Int J Pharm Bio Sci 2012 July; 3(3): (B) 863 - 874

Research Article **Bio Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ACUTE TOXICITY OF HEAVY METALS (CADMIUM CHLORIDE, CHROMIUM TRIOXIDE AND LEAD NITRATE) AND THEIR EFFECTS ON THE FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII*

R. SOWDESWARI, P. SARAVANA BHAVAN* AND P. ANANTHI

Crustacean Biology Laboratory, Department of Zoology, Bharathiar University, Coimbatore- 641046, Tamil Nadu, India.

ABSTRACT

The post larvae (PL) of *Macrobrachium rosenbergii* (2.5 cm and 0.15g) were subjected to static renewal type acute toxicity tests against cadmium chloride ($CdCl_2$), chromium trioxide (CrO_3) and lead nitrate ($Pb(NO_3)_2$). The 96 h LC_{50} values were calculated to be 58.93 µg/L, 2.08 mg/L and 25.97 mg/L for $CdCl_2$, CrO_3 and $Pb(NO_3)_2$ respectively. PL were exposed to the 96 h LC_{50} concentrations of these heavy metals for a period of 96 h to study their acute impacts on whole body concentrations of basic biochemical constituents, such as total carbohydrate, lipid, protein and amino acids, activities of metabolic enzymes, such as glutamate–oxaloacetate transaminase (GOT) and glutamate–pyruvate transaminase (GPT) and activity of enzymatic antioxidant, catalase. The levels of these biochemical constituents were found to be significantly ($P<0.102 - 0.00$) decreased in test prawns when compared with control. Among these heavy metals the recorded impact in contents of biochemical constituents was severe in test prawns that had been exposed to $CdCl_2$, followed by $Pb(NO_3)_2$ and CrO_3. These results indicated the fact that energy was utilized to mitigate the toxic stress induced through these heavy metals. The energy utilization percentage was found to be higher in lipid followed by carbohydrate and protein. The activities of GOT and GPT were found to be significantly ($P<0.020 - 0.00$) decrease in test prawns when compared with control. The percentage decrease in GOT and GPT was higher in prawns exposed to $CdCl_2$, followed by $Pb(NO_3)_2$ and CrO_3. Among these two enzymes, the impact was more on GPT than GOT. These results indicated the presence of metabolic disturbances in test prawn due to heavy metal toxicity. The activity of catalase was found to be significantly ($P<0.020 - 0.00$) elevated in test prawns when compared with control. Such an elevation was higher in $CdCl_2$ followed by $Pb(NO_3)_2$ and CrO_3. Therefore, elimination of oxidative stress was in operation in test prawns exposed to these heavy metals. The overall result of this acute exposure to lethal concentrations of these heavy metals indicates the fact that the test prawns were under metabolic disturbances and tried to eliminate the oxidative stress.

KEY WORDS: ***M. rosenbergii*, $CdCl_2$, CrO_3, $Pb(NO_3)_2$, Protein, Carbohydrate, Lipid, GOT, GPT, Catalase.**

P. SARAVANA BHAVAN
Crustacean Biology Laboratory, Department of Zoology, Bharathiar University, Coimbatore- 641046, Tamil Nadu, India.
bhavan@buc.edu.in, psbhavan67@gmail.com

Corresponding author

INTRODUCTION

The giant freshwater prawn, *Macrobrachium rosenbergii* has been regarded as prime aquaculture prospect in many countries. It is a nutritious delicacy for man (Bhavan *et al*., 2010). Now-a-days the 'mother nature' is facing serious threat in all kinds of life due to increased industrialization, urbanization, population growth and consequent overall man's greed to overexploitation. The aquatic pollution due to heavy metals is one among them. The toxicity of heavy metals to aquatic resources particularly on fishes and prawns are well studied (Kabila *et al*., 1999, 2000a, b; Yamuna *et al*, 2002, 2009, 2012; Carpene *et al*., 2003; Lodhi *et al*., 2006; Sobha *et al*., 2007; Bhavan *et al*., 2008; Asagba *et al*., 2008; Palaniyappan and Vijayasundaram, 2008; Ates *et al*., 2008; Kalyanaraman and Kumaar, 2009). Cadmium, chromium and lead are heavy metals with limited biological function (Vallee and Ulmer, 1972; Lane and Morel, 2000; Metwally and Fouad, 2008; Golovanova, 2008) are widely distributed in the environment as results of natural and anthropogenic activities. However, no data is available pertaining to the acute toxicity of these metals on *Macrobrachium*. We have summarized the available 96 h LC_{50} in the form of table for these metals in other crustaceans (Table 1). .

Table1
Available reported LC_{50} values of $CdCl_2$, CrO_3 and $Pb(NO_3)_2$ in crustacean species

Heavy metal	Organism	96 h LC_{50} (mg/l)	Author (s)
$CdCl_2$	*Clibanarius africanus* (hermit crab)	13.423	Otitoloju and Perdo, 2002
	Sesarma huzardi (mangrove crab)	122.998	Otitoloju and Perdo, 2002
	Litopenaeus vannamei (white shrimp)	1.07	Wu and Chen, 2004
	Corophium insidiosum (brackish water crustacean)	2.11	Prato *et al*., 2008
	Litopenaeus vannamei (white shrimp)	1.07	Wu *et al*., 2009
	Brachionus plicatilis (rotifer)	0.8	Arulvasu *et al*., 2010
CrO_3	*Carcinus meanas* (shore Crab)	15.0	Elumalai *et al*., 2002
	Ceriodaphnia dubia (water fleas)	3.711	Baral *et al*., 2006
$Pb(NO_3)_2$	*Scylla serrata* (mud crab)	370.0	Krishnaja *et al*., 1987
	Clibanarius africanus (hermit crab)	370.76	Otitoloju and Perdo, 2002
	Sesarma huzardi (mangrove crab)	2320.13	Otitoloju and Perdo, 2002
	Thenus orientalis (sand lobster)	0.120	Kalyananaraman and Senthilkumar, 2009
	Callinectes amnicola (blue crab)	0.041mM (13.57 mg/l)	Adebayo *et al*., 2009

