify intra- and inter-specific variation among species.

According to Svobodova et al., more active species have greater haematological parameter values than less active forms. Fast mobility, a predatory disposition, and high activity levels with streamlined bodies are all correlated with high RBCs values (Rambhaskar; 1986). The ranges of serum biochemistry differed from species to species and were affected by a variety of biotic and abiotic elements, including diet, age, and sex of the fish, water temperature, seasonal patterns, and patterns of feeding.

Haematological and biochemical methods have recently been used in numerous research to investigate the effects of UVA (Sayed; 2017), gamma radiation (Sayed; 2017), heavy metals (Mekaway; 2013), 4-nonylphenol (Sayed; 2017), and pesticides (Mekaway; 2013). Growing attention is being paid to the structural characteristics of fish blood cells, which are thought to be crucial for aquaculture and studies of environmental pollution.

Since proteins are species-specific and electrophoretic separations are simple to carry out, electrophoresis is a useful technique for producing systematic data from macromolecules (Jesslin G; 2013). Numerous studies have used protein electrophoresis as a reliable tool to identify intra- and inter-specific variation among species, which may reflect the metabolic level of the organism, its adaptations to environmental changes, the various nutritional statuses of the fish, and feeding habits (Navarro and Gutiérrez;1995).

## **2. Materials And Methods**

### **SDS - polyacrylamide gel electrophoresis**

#### **Preparation of Samples**

Protein content of the liver kidney and brain were quantified by a standard method (Bradford, 1976). The samples were then subjected to SDS- PAGE under 6% stocking gel and 10 % separating gel (Laemmli, 1970).

Electrophoretic study of protein patterns in different tissues of *Cyprinus carpio* was carried out in the abovementioned tissues.

#### **Preparation and Casting of Gel**

The gel plates were assembled according to the manufacture's instruction and the volume of the gel mould, was determined. In a conical flask, the acrylamide mixture for 10% resolving gel was prepared (10ml acrylamide + 7.5ml resolving gel buffer, pH 8.8 + 12.3 ml distilled water + 150 µl ammonium per sulphate - freshly prepared (10% stock and 50 µl TEMED ( N,N,N,N' Tetra Methylene diamine).

The components were mixed and without delay the acrylamide mixture was poured into the glass mould till the lower mark. A layer of distilled water or iso propyl alcohol was overlaid to facilitate proper polymerization. After polymerization (30 minutes) the layer was removed and the gel top was washed with distilled water.

#### **Stocking Gel**

6% stocking gel was then prepared using 2ml acrylamide (30 % stock) + 3ml stocking gel buffer (pH 6.8) + 4.9 ml distilled water + 75µl APS (10% stock) + 25 µl TEMED.

The components were mixed properly and poured over the resolving gel, the comb was immediately inserted and the gel was allowed to polymerize.

#### **Loading of Sample**

Samples were prepared using required volume of sample (100 µg protein / lane) + equal volume of sample buffer (7.25 ml distilled water + 1.25 ml stocking gel buffers + 1ml Glycerol + 0.5ml β mercaptoethanol + 150 mg SDS and a pinch of Bromophenol blue).

The samples were heated in boiling water bath for 2 minutes. Denatured the protein and kept on ice to retain the denatured stage. The comb was then removed from the mould and the wells were washed with distilled water. The gel was mounted on electrophoretic apparatus. Electrophoretic buffer (Tris 3gm, Glycine 14.4gm and SDS 1gm in 1000ml distilled water, pH 8.3) was added to the top and bottom reservoir of the electrophoretic apparatus. The samples were loaded along with marker proteins into the lanes/ wells).

#### **Electrophoresis**

The apparatus was attached to power supply unit, 8v/cm for gel (70v) and 15v/cm for resolving gel (150- 200 v) was applied. The electrical contact between the two buffer tanks was through the slab gel, care was taken to avoid air bubbles, while adding electrode buffer in the tanks as the air bubbles, inhibit electrophoretic mobility.

The gel was run until the Bromophenol blue dye reached the bottom of the resolving gel. The power supply was turned off and the gel was removed from the sandwiched plates from the apparatus and placed on a paper towel.

