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Cadmium Stress On Protein Content In *Portunus Sanguinolentus* (Marine Crab)

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ABSTRACT

Marine water studies were carried out to quantify the cadmium distribution and toxic effect on total protein metabolism in marine crab to identify the impacts of potential sources in the vicinity of Visakhapatnam coastal environment. Grab samples were collected at 5 polluted sites and 1 unpolluted site which was chosen as reference during pre monsoon, monsoon and post monsoon seasons in 2003 –2004. The observations revealed that the metal concentrations were low in pre monsoon and high in post monsoon. Were observed cadmium concentrations inversely proportional to total protein content in marine crab, cadmium low PRW1 (0.019 μg/g) bioaccumulation were found in pre-monsoon and high MPS (0.141 μg/g). The amounts of total protein in the dry tissues of marine crabs were found to be high in post-monsoon (168.2 mg/gm) low in pre-monsoon (154.0 mg/gm). In all sites tested, the reduction percent change of proteins in the dry tissues of marine crabs over the control was found, post-monsoon high reduction percent change in MPS (28.41%) and pre-monsoon low reduction percent change in PRW1 (11.10%). Results indicated that the marine environment near Visakhapatnam coast are potentially influenced by Mehadrigedda and Gosthani rivers that receive domestic, agricultural and industrial effluents and by the city storm water drain. Ship harvester activity is also contributing to the elevated metal concentration in the marine water.

KEYWORDS

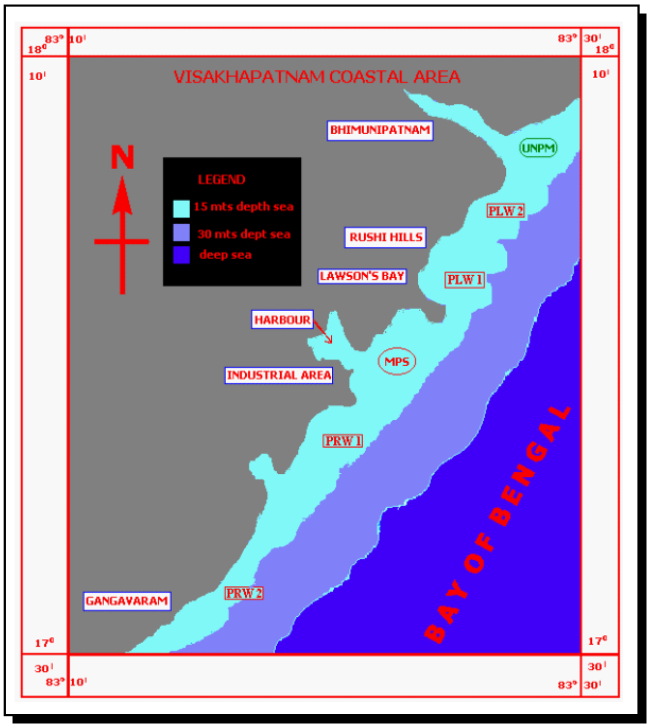
Marine water;
Heavy metal pollution;
Coastal environment;
Total protein

INTRODUCTION

Metals occur naturally and several of them are essential components of global ecosystems. They are present in the environment with a wide range of oxidation states and coordination number and these differences are related to their toxicity. Metals such as copper (Cu) and zinc (Zn) are essential to life, whereas others such as mercury (Hg), lead (Pb) and cadmium (Cd) are not known to perform a useful biochemical function[18]. Heavy metals are released either in combined form with ions like chlorides, sulphates etc., or in free ionic form (Cd^{+2}, Zn^{+2}, Pb^{+2}). The detailed analysis of the effluents of different industries of Visakhapatnam has been done and reported by Someswara Rao[38]. Many others also reported the concentration of these heavy metals in harbour water and their accumulation in phytoplankton, zooplankton etc[35].

