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# ALTERATIONS IN BEHAVIOUR, SCAPHOGNATHITE OSCILLATION AND HEART BEAT RATE OF FRESHWATER PRAWN, *MACROBRACHIUM DAYANUM* (CRUSTACEA : DECAPODA), INDUCED BY CADMIUM CHLORIDE EXPOSURE

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**ABSTRACT** – Alterations in behaviour, scaphognathite oscillation and heart beat rate of *Macrobrachium dayanum* exposed to cadmium chloride (CdCl<sub>2</sub>) were recorded. Bioassay tests were conducted to determine the LC<sub>50</sub> values of the toxicant for 24, 48, 72 and 96 h for males which were 0.24, 0.19, 0.17 and 0.15 mg/l while 0.24, 0.22, 0.17 and 0.16 mg/l for females, respectively. For acute toxicity studies, males were exposed to 0.15 mg/l (96 h LC<sub>50</sub>) while for sub-acute toxicity tests (25% of 96 h LC<sub>50</sub>) value of CdCl<sub>2</sub> was 0.0375 mg/l. For acute toxicity observations, females were exposed to 0.16 mg/l (96 h LC<sub>50</sub>) and 0.04 mg/l for sub-acute studies. Variations in behaviour, scaphognathite oscillation and heart beat rate were recorded for both the sexes. Changes in aggressive behaviour and body colouration were more pronounced in males as compared to the females. Behavioural changes, scaphognathite oscillation and heart beat rate were significantly different in males and females. Variations in these parameters may probably be due to the impairment in functioning of central nervous system (CNS) due to accumulation of the heavy metal in exposed prawns.

**Key words** : Cadmium chloride, scaphognathite oscillation, heart beat rate, *Macrobrachium dayanum*.

## INTRODUCTION

Prawns are the rich source of protein, free amino acids and vitamins A and D. Owing to less fat content, they are advised for diabetic people and used in Unani and Ayurvedic systems of medicine (Proudfit and Robinson, 1955; Singh, 1977). Thus, prawns are in much demand in both the national and international markets (Khan *et al.*, 1994; Martin, 1996; Sankar *et al.*, 2011). These organisms are very sensitive to pollutants and considered as good bio-indicators too (Brown *et al.*, 2004; Camus *et al.*, 2004). *M. dayanum* and *M. lamarrei* are found in river Gomti, a stretch of about 940 km from its origin in Pilibhit to the confluence with the Ganges near Rajwari (Ghazipur-Varanasi border), Uttar Pradesh. This river is exposed to pollution due to industrialization and urbanization and high concentration of the seven heavy metals-cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) have been recorded in this tributary at Lucknow (Singh *et al.*, 2005a, b; Gaur *et al.*, 2005; Lohani *et al.*, 2008). The heavy metals are toxic, persistent contaminant and their presence in water bodies are serious threat to existence of the aquatic life (Mason, 1991; Jackson *et al.*, 2005; Dutta *et al.*, 2011). Cadmium, being readily soluble and poor biodegradability, gets accumulated in the organisms

making it environmentally stable and mobile.

Changes in behaviour are the earliest response of organisms to the toxicants. Though the behavioural changes are difficult to quantify, stress can be measured by cardiac and ventilatory responses (Kannupandi *et al.*, 2001; Prasanth *et al.*, 2005; Bierbower and Cooper, 2009). Ventilation rate (VR) and heart beat rate (HR) are the direct measures of physiological response and increase in both these parameters have been reported in crustaceans exposed to the pesticides (Lundebye *et al.*, 1997; Medesani *et al.*, 2011). Since the crustaceans can be used as biomarkers of pollution (Depledge *et al.*, 1995; Pal *et al.*, 1995; Brown *et al.*, 2004; Camus *et al.*, 2004; Ansari *et al.*, 2012), an attempt has been made to record the CdCl<sub>2</sub>-induced stress-related changes in the behaviour, scaphognathite oscillation and heart beat rate of both the sexes of *Macrobrachium dayanum*.