Cadmium chloride is widely used in cadmium-nickel battery production, pigments for plastics and enamels, fumicides, electroplating and metal coating. The principle uses of chromium are in the metallurgical processing of ferrochromium and other metallurgical products to impart corrosion resistance, chiefly stainless steel. Chromium is used in chemical processing to produce chromic acid and chromates. Lead nitrate is widely used in battery manufacture, fuel additives, manufacturing of ammunition, caulking, compounds, solders, pigments, paints, herbicides and insecticides.

Different studies revealed on biochemical effects of heavy metals on fishes and prawns to establish the potential of predictive biomarkers for use in water pollution monitoring (Kabila *et al*., 1999, 2000a, b; Yamuna *et al.*, 2002, 2009, 2012; Carpene *et al*., 2003; Sobha *et al*., 2007; Ahmed *et al.,* 2007; Ogueji *et al*., 2007; Bhavan *et al*., 2008; Asagba *et al*., 2008; Palaniyappan and Vijayasundaram, 2008; Ates *et al*., 2008; Metwally and Fouad, 2008; Golovanova, 2008; Kalyanaraman and Kumaar, 2009). In this context it is necessary to obtain scientific information about the effects of heavy metal, cadmium chloride ($CdCl_2$), chromium trioxide (CrO_3) and lead nitrate $Pb(NO_3)_2$ on local, commercially viable freshwater prawn species, *Macrobrachium rosenbergii* to improve risk-assessment studies and to prevent the irreversible effects of these heavy metals on economically important organisms. Therefore, in this study the 96 h acute toxicity of $CdCl_2$, CrO_3 and $Pb(NO_3)_2$ were assessed on *M. rosenbergii* post larvae (PL). Further their possible effects on concentrations of basic biochemical constituents (total carbohydrate, lipid, protein and amino acid), activities of metabolic enzymes (glutamate–oxaloacetate transaminase (GOT) and glutamate–pyruvate transaminase (GPT), and activity of a enzymatic antioxidant, catalase were evaluated on *M. rosenbergii* that had been exposed to 96 h LC_{50} concentrations of these heavy metals.

MATERIAL AND METHODS

The post larvae of *M. rosenbergii* (PL-10) were purchased from Government prawn hatchery, Azhikkode, Thrissur, Kerala, India. They were safely brought to the laboratory in well-oxygenated plastic bags. They were stocked in large cement tank (6' x 4' x 3') and acclimatized for 2 weeks in ground water. During which they were fed with boiled egg albumin, *Artemia* nauplii and commercially available scampi crumble feed alternatively thrice a day. The excreta, unfed feed and exuvia if any were removed daily, three fourth of the water was renewed daily and adequately aerated. The hatchery water had these physicochemical characteristics: pH, 6.7±0.25; total dissolved solids, 1.2±0.05g L^{-1}; dissolved oxygen, 6.5±0.45 mg L^{-1}; BOD, 42.0±1.25mg L^{-1}; COD, 140.0±4.55mg L^{-1}; ammonia, 1.20±0.05 mg L^{-1}. Similarly, the ground water used had these physicochemical characteristics as pH, 7±0.15; total dissolved solids, 0.9±0.005 g L^{-1}; dissolved oxygen, 7.2 ±0.55mg L^{-1}; BOD, 30.0±1.30 mg L^{-1}; COD, 125.0 ±3.2mg L^{-1}; ammonia, 0.028±0.004mg L^{-1}.

Cadmium chloride (98% pure, $CdCl_2.H_2O$; Merck Specialities Pvt. Ltd., Mumbai, India), Chromium trioxide (99% pure, CrO_3; Nice chemicals Pvt. Ltd., Kochi, Kerala, India) and Lead nitrate (99% pure, $Pb(NO_3)_2$; Nice chemicals Pvt. Ltd., Kochi, Kerala, India) were purchased from a local scientific chemical company and used in this study. As these metals are in solid form were dissolved with double distilled water to prepare solutions of the required concentrations. Eleven groups of 10 PL prawns each were taken for each heavy metal in a triplicate exposure set-up of static renewal type. One group served as control and other ten groups were exposed to ten different known concentrations of heavy metal for 96 h to assess the median lethal concentration, 96 h LC_{50} value (ASTM, 1980). Toxic medium was renewed daily by siphoning method and freshly prepared concentrations of heavy metal were added to maintain the toxic level in a steady state. During the experiment the prawns were

neither fed nor aerated. The concentrations and their respective mortality percentage were subjected to probit analysis for calculating the 96 h LC_{50} with 95% confidence limit (Finney, 1971) by adopting SPSS software version-13.

Four groups each with 60 similar sized PL of *M. rosenbergii* in triplicate were taken. One group was served as control and the remaining three groups were subjected to exposure to the 96 h LC_{50} concentrations of these heavy metals for a period of 96 h. During which they were fed *ad libitum* with boiled egg albumin. The excreta, unfed feed and exuvia if any were removed daily. Toxic medium was renewed daily by siphoning method and freshly prepared concentrations of heavy metal were added to maintain the toxic level in a steady state. The mortality of PL was recorded up to 96 h. The remaining PL was sacrificed for estimation of concentrations of basic biochemical constituents, such as total carbohydrate (Roe, 1955), lipid (Folch *et al.,* 1957), protein (Lowry *et al*., 1951) and amino acid (Moore and Stein, 1948), activities of metabolic enzymes, such as GOT and GPT (Reitman and Frankel, 1957) and activity of a enzymatic antioxidant, catalase (Sinha, 1972) in the whole body. The data obtained were analyzed statistically by adopting 'Student t-test' (Zar, 1984) between control and experiments using SPSS, version-13.0 of IBM software.