Proteins are the abundant organic constituents and they are concerned with the structural organization and functional dynamics of the living organisms[27]. The levels of tissue proteins are determined by their rates of synthesis and degradation resulting in continuous renewal which is known as protein turn over[36]. According to[16] on the protein composition level based on spawning seasons[20]; on the chemical composition of fish[4]; on the heavy metal composition of the mummichog (Fundulus heteroclitus)[37]; on the effect of heavy metal concentrations on the biochemical composition of Mystus spp[41]; on the impact of industrial pollution on the biochemical composition of Macrobrachium rude[23]; on the impact of heavy metal concentration in muscle protein of Mystus vittatus.

MATERIALS AND METHODS



The present work was carried out to assess the seasonal variations of the effect of cadmium stress on the protein in the dry tissue of the selected marine organism, namely marine crab, Portunus sanguino lentus in the Visakhapatnam coastal waters during Feb. 2003 and Jan. 2004. Six sites were selected for the study viz., the selected location for sample collection is located in between Bheemuni patnam and Gangavaram (Lat 17^0 30| to 18^0 10| N and Long 83^0 10| to 83^0 30| E) adjoining the industrial area and harbour township of Visakhapatnam on East coast of India.

The main sampling location was in between the industrial and the harbour area of the Visakhapatnam coast that is considered as the main pollution source (MPS). Remaining four polluted sites were selected left wing of the MPS named as PLW1, PLW2 and right wing of the MPS named as PRW1, PRW2, each polluted sites distance of around 6 km. The control site was identified away from the polluted sites, named as UNPM (Un-polluted Marine).

The sediment samples analysis was done according to the method described by[3,14]. The samples were oven-dried at 60°C for 48 hrs, and 10 g portion of muscle from each sample was washed and re-weighed after dry sample, were taken 5 g of dry weighed samples were placed in a crucible with 2 ml HNO_3 and allowed stand overnight. The mixture was heated to near dryness and allowed to cool before 5 ml of HNO_3 solution was added. They were transferred to 25 ml volumetric flask and were added HCl as necessary. Then they were cooled and diluted to volume and filtered. The samples were analyzed with in three hours of digestion. The digestion solution was assayed by AAS (Perkin - Elmer 2380).

The total crude protein content of muscle tissues was estimated by using[21] method which involves two steps. The carbohydrate groups of protein molecules react with copper and potassium of the reagent to give a blue coloured copper potassium biuret complex; this complex together with tyrosine and phenolic compounds present in the protein reduce the phosphomolybdate of the foline reagent to intensify the colour of the solution. The optical density of colour developed in measured at 540 nm and the protein content of the samples was calculated with the help of a standard graph prepared by using known quantity of bovine serum albumin (B.A.S.).

RESULTS

Show that the data on the concentration of cadmium (TABLE and Figure - 1) presented in the marine crab in 5 selected polluted sites and 1 control (un-polluted) site of Visakhapatnam coast in different seasons like pre-monsoon, monsoon and post monsoon.

The metal, cadmium was not noticed in the dry tissues of marine crabs in control site in all three seasons, which is indicated in the table as below detection limit (BDL). Cadmium low bioaccumu lation were found in pre-monsoon and high bioaccumulation in post-monsoon, the trend was followed by MPS (0.046 - 0.141 μg/g) > PLW2 (0.035 - 0.118 μg/g) > PRW2 (0.032 - 0.112 μg/g) > PLW1 (0.023 - 0.096 μg/g)> PRW1 (0.019 - 0.086 μg/g). The amounts of total proteins (sea TABLE and Figure 2, 3) in the dry tissues of marine crabs were found to be high in post-monsoon (168.2 mg/gm) in the control sites followed by monsoon (160.6 mg/gm) and pre-monsoon (154.0 mg/gm).