The plates were removed using a spatula and the orientation was marked. The temperature for electrophoresis was kept, constant in an airconditioned room at 25oC.

### Staining and Destaining

This Gel was immersed in 5 volume of staining solution (200 mg Coomassive Brilliant Blue R + 250 + 50 MeOH+ 7ml Acetic acid solution + 30 ml MeOH + 63 ml distilled water) every half an hour. This was followed by two to three washes. The gel was then stored in 7% Acetic acid solution.

When visualized under the illuminated, the protein zones were visible as dark blue bands after 24 to 48h of destaining. The results were recorded by observing the relative electrophoretic mobility of protein zones for each sample and the run was repeated for the samples that did not show clear cut zones.

The protein profiles of the gel obtained out of the prepared cell-lysates are manually observed and compared with the various protein bands in the standard, control and the treated. Subsequently treated with staining and de-staining solution and identified the additional bands with varied KDa protein.

### 3. Results and Discussion

#### Physicochemical analysis of freshwater

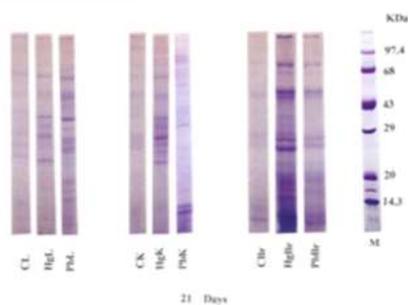
The physical and chemical properties of the freshwater in which *C. Carpio* was found and analyzed for the presence of dissolved salts and toxic metals. The dissolved oxygen content was found to be 6.2 0.4 mg/l, with a neutral pH of 7.3 0.01. The total hardness of the water was determined to be 345 99 mg/l, whereas the free CO<sub>2</sub> concentration was calculated to be 2.1-0.12 mg/l. In the tested water, there were no residues of mercury or cadmium, though there were traces of calcium (81 88 mg/l) and magnesium (34 mg/l). In addition, the water sample contained high levels of sulfates and chlorides (Table 1). This test showed that *C. carpio* had not been exposed to mercury before the trial began.

Table 1. The physicochemical characteristics of water were analyzed by using standard methods (APHA, 1995 and 2005).

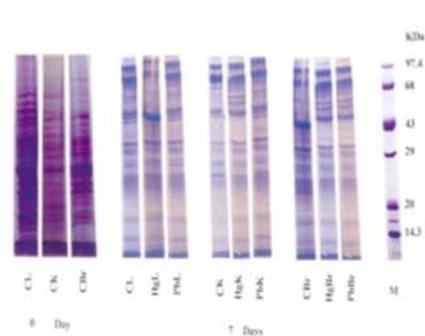
Parameters	Values
Dissolved Oxygen	6.2 ± 0.4 mg/l
pH	7.3 ± 0.01 m
Temperature	28 ± 2°C
Total hardness	345 ± 99 mg/l
Free CO <sub>2</sub>	2.1 ± 0.12 mg/l
Ca	81 ± 88 mg/l
Mg	34 ± 0.0 mg/l
Hg	Nil
Sulphates	112 ± 0.9 mg/l
Chlorides	234 ± 22 mg/l
Cd	Nil
Specific conductance	2340 (Micro siemens/cm) at 2°C

SDS Results: Table 2. The protein content of the liver kidney and brain was quantified by a standard method (Bradford, 1976).

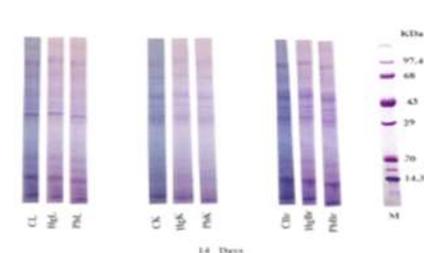
Protein profile showing the difference in protein fractions in comparison between the Standard, Control, Mercury and Lead treated tissues of *Cyprinus carpio* on 0 Day and 7 Days.



