A study on Behavioral responses to sub lethal and lethal concentrations of cadmium chloride (CdCl2.H2O) in freshwater crab *Paratelphusa hydrodromous* (Decapoda: Brachyura)

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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 Paratelphusa hydrodromous which was chosen for its abundance and commercial and ecological importance. 96 hrs LC50 values for pre-moult and post moult male crabs were found to be 158.49 ppm and 156.68 ppm. For pre-moult and post moult 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 *Paratelphusa 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 *Cragonon cragonon* (Partman and Wildson, 1971), *Homarus americanus* (Johnson and Gentile, 1979), *Palaemon serratus* (Wilson and Cannor 1971) and *P. japonicus* (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 (ZnCo3). 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 Fe2+, Zn2+, Cu2+, Ca2+ etc (Wright and Frain 1981).

*Paratelphusa 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 be 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 supposed 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 investigation was focused on the determination of acute toxicity (LC50 for 96 hrs) of premoult and postmoult a crabs belonging to both sexes exposed to metal cadmium.

**MATERIALS AND METHOD**

Healthy and active crabs *Paratelphusa 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 (CdCl2). 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 LC50 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...
In this comparative study, reported that the lawal of spider crab to 2.1979. The actual LC50 value was found to be 132.43 ppm. The fiducial limits with 95% confidence level ranged from 2.0460 to 2.4050. The actual LC50 value for this animal was ranging at 28°C ± 2°C 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 locomotory 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 LC50 values for cadmium calculated by probit analysis for premoult and postmoult male and female crabs of Paratelphusa hydrodomous are presented in the Tables: 1 to 4.

The LC50 log dose value of cadmium for the premoult 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 LC50 value of cadmium was found to be 158.49 ppm (Table: 1). In the case of postmoult male crab LC50 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 LC50 value was 136.68 ppm (Table: 2).

The LC50 log dose value for the premoult female crab was 2.142 and the fiducial limits with 95% confidence level was ranging from 1.8790 to 2.4050. The actual LC50 value of cadmium was found to be as 138.68 ppm. (Table: 3). For the post moult female crab, LC50 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 LC50 value was found to be 132.43 ppm. (Table: 4).

In the present investigation it was noted that 96 hrs LC50 value for cadmium for premoult and postmoult male and female crabs of Paratelphusa hydrodomous did not show much variation. LC50 value for premoult male crab 158.49 ppm and for postmoult male crab 156.68 ppm. Similarly, these two stages did not have much impact on lethality in female crabs also (LC50 value for premoult female, 138.68 ppm; LC50 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 LC50 values for different species of crabs. (Narayanan et al. 1997) reported 96 hrs. LC50 value as 8 ppm for cadmium in the mud crab Scylla serrata. As observed for an estuarine crab Chasmagnathus granulatus, 96 hrs LC50 value of 2.69 mg/l and its subsequent exposure to sub lethal level indicates 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 lawal of spider crab were remarkably sensitive to capper and cadmium. On his further observation, the duration of exposure was found to be a crucial parameter for obtaining standard measures of LC50 (Johnson and Gentile 1979) for the increase of resistance, size and stage of development in lawae 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 or 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 the third maxillepeds 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 maxillepeds 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 ad 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=1.107, p<0.05 in control Vs 800 ppm; F=0.16, p<0.05 in 20 ppm Vs 800 ppm). This was also noticed in lethal concentration of 200 ppm and 400 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 ANTENAE
The antennae were noticed to be flicked up and down frequently. The erratic movements of antennae were 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.

RETRACTION OF ANTENULES
Antennules showed continuous rapid flicking movements but frequently they were folded backwards into a groove of the car-
The rate of response increased in the lethal concentration of cadmium (800 ppm) was found to be an attempt to escape from the adverse medium for *Phydrorumous*. 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 *Phydrorumous* 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 bioacculmulator as well as indicator organism of aquatic pollution.