TABLE 1: Seasonal variations of cadmium (μg/g) accumulation in marine crab in the selected sites of Visakhapatnam coast

Sites	Seasons					
	Pre-monsoon		Monsoon		Post-monsoon	
	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
Control	BDL	-	BDL	-	BDL	-
PLW2	0.035	0.0158	0.092	0.0251	0.118	0.0261
PLW1	0.023	0.0132	0.067	0.0143	0.096	0.0153
MPS	0.046	0.0152	0.107	0.0215	0.141	0.0252
PRW1	0.019	0.0138	0.062	0.0138	0.086	0.0138
PRW2	0.032	0.0142	0.090	0.0152	0.112	0.0154

SD (±) : Standard Deviation
BDL : Below Detection Limit

In all sites tested, the reduction percent change of proteins in the dry tissues of marine crabs over the control was found and the trend was followed by PRW1 (11.10%) < PLW1 (11.81%) < PRW2 (13.24%) < PLW2 (14.15%) < MPS (15.58%) in pre-monsoon, PRW1 (17.43%) < PLW1 (18.49%) < PRW2 (19.73%) < PLW2 (20.23%) < MPS (22.16%) in monsoon and PRW1 (22.59%) < PLW1 (24.01%) < PRW2 (26.21%) < PLW2 (26.63%) < MPS (28.41%) in post-monsoon. The above biochemical changes in all seasons were found to be statistically significant ($p< 0.001$).

DISCUSSION

The protein plays a vital role in biological functions in any organism. Cadmium (Cd) was showed a negative effect on protein content in the muscle tissue of the marine crab, *Portunus sanguinolentus*. Alterations in structural and biological functions of an organism may cause due to the certain toxic conditions[15]. Cadmium, a non-essential element with no biological function[42] and highly toxic to man[7] and other living organisms critical effect is the first physiological or biochemical change which can be detected [26]. According to the Department of Water Affairs and Forestry[8], cadmium is a metal element that is highly toxic to marine and freshwater aquatic life. The routes of uptake of cadmium by fish are mainly through ingestion (food and water or both). Cadmium causes mutagenic, carcinogenic and teratogenic effects[10,11,17] and it has been found to be toxic to fish and other aquatic organisms[32,43].

In the present investigation, the polluted sites are with control site protein percent change significant value of $p<0.001$ in all sites and all seasons. The protein reduction percent change over the control site in post-monsoon protein high reduction percent change (28.41%) post-monsoon and low reduction percent change (15.58%) in pre-monsoon were observed in MPS site when assayed the muscle tis-

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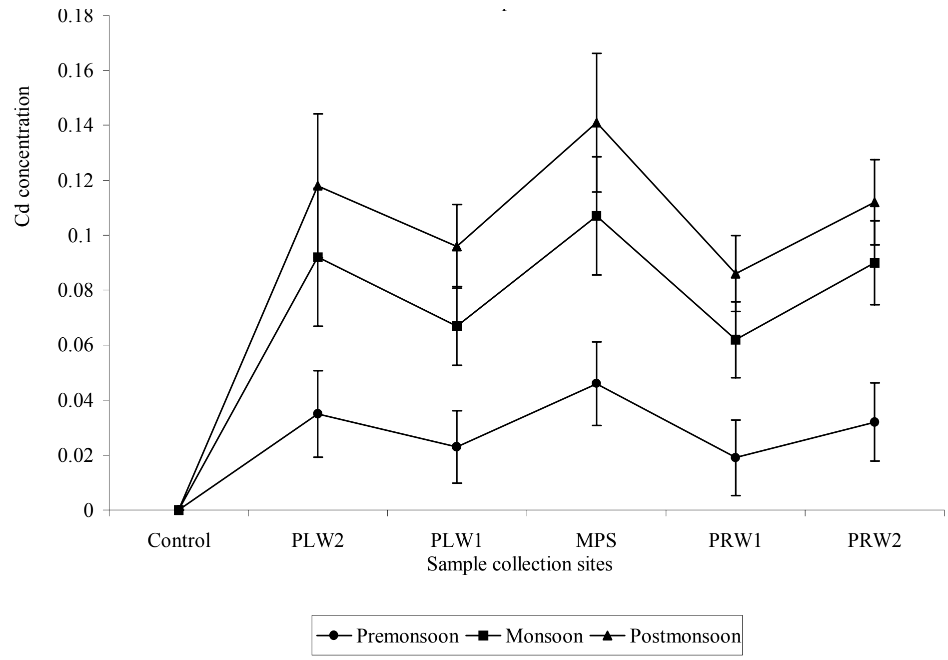

