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A comparative study of changes in protein contents in freshwater bivalves after chronic exposure to cadmium

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ABSTRACT

The objective of our present research was to investigate changes in protein contents in different tissues like mantle, gill, digestive glands and whole soft body of freshwater bivalves *Corbicula striatella, Parreysia corrugata* and *Parreysia cylindrica* after exposure to cadmium. Bivalves were exposed to chronic concentration of cadmium (0.1284 ppm) upto 20 days. Changes in protein contlents were found to be exposure dependent. *Parreysia cylindrica* shows highest protein depletion in different soft tissues as compared to the other studied bivalve species. Among the different studied tissues the digestive glands shows highest depletion in protein content. Thus, the changes in protein contents can be used for early diagnosis of stress or as a probable biomarker for assessment of cadmium metal pollution in aquatic ecosystem.

Key words: protein, freshwater, *Corbicula striatella, Parreysia corrugata*, *Parreysia cylindrica*, cadmium, chronic concentration

INTRODUCTION

Maintaining the quality of aquatic ecosystems is one of the most difficult challenges in the 21st century. Natural processes and anthropogenic activities lead to input of heavy metals in aquatic environments (Franca et al. 2005). Heavy metals are non-biodegradable, highly toxic, have the ability to bioaccumulate and have deleterious effect to most organisms (Kaoud and Dahshan, 2010) and also influence the structure and function of the ecosystems. Thus, contamination of water bodies due to heavy metal pollution has turn out to be a topic of concern all over the world. Among the chemicals reaching aquatic ecosystems cadmium is a persistent, non-essential, non-biodegradable, potentially toxic even at trace concentrations, it retained for long periods of time in organisms after bioaccumulation and ranks eighth in the priority list of top 20 hazardous substances (Glenn, 2001) and therefore it is a serious environmental contaminant.

Biomarkers have recently generated significant interest as a diagnostic tool to assess the environmental pollution. Biomarkers are measurable changes in a biological system in response to a chemical, physical or biological agent.

They have been used to indicate stress in aquatic organisms or the level of environmental pollution. An ideal biomarker should be easily measurable, consistent and its levels must correlate with increase in response to stress or pollution. The changes occurring in the organisms can be measured at molecular, biochemical, physiological or histological level. Among these the molecular and cellular biomarkers play an important role in ecotoxicology and environmental pollution monitoring. They are useful for diagnosing the impact of pollution, for the detection of sublethal and chronic effects with their relation to ecological alterations (Relyea and Hoverman, 2006). The assessment of biochemical changes at organism level will help to develop a reliable approach for environmental risk assessment, to predict the early detection and effects of heavy metal water pollution and our understanding of organism response after exposure to heavy metal stress.

The bivalves are considered as useful bioindicator organisms in assessment of risk associated with water contamination and thus used for biomonitoring purpose worldwide (Otchere, 2003, Tay et al. 2004). Bivalves are benthic, widespread in distribution and ecologically important because of their biological filtration activity. They can accumulate heavy metals in their tissues at concentrations in excess of the ambient water (Poteat et al. 2013) through ingestion of sediment particles, food and directly from overlaying water. Accumulated heavy metal stress causes biochemical alterations in the organs involved in detoxification mechanisms (Zhang et al. 2010; Rajkumar and Milton, 2011). Thus, the study on biochemical changes in bivalves is essential to understand the mechanism of metal toxicity as well as to know how bivalves deals with the stress of pollutants at the biochemical level which can be serves as biomarkers of environmental pollution.

MATERIALS AND METHODS

The freshwater bivalves, *Corbicula striatella*, *Parreysia corrugata* and *Parreysia cylindrica,* were collected from Girna dam 39.6 km away from Chalisgaon city of Jalgaon district of Maharashtra state, India. After collection animals were brought to laboratory and were cleaned and acclimatized in aquarium containing dechlorinated tap water for 10 days. During acclimatization and experimentation, the animals were fed with fresh water algae and water of aquarium was changed after every 24 hours.

Experimental design

After acclimatization, the active, medium, uniform size and healthy bivalves of each species were selected by measuring their shell length and width and divided into two groups. Each species was separately exposed to fixed average chronic concentrations ($LC_{50/10}$) of ionic form of cadmium. 1st group was maintained as control and 2nd group was exposed to chronic concentration of cadmium (0.1284 ppm) upto 20 days. On 10th and 20th days of exposure period animals of each species from experimental and control groups were dissected and their mantle, gills, digestive glands and whole soft body tissues were removed and dried in oven at 70^0-80^0C till constant weight was obtained. After oven drying, dry tissues were blended into dry powder. These powders were used for the estimation of protein.

Protein estimation:

Protein content of the tissues was estimated by Lowry's method (Lowry et al. 1951). The optical density of blue colour developed was read at 530nm on a double beam Spectrophotometer (Elico BL 200). The blank was prepared in same way without dissolving protein precipitate. The protein content in different tissues was calculated referring to standard graph value and it was expressed in terms of mg protein/100 mg of dry tissue. The Bovine serum albumen was used as a standard.

RESULTS & DISCUSSION

The changes in protein content of all the studied soft tissues of experimental bivalve species, *C. striatella*, *P. corrugata* and *P. cylindrica* after chronic exposure to cadmium for 10 and 20 days are summarized in table no.1. The obtained results revealed that, the Cd causes significant depletion in protein contents in all studied tissues of the experimental bivalve species as compared to those of control bivalves. A maximum depletion in protein contents in response to cadmium exposure was observed in different soft tissues of *P. cylindrica* as compared to *C. striatella* and *P. corrugata*. The organ wise order of protein depletion observed in experimental bivalve species was, digestive glands > whole soft body > gills > mantle.

