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Impact of Cadmium and Zinc on Protein Profile of Fresh Water Bivalve, *Lamellidens marginalis*

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Abstract: Cadmium (Cd) and zinc (Zn) are pervasive heavy metals potentially toxic to aquatic life. This study evaluates their impact on the protein profile of the freshwater bivalve *Lamellidens marginalis*, significant for its ecological and economic roles. Using SDS-PAGE, we analyzed protein alterations in *L. marginalis* exposed to various Cd and Zn concentrations (winter season). Results revealed significant protein profile changes, with Cd exposure inducing more pronounced alterations than Zn. Cd led to new protein bands and the loss of several existing ones, indicating a greater disruption of protein integrity. These findings suggest Cd poses a higher risk than Zn and highlight the potential of protein profile changes as biomarkers for environmental monitoring. Future research should further investigate affected proteins and the long-term impacts of metal exposure.

Keywords: *Lamellidens marginalis*, Cadmium chloride, Zinc chloride, Protein profile, Toxicity, SDS-PAGE.

1.INTRODUCTION

Cadmium (Cd) and zinc (Zn) frequently coexist in aquatic environments due to industrial and anthropogenic activities [1-2]. Cd is highly toxic [3], while Zn, although essential, shares similar properties with Cd [4-5]. Understanding their interactions and impacts on aquatic organisms is essential [6]. Over the past 20 years, analyzing living organisms has become preferred because it better reflects local metal bioavailability over time. Additionally, sentinel species can accumulate high metal concentrations in soft tissues, making them more effective for analysis than direct abiotic sample testing. Aquatic organisms encounter a range of pollutants in their natural habitats, yet metal risk assessments typically rely on data derived from laboratory experiments involving individual metals [7-10].

Freshwater animals exposed to heavy metals can cause biochemical alterations such as genetic damage, metabolic disorders, inhibition of enzymes, hypertension, and cancer [11-14]. Heavy metal ions

interact with biomolecules, impairing physiological processes. To cope with this stress, organisms develop mechanisms that alter chemical profiles in the body, which serve as early stress indicators [15]. Pollutants impact biologically active molecules like amino acids, coenzymes, and sulfur- and phosphorus-containing proteins, affecting physiological functions. Proteins, with their complex amino acid structures, play crucial roles in cellular and extracellular processes, being essential for metabolism and interactions involving proteins, amino acids, enzymes, and coenzymes [16-17]. Bivalves were chosen for this study as they make effective biological monitors [18]. They are sedentary, widely distributed, long-lived, and have ample tissue for analysis. Their ability to accumulate metals allows them to reflect average exposure levels over time.

This study examines the impact of cadmium and zinc exposure on the protein profile of *Lamellidens marginalis*. By employing SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis), we aim to analyze variations in protein bands and molecular weight shifts, which can reveal alterations in protein expression and structural modifications induced by metal exposure. The results from SDS-PAGE will offer a detailed view of how cadmium and zinc affect the protein profiles of these bivalves, highlighting any significant changes in band patterns and molecular weight. Such changes can indicate underlying biochemical and physiological responses to heavy metal stress.

This research enhances our understanding of how metal contamination impacts aquatic organisms and evaluates the efficacy of bivalves as bioindicators. Future studies should focus on identifying specific proteins affected by metal exposure, examining the long-term consequences of chronic exposure, and assessing whether changes in protein profiles can serve as reliable biomarkers for environmental monitoring and risk assessment.

1. MATERIAL AND METHODS

2.1. Collection: Experimental bivalves, *Lamellidens marginalis* (Manjra dam, Dharashiv) exposed to cadmium chloride and zinc chloride respectively (LC0 and LC50 values of 96 hrs). After the exposure, the samples (bivalves), were dissected to remove body parts (foot, gill, gonad, hepatopancreas, and adductor muscle) and the whole body. The protein profile of the tissue was studied using polyacrylamide sodium dodecyl sulfate electrophoresis (SDS-PAGE).

2.2. Sample preparation:

2.2.1. Sample preparation was done by using suitable extraction methods [19-20]

2.2.2. 1 volume of sample (1gm weight tissue) was ground in five volumes of prechilled 100% acetone (5ml) with the help of mortar and pastel to convert into a fine paste.