Table 2
96 hr LC_{50} of $CdCl_2$ on M. rosenbergii PL

Concentration (µg/l)	Observed mortality					Expected mortality (%)	Residual	Probit value	LC_{50} (µg/l)	95% Confidence limits		χ^2
	T1	T2	T3	Mean	%					Upper (µg/l)	Lower (µg/l)	
20	0	1	1	0.66	6.6	7.79	-1.797	0.077	--	--	--	--
30	2	2	1	1.66	16.6	14.58	1.415	0.145				
40	3	2	3	2.66	26.6	24.51	1.489	0.245				
50	5	3	4	4.00	40.0	37.24	2.760	0.372	58.93	61.48	56.32	11.46
60	5	5	6	5.33	53.0	51.55	1.447	0.515				
70	7	7	6	6.66	66.6	65.66	0.333	0.656				
80	8	7	7	7.33	73.3	77.87	-4.871	0.778	--	--	--	--
90	8	8	8	8.00	80.0	87.12	-7.124	0.871				
100	10	10	9	9.66	96.0	93.27	2.723	0.932				
110	10	10	10	10.0	100	96.86	3.136	0.968				

T1, T2, and T3 represented triplicates of exposure each with 10 post larvae of M. rosenbergii.

Table 3
96 hr LC_{50} of CrO_3 on M. rosenbergii PL

Concentration (mg/l)	Observed mortality					Expected mortality (%)	Residual	Probit value	LC_{50} (mg/l)	95% Confidence limits		χ^2
	T1	T2	T3	Mean	%					Upper (mg/l)	Lower (mg/l)	
0.4	0	0	0	0.00	0.0	4.55	-4.557	0.045	--	--	--	--
0.8	2	1	1	1.33	13	9.87	3.127	0.098				
1.2	2	2	2	2.00	20.0	18.72	1.279	0.187				
1.6	3	3	4	3.33	33.3	31.29	1.709	0.312	2.08	2.23	1.93	14.62
2.0	5	5	5	5.00	50.0	46.53	3.467	0.465				
2.4	7	7	6	6.66	66.6	62.30	3.692	0.623				
2.8	8	7	7	7.33	73.3	76.24	-3.244	0.762	--	--	--	--
3.2	8	8	8	8.00	80.0	86.75	-6.753	0.867				
3.6	9	9	10	9.33	93.3	93.51	-0.516	0.935				
4.0	10	10	10	10.00	100	97.23	2.768	0.972				

T1, T2, and T3 represented triplicates of exposure each with 10 post larvae of M. rosenbergii

Table 4
96 hr LC_{50} of $Pb(NO_3)_2$ on M. rosenbergii PL

Concentration (mg/l)	Observed mortality					Expected mortality (%)	Residual	Probit value	LC_{50} (mg/l)	95% Confidence limits		χ2
	T1	T2	T3	Mean	%					Upper (mg/l)	Lower (mg/l)	
5	0	0	0	0.00	0.0	1.94	-1.941	0.019				
10	0	0	1	0.33	3.3	5.78	-2.780	0.057	--	--	--	--
15	2	2	2	2.00	20.0	13.98	6.015	0.139				
20	2	4	3	3.00	30.0	27.80	2.191	0.278				
25	4	6	5	5.00	50.0	46.17	3.825	0.461	25.97	28.22	24.56	15.76
30	6	8	6	6.66	66.6	65.41	0.587	0.654				
35	7	8	7	7.33	73.3	81.29	-5.299	0.812				
40	8	9	8	8.33	83.3	91.64	-5.643	0.916	--	--	--	--
45	10	10	9	9.66	96.6	96.95	3.047	0.969				
50	10	10	10	10.00	100	99.10	0.898	0.991				

T1, T2, and T3 represented triplicates of exposure each with 10 post larvae of M. rosenbergii

Table 5
Concentrations of biochemical constituents in M. rosenbergii PL exposed to 96 h LC_{50} concentrations of heavy metals

Parameters (mg/g^{-1} wet wt.)	Control	$CdCl_2$ 58.93 µg/L	t/ P< (Control Vs. $CdCl_2$)	CrO_3 2.08 mg/L	t/ P< (Control Vs. CrO_3)	$Pb(NO_3)_2$ 25.97 mg/L	t/ P< (Control Vs. $Pb(NO_3)_2$)	F-value
Carbohydrate	30.20 ±1.22	17.88±1.85 (40.7↓)	33.87/ 0.001	18.71 ±1.77 (38.0↓)	36.18/ 0.001	18.18 ±1.90 (39.8↓)	30.61/ 0.001	36.84
Lipid	4.21 ± 0.75	1.84 ± 0.17 (56.2↓)	3.15/ 0.087	1.98 ± 0.13 (52.9↓)	2.88/ 0.102	1.86 ± 0.11 (55.8↓)	2.99/ 0.096	7.27
Protein	40.96±2.23	26.98 ±2.64 (34.1↓)	58.80/ 0.00	29.52 ±2.65 (27.9↓)	46.93/ 0.00	27.37± 2.15 (33.1↓)	292.93/ 0.00	21.94
Amino acids	28.32 ±1.16	17.30 ±1.88 (38.9↓)	26.51/ 0.001	19.45 ±1.62 (31.3↓)	31.35/ 0.001	18.74 ±1.63 (33.8↓)	35.30/ 0.001	29.15

Each value is mean ± SD of 3 individual observations. All the values are significant between P< 0.102 - 0.00 Values in parentheses represent percentage decrease over control.