The results obtained during the present study were clearly revealed progressive depletion in protein content along with the increase in exposure period. The obtained results are in harmony with the results reported by various researchers (Nawale, 2008; Andhale and Zambare, 2011; Patil, 2011; Waykar and

Pulate, 2012; Tripathi et al. 2012). The presence or absence of biochemical changes in laboratory animals exposed to environmental chemicals/xenobiotics is an important diagnostic tool in the overall assessment of the risk and hazards of potential human or animal exposure (Krishna and Ramachandran, 2009). The effects of long term stress on animals specifically affects metabolism and various biochemical processes occurring in cells leads to an imbalance of the cellular redox status and causes damages in different biomolecules and mutagenic and carcinogenic processes can occur (Sies and Stahal,1992).

Proteins represent the molecular phenotype of cells which have a direct effect on organismal physiology (Feder and Walser, 2005). Protein responds for better survival by either increasing or decreasing their levels. These are the key substance to show the effects of heavy metals. The heavy metals have affinity for metal sensitive groups, such as thiol groups. It blocks functional groups of proteins, displace and/or substitute essential metals, induce conformational changes, denature enzymes and disrupt cell and cell organelle integrity (Hall, 2002).

Bioaccumulated heavy metal Cd can indirectly induce oxidative stress by generation of reactive oxygen species (ROS) which results in strong defenses, tissue destruction, altered enzyme activities and alteration in protein metabolism by direct oxidation of their amino acid residues and cofactors or by secondary attack via lipid peroxidation (Requena et al. 2003), possible utilization of the products of their degradation for metabolic purposes (Ambrose et al. 1994) and blocking of protein synthesis (Somnath, 1991). Oxidative stress also affects protein folding and protein ubiquitination in molluscs (Jie Meng et al. 2017).

Table 1: Profile of protein content in different tissues of freshwater bivalve, *Corbicula striatella, Parreysia corrugata* and *Parreysia cylindrica* after chronic exposure to different heavy metals (Values are in mg/100mg of dry weight).

Bivalve species	Treatment	Mantle		Gill		Digestive gland		Whole soft body	
		10th day	20th day	10th day	20th day	10th day	20th day	10th day	20th day
Corbicula striatella	**Control**	49.89 ±3.44	49.57 ±2.02	61.71 ±3.37	60.98 ±3.53	54.09 ±1.80	53.60 ±1.98	57.22 ±1.36	56.54 ±3.39
	Cd	41.42 NS ±2.44 (-16.98)	35.62* ±2.84 (-28.14)	47.40** ±1.66 (-23.18)	40.12* ±2.63 (-34.21)	39.57 NS ±3.04 (-26.84)	33.35** ±1.86 (-37.77)	46.30** ±1.80 (-19.08)	37.91** ±2.56 (-32.95)
Parreysia corrugata	**Control**	46.39 ±2.32	45.60 ±3.52	60.14 ±3.57	59.66 ±3.73	51.18 ±1.69	50.46 ±1.78	55.46 ±1.18	54.88 ±2.54
	Cd	37.79* ±2.95 (-18.52)	32.69 NS ±3.13 (-28.31)	45.99** ±2.33 (-23.54)	38.28* ±2.14 (-35.84)	37.05 NS ±1.97 (-27.61)	29.73* ±3.53 (-41.08)	42.05** ±1.98 (-24.19)	34.53 NS ±3.68 (-37.07)
Parreysia cylindrica	**Control**	50.37 ±3.27	49.78 ±2.46	64.48 ±2.93	64.07 ±3.45	57.38 ±1.96	56.60 ±3.71	59.95 ±3.70	59.33 ±3.08
	Cd	39.64* ±3.79 (-21.31)	33.68* ±2.96 (-32.35)	47.74* ±3.63 (-25.97)	38.52** ±3.97 (-39.88)	40.53 NS ±2.22 (-29.37)	32.21** ±2.57 (-43.09)	44.22* ±3.10 (-26.25)	35.49* ±3.54 (-40.17)

1. (+) or (-) indicate percent change over control
2. (±) value indicates S.D. of three observations
3. Values are significant at ****P*<0.001, ***P*<0.01, **P*<0.05, NS- not significant

Oxidation of amino acid side chains consequences in the formation of carbonyl derivatives which are non-reversible, causing conformational alterations, declined catalytic activity of enzymes and finally resulting into breakdown of proteins by proteases as a result of increased susceptibility (Almroth et al. 2005). Increased protease activity in fresh water bivalves after exposure to pollutants was observed by Waykar and Lomte (2002, 2004). Another possible reason for the depletion in protein might be the diversification of energy to meet the impending energy demand when the animal is under stress (Waykar and Lomte, 2001a; Satya Parameshwar et al. 2006). Hence, depleted protein contents in the bivalves exposed to the chronic concentration of Cd indicates interruption of usual metabolic processes.

CONCLUSION

The alterations in protein contents in different tissues of the bivalves exposed to the chronic concentration of Cd can be used as biomarker and considered as a diagnostic tool to determine the physiological responses of the cells and organs for its toxic effect. Among the studied bivalves, *Parreysia cylindrica* is a good bioindicator species for biomonitoring of pollution while the digestive glands can be used as a potential bioindicator organ.

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Conflicts of interest: The authors stated that no conflicts of interest.

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