2.2.3. The paste was transferred in microtubes which were then vortexed for 30 sec. These microtubes were placed in a -20°C freezer overnight. Microtubes were centrifuged at 4°C for 20min with 15000 rpm.

2.2.4. Supernatants were discarded and 5 volumes of prechilled 50% acetone were added to pellets and vortexed for 30 sec.

2.2.5. Microtubes were centrifuged with the above-mentioned conditions. Supernatants were discarded and Pellets were air-dried.

2.2.6. 15 mg from Pellets was transferred to a microcentrifuge tube containing 300ul rehydration solution (sample buffer; 7 M urea, 2 M thiourea, 40 mm tris-Hcl, 4%

CHAPS, and 1 % DTT) and homogenized in a vortex for 5 min at 25°C.

2.2.7. Then these tubes were kept in an ice tray (4ºc) for 2 hrs. The homogenized sample was centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant was collected and transferred to a new 1.5 ml tube.

Sample preparation of all desired tissues was prepared by following the above-mentioned process.

2.3. **Quantification of protein:** The protein concentrations were determined by Bradford assay [21] with bovine serum albumin (BSA) as a standard of measurement and absorbance at 595 nm. The sample and the standard reading were taken in triplicate.

2.4. SDS PAGE: SDS-PAGE was performed by following the method of Laemmli [22] (1970). 30 µg of protein were separated in a 12% acrylamide resolving gel with stacking at 5% for SDS-PAGE on Bio-Rad mini Vertical Unit. The acrylamide gel was run at 200 mA for 35 min and then at 120 mA for 2 hr in SDS buffer (running buffer) (3 gm Tris; 14.4 glycine; 1gm SDS). The MAGSPIN-34 MAGUniversal blU Protein Ladder from the APS lab was used. At the end of electrophoresis, the gels were visualized by staining with Coomassie brilliant blue CBB G-250 and then destained with 50 ml water; 40 ml crude methanol; 10 ml glacial acetic acid. The molecular weight in daltons of proteins with variation was determined by using a standard marker. The correlation between molecular weight and relative mobility was determined [23].

3. RESULTS

The experimental freshwater bivalve species *L. marginalis* were exposed to heavy metals; zinc and cadmium showed significant variations in protein profile compared to that of the control bivalve. A whole body of bivalve (control) and the metal exposed LC0 and LC50 bivalve tissue samples like foot, gill, gonad, hepatopancreas, and adductor muscle were resolved onto SDS-PAGE. The results are presented in the form of Figure 1, figure 2, Table 1, and Table 2.

Analysis of results (fig. 1) revealed that the experimental control whole body (lane 1) showed seven polypeptide bands with molecular weight 170.50KDa, 137.33KDa, 89.10KDa, 57.81KDa, 41.79KDa, 33.66KDa, 14.17KDa. Whereas the treated whole body with heavy metal zinc (lane 2) showed six polypeptide bands observed with molecular weight 137.33KDa, 99.28 KDa, 79.96KDa, 41.79KDa, 33.66KDa, 24.33KDa. Among these three new polypeptides with a molecular weight of 99.28KDa, 79.96KDa, and 24.33KDa were seen. In cadmium-treated whole body (lane 3) only three bands were observed with molecular weight 137.33KDa, 99.28KDa, and 41.79KDa. Here, a new polypeptide with a molecular weight of 79.96KDa was seen. Five bands disappeared in cadmium-treated LC50 bivalve as compared to LC0 bivalve.

The experimental control LC0 foot (lane 4) showed 10 polypeptide bands with molecular weight 153.02KDa, 137.33KDa, 123.25KDa, 99.28KDa, 79.76KDa, 57.81KDa, 46.56KDa, 37.50KDa, 27.11KDa and 11.41KDa. In the zinc-treated LC50 foot (lane 5), all the ten polypeptide bands with molecular weight 153.02KDa, 137.33KDa, 123.25 KDa, 99.28KDa, 79.76KDa, 57.81KDa, 46.56KDa, 37.50KDa, 27.11KDa, 11.41KDa were seen with slightly reducing their intensity. In the cadmium-treated LC50 foot (lane 6), seven bands with molecular weight 153.02KDa, 137.33KDa,

110.62KDa, 79.96KDa, 41.79KDa, 33.66 KDa, and 12.71KDa were observed, among which four new polypeptides with a molecular weight of 110.62KDa, 41.79KDa, 33.66 KDa, and 12.71KDa were seen. Nine bands disappear in cadmium LC50 foot as compared to LC0 foot.

