

## A study on Behavioral responses to sub lethal and lethal concentrations of cadmium chloride (cdcl<sub>2</sub>.H<sub>2</sub>O) in freshwater crab *paratelpusa hydrodromous* (Decapoda: Brachyura)



### Zoology

**KEYWORDS :** Heavy metal, Cadmium chloride, acute toxicity, paratelpusa hydrodromous.

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### ABSTRACT

*Cadmium (Cd), one of the twenty three heavy metal toxicants, is widely used in Ni-Cd batteries manufacture, metal and mining industry, dentistry etc. These excess amounts in addition to naturally occurring levels gradually build up to toxic levels causing damage to the biota of the aquatic ecosystem. The test species for this study was Paratelpusa hydrodromous which was chosen for its abundance and commercial and ecological importance. 96 hrs LC<sub>50</sub> values for premoult and postmoult male crabs were found to be 158.49 ppm and 156.68 ppm. For premoult and postmoult female crabs, these values were 138.68 ppm and 132.43ppm. Each batch of 3 crabs was exposed to a sub lethal concentration (20 ppm) and lethal concentrations (200 to 800 ppm). The present study evaluates toxicity of Cd and its impact on behavioral responses in the fresh water field carp paratelpusa hydrodromous.*

### INTRODUCTION

The problem of heavy metal pollution on aquatic organisms draws much attention. Information concerning toxicities of heavy metals are widespread and however seem to be limited only to certain animals. The rivers are known to transport and accumulate significant amount of persistent pollutants. Metals particularly mercury, copper, cadmium and chromium are common aquatic pollutants of urban and industrial origin (Abel 1989; Kennish, 1992). Many crustaceans are widely used as toxicity test species since they are being considered as ecologically important and commercially relevant for mankind. Earlier findings have been extended towards many decapod crustaceans such as *Crangon crangon* (Partman and Wildson, 1971), *Homarus americanus* (Johnson and Gentile, 1979), *Palaemon serratus* (Wilson and Cannor 1971) and *Pjaponicus* (Bombang *et al*; 1995).

Cadmium is a silver white metal with an (Atomic weight of 112.4 and a low melting point of 321°C). It is rare and not found in pure state in nature and is a constituent of smithsonite (ZnCo<sub>3</sub>). Cadmium (Cd) is a well known heavy metal toxicant with a specific gravity 8.65 times greater than water (Lide 1992). Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. The target organs for Cd toxicity have been identified as liver, placenta, kidneys, lungs, brain and bones (Roberts 1999). If the laboratory testing procedures indicate blood levels of cadmium above 5 mcg/dL and creatinine levels in urine above 10 mcg/dL, then it can be considered to be suggestive of Cd toxicity (Dupler 2001).

The occurrence of Cd in considerably toxic amounts was reported by earlier workers in various aquatic ecosystems (Arno Kaschl *et al.*, 2002; BR Kiran *et al.*, 2006). Cd was found to be teratogenic, embryotoxic, carcinogenic, nephrotoxic in humans and the risk is greater among smokers (Sunderman *et al.*, 1991). Cd can be taken up from the environment into the body through pulmonary and enteric pathways. Cd, like many other heavy metals, is antagonistic to essential trace elements like Fe<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, Ca<sup>2+</sup> etc (Wright and Frain 1981).

*Paratelpusa hydrodromous* (Decapoda: Brachyura) exhibiting a wide distribution in freshwater, inhabiting a habitat with gravel of stones in waterways and also among the vegetation around the channels of paddy fields. This species is considered to an opportunistic omnivorous in feeding. This is one of the important crustacean in the freshwater food chain due to their higher abundance and its multiple role as scavenger, predator and also as a prey to higher vertebrates. This crab is exposed to various contaminants and is suppose to potentially accumulate considerable amount of metallic pollutants. This is ecologically and economically important tropical species with outstanding potential as sentinel organism. The aim of this present investi-

gation was focused on the determination of acute toxicity (LC<sub>50</sub> for 96 hrs) of premoult and postmoult a crabs belonging to both sexes exposed to metal cadmium.

### MATERIALS AND METHOD

Healthy and active crabs *Paratelpusa hydrodromous* were collected from the river bed, canals, paddy fields, etc., situated in and around the rivers of Cauvery and Bhavani. Both sexes of crab at their intermoult stage having an average carapace length of 3.0 ± 0.5 cm and breadth of 4.0 ± 0.5 cm were used for this study. They were maintained in a large cement tank (size : Length - 120 cm; Breadth - 60 cm; Height - 100cm) and were acclimatized to laboratory conditions for a week before the experiment in freshwater (salinity - 0.5 ± 0.1 ‰; pH - 7.1 ± 0.2 ; Temperature - 28° C ± 2°C) water was changed daily and aerated continuously. The animals were fed daily around 08.00 hrs with soya beans (pre soaked in water). The supply of food was stopped, 24 hrs before the start of dose mortality test to synchronize the physiology of the experimental animal.