Figure 1: Seasonal variations of cadmium (μg/g) accumulation in marine crab in the selected sites of Visakhapatnam coast

TABLE 2: Seasonal variations of total protein content (mg/g) in dry tissue of marine crab in the selected sites of Visakhapatnam coast

Sites	Seasons					
	Pre-monsoon		Monsoon		Post-monsoon	
	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
Control	154.210	15.2561	160.602	14.2351	168.210	14.3252
PLW2	132.201*	12.3351	128.102*	10.2362	123.402*	11.2350
PLW1	135.810*	12.3651	130.912*	11.2360	127.802*	11.1220
MPS	130.012*	10.1252	125.312*	10.2651	120.402*	11.2361
PRW1	136.902*	11.2352	132.601*	12.3250	130.220*	11.2360
PRW2	133.621*	10.3253	128.902*	13.2251	124.121*	10.2362

SD (±) : Standard Deviation, * : p <0.001

TABLE 3: Total protein seasonal reduction percent change over the control in dry tissue of marine crab in the selected sites of Visakhapatnam coast

Sites	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
	RPC	RPC	RPC
PLW2	14.1558	20.2366	26.6349
PLW1	11.8181	18.4931	24.0190
MPS	15.5844	22.1668	28.4185
PRW1	11.1039	17.4346	22.5921
PRW2	13.2467	19.7384	26.2187

RPC : Reduction Percent Change Over the Control

sue of the marine crab. However, cadmium was high concentration (0.141 μg/g) in post-monsoon and low concentrations (0.046 μg/g) in pre-monsoon were observed in MPS site when compare the remaining sites. A heavy metal becomes toxic when a level is reached where it damages the life functions of an organism[1] and leads to the decrease levels of proteins. The main pollution site (MPS) is located at main polluted area in the Visakhapatnam harbour and there are two monsoonal streams include industrial and agricultural run off namely Gosthani river water and an industrial water storm, which entered in PLW2 and PRW2 respectively.

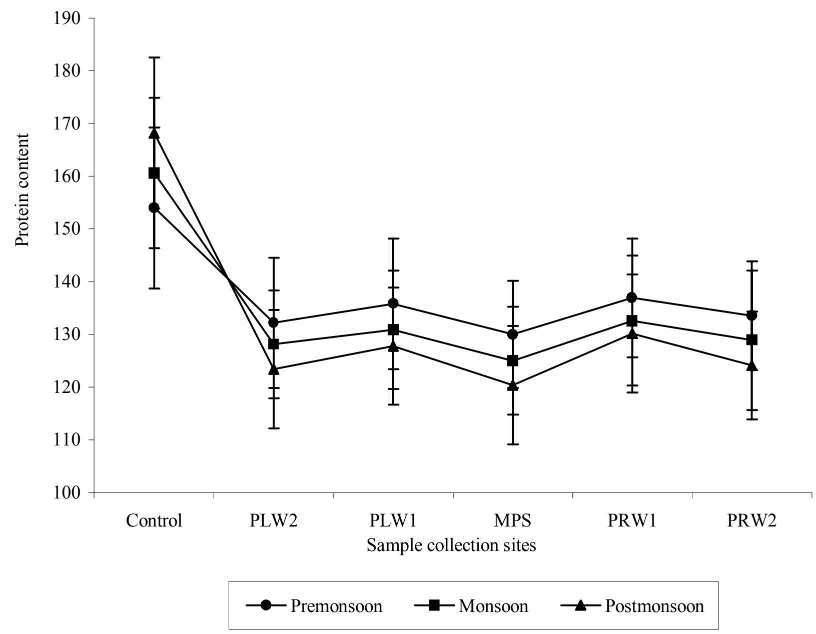

Figure 2: Seasonal variations of total protein content (mg/g) in dry tissue of marine crab in the selected sites of Visakhapatnam coast