The experimental control LC0 gill (lane 7) showed seven polypeptide bands with molecular weight 110.62KDa, 79.96KDa, 57.81KDa, 41.79KDa, 24.33KDa, 6.64KDa, and 4.80KDa. In zinc-treated LC50 gill (lane 8), four polypeptide bands with molecular weights 110.62KDa, 89.10KDa, 41.79KDa, and 14.17KDa, among which two new polypeptides with a molecular weight of 89.10KDa and 14.17KDa, were seen. In cadmium-treated LC50 gill (lane 9), four bands with molecular weight 110.62KDa, 71.77KDa, and 41.79KDa were observed. Here, a new polypeptide with a molecular weight of 71.77KDa was seen. The dark band (41.79KDa) reduces its intensity in cadmium LC50 gill. Five bands disappear in both metal LC50 gill as compared to LC0 gill

The analysis of results (fig. 2) revealed that the experimental control LC0 gonad (lane 10) showed 11 polypeptide bands with molecular weight 137.52KDa, 125.52KDa, 104.56KDa, 95.43KDa, 72.56KDa, 60.45KDa, 50.36KDa, 45.96KDa, 38.29KDa, 22.13KDa and 12.79KDa. In the zinc-treated LC50 gonad (lane 11), six polypeptide bands with molecular weight were 137.52KDa, 125.52KDa, 114.56 KDa, 79.50KDa 50.36KDa, 34.94KDa, among which three new polypeptides with a molecular weight of 114.56KDa, 79.50KDa, and 34.94KDa were seen. In cadmium-treated LC50 gonad (lane 12), seven bands were observed with molecular weight 137.52KDa, 104.56KDa, 72.56KDa, 55.17KDa, 41.95KDa, 31.89KDa, and 11.68KDa were observed, among which four new polypeptides with a molecular weight of 55.17KDa, 41.95KDa, 31.89 KDa, and 11.68KDa were seen. In zinc-treated LC50 gonad dark bands slightly lost intensity whereas in cadmium-treated LC50 gonad, all dark bands become light.

The experimental control LC0 hepatopancreas (lane 13) showed eight polypeptide bands with molecular weight 137.52KDa, 104.56KDa, 79.50KDa, 66.23KDa, 50.36KDa, 34.94KDa, 26.57KDa, and 24.25KDa. In zinc-treated LC50 hepatopancreas (lane 14), four polypeptide bands with molecular weight 137.52KDa, 72.56KDa, 50.36KDa, and 38.29KDa were observed, among which two new polypeptides with a molecular weight of 72.56KDa and 38.29KDa were seen. All dark bands become light in zinc-treated LC50 hepatopancreas as compared to LC0 hepatopancreas. In cadmium-treated LC50 hepatopancreas (lane 15), four bands observed with molecular weight 137.52KDa, 79.50KDa, 50.36KDa, and 41.95KDa were observed. Here, a new polypeptide with a molecular weight of 41.95KDa was seen. Four bands disappear in both treated metal LC50 hepatopancreas as compared to LC0 hepatopancreas.

The experimental control LC0 adductor muscle (lane 16) showed twelve polypeptide bands with molecular weight 150.67KDa, 137.52KDa, 114.56KDa, 104.56KDa, 95.43KDa, 87.10KDa, 72.56KDa, 60.45KDa, 55.17KDa, 45.96KDa, 38.29KDa and 24.25KDa. In zinc-treated LC50 (lane 17) adductor muscle, ten polypeptide bands with molecular weights 150.67KDa, 137.52KDa, 114.56KDa, 104.56KDa, 95.43KDa, 87.10KDa, 60.45KDa, 45.96KDa, 38.29KDa, 26.57KDa, among which two new polypeptides with a molecular weight of 60.45KDa and 26.57KDa were seen. In cadmium-treated LC50 (lane 18) adductor muscle, nine bands with molecular weight 150.67KDa, 137.52KDa, 104.56KDa, 95.43KDa, 79.50KDa, 50.36KDa, 45.96KDa, 38.29 KDa, and 22.13KDa were observed, among which three new polypeptides with a molecular weight of 79.50KDa,

50.36KDa, and 22.13KDa were seen. As compared to LC0 adductor muscle, the intensity of some dark bands increases in both treated metal LC50 adductor muscle. In the tissue samples, treated bivalve polypeptide bands were significantly decreased compared to control bivalve tissue samples.