The dose mortality tests were carried out based on the differential concentration of cadmium chloride (CdCl<sub>2</sub>). Stock solution was prepared from the analytical grade of cadmium chloride (E. Merek, India). Higher and lower concentrations ranging from 50 ppm to 1000 ppm were prepared and tested to determine approximate mortality rate. After this approximation, dose mortality rate experiments were further preceded. Normal, healthy and active crabs with average size (Length - 3.0 ± 0.5 cm; Breadth - 4.0 ± 0.5 cm) acclimatized previously were selected. Both males and females were segregated for dose mortality test separately. Both were sorted out from the stock and grouped into a number of required batches of 10 in number belonging to premoult and postmoult stages. Each batch of crabs according to sex and molting stage were exposed to different concentrations of cadmium chloride (100 ppm to 300 ppm) prepared from the stock solution separately. One control group (10 in number) was also maintained simultaneously to determine the corrected percentage mortality.

A toxicity evaluation was carried out following bioassay method (Doudoroff *et al.*, 1951). These experiments were started during the early hours of the day under the normal laboratory conditions as mentioned above. Mortality and survival rate in both control and experimental crabs were noted for 96 hrs. Further observations were made on any adverse behavioral changes such as cessation of movement of the body and appendages, erratic respiratory activity, lack of response to external stimuli along with the formation of opaqueness of the body and ultimate death of the animal as the indications of toxic effects of test solutions. Then LC<sub>50</sub> values for the crabs were determined for the crabs from the graphical interpolations and by adapting probit method of analysis (Finney 1971 ; Busvine 1971 ; APHA

– AWWA – WEF 1992). The results on this toxicity evaluation were represented by tabulations with the concentrations being recorded in log scale and the mortality in arithmetic scale. Based on this probit analysis, the actual dose mortality value for each group of crabs due to toxicity of cadmium was determined. The fiducial limits ( $m_1$  – lower limit;  $m_2$  – upper limit) with 95 % confidence of the concentrations were also estimated from the variance.

The crabs were collected and reared in the laboratory. They were acclimatized to laboratory conditions. In order to determine the behavioral responses, the adult crab (inter moult stage) were randomly selected (Carapace length of  $3.0 \pm 0.5$  cm and breadth of  $4.0 \pm 0.5$  cm). Each batch of 3 crabs were exposed to a sub lethal concentration (as per 10%  $LC_{50}$  value of toxicity evaluation; 20 ppm) and lethal concentrations (200, 400, 600 and 800 ppm) of the medium kept in separate round plastic bowls (10 litre). (A control group of 3 crabs was also maintained at  $28_0C \pm 2_0C$ ) was similar to those in the holding storage tank. Behavioral responses of the crabs were observed for a total of 3 hrs. Seven different behavioral activities such as locomotors activity, mouth parts movement, mouth parts cleaning, antennae movement, antennae flicking, antennule retraction, and abdomen extension were observed. These behaviors were recorded for 1 minute at set time intervals in constant light, over a period of 3 hrs (McGaw *et al.*, 1999). Student – Newman pair wise tests for significant differences in behavior between control and each experimental concentration of test solution were performed.

## RESULTS AND DISCUSSION

The 96 hrs  $LC_{50}$  values for cadmium calculated by probit analysis for pre-moult and postmoult male and female crabs of *Paratelphusa hydrodomous* are presented in the Tables: 1 to 4.

The  $LC_{50}$  log dose value of cadmium for the pre-moult male crab was found to be 2.20 and the fiducial limits with 95% confidence level ranged from 2.053 to 2.348. The actual  $LC_{50}$  value of cadmium was found to be 158.49 ppm (Table: 1). In the case of postmoult male crab  $LC_{50}$  log dose value was calculated as 2.195 and the fiducial limits with 95% confidence level were found to be ranging from 1.974 to 2.416. Hence, the actual  $LC_{50}$  value was 156.68 ppm. (Table: 2).