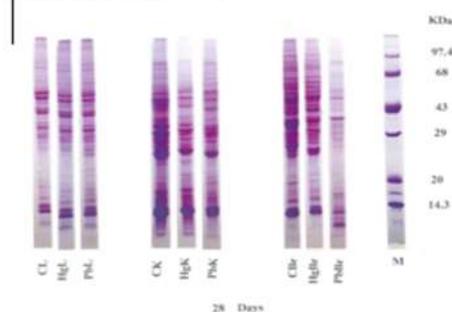
Protein profile showing the difference in protein fractions in comparison between the Standard, Control, Mercury and Lead treated tissues of *Cyprinus carpio* on 21 Days.



Protein profile showing the difference in protein fractions in comparison between the standard, control, mercury and lead treated tissues of *Cyprinus carpio* on 14 Days.



Protein profile showing the difference in protein fractions in comparison between the Standard, Control, Mercury and Lead treated tissues of *Cyprinus carpio* on 28 Days.



The gene expression, serum profiles, tissue histology, and bioindices of fish exposed to such metals are altered, and these changes serve as general health biomarkers. The heavy metals (Ni, Cd, and Cr) accumulated in water and fish tissues, were beyond the permissible limits defined by the Central Pollution Control Board/World Health Organization. According to Sadiya et al, 2020 Metallothionein (MT) and glutathione peroxidase (GPX) genes expression patterns highlighted the metal-specific exposure of fish. Fish are subject to a variety of stressors in the water, with heavy metals being the most commonly reported one (Banday et al., 2019). The constant accumulation leads to metabolic and genetic changes, and they are typically hazardous at larger concentrations.

SDS strives to achieve highly resolved separation of complex protein combinations. This method denatures the protein that will be exposed to electrophoresis. Since the protein fraction expression on the protein profile is significantly lower on the seventh day than it is on the eighth, the protein fraction expression is directly inversely related to the number of days. In order to determine the number of protein bands expressed in different tissues of *Cyprinus carpio* exposed to mercury and lead as the number of days rose, electrophoretic research was conducted. The difference between the 27 bands for lead and 36 bands for mercury on the seventh day and the 29 bands for lead and 40 bands on the thirty-first day suggests that as the number of exposure days to lead and mercury rises, so do the number of protein bands that are expressed. Using gel electrophoresis, a mixture of proteins is categorized by kind and molecular weight in the Western Blotting method. The results are then put into a membrane, which results in the formation of a band for each protein. Two HSP70 gene bands were found in *Cyprinus carpio* tissues after exposure to lead and mercury. Two genes may be seen on the plate on the 28th day, when the HSP70 gene began to express itself due to antigenic similarities and polypeptide variances. Because the liver is an organ that detoxifies the body, the buildup is lessened. As a result, the gene's strength greatly reduces as accumulating strength does. The brain is neurosensitive, while the kidney is the organ of excretion. Zang et al 2018 proved, HSP70, one of the most sensitive proteins that are generated under stress conditions and important for normal cell function, is upregulated in response to cadmium exposure in animals as a result of the hepatotoxicity that is brought on by cadmium exposure. According to the investigations, after being exposed to cadmium through water, *P. olivaceus* dramatically increased hepatic heat shock protein 70 (Deok-Chan Lee, 2022).

#### 4. Conclusion

Fish physiology is affected by heat shock protein genes in a number of ways, including growth and aging, stress physiology and endocrinology, immunology, environmental physiology, stress tolerance, and acclimatization (Basu et al. 2003). The reaction of the HSP gene can change depending on the tissue, different HSP families, and stresses. Season, developmental stage, and species can all affect how sensitively the Hsp gene is expressed. Prior research on fish examined the expression of the Hsp gene after bacterial infection. The investigations, however, mostly focused on Hsp90 and Hsp70. In the current communication, a variety of Hsp genes are investigated to determine their potential function in immunity in a comparative manner, and the findings regarding the expression of the Grp78 gene in liver tissue highlighted the crucial roles that these molecules played during bacterial pathogenesis. The Hsp90 gene appeared to be important in bacterial immunity, and fish virus infections were associated with higher expression levels of the gene.

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