Table 6
Activities of enzymes in M. rosenbergii PL exposed to 96 h LC_{50} concentrations of heavy metals

Parameters	Control	$CdCl_2$ 58.93 µg/L	t/ P< (Control Vs. $CdCl_2$)	CrO_3 2.08 mg/L	t/ P< (Control Vs. CrO_3)	$Pb(NO_3)_2$ 25.97 mg/L	t/ P< (Control Vs. $Pb(NO_3)_2$)	F-value
Catalase (µmol/mg protein)	44.17±1.47	106.57±5.89 (141.2↑)	-24.45/ 0.002	68.01±3.49 (69.9↑)	-20.44/ 0.002	78.42±4.85 (77.5↑)	-17.55/ 0.003	110.57
GOT (IU/L)	26.33 ±2.60	16.87 ± 2.76 (35.9↓)	102.40/ 0.00	18.60±2.70 (29.3↓)	133.88/ 0.00	17.33±2.50 (34.18↓)	6.92/ 0.020	5.20
GPT (IU/L)	22.27 ±1.10	15.69 ±1.48 (29.5↓)	29.99/ 0.001	17.29±1.97 (22.3↓)	9.91/ 0.010	15.94±1.32 (28.4↓)	49.83/ 0.00	12.47

Each value is mean ± SD of 3 individual observations. All the values are significant between P< 0. 020 - 0.00. Values in parentheses represent percentage increase or decrease over control.

RESULTS AND DISCUSSION

The 96 h LC_{50} values of $CdCl_2$, CrO_3 and $Pb(NO_3)_2$ for *M. rosenbergii* PL were assessed to be 58.93 µg/L, 2.08 mg/L and 25.97 mg/L respectively (Tables 2-4). These result indicate the fact that toxicity of these heavy metals to *M. rosenbergii* was in the order of $CdCl_2$ > CrO_3 > $Pb(NO_3)_2$. Literature survey revealed that the reported 96 h LC_{50} values for $CdCl_2$ and CrO_3 in other crustacean species are many fold higher than that of the values recorded in the present study. In the case of $Pb(NO_3)_2$ some of the reported values are lower than that of the value recoded in the present study (see the table 1 summarized in introduction). It is important to mention here that toxicity of a xenobiotic is governed by many factors, like water temperature, purity of the toxin, life stage of an organism etc.

The levels of biochemical constituents, such as concentrations of total carbohydrate, lipid, protein and amino acid were found to be significantly (P<0.102 - 0.00) lower in test prawns when compared with control (Table 5). Among these heavy metals the recorded impact in contents of these biochemical constituents was severe in test prawns that had been exposed to $CdCl_2$, followed by $Pb(NO_3)_2$ and CrO_3. These results indicated the fact that energy was utilized to mitigate the toxic stress induced through these heavy metals. Among these biochemical reserves total lipid was found to be utilized in higher quantity followed by carbohydrate and protein.

Carbohydrate represents the principle and immediate energy precursors for organisms exposed to stress. Heavy metals and pesticides toxicity have been reported to cause a hypoxic/anoxic condition, which promotes anaerobic glycolysis and decrease oxidative metabolism in fishes and prawns (Bhavan and Geraldine, 1997, 2001, 2002, 2009; Geraldine *et al*., 1999; Vijayavel *et al*., 2006; Bhavan *et al*., 2008; Logaswamy and Remia, 2009; Suryavanshi *et al*., 2009). Operations of such conditions in the present study necessitating the utilization of carbohydrate to meet the energy demand occurred due to heavy metal toxicity.

Lipid is reported to severe as an alternate source of energy in crustaceans, particularly during stress conditions (Gilbert *et al*., 1970; Chang and O' Conner *et al.,* 1983; Bhavan and Geraldine, 1997, 2001, 2002, 2009). In this study, hydrolysis of lipid was takes place in order to cope with the increased energy demand occurring due to heavy metal toxicity. Similar observations have been reported in fishes and prawns due to heavy metals and pesticides toxicity (Roe *et al.,* 1981; Pant *et al.,* 1987; Bhavan and Geraldine, 1997, 2001, 2002, 2009; Carpene *et al*., 2003; Vijayavel *et al*., 2006; Sobha *et al*., 2007; Bhavan *et al*., 2008; Palaniyappan and Vijayasundaram, 2008; Kalyanaraman and Kumaar, 2009; Logaswamy and Remia, 2009).

As carbohydrate and lipid reserves were exhausted in fast rate, the utilization of protein was necessitated in prawns that had been exposed to $CdCl_2$, CrO_3 and $Pb(NO_3)_2$. Decrease in total protein have also been reported in fishes and prawns due to heavy metals and pesticides toxicity (Nagabhushanam *et al*., 1987; Jaiswal *et al*., 1991; Reddy *et al.,* 1991; Yasmeen *et al*., 1991; Bhavan and Geraldine, 1997, 2001, 2002, 2009; Geraldine *et al*., 1999; Carpene *et al*., 2003; Sobha *et al*., 2007; Bhavan *et al*., 2008; Palaniyappan and Vijayasundaram, 2008; Logaswamy and Remia, 2009; Suryavanshi *et al*., 2009). Utilization of protein in test prawn was confirmed through parallel decrease in total amino acid level recorded (Table 5). Therefore, it can be hypothetically explained that protein degradation/ proteolysis was induced due to heavy metal stress to activate physiological compensatory mechanisms for providing intermediates to derive energy through Kreb's cycle, and to compensate osmoregulatory problems (arising out of leakage of ions and other essential molecules) by enhancing the free amino acid level in the hemolymph of *Macrobrachium malcolmsonii* (Bhavan and Geraldine, 1997, 2001, 2002, 2009). Similar compensatory mechanisms were possibly operative in test prawns exposed to heavy metals in this study.