The  $LC_{50}$  log dose value for the pre-moult female crab was 2.142 and the fiducial limits with 95% confidence level were ranging from 1.8790 to 2.4050. The actual  $LC_{50}$  value for this animal was determined as 138.68 ppm. (Table: 3). For the post moult female crab,  $LC_{50}$  log dose value was shown to be as 2.122 and the fiducial limits with 95% confidence level ranged from 2.0460 to 2.1979. The actual  $LC_{50}$  value was found to be 132.43 ppm. (Table: 4).

In the present investigation it was noted that 96 Hrs  $LC_{50}$  value for cadmium for pre-moult and postmoult male crab *Phydrodromous* did not show much variation ( $LC_{50}$  value for pre-moult male crab 158.49 ppm and for postmoult male crab 156.68ppm). Similarly, these two stages did not have much impact on lethality in female crabs also ( $LC_{50}$  value for pre-moult female. 138.68 ppm;  $LC_{50}$  value for postmoult female 132.43 ppm). These results expressed sexual differences on lethality. The females were found to be comparatively lesser tolerant than males.

Tolerance of freshwater organisms would differ due to cationic actions, and there by altering toxicities (Spear and Pierce 1979). Several authors noted the variations of  $LC_{50}$  values for different species of crabs. (Narayanan *et al.*, 1997) reported 96 hrs.  $LC_{50}$  value as 8 ppm for cadmium in the mud crab *Scylla serrata*. As observed for an estuarine crab *Chasmagnathus granulata*, 96 hrs  $LC_{50}$  value of  $2.69 \text{ mg l}^{-1}$  and its subsequent exposure to sub lethal level brought differential permeability in membrane potential (Vitale *et al.*, 1999).

Some other toxicity studies were conducted on the larval forms of the crabs and some other crustaceans. (Balsa *et al.*; 2000) in his comparative study, reported that the larval of spider crab

were remarkably sensitive to copper and cadmium. On his further observation, the duration of exposure was found to be a crucial parameter for obtaining standard measures of  $LC_{50}$  (Johnson and Gentile 1979) for the increase of resistance, size and stage of development in larvae would be influential criteria (Bambang *et al.*; 1994). Based on these limited biological data the sexual variation on toxicity would need some further investigation.

## BEHAVIORAL RESPONSE TO SUB LETHAL CONCENTRATION OF CADMIUM LOCOMOTOR ACTIVITY

In this study the locomotor activity was quantified with the effect of cadmium. The locomotor activity was decreased progressively in all the concentrations either in sub lethal (20 ppm) or in all the lethal concentrations (200 ppm, 400 ppm, 600 ppm and 800 ppm) with the increase in the duration of the experiment from 30 mts to 120 mts. However the concentration increased from sub lethal 20 ppm to lethal 200 to 800 ppm there was a Rain of rate of locomotor activity at the initial stage 30 mts. After that the locomotor response was gradually decreased (Figure:1). The change of locomotary activity was found to be highly significant ( $F=14.17$ ;  $p<0.01$ ) between 20 ppm and 800 ppm and for the exposed concentrations between control and 800 ppm, 200 ppm versus 800 ppm and for higher concentrations between 600 ppm and 800 ppm.

## MOVEMENT OF MOUTH PARTS

The opening and closing of third maxillipedes laterally and the rapid flicking of mouth parts were counted. There was a clear increase of the rate of movement of mouth parts from sub lethal level to lethal level (upto 800 ppm) as an initial response during first 30 mtrs. But in each concentration with increase of duration brought a noticeable decrease of rate of mouth parts (Figure: 2) which was statistically significant ( $F = 13.22$ ,  $p<0.05$  in control Vs 800 ppm;  $F=11.85$ ,  $p<0.05$  in 20 ppm Vs 800 ppm;  $F=10.36$ ,  $p<0.05$  in 200 ppm Vs 800 ppm).

## CLEANING OF MOUTH PARTS

The third maxillipedes and exopodites of the mouth parts were found 10 scrapped as a response for cleaning. This behaviour was observed for earlier duration (30 mts) for each sub lethal and lethal concentrations. However, as the duration proceeded from 30 mts to 120 mts this activity was found to be gradually reduced which was statistically significant ( $F=11.07$ ,  $p<0.05$  in control Vs 800 ppm;  $F=8.16$ ,  $p<0.05$  in 20 ppm Vs 800 ppm). This was found to a similar response recorded for locomotor activity and movement of mouth parts (Figure: 3).