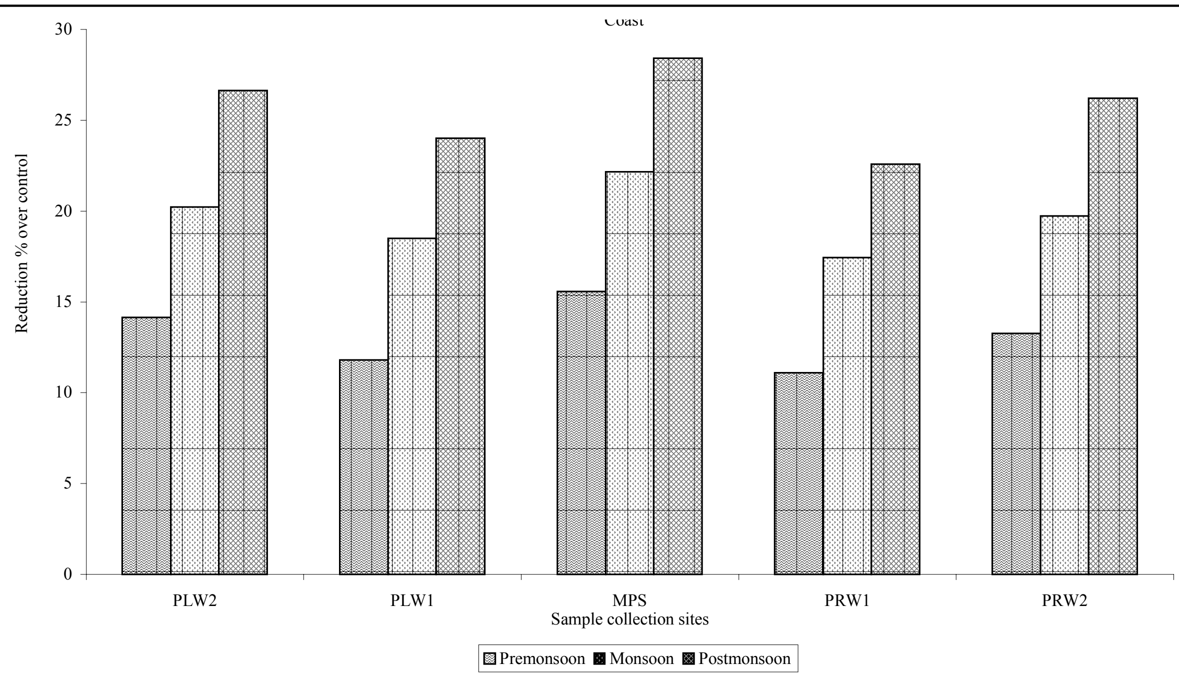

Figure 3: Seasonal variations of total protein reduction change over the control in dry tissue of marine crab in the selected sites of Visakhapatnam coast

In the present investigation, the amounts of Cd in crab were found to be more in MPS when compared to the remaining four polluted sites in all three seasons. The observed reasons for more metal pollution in MPS were due to discharge of industrial effluents[2,30] solid waste, domestic effluents[31] urban storm - water runoff[9], spoil heaps[13], weathering of minerals and soils[24] and shipping activities than the other. Similar conditions were reported in the past by[28,41,23], from the same habitat i.e., the harbour waters of Visakhapatnam.

PLW2 low reduction percent change in pre-monsoon (14.15%) high reduction percent change in post-monsoon (26.63%) and PRW2 low reduction percent change in pre-monsoon (13.24%) and high reduction percent change in post-monsoon (26.21%). Protein reduction percent change next to the MPS, Cd toxicity due to river the agriculture runoff enter in the sites through Gosthani. However, the metal accumulation of crab was more, next to MPS in PLW2 low concentration in pre-monsoon (0.035 μg/g) and post-monsoon (0.118 μg/g). Were observed PRW2 low amount of Cd in pre-monsoon (0.032 μg/g) and high in post-monsoon (0.112 μg/g) than the other sites in all seasons studied respectively. This was only due the polluted water from Gosthani river water and an industrial water storm entering into these sites. A similar observation was reported[12]. As the results indicated in TABLE - 1, the higher bioaccumulation of metals by the crab was found in all the seasons when compared to the concentration of metals in coastal marine water. Similar observations were reported that the metal concentrations Cd was very high in sea snail, mussel and fish in Turkish coast of the Black sea[40].