The activities of metabolic enzymes, GOT and GPT were found to be significantly ($P<0.020$ - 0.00) decrease in test prawns when compared with control **(Table 6).** The percentage decrease in GOT and GPT was higher in prawns exposed to $CdCl_2$, followed by $Pb(NO_3)_2$ and CrO_3. Among these two metabolic enzymes, the impact was more on GOT than GPT. These results indicated the presence of metabolic disturbances in test prawn due to inhibitions of these enzymes owing to the lethal concentrations of these heavy metals. GOT also known as aspartate aminotransferase (ASAT), catalyzes an important reaction of the molecular rearrangement involving amino acids linked to the citric acid cycle at two points (oxaloacetic and ketoglutaric acids), being the most important mechanism for introducing reduction equivalents into mitochondria (Urich,1994). GPT, also known as alanine aminotransferase (ALAT), predominates in organs with intensive glycogenesis, such as the liver (Urich, 1994; Torre *et al*., 2000). GOT and GPT are important diagnostic tools in medicine and clinics, and are used to detect the toxic effects of various pollutants (Nelson and Cox, 2000).

The effects of heavy metals on fish serum and liver GOT and GPT activities have been reported (Vaglio and Landriscina, 1999; Torre *et al*., 2000; Kim and Kang, 2004). The liver of vertebrate is usually rich in GOT and GPT, and damage in it initially can result in the liberation of large quantities of these enzymes in to the blood. It has been reported in *Sparus aurata* that Cd exposure decreased GOT and GPT activities in liver cells (Vaglio and Landriscina, 1999). In fact, related studies on crustaceans are fairly limited, except a few. Galindo-Reyes *et al.,* (2000) reported insignificant alterations in GOT and GPT activities in *Litopenaeus vannamei* exposed to pesticides. In this study, the recorded significant decrease in activities of GOT and GPT were due to the inhibitory effects of lethal concentrations of these heavy metals. Further, it is suggested severe damage in structural integrity of endoplasmic reticulum and membrane transport system as reported in *Tilapia zilli* due to lead (Karatas and Kaley, 2002) in *Boleopthalmous dussumieri* due to chlorpyrifos (Roy, 2002) and in *Perna viridis* due to Zinc (Anand *et al.,* 2010) induced toxicities.

The antioxidant enzyme, catalase activity is considered as a sensitive biomarker of oxidative stress before hazardous effects occur in organisms exposed to pollutants (Gul *et al.,* 2004). Alterations in activity of catalase (either induced or inhibited depending on the dose, the species or the route of exposure) have been reported in animals exposed to organic or metallic contaminants under field and laboratory conditions (Romeo *et al*., 2000; Sanchez *et al*., 2005). In the present study, activity of catalase was found to be significantly ($P<0.020$ - 0.00) elevated in test

prawns when compared with control (Table 6). Such an elevation was higher in $CdCl_2$ followed by $Pb(NO_3)_2$ and CrO_3. This result suggests production of H_2O_2 and thereby elimination of oxidative stress induced by these metal toxins was in operation in test prawns. The elevation of catalase activity have also been reported in the following animals: in *Carassius auratus* treated with cadmium (Zikic *et al*., 2001), in rainbow trout administered with (ROS-generating) ciprofibrate (Yang *et al*., 1990), in *Brachydanio rerio* (Paris-Palacios *et al*. 2000) exposed due to copper sulfate, in *Oriochromis niloticus* exposed to metal (Atli and Canli, 2007), in *Cyprinus carpio* exposed to Cu (Dautremepuits *et al.,* 2004), in *Oriochromis mossambicus* exposed to Cu (Basha and Rani, 2003) and in fish samples (taken in the wild) due to heavy metal contaminations (Metwally and Fouad, 2008).

From the above discussion, it is clear that $CdCl_2$, CrO_3 and $Pb(NO_3)_2$ toxicity adversely affects some metabolic functions in *M. rosenbergii* PL and these were reflected in alterations in concentrations of total carbohydrate, lipid, protein and amino acids, and activities of GOT, GPT and catalase. This study needs further clarification at various life stages of this organism to conclude that whether these changes can be considered as sensitive biomarkers for environmental monitoring and this species of prawn as a biological indicator of heavy metal pollution. However, the data presented in this study will definitely serve as reference material for future investigators.

ACKNOWLEDGEMENT

The University Grants Commission (UGC), Government of India, New Delhi is gratefully acknowledged for the financial support provided in the form of Research Fellowship in Science for Meritorious Students (RFSMS) to the first author. This agency is also acknowledged for utilizing the facility acquired through Major Research Project sanctioned to the second author, Dr. P. Saravana Bhavan. The first author is sincerely thanking her colleagues Mr. S. Radhakrishnan, T. Muralisankar and N. Manickam for rendering their supports in preparation of this manuscript.