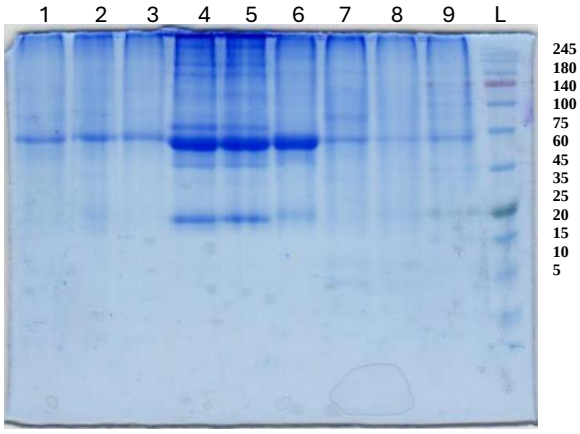

Fig. 1: protein profile from whole body and body parts of zinc and cadmium exposed freshwater bivalve, *Lamellidens marginalis* (96hrs exposure, LC0, and LC50)

Legends:
Lane 1. Whole body (LC0)
Lane 2. Whole body zinc treated (LC50)
Lane 3. Whole body cadmium treated (LC50)
Lane 4. Foot (LC0)
Lane 5. Foot zinc treated (LC50)
Lane 6. Foot cadmium treated (LC50)
Lane 7. Gill (LC0)
Lane 8. Gill zinc treated (LC50)
Lane 9. Gill cadmium treated (LC50)
L. Protein ladder

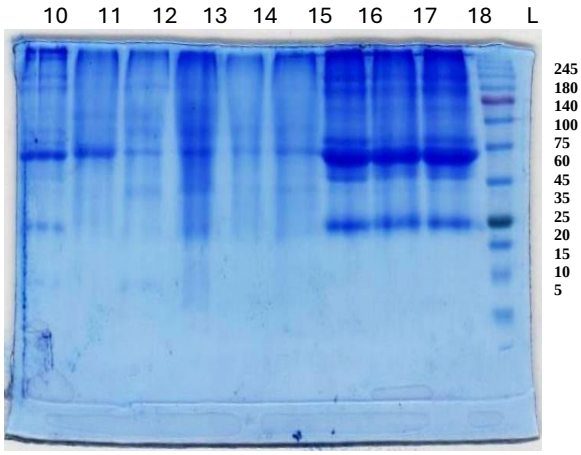

Fig. 2: protein profile from body parts of zinc and cadmium exposed freshwater bivalve, *Lamellidens marginalis* (96hrs exposure, LC0, and LC50)

Legends:
Lane 10. Gonad (LC0)
Lane 11. Gonad zinc treated (LC50)
Lane 12. Gonad cadmium treated (LC50)
Lane 13. Hepatopancreas (LC0)
Lane 14. Hepatopancreas zinc treated (LC50)
Lane 15. Hepatopancreas cadmium treated (LC50)
Lane 16. Adductor muscle (LC0)
Lane 17. Adductor muscle zinc treated (LC50)
Lane 18. Adductor muscle cadmium treated (LC50)
L. Protein ladder

4. DISCUSSION

Exposure of bivalves to heavy metals can produce toxic effects like changes in biochemical content [24-25], reduction in fecundity[26], variations in behaviour [27], damage of tissues [28], changes in enzyme activities[29-31] and changes in proteins expression [32-33]. All the major structural and functional aspects of the body are carried out by protein molecules. Any abnormality in protein structure will lead to adverse influence with profound alterations in metabolic functions[34]. The present investigation was designed to evaluate the alteration in protein profile due to the toxic effect of heavy metal zinc chloride and cadmium chloride on the freshwater mussel Lamellidens *marginalis* by using the SDS PAGE technique. In all of the tissue samples, there was a reduction of polypeptide bands in treated mussels as compared to the protein profile of control mussels. The results of the SDS-PAGE analysis of the protein extracts are presented in Figur 1, Figur 2, Table 1, and Table 2.