According to the present research work as represented protein low reduction percent change were observed in the sites of PLW1 (low reduction percent change in pre-monsoon (11.81%) and high reduction percent change in post-monsoon (24.01%) and PRW1 low reduction percent change in pre-monsoon (11.10%) and high reduction percent change in post-monsoon (22.59%) and low bioaccumulation in the sites of PLW1 in pre-monsoon (0.023 μg/g) and high in the post-monsoon (0.096 μg/g). PRW1 low amount of Cd in pre-monsoon (0.019 μg/g) and high amount of post-monsoon (0.086 μg/g), reason of very less contamination are enter in the sea when compare the other polluted sites.

The amount of cadmium in the tissue of marine crab was exceeded the tolerance limits in the present study in all experimental sites and seasons. The higher accumulation of cadmium by marine organisms like crab, fish etc. leads to damage the liver functions and ultimately decreased levels of mostly carbohydrates due to the absence or less process of the gluconeogenesis and in low reduction percentage change of the lipid and protein contents. Cadmium is extremely toxic to marine organisms and it induces a variety of biochemical and hematological changes [19] and ultimately it causes to decrease in the protein content. Marine organisms with low fat content subjected to high levels of pollutants show marked picture of pollutant accumulation.

According to[41,23] increased cadmium level, correlated with decreased protein level in *M.rude*, and *Mystus vittatus* respectively from the same habitat. It indicates that there is a negative relationship between the protein content and the heavy metal concentration in the body[25]. Reported that Cd effect on *Poecilia reticulate* leads to growth reduction, RNA decrease and ultimately reduced in protein content at a high level than the carbohydrate and lipid contents[5]. Observed the disrupted lactic activity and blood glucose levels in *Salvelinus fontinalis* with higher accumulation of Cd. Reduction in muscle proteins and growth in *Salmo salar* with high Cd concen tration than the tolerance limit was observed[29] .

The amount of lead in the tissue of marine crab was exceeded the tolerance limits in the present study in all experimental sites and seasons. The higher accumulation of lead by marine organisms like crab, fish etc. leads to damage the liver functions and ultimately decreased levels of mostly carbohydrates due to the absence or less process of the gluconeogenesis and in low reduction percentage of the lipid and protein contents. Several investigators were observed the similar reports regarding to changes of proteins, carbohydrates and lipids in different marine species like *G.pseudolimnaeus*, *T.abyssorum*, *G.zaddachi* etc. expore to lead[22,33,34,6].

CONCLUSION

Visakhapatnam coastal water is prone to seasonal variations in Cd concentration, due to the industrialization, urbanization and agricultural activities in the vicinity or catchments of Visakapathanam coast are definitely influencing the marine water quality. Metal concentrations exceeding the tolerance limit during all seasons indicates threat to the marine aquatic ecosystems and commercial activity in this region. The results indicated wide bioaccumulation variation in the levels of metals between the sites. However the worked-out ratios indicated as better picture on the bioaccumulation/bio-toxicity to the marine crab Portunus sanguinolentus it's caused to the biological abnormal are indicated. In the present investigation reveal that the concentration of cadmium, accumulation (Portunus sanguinolentus) in the control site would be extremely lower than the polluted/experimental sites values and according, the human consumption of crab caught from the control/unpolluted site region is safe. The harbor area was deal with the high pollutants it my caused to toxicity in marine ecosystem but protection of the aquatic environment is warranted to preserve this important part of the traditional diet. Moreover, regular monitoring of pollutants in crab and other biota are essential from the point of view environmental toxicology.

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