## MOVEMENT OF ANTENNAE

The antennae were found to be folded down and raised up frequently. The erratic movements of antennae were found 10 be noticeable events. Their rate of movement was almost equal during the first 30 mts of the experiment at a sub lethal (20 ppm) and a lethal concentration of 600 ppm. Similar trend was also noticed in lethal concentration of 200 ppm and 400 ppm. At 800 ppm once again there was a decline of the rate of their movements (Figure: 4). The rate of change of movement of antennae was found to be significant between control Vs 800 ppm ( $F=9.138$ ;  $p<0.05$ ).

## FLICKING OF ANTENNAE

The antennae were noticed to be flicked up and down independently of each other which were counted down as another response. This response was almost similar for sub lethal concentration of 20 ppm and lethal concentrations of 200 ppm and 400 ppm at earlier stage. A sudden increase of the rate of flicking for the remaining lethal concentrations of 600 ppm and 800 ppm (Figure: 5). A noticeable significant variations of antennae flicking were found at higher concentration of 800 ppm ( $F=7.927$ ,  $p<0.05$  in control Vs 800 ppm;  $F=12.297$ ,  $p<0.05$  in 200 ppm Vs 800 ppm;  $F=13.284$ ,  $p<0.05$  in 200 ppm Vs 800 ppm;  $F=17.627$ ,  $p<0.01$  in 400 ppm Vs 800 ppm).

## RETRACTION OF ANTENNULES

Antennules showed continuous rapid flicking movements but frequently they were folded backwards into a groove of the car-

apace as another response as retraction. The rate of response was higher in the concentrations of 400 ppm initially but it was found to be decreased in the newline in lethal concentrations of 600 ppm and 800 ppm which were statistically insignificant (Table 5). This erratic trend was similar as observed for antennae movement (Figure 6). The higher lethal concentration (800 ppm) caused significant variations ( $F=5.992, p<0.05$  in control Vs 200 ppm;  $F=7.697, p<0.05$  in control Vs 800 ppm;  $F=7.740, p<0.05$  in 20 ppm Vs 800 ppm).

**EXTENSION OF ABDOMEN**

In the movement of abdomen, the last abdominal sequent was opened and closed initially and the crab raised up itself on its legs. During the first 30 mts, there was a gradual increase of rate of activity in the concentrations of 20 ppm (sub lethal) which was statistical significant ( $F=14.045, p<0.01$ ) and in the lethal level of 200 ppm and 400 ppm. However, the rate of abdominal movement was found to be decreased in the concentrations of 600 ppm and 800 ppm which was statistically insignificant (Table 5). The duration of the experiment from 30 mts to 120 mts was also found to influence this behavior erratically which ultimately resulted in the increase of the rate of activity in the sub lethal level of 20 ppm and lethal level of 800 ppm. (Figure: 7).

The pollutants can exert effects at all levels of biological organization. It is generally believed that the effects at the cellular level could proceed with detectable effects at behavioral level. Pollutants on exerting stress could impair the processes of motivation, orientation and co-ordination. (Weis *et al*; 2000). The effects of anoxia were found to be associated with a number of behavioral responses (Rychter 1997).

The increased duration and the increased concentration caused significant reduction in the activities of the appendages. The higher lethal concentration of cadmium would perhaps exceed the tolerable limit. The erratic movement's antennae and antennules noted with the effect of cadmium indicated the stress which was similar for the crabs *Callinectes sapidus*, *Carcinus maenas*, *Cancer magister* and *Libinia emarginata* exposed to

the differential salinity (McGraw *et al*, 1999). The antennae and antennules were implicated as having chemosensory role in salinity detection (Gleeson *et al*, 1997) which would thus detect the change of external media with cadmium in our experimental animal *P.hydrodromous*.

This startle response was possibly due to irritation of setae of mouth parts which were suppose to be chemosensory in function (Shelton and Laverack 1970). The erratic movement of retraction of antennules loss or irritation with the effect of cadmium in *Phydrodromous* as observed earlier (Gleeson *et al*; 1996). The rate increase of the abdominal extension at higher concentration of cadmium (800 ppm) was found to be an attempt to escape from the adverse medium for *Phydrodromous*. A possible role of hindgut and rectum of the crab to be contacted with water on abdominal extension for ionic regulation could be attributed (Heeg and Cannoe 1966). A major mechanism under the altered behavior would be a possible damage to the nervous system (Weis *et al*; 2000).

**CONCLUSION**

The present study on the crab *P.hydrodromous* with the sub lethal and lethal effect of cadmium under laboratory condition generally showed stressful response increasing the activities of the appendages. The flicking of antennae and antennules retraction was found to be significant. The locomotors activity and the cleaning of mouth parts initially raised. The toxic chemical like cadmium could alter both the structure and function of nerve cells. It could alter the synthesis and release of neurotransmitters, associated with such behavioral changes. The crab *paratelphusa hydrodromous* could be an effective bioaccumulatur as well as indicator organism of aquatic pollution.