## MATERIALS AND METHODS

Freshwater prawn, *Macrobrachium dayanum* (Henderson) (Crustacea : Decapoda), were collected from river Gomti at different locations in Lucknow (26° 55'N; 80° 59'E), India. Freshly collected animals were brought to the laboratory and acclimatized under the ambient conditions for one week before initiation of the experiment. Intermolt prawns of both the sexes - males

(length  $4.86 \pm 0.18$  cm; weight  $0.92 \pm 0.06$  g) and females (length  $3.12 \pm 0.14$  cm; weight  $0.52 \pm 0.03$  g) were selected for the experimental purpose. Static bioassay tests were carried out to evaluate median lethal concentration ( $LC_{50}$  values) of  $CdCl_2$  (molecular weight: 228.35, Analytical Grade, Thomas Baker Chemical Ltd, Mumbai, India) following the standard methods (APHA, 1998). The  $LC_{50}$  values of  $CdCl_2$  for 24, 48, 72 and 96 h for males were 0.24, 0.19, 0.17 and 0.15 mg/l and for females 0.24, 0.22, 0.17 and 0.16 mg/l, respectively. The prawns were subjected to acute (0.15 mg/l and 0.16 mg/l) and sub-acute exposure (0.0375 mg/l and 0.040 mg/l; 25% of 96 hr  $LC_{50}$ ) for males and females, respectively. Experiments were carried out in glass aquaria of 20 liters capacity containing 10 liter dechlorinated tap water. All the experimental tests were run up to 96 h (acute) and 30 days (sub-acute) exposures. 10 healthy intermolt prawns were transferred in each test aquarium and the experiment repeated thrice. Separate sets of the experimental as well as control aquaria were maintained for males and females. Behavioural alterations like hyperactivity, swimming patterns, surfacing movements, responsiveness, food grasping as well as change in body colouration were recorded in the experimental and control prawns. Scaphognathite oscillation and heart beat rate of 10 prawns of both the groups were observed separately for males and females with help of a stop watch by wrapping the prawns with minimal pressure by thread upon a glass slide under stereo-binocular.

## RESULTS AND DISCUSSION

### Acute exposure

Alterations in behaviour in both the sexes of *Macrobrachium dayanum* after acute exposure of  $CdCl_2$  have been summarized in Table 1. The prawns displayed an initial hyperactivity just after being exposed to the toxicant, the response being more pronounced among males as compared to the females. They were distributed throughout the entire aquaria, exhibited enhanced pleopod movements and for rapid scrapping of gill and antennae, chelepedes were used. After 24 h of the exposure, there was copious secretion of mucus in the gill and carapace region, cleaning became rapid, prawns were scattered throughout bottom of the aquaria and most of the animals were lethargic and fighting tendency in females decreased considerably as compared to the males. Slight discolouration of body was also noticed in both the sexes. After 48 h of exposure, activity was declined drastically in prawns as they resided at the bottom and displayed frequent scrapping, loss of balance and enhanced mucous secretion. The fighting tendency decreased in females as compared to males. Body discolouration was prominent

in males whereas it was slight in case of females. At 72 h, the movement of animals was slowed down, loss of balance was prominent and scrapping activity increased. Body colouration in males decreased as compared to females. Experimental prawns exhibited soft body while mucous secretion increased in both the sexes which was more profuse in males in comparison to females. At 96 h of exposure, the activities like surfacing, swimming and fighting ceased, loss of balance and mucous secretion became very prominent. Fading of body colour was comparable in both the sexes.

Effects of acute  $CdCl_2$  exposure on scaphognathite oscillation/min of male *M. dayanum* have been given in Fig. 5. The males recorded a highly significant ( $P < 0.001$ ) increase ( $280.3 \pm 2.48$ /min) in this response after 24 h of exposure than the controls ( $259.8 \pm 3.13$ /min). Thereafter, the scaphognathite oscillation/min is declined at 48 h and remained insignificant ( $P > 0.05$ ) in both the groups. However, it increased again significantly ( $P < 0.05$ ) in exposed males ( $272.3 \pm 2.81$ /min) at 72 h. The scaphognathite oscillations exhibited declining trend and came below in the test animals ( $260.6 \pm 1.81$ /min) than the controls ( $264.9 \pm 1.75$ /min) at 96 hr and the difference between the two values were non-significant ( $P > 0.05$ ). The overall variations due to acute exposure of  $CdCl_2$  up to 96 h were highly significant in test prawns ( $F = 11.8$ ,  $P < 0.001$ ) while insignificant in the controls ( $F = 0.76$ ;  $P > 0.05$ ).

Scaphognathite oscillation/min in female *M. dayanum* exposed to  $CdCl_2$  showed variability in different periods (Fig. 6). A highly significant ( $P < 0.001$ ) increased response in test animals ( $269.4 \pm 1.63$ /min) was observed after 24 h as compared to the controls ( $256.8 \pm 1.55$ /min). At 48 h, the scaphognathite oscillations of experimental prawns declined but remained above the controls, the difference between the two groups was insignificant ( $P > 0.05$ ). At 72 h, the scaphognathite oscillation/min exhibited a rising trend with significant ( $P < 0.05$ ) increase in test prawns ( $264.7 \pm 1.68$ /min) than controls ( $259 \pm 1.39$ /min). Thereafter, the response started declining in test animals and came below ( $259 \pm 1.62$ /min) the control level ( $260.3 \pm 2.04$ /min) at 96 h, the difference between two values was insignificant ( $P > 0.05$ ). Overall, the experimental values for the test animal exposed to acute  $CdCl_2$  toxicity varied significantly ( $F = 8.40$ ;  $P < 0.001$ ) than the controls ( $F = 0.92$ ;  $P > 0.05$ ).