**ACKNOWLEDGEMENTS**

We express our sincere thanks to the Sri Vasavi college, Erode for their Continued encouragement and support. We also express our deep sense of gratitude to Dr. M.Sultan Ali, M, Sc., M.Phil, Ph.D, PG and Research Department of zoology, Sri Vasavi college, Erode for having provided us the laboratory facilities and the moral support.

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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**

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<th>Corrected % of Mortality</th>
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<th>Empirical probit</th>
<th>Expected probit</th>
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**Sum of the products =**

48.43 111.78 240.94 561.27 258.85 1232.19

\[
X = 2.308 \\
Y = 4.975 \\
b = 6.023 \\
a = 8.929
\]

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

Chi square (X2) = 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 HYDROMOMOUS) EXPOSED FOR 96 HRS. DURATION TO DIFFERENT CONCENTRATIONS OF CADMIUM CHLORIDE

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<th>No. of crabs exposed</th>
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<th>Corrected percentage of mortality</th>
<th>Log dose X</th>
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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 HYDROMOMOUS) EXPOSED FOR 96 HRS. DURATION TO DIFFERENT CONCENTRATIONS OF CADMIUM CHLORIDE

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<th>Concentration of cadmium (ppm)</th>
<th>No. of Crabs exposed</th>
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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
TABLE 4: PROBIT ANALYSIS FOR THE POSTMOLT FEMALE CRAB (PARATELPHUSA HYDRODROMOUS) EXPOSED FOR 96 HRS. DURATION TO DIFFERENT CONCENTRATIONS OF CADMIUM CHLORIDE

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 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 Paratelphusa 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 *P. hydrodromous* between each difference concentration of cadmium chloride |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1                             | 0.094            | 1.9074           | 1.987            | 3.365            | 0.0002           | 1.233           |
| 2                             | 0.349            | 2.665            | 3.291            | 0.2072           | 5.992*           | 4.377           |
| 3                             | 0.0160           | 1.48             | 3.77             | 0.2179           | 0.921            | 5.79            |
| 4                             | 0.046            | 3.97             | 1.999            | 0.4968           | 0.849            | 2.83            |
| 5                             | 11.20*           | 11.07*           | 9.138*           | 7.927*           | 7.697*           | 13.49*          |
| 6                             | 1.18             | 0.575            | 0.8732           | 0.1296           | 0.77             | 3.99            |
| 7                             | 0.025            | 1.114            | 0.1385           | 0.766            | 1.89             |
| 8                             | 0.004            | 2.041            | 0.048            | 0.4646           | 0.849            | 2.207           |
| 9                             | 14.17**          | 8.16*            | 4.84             | 12.297*          | 7.74*            | 14.045**        |
| 10                            | 0.512            | 0.064            | 0.0048           | 0.0004           | 0.733            | 1.384           |
|                               | 0.659            | 0.0061           | 0.03             | 0.1916           | 1.0068           | 0.205           |
| 11                            | 12.19*           | 1.87             | 0.77             | 13.284*          | 12.299           | 5.404           |
|                               | 0.008            | 0.12             | 0.044            | 0.226            | 0.0078           | 0.246           |
| 12                            | 6.21*            | 2.45             | 0.914            | 17.627**         | 0.277            | 0.233           |
| 13                            | 11.6*            | 1.98             | 0.302            | 4.066            | 0.467            | 1.05            |
|                               | 15               | 2.67             | 15               | 15               | 15               | 15              |

1. control x 20 ppm 6.20 ppm x 200 ppm 11.200 ppm x 600 ppm
2. control x 200 ppm 7.20 ppm x 400 ppm 12.200 ppm x 600 ppm
3. control x 400 ppm 8.20 ppm x 600 ppm 13.400 ppm x 600 ppm
4. control x 600 ppm 9.20 ppm x 800 ppm 14.400 ppm x 800 ppm
5. control x 800 ppm 10.200 ppm x 400 ppm 15.600 ppm x 800 ppm

* 'F' = 0.05 (1,6) = 5.99
** 'F' = 0.01 (1,6) = 13.74
REFERENCES