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**TABLE 1: PROBIT ANALYSIS FOR THE PREMOULT MALE CRAB (PARATELPHUSA HYDRODROMOUS) EXPOSED FOR 96 HRS. DURATION TO DIFFERENT CONCENTRATIONS OF CADMIUM CHLORIDE**

Concentration of cadmium chloride (ppm)	No. of crabs exposed	% of Mortality	Corrected percentage of mortality	Log dose X	Empirical probit	Expected probit Y	Working probit y	Weiging co-efficient	Weight W	WX	Wy'	WXy'	WX <sup>2</sup>	Wy' <sup>2</sup>	y'
100	10	10	10.00	2.00	3.72	3.5	3.75	0.269	2.69	5.38	10.09	20.18	10.76	37.83	3.12
125	10	20	11.11	2.10	3.77	3.9	3.79	0.405	4.05	8.51	15.35	32.25	17.86	58.17	3.72
150	10	30	22.22	2.18	4.23	4.2	4.24	0.503	5.03	10.97	21.33	46.51	23.90	90.43	4.21
175	10	30	22.22	2.24	4.23	4.4	4.25	0.558	5.58	12.50	23.72	53.12	28.00	100.79	4.57
200	10	50	44.44	2.30	4.85	4.6	4.87	0.601	6.01	13.82	29.27	67.30	31.79	142.54	4.93
225	10	50	44.44	2.35	4.85	4.8	4.86	0.627	6.27	14.73	30.47	71.59	34.63	148.09	5.23
250	10	70	66.67	2.40	5.44	5.0	5.42	0.637	6.37	15.29	34.53	82.87	36.69	187.13	5.53
275	10	90	87.50	2.44	6.18	5.2	5.96	0.627	6.27	15.30	37.37	91.19	37.33	222.72	5.77
300	10	100	100.00	2.48	-	5.3	6.30	0.616	6.16	15.28	38.81	96.26	37089	244.49	6.01
Sum of the products =									48.43	111.78	240.94	561.27	258.85	1232.19	

X̄ = 2.308

Ȳ = 4.975

b = 6.023

a = - 8.925

Regression Equation = Y = - 8.925 + (6.023X)

Chi square (X<sup>2</sup>) = 2.309 (P> 0.05) df = 8, 5% level = 15.507 (Value Not significant)

Variance = 0.0057

Fiducial limit = m1 = 2.053

= m2 = 2.347

LC50 log dose mean value = 2.2

Actual LC50 value = 158.49 ppm

**TABLE 2: PROBIT ANALYSIS FOR THE POSTMOULT MALE CRAB (*PARATELPHUSA HYDRODROMOUS*) EXPOSED FOR 96 HRS. DURATION TO DIFFERENT CONCENTRATIONS OF CADMIUM CHLORIDE**

Concentration of cadmium (ppm)	No. of crabs exposed	% of Mortality	Corrected percentage of mortality	Log dose X	Empirical probit	Expected probit Y	Working probit y	Weighing co-efficient	Weight W	WX	Wy'	WXy'	WX <sup>2</sup>	Wy <sup>2</sup>	y'
100	10	10	10.00	2.00	3.72	3.7	3.72	0.336	3.36	6.72	12.50	25.00	13.44	46.50	3.27
125	10	20	11.11	2.10	3.77	4.0	3.80	0.439	4.39	9.22	16.68	35.03	19.36	63.39	3.79
150	10	30	22.22	2.18	4.23	4.2	4.24	0.503	5.03	10.97	21.33	46.51	23.90	90.43	4.20
175	10	40	33.33	2.24	4.56	4.4	4.58	0.558	5.58	12.50	25.56	57.25	28.00	117.05	4.51
200	10	50	33.33	2.30	4.56	4.5	4.57	0.581	5.81	13.36	26.55	61.05	30.73	121.34	4.82
225	10	60	37.50	2.35	4.69	4.7	4.68	0.616	6.16	14.48	28.83	67.77	34.02	134.92	5.08
250	10	70	50.00	2.40	5.00	4.8	5.00	0.627	6.27	15.05	31.35	75.25	36.11	156.75	5.33
275	10	80	75.00	2.44	5.67	5.0	5.63	0.637	6.37	15.54	35.86	87.49	37.92	201.91	5.54
300	10	100	100.00	2.48	-	5.1	6.26	0.634	6.34	15.72	39.69	98.41	38.99	248.45	5.75
Sum of the products =									49.31	113.56	238.35	553.76	262.47	1180.74	