The formation of new polypeptide bands takes place in both metal-treated bivalves. These new proteins could be stress proteins (like metalotheionine) to overcome the toxic effect of heavy metals [35]. It is observed that the synthesis of new protein is more seen in the gonad followed by the foot, hepatopancreas, adductor muscle, and gills. Similar to these observed results, several findings of authors are in agreement stating that the hepatopancreas and gonads of exposed bivalve show more synthesis of protein when compared with the gills of exposed bivalve[36-40]. The digestive gland (hepatopancreas) of the Mollusca is the main centre

for metabolic regulation, participating in the mechanisms of immune defence and homeostatic regulation of the internal medium as well as in the elimination of xenobiotics and the processes of detoxification.

The intensity of dark bands reduced to become light is mostly observed in cadmium-treated bivalves as compared to the zinc-treated bivalves. This may be due to the denaturation of protein during the exposure period in which the destruction of the tertiary structure of the protein molecule takes place and the formation of a random polypeptide chain occurs [41]. such kinds of misfolded proteins are cytotoxic, where they aggregate and interact inappropriately with other cellular components which then results in Age-related and several neurodegenerative disorders [42-47]. Zinc is an essential metal but its high dose causes pancreatitis, anaemia, muscle pain, and acute renal failure[48]. As compared to the LC0 bivalve tissues, in LC50 cadmium-treated bivalve tissues, the deduction of polypeptide bands takes place more in the gill followed by hepatopancreas, gonads, foot, and adductor mussel. In zinc-treated bivalve deduction in polypeptide bands takes place more in the gill followed by hepatopancreas, gonads, adductor muscle, and foot. In both metal-exposed tissues, it was observed that significant deduction in bands occurred in gills and hepatopancreas whereas a minimum effect was observed on adductor muscle. The gills of aquatic organisms constitute a key interface for the uptake of dissolved metal ions from water. The depletion of the protein in the tissues is indication of proteolytic activity which may due to the demands of excess energy under toxic conditions[49-50]. Depletion in protein fraction occurred more in cadmium-treated as compared to the zinc-treated bivalves. All the observations suggest that cadmium is more toxic than zinc for bivalves because maximum band disappearance and formation of new bands take place in cadmium-treated bivalves. The protein profile provides fundamental insights into the underlying mechanism of toxicity based on polypeptide bands quantifying.

5. CONCLUSIONS

This study demonstrates that exposure to cadmium and zinc chloride significantly reduces the protein profile in the soft tissues of the freshwater bivalve *Lamellidens marginalis*. This reduction, coupled with the formation of abnormal polypeptides, likely disrupts critical biological processes such as reproduction, growth, development, and shell formation. Consequently, these disturbances may lead to bivalve population declines. Future research should focus on the long-term ecological impacts of heavy metals and strategies to mitigate pollution, essential for conserving freshwater ecosystems and bivalve sustainability.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

Table. 1:
Relative mobility values and their molecular weight of Lamellidens marginalis when exposed to LC0 and LC50 values of Zinc chloride during the winter season