REFERENCES

1. Adebayo, A.O., K.E. Olugbenga, and O.O. Adesola, 2009. Biological responses in edible crab, *Callinectes amnicola* that could serve as markers of heavy metals pollution. *Environmentalist*, 29: 37–46.
2. Ahmed, M., E.F. Shehawi, K. Ali, and A.S. Mohamed, 2007. Estimation of water pollution by genetic biomarkers in tilapia and catfish species shows species-site interaction. *African J. Biotech.*, 6: 840-846.
3. Anand, S., C.S. Purushothaman, A. Pal, and K.V. Rajendran, 2010. Toxicological studies on the effect of copper, lead and zinc on selected enzymes in adductor muscle and intestinal diverticula of the green mussel *Perna viridis*. *Indian J. Mari.,* 39: 299-302.
4. Arulvasu, C., K. Padmini, P. Prabu, N. Thangaraju, D. Dinesh, and S. Sellamuthu, 2010. Evaluation of cadmium toxicity on the population growth of *Brachionus plicatilis* (O. F. Muller). *Indian J. Sci. Technol.,* 3: 90-93.
5. Asagba, S.O., G.E. Eriyamremu, and M.E. Igberaese, 2008. Bioaccumulation of cadmium and its biochemical effect on selected tissues of the cat fish (*Clariasga riepinus*). *Fish Physiol. Biochem.*, 34: 61–69.
6. ASTM, 1980. American Society for Testing and Materials, Standard practice for conducting acute toxicity test with fishes, macro invertebrates and amphibians. In: *Annual Book of ASTM Methods*. American Society for Testing and Materials, Philadelphia, 1: 272-296.

7. Ates, A.S., T. Ozcan, and T. Katağan, 2008. On the occurrence of the mediterranean endemic majid crab, *Inachus parvirostris* (Risso, 1816) (Decapoda, Brachyura, Majidae) in Turkish Seas. *Crustaceana,* 81: 503-506.
8. Atli, G. and M. Canli, 2007. Enzymatic responses to metal exposures in freshwater fish *Oreochromis niloticus*. *Comp. Biochem. Physiol*.C., 145: 282-287.
9. Baral, A., R. Engelken, W. Stephens, J. Farris, and R. Hannigan, 2006. Evaluation of aquatic toxicities of chromium and chromium-containing effluents in reference to chromium electroplating industries. *Arch. Environ. Contam. Toxicol., 50*: 496–502.
10. Basha, P.S. and A.U. Rani, 2003. Cadmium-induced antioxidant defense mechanism in freshwater teleost *Oreochromis mossambicus* (Tilapia). *Ecotoxicol. Environ. Saf.,* 56: 218-221.
11. Bhavan, P.S. and P. Geraldine, 2001. Biochemical stress responses in tissues of the prawn *Macrobrachium malcolmsonii. Pesitc. Biochem. Physiol.,* 70: 27–41.
12. Bhavan, P.S. and P. Geraldine, 1997. Alterations in concentrations of protein, carbohydrate, glycogen, free sugar, and lipid in the prawn, *Macrobrachium malcolmsonii* on exposure to sublethal concentrations of endosulfan. *Pestic. Biochem. Physiol.,* 58: 89–101.
13. Bhavan, P.S. and P. Geraldine, 2002. Carbaryl-induced alterations in biochemical metabolism of the prawn *Macrobrachium malcolmsonii*. *J. Environ. Biol.,* 23: 157-162.
14. Bhavan, P.S. and P. Geraldine, 2009. Manifestation of carbaryl toxicity on soluble protein and histopathology in the hepatopancreas and gills of the prawn *Macrobrachium malcolmsonii. J. Environ. Biol.,* 30: 533–538.
15. Bhavan, P.S., A. Yamuna, and P. Geraldine, 2008. Mercury induced metabolic changes in the juveniles of the economically important freshwater prawn *Macrobrachium malcolmsonii. Asian J. Anim. Sci.,* 3: 60–65.
16. Bhavan, P.S., S. Radhakrishnan, C. Seenivasan, R. Shanthi, R. Poongodi, and S. Kannan, 2010. Proximate composition and profiles of amino acids and fatty acids in the muscle of adult males and females of commercially viable prawn species *Macrobrachium rosenbergii* collected from natural culture environments. *Int. J. Biol.,* 2: 107-119.
17. Carpene, E., G. Andreani, M. Monari, M. Kindt, and G. Isani, 2003. Biochemical changes during post larval growth in white muscle of Gilthead sea bream *Sparus aurata* fed Zinc fortified diets. *Vet. Res. Commun*. 27: 215-281.
18. Chang, E. and J. O' Connor, 1983. Metabolism and transport of carbohydrates and lipids. In: *The Biology of Crustacea*, (ed. by D.E. Bliss), Academic Press, New York. Vol.5: 263–287.
19. Dautremepuits, C., S. Paris-Palacios, S. Betoulle, and G. Vernet, 2004. Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio* L.) induced by copper and chitosan. *Comp. Biochem. Physiol.,* 137C: 325-333.
20. Elumalai, M., C. Antunes, and L. Guilhermino, 2002. Effects of single metals and their mixtures on selected enzymes of *Carcinus maenas*. *Water Air Soil Poll.,* 141**:** 273–280.
21. Finney, D.J., 1971. Probit Analysis. Cambridge, Cambridge University Press. UK.
22. Folch, J., M. Lees and G.H. Bloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.,* 266: 497-509.
23. Galindo-Reyes, J.G., L.D. Venezia, G. Lazcano-Alvarez, and H. Rivas-Mendoza, 2000. Enzymatic and osmoregulative alterations in white shrimp *Litopenaeus vannamei* exposed to pesticides. *Chemosphere,* 40: 233–237.
24. Geraldine, P., P.S. Bhavan, J. Kaliamurthy, and Z. Zayapragasaarazan, 1999. Effects of dichlorvos intoxication in the fresh water prawn *Macrobrachium*