X- = 2.303  
 Y- = 4.834  
 b = 5.149  
 a = -7.024  
 Regression Equation =  $Y- = -7.024 + (5.149X)$   
 Chi square (X2) = 3.64 (P> 0.05) df = 8, 5% level = 15.507 (Not significant)  
 Variance = 0.01276  
 Fiducial limit = m1 = 1.974  
 = m2 = 2.416  
 LC50 log dose mean value = 2.195  
 Actual LC50 value = 156.68 ppm

**TABLE 3: PROBIT ANALYSIS FOR THE PREMOULT FEMALE CRAB (*PARATELPHUSA HYDRODROMOUS*) EXPOSED FOR 96 HRS. DURATION TO DIFFERENT CONCENTRATIONS OF CADMIUM CHLORIDE**

Concentration of cadmium (ppm)	No. of crabs exposed	% of Mortality	Corrected percentage of mortality	Log dose X	Empirical probit	Expected probit Y	Working probit y	Weighing co-efficient	Weight W	WX	Wy'	WXy'	WX <sup>2</sup>	Wy <sup>2</sup>	y'
100	10	10	10.00	2.00	3.72	3.7	3.72	0.336	3.36	6.72	12.50	25.00	13.44	46.50	3.60
125	10	10	10.00	2.10	3.72	4.2	3.82	0.503	5.03	10.56	19.21	40.34	22.18	73.40	4.09
150	10	20	20.00	2.18	4.16	4.6	4.20	0.601	6.01	13.10	25.24	55.02	28.56	106.02	4.47
175	10	30	22.22	2.24	4.23	4.9	4.30	0.634	6.34	14.20	27.26	61.06	31.81	117.23	4.76
200	10	50	44.44	2.30	4.85	5.2	4.86	0.627	6.27	14.42	30.47	70.08	33.17	148.09	5.06
225	10	70	66.67	2.35	5.44	5.4	5.43	0.601	6.01	14.12	32.63	76.67	33.19	177.20	5.30
250	10	80	75.00	2.40	5.67	5.7	5.67	0.532	5.32	12.77	30.16	71.13	30.64	171.03	5.54
275	10	90	87.50	2.44	6.18	5.9	6.12	0.471	4.71	11.49	28.83	70.32	28.04	173.41	5.73
300	10	100	100.00	2.48	-	6.0	6.65	0.439	4.39	10.89	29.19	72.42	27.00	194.14	5.93
Sum of the products=									47.44	108.27	235.49	542.04	248.03	1210.02	

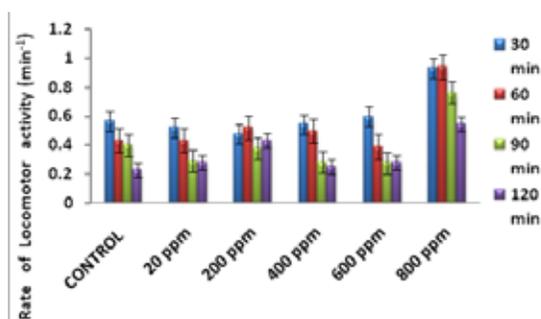
X- = 2.282  
 Y- = 4.964  
 b = 4.844  
 a = -6.086  
 Regression Equation =  $Y- = -6.086 + (4.844X)$   
 Chi square (X2) = 18.52 (P> 0.05) df = 8, 5% level = 15.507 significant 5% level  
 Variance = 0.0180  
 Fiducial limit = m1 = 1.8790  
 = m2 = 2.4050  
 LC50 log dose mean value = 2.142  
 Actual LC50 value = 138.68 ppm

Concentration of cadmium (ppm)	No. of crabs exposed	% of Mortality	Corrected percentage of mortality	Log dose X	Empirical probit	Expected probit Y	Working probit y	Weighing co-efficient	Weight W	WX	Wy'	WXy'	WX <sup>2</sup>	Wy <sup>2</sup>	y'
100	10	10	10.00	2.00	3.72	3.7	3.72	0.336	3.36	6.72	12.50	25.00	13.44	46.50	3.56
125	10	30	30.00	2.10	4.48	4.1	4.53	0.471	4.71	9.89	21.34	44.80	20.77	96.65	4.15