Body parts \ Bands			1	2	3	4	5	6	7	8	9	10	11	12
Whole Body Control	LC0	REM	0.018	0.056	0.132	0.207	0.264	0.301	0.452					
		MW	170.50	137.33	89.10	57.81	41.79	33.66	14.17					
	LC50	REM	0.056	0.113	0.150	0.264	0.301	0.358						
		MW	137.33	99.28	79.96	41.79	33.66	24.33						
Foot	LC0	REM	0.037	0.056	0.075	0.113	0.150	0.207	0.245	0.283	0.339	0.490		
		MW	153.02	137.33	123.25	99.28	79.96	57.81	46.56	37.50	27.11	11.41		
	LC50	REM	0.037	0.056	0.075	0.113	0.150	0.207	0.245	0.283	0.339	0.490		
		MW	153.02	137.33	123.25	99.28	79.96	57.81	46.56	37.50	27.11	11.41		
Gill	LC0	REM	0.094	0.150	0.207	0.264	0.358	0.584	0.641					
		MW	110.62	79.96	57.81	41.79	24.33	6.64	4.80					
	LC50	REM	0.094	0.132	0.264	0.452								
		MW	11.62	89.10	41.79	14.17								
Gonad	LC0	REM	0.044	0.066	0.111	0.133	0.2	0.244	0.288	0.311	0.355	0.488	0.622	
		MW	137.52	125.52	104.56	95.43	72.76	60.45	50.36	45.96	38.29	22.13	12.79	
	LC50	REM	0.044	0.066	0.088	0.177	0.288	0.377						
		MW	137.52	125.52	114.56	79.50	50.36	34.94						
Hepato Pancreas	LC0	REM	0.044	0.111	0.177	0.222	0.288	0.377	0.444	0.466				
		MW	137.52	104.56	79.50	66.23	50.36	34.94	26.57	24.25				
	LC50	REM	0.044	0.2	0.288	0.355								
		MW	137.52	72.56	50.36	38.29								
Adductor Muscle	LC0	REM	0.022	0.044	0.088	0.111	0.133	0.155	0.2	0.244	0.266	0.311	0.355	0.466
		MW	150.67	137.52	114.56	104.56	95.43	87.10	72.56	60.45	55.17	45.96	38.29	24.75
	LC50	REM	0.022	0.044	0.088	0.111	0.133	0.155	0.244	0.311	0.355	0.044		
		MW	150.67	137.52	114.56	104.56	95.43	87.10	60.45	45.96	38.29	26.57		

Body parts \ Bands			1	2	3	4	5	6	7	8	9	10	11	12
Whole Body Control	LC0	REM	0.018	0.056	0.132	0.207	0.264	0.301	0.452					
		MW	170.50	137.33	89.10	57.81	41.79	33.66	14.17					
	LC50	REM	0.056	0.113	0.264									
		MW	137.33	99.28	99.28									
Foot	LC0	REM	0.037	0.056	0.075	0.113	0.150	0.207	0.245	0.283	0.339	0.490		
		MW	153.02	137.33	123.25	99.28	79.96	57.81	46.56	37.50	27.11	11.41		
	LC50	REM	0.037	0.056	0.094	0.150	0.264	0.301	0.471					
		MW	153.02	137.33	110.62	79.96	41.79	33.66	12.71					
Gill	LC0	REM	0.094	0.150	0.207	0.264	0.358	0.584	0.641					
		MW	110.62	79.96	57.81	41.79	24.33	6.64	4.80					
	LC50	REM	0.094	0.169	0.264									
		MW	110.62	71.77	41.79									
Gonad	LC0	REM	0.044	0.066	0.111	0.133	0.2	0.244	0.288	0.311	0.355	0.488	0.622	
		MW	137.52	125.52	104.56	95.43	72.76	60.45	50.36	45.96	38.29	22.13	12.79	
	LC50	REM	0.044	0.111	0.2	0.266	0.333	0.4	0.644					
		MW	137.52	104.56	72.56	55.17	41.95	31.89	11.68					
Hepato Pancreas	LC0	REM	0.044	0.111	0.177	0.222	0.288	0.377	0.444	0.466				
		MW	137.52	104.56	79.50	66.23	50.36	34.94	26.57	24.25				
	LC50	REM	0.044	0.177	0.288	0.333								
		MW	137.52	79.50	50.36	41.95								
Adductor Muscle	LC0	REM	0.022	0.044	0.088	0.111	0.133	0.155	0.2	0.244	0.266	0.311	0.355	0.466
		MW	150.67	137.52	114.56	104.56	95.43	87.10	72.56	60.45	55.17	45.96	38.29	24.75
	LC50	REM	0.022	0.044	0.111	0.133	0.177	0.288	0.311	0.355	0.488			
		MW	150.67	137.52	104.56	95.43	79.50	50.36	45.96	38.29	22.13			

Table 2:
Relative mobility values and their molecular weight of Lamellidens marginalis when exposed to LC0 and LC50 values of Cadmium chloride during the winter season