malcolmsonii. *J. Environ. Biol.,* 20: 141–148.
25. Gilbert, L.I. and J.D.O. Conner, 1970. Lipid metabolism and transport in arthropods. In; Chemical Zoology (ed. by M. Florkin, & B.T. Sheer), Academic press, New York. Vol. 5: 229-254.
26. Golovanova, I. L., 2008. Effects of heavy metals on the physiological and biochemical status of fishes and aquatic invertebrates. *Inland Water Biology* (*Aquatic Toxicology*), 1: 93–101.
27. Gul, S., E. Belge-Kurutaş, E. Yıldız, A. Sahan, and F. Doran, 2004. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living in Seyhan Dam Lake, Turkey. *Environ. Int.,* 30: 605- 609.
28. Jaiswal, K., R. Sarojini, and R. Nagabhushanam, 1991. Chronic effects naphthalene on total protein, free amino acids, RNA and DNA in certain tissue of freshwater prawn *Macrobrachium kistensis*, *J. Environ. Biol.,* 12: 51-56.
29. Kabila, V., P.S. Bhavan, and P. Geraldine, 2000a. Tissue specific expression of stress 70 in the freshwater prawn *Macrobrachium malcolmsonii* following exposure to nickel. *Asian J. Microbiol. Biotech. Environ. Sci.,* 2: 91–94.
30. Kabila, V., P.S. Bhavan, and P. Geraldine, 2000b. Induction of metal binding protein in the freshwater prawn *Macrobrachium malcolmsonii* following exposure to nickel. *J. Nat. con.,* 12:95–102.
31. Kabila, V., P.S. Bhavan, and P. Geraldine, 1999. The freshwater prawn *Macrobrachium malcolmsonii* an accumulator of nickel. *J. Environ. Boil.,* 20: 307–312.
32. Kalyanaraman, V. and P.S. Kumar, 2009. Effect of lead on the expression of nutritional content in edible lobster, *Thenus orientalkis. Indian J. Sci. Tech.,* 2: 17-22.
33. Kaoud, H.A. and R.A. Eldahshan, 2010. Bioaccumulation of cadmium in the fresh water prawn *Macrobrachium rosenbergii. Nature and Science*, 8: 157-168.
34. Karatas, S. and M. Kaley, 2002. Accumulation of lead in the gill, liver, kidney and brain tissues of *Tilapia zilli. Turkish J. Veteri. Anim. Sci.,* 26: 471–477.
35. Kim, B.H., K.S. Kang, and Y.S. Lee, 2004. Effect of retinoids on LPS-induced COX-2 expression and COX-2 associated PGE_2 release from mouse peritoneal macrophages and TNF-α release from rat peripheral blood mononuclear cells. *Toxicol. Lett.,* 21: 191-201.
36. Lane, T.W. and F.M.M. Morel, 2000. A biological function for cadmium in marine diatoms. *Proc Natl. Acad. Sci.,* USA, 97, 4627-4631.
37. Lodhi, H.S., M.A. Khan, R.S. Verma, and U.D. Sharma, 2006. Acute toxicity of copper sulphate to fresh water prawns. *J. Environ. Biol.,* 27: 585-588.
38. Logaswamy, S. and K.M. Remia, 2009. Impact of cypermethrin and Ekalux on respiratory and some biochemical activities of a freshwater fish *Tilapia mossambica. Curr. Biotech.,* 3: 65-73.
39. Lowry, O. H. and W. J. Rosenberg, 1951. A.L. Fair, and R.J. Randall, 1951. Protein measurement with Folin- phenol reagent. *J. Biol. Chem.,* 193: 265- 275.
40. Metwally, M.A.A. and I.M. Fouad, 2008. Biochemical changes induced by heavy metal pollution in marine fishes at Khomse, Limya. *Global Veterinaria,* 2: 308-311.
41. Moore, S. and W.H. Stein, 1948. Photometric ninhydrin method for use in the chromatography of amino acid. *J. Biol. Chem.,* 176: 367-388.
42. Nagabhushanam, R., J. Deshpande, and R. Sarojini, 1987. Effects of some pesticides on biochemical constituents of the freshwater prawn *Macrobrachium kistensis*, *Proc. Natl. Symp. Ecotoxicol.* pp. 73.
43. Nelson, D.L. and M.M. Cox, 2000. Lehninger Principles of Biochemistry (eds. By D.L. Nelson, and M.M. Cox). Worth Publishers, New York. pp. 623.
44. Ogueji, E. and O.J. Auta, 2007. The effects of sub-lethal doses of Lambda-cyhalothrin on some biochemical characteristics of the African catfish,

Clarias gariepinus. *J. Biol. Sci.,* 7: 1473-1477.