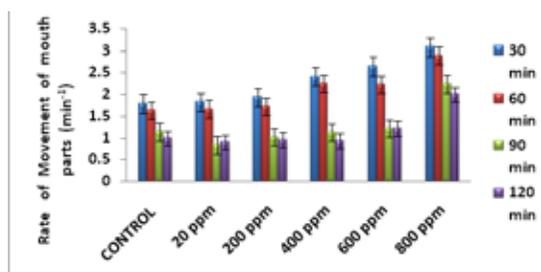
150	10	30	30.00	2.18	4.48	4.4	4.48	0.558	5.58	12.16	25.00	54.48	26.52	111.99	4.62
175	10	40	40.00	2.24	4.75	4.8	4.74	0.627	6.27	14.05	29.72	66.60	31.46	140.87	4.97
200	10	50	50.00	2.30	5.00	5.0	5.01	0.637	6.37	14.65	21.91	73.40	33.70	159.89	5.32
225	10	70	66.67	2.35	5.44	5.2	5.43	0.627	6.27	14.73	34.05	79.98	34.63	184.87	5.62
250	10	90	88.89	2.40	6.23	5.4	6.04	0.001	6.01	14.42	36.30	87.10	34.62	219.25	5.90
275	10	100	100	2.44	-	5.6	6.42	0.558	5.58	13.62	35.82	87.44	33.22	229.99	6.14
300	10	100	100	2.48	-	5.7	6.47	0.532	5.32	13.19	34.42	85.34	32.72	222.70	6.37
Sum of the products =									49.47	113.43	261.06	604.14	261.08	1412.71	

**TABLE 4: PROBIT ANALYSIS FOR THE POSTMOLT FEMALE CRAB (*PARATELPHUSA HYDRODROMOUS*) EXPOSED FOR 96 HRS. DURATION TO DIFFERENT CONCENTRATIONS OF CADMIUM CHLORIDE**

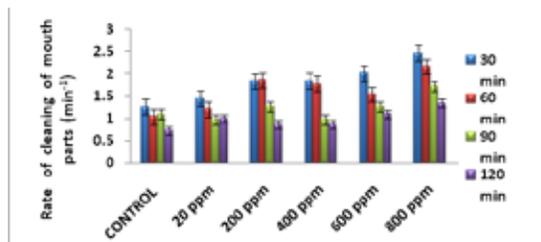
X- = 2.293  
 Y- = 5.277  
 b = 5.85  
 a = -8.137  
 Regression Equation = Y- = -8.137 + (5.85X)  
 Chi square (X2) = 2.75 (P> 0.05) df = 8, 5% level = 15.507 (Not significant)  
 Variance = 0.0015  
 Fiducial limit = m1 = 2.0460  
 = m2 = 2.1979  
 LC50 log dose mean value = 2.122  
 Actual LC50 value = 132.43 ppm



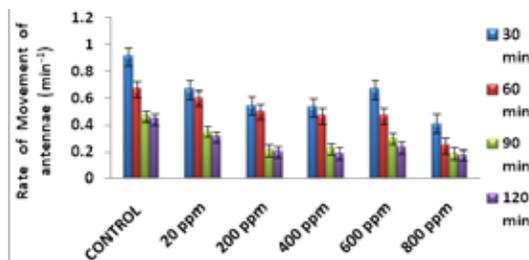
**Fig: 1: Rate of locomotor activity of the crab *Paratelphusa hydrodromous* during three hours of exposure to lethal and sub lethal concentrations of cadmium. (Values in Mean ± SEM)**



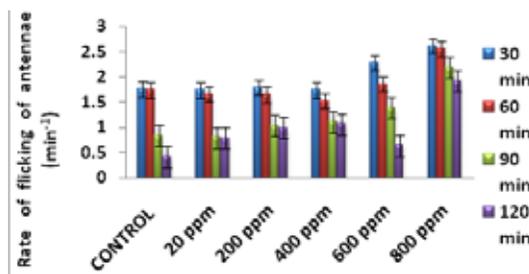
**Fig: 2: Rate of Movement of mouth parts of the crab *Paratelphusa hydrodromous* during three hours of exposure to lethal and sub lethal concentrations of cadmium. (Values in Mean ± SEM)**



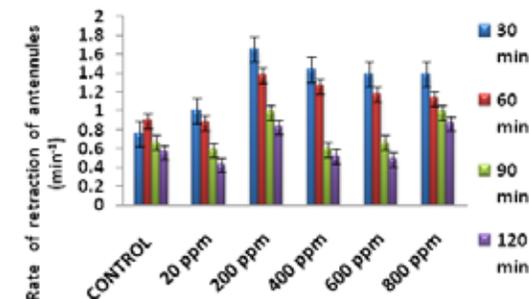
**Fig: 3: Rate of Cleaning of mouth parts of the crab *Paratelphusa hydrodromous* during three hours of exposure to lethal and sub lethal concentrations of cadmium. (Values in Mean ± SEM)**