45. Otitoloju, A.A. and K.N. Don Petro, 2002. Establishment of the toxicity ranking order of heavy metals and sensitivity scale of benthic animals inhabiting the *Lagos lagoon.West African J. Appl. Ecol.,* 3: 31-41.
46. Palaniyappan, P.L.R.M. and V. Vijayasundaram, 2008. Ftir study of arsenic induced biochemical changes on the liver tissues of freshwater fingerlings *Labeo rohita. Roomanian J. Biophys.,* 18: 135-144.
47. Pant, J., H. Tewari, and T.S. Gill, 1987. Effects of aldicarb on the blood and tissues of a fresh water fish. *Biol. Environ. Contam. Toxicol*., 38: 36-40.
48. Paris-Palacios, S., S. Biagianti-Risbourg, and G.Vernet, 2000. Biochemical and (ultra) structural hepatic perturbations of *Brachydanio rerio* (Teleostei, Cyprinidae) exposed to two sub lethal concentrations of copper sulfate. *Aquat. Toxicol.,* 50: 109-124.
49. Prato, E., C. Scardicchio., and F. Biandolino, 2008. Effects of temperature on the acute toxicity of cadmium to *Corophium insidiosum*. *Environ. Monit. Assess*., 136: 161-166.
50. Reddy, A.N., N.B.R.K. Venugopal, and S.L.V. Reddy, 1991. Effects of endosulfan 35 EC on certain aspects of protein metabolism in various tissues of a freshwater field crab *Barytephusa guerini. Pestic. Biochem. Physiol.,* 39: 121-129.
51. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *American J. Clin. Pathol.,* 28: 56-63.
52. Roe, J.H., 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. *J. Biol. Chem.*, 153: 335-343.
53. Roe, J.R. and K.V.R. Roa, 1981. Lipid derivation in the tissue of fresh water teleost, *Sarotheerodon mosambicus* (Peter), effects of methyl parathion, *Proc. Indian Natl. Sci. Acad.*, 47: 53-58.
54. Romeo, M., N. Bennani, M. Gnassia-Barelli, M. Lafaurie, and J.P. Girard, 2000. Cadmium and copper display different responses towards oxidative stress in the kidney of the sea bass *Dicentrarchus labrax*. *Aquat. Toxicol*., 48: 185-194.
55. Roy, S.S., 2002. Some toxicological aspects of chlorpyrifos to the intertidal fish *Boleopthalmus dussumieri*. Ph.D., thesis, University of Mumbai, India. pp. 52–71.
56. Sanchez, W., O. Palluel, L. Meunier, M. Coquery, J.M. Porcher, and S. Ait-Aissa, 2005. Copper-induced oxidative stress in three-spined stickleback: relationship with hepatic metal levels. *Environ. Toxicol. Pharmacol.,* 19: 177-183.
57. Sinha, A.K., 1972. Colorimetric assay of Catalase. *Anal. Biochem.,* 47: 389-394.
58. Sobha, K., A. Poornima, P. Harini, and K. Veeraiah, 2007. A study on biochemical changes in the fresh water fish *Catla catla* exposed to the heavy metal toxicant cadmium chlorid. *J. Sci. Engine. Technol.,* 24:77-87.
59. Suryavanshi, U., R.A. Sreepada, Z.A. Ansari, S. Nigam, and S. Badesab, 2009. A study on biochemical changes in the penaeid shrimp, *Metapenaeus monoceros* (Fabricius) following exposure to sublethal doses of organochlorine pesticide (Endosulfan). *Chemosphere,* 77: 1540-1550.
60. Torre, F.R., A. Salibian, and L. Ferrari, 2000. Biomarkers assessment in juvenile *Cyprinus carpio* exposed to waterborne cadmium, *Environ. Poll.,* 109: 277-282.
61. Urich, K., 1994. The structure and metabolism of carbohydrates. In: *Comparative Animal Biochemistry*, *Berlin*, *Springer*. pp. 463-513
62. Vaglio, A. and C. Landriscina, 1999. Change in liver enzyme activity in the teleost *Sparus aurata* in response to cadmium intoxication. *Ecotoxicol. Environ. Saf*., 43: 111-116.
63. Valee, B.L. and D.D. Ulmer, 1972. Biochemical effect of mercury, cadmium and lead. *Annu. Rev. Biochem.,* 41: 91-128.
64. Vijayavel, K., C. Anbuselvam, M.P. Balasubramanian, S.V. Deepak, and S.

Gopalakrishnan, 2006. Assessment of biochemical components and enzyme activities in the estuarine crab *Scylla tranquebarica* from naphthalene contaminated habitants. *Ecotoxicol.,* 15: 469-476.

65. Wu, J.P. and H.C. Chen., 2004. Effects of cadmium and zinc on oxygen consumption, ammonium excretion, and osmoregulation of white shrimp (*Litopenaeus vannamei*). *Chemosphere,* 57: 1591–1598.
66. Wu, J.P., H.C. Chen, and D.J. Huang, 2009. Histopathlogical alternations in gills of white shrimp *Litopenaeus vannamei (Boone*) after acute exposure to cadmium and Zinc. *Bull. Environ. Contam. Toxicol.,* 82: 90–95.
67. Yamuna, A., P.S. Bhavan, and P. Geraldine, 2002. Effects of Hg and Cu on hemocytes mediated functions in the prawn *Macrobrachium malcolmsonii*. *J. Environ. Biol.,* 23: 7–13.
68. Yamuna, A., P.S. Bhavan, and P. Geraldine, 2009. Ultrastructural observations in gills and hepatopancreas of prawn *Macrobrachium malcolmsonii* exposed to mercury. *J. Environ. Biol.,* 30: 693–699.
69. Yamuna, A., P.S. Bhavan, and P. Geraldine, 2012. Glutathione S-transferase and metallothionein levels in the freshwater prawn *Macrobrachium malcolmsonii* exposed to mercury. *J. Environ. Biol*., 33: 133-137.
70. Yang, J.F., R.B. Stein, J. Jhamandas, and T. Gordon, 1990. Motor unit numbers and contractile properties after spinal cord injury. *Ann. Neurol*., 28: 496-502.
71. Yasmmen, R., S.J. Tulasi, and J.V.R. Rao, 1991. Metabolic changes in the air-breathing fish *Anabas scandens* on long term exposure to endosulfan, *Pestic. Biochem. Physiol.,* 40: 205-211.
72. Zar, J.H. 1984. Biostatistical analysis, (ed. by E. Kurtz), 3rd Edn. Prentice Hall, Inc., New Jersey. pp. 184.
73. Zikic, R.V. and S. Stajn, 2001. Activities of superoxide dismutase and catalase in erythrocyte and plasma transaminases of goldfish *(Carassius auratus gibelio Bloch*) exposed to cadmium. *Physiol. Res*., 50: 105-111.