**Fig: 4: Rate of Movement of antennae of the crab *Paratelphusa hydrodromous* during three hours of exposure to lethal and sub lethal concentrations of cadmium. (Values in Mean ± SEM)**



**Fig: 5: Rate of of flicking of antennae of the crab *Paratelphusa hydrodromous* during three hours of exposure to lethal and sub lethal concentrations of cadmium. (Values in Mean ± SEM)**



**Fig: 6: Rate of retraction of antennules of the crab *Paratelphusa hydrodromous* during three hours of exposure to lethal and sub lethal concentrations of cadmium. (Values in Mean ± SEM)**

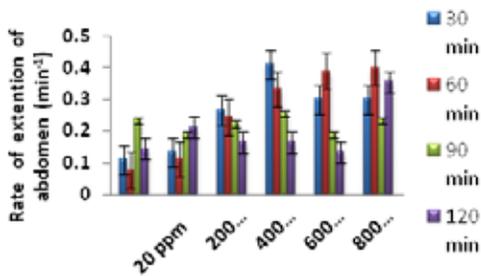


Fig: 7: Rate of extension of abdomen of the crab *Paratelson hydrodromous* during three hours of exposure to lethal and sub-lethal concentrations of cadmium. (Values in Mean ± SEM)

Table:5: Student Newman-keuls pairwise tests for significant differences in behaviour of *Phydrodromous* between each difference concentration of cadmium chloride

	F' Ratio		F' Ratio		F' Ratio		F' Ratio		F' Ratio		F' Ratio		F' Ratio	
Locomotor Activity	1	0.094	1	0.067	1	0.7074	1	0.987	1	3.365	1	0.0002	1	0.233
	2	0.349	2	0.004	2	2.665	2	3.291	2	0.2072	2	5.992*	2	4.377
	3	0.0160	3	0.482	3	1.48	3	3.77	3	0.2179	3	0.921	3	5.79
	4	0.046	4	1.16	4	3.97	4	1.999	4	0.4968	4	0.849	4	2.83
	5	11.20*	5	13.22*	5	11.07*	5	9.138*	5	7.927*	5	7.697*	5	13.49*
	6	1.18	6	0.0832	6	0.575	6	0.8732	6	0.1296	6	0.77	6	3.99
	7	0.025	7	0.682	7	0.567	7	1.114	7	0.1385	7	0.766	7	1.89
	8	0.004	8	1.3976	8	2.041	8	0.048	8	0.4646	8	0.849	8	2.207
	9	14.17**	9	11.85*	9	8.16*	9	4.84	9	12.297*	9	7.74*	9	14.045**
	10	0.512	10	0.37	10	0.064	10	0.0048	10	0.0004	10	0.733	10	1.384
	11	0.659	11	0.0925	11	0.0061	11	0.03	11	0.1916	11	1.0068	11	0.205
	12	12.19*	12	10.36*	12	1.87	12	0.77	12	13.284*	12	0.299	12	5.404
	13	0.008	13	0.0768	13	0.12	13	0.044	13	0.226	13	0.0078	13	0.246
	14	6.21*	14	3.6	14	2.45	14	0.914	14	17.627**	14	0.277	14	0.233
	15	11.6*	15	2.67	15	1.98	15	0.302	15	4.066	15	0.467	15	1.05
		Mouth Parts Movement			olith Parts Cleaning		Antenna Movement		Antenna Flicking		Antennules Retraction		Abdomen Extension	

- 1. control x 20 ppm
  - 2. control x 200 ppm
  - 3. control x 400 ppm
  - 4. control x 600 ppm
  - 5. control x 800 ppm
  - 6. 20 ppm x 200 ppm
  - 7. 20 ppm x 400 ppm
  - 8. 20 ppm x 600 ppm
  - 9. 20 ppm x 800 ppm
  - 10. 200 ppm x 400 ppm
  - 11. 200 ppm x 600 ppm
  - 12. 200 ppm x 800 ppm
  - 13. 400 ppm x 600 ppm
  - 14. 400 ppm x 800 ppm
  - 15. 600 ppm x 800 ppm
- \* 'F' = 0.05 (1,6) = 5.99  
 \*\* 'F' = 0.01 (1,6) = 13.74

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