Variations in heart beat/min of the control and experimental *M. dayanum* males due to acute  $CdCl_2$  toxicity have been summarized in Fig. 7. At 24 h of exposure, highly significant ( $P < 0.001$ ) increase in heart beat rate ( $213.30 \pm 2.53$ /min) was observed in experimental

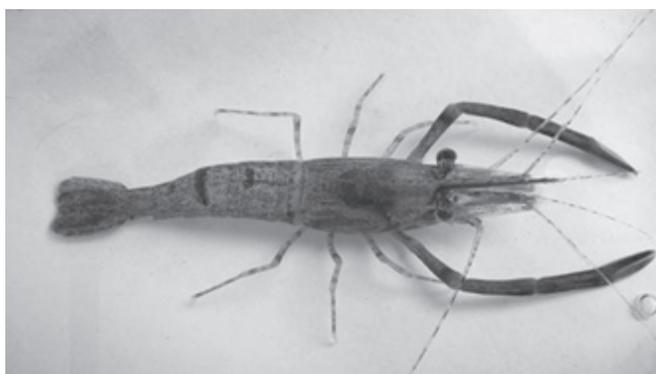


Fig. 1 : Control male *M. dayanum*.

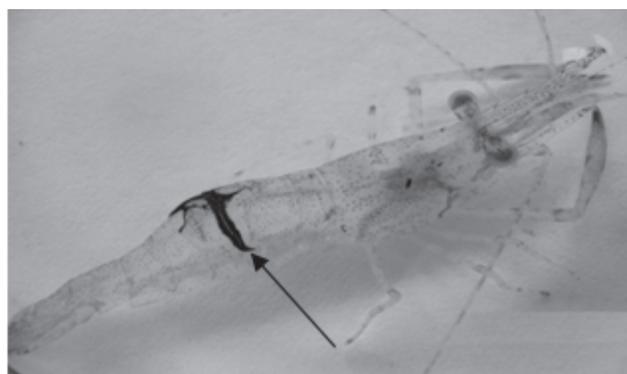


Fig. 2: Male *M. dayanum* on day 30 of exposure showing blackening.



Fig. 3: Control female *M. dayanum*

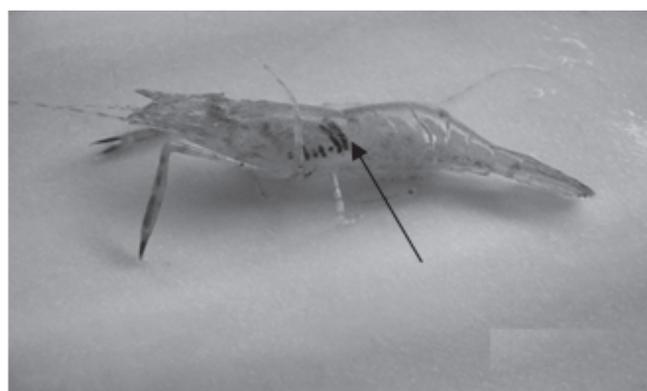


Fig. 4: Female *M. dayanum* on day 30 of exposure exhibiting blackening.

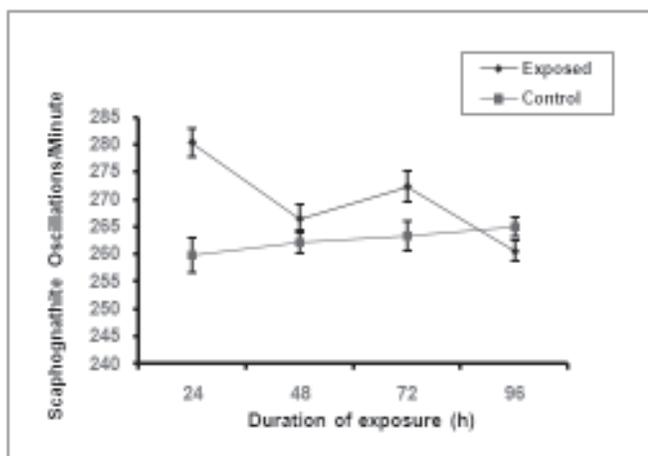


Fig. 5: Scaphognathite oscillations/min of male *M. dayanum* after acute exposure of  $\text{CdCl}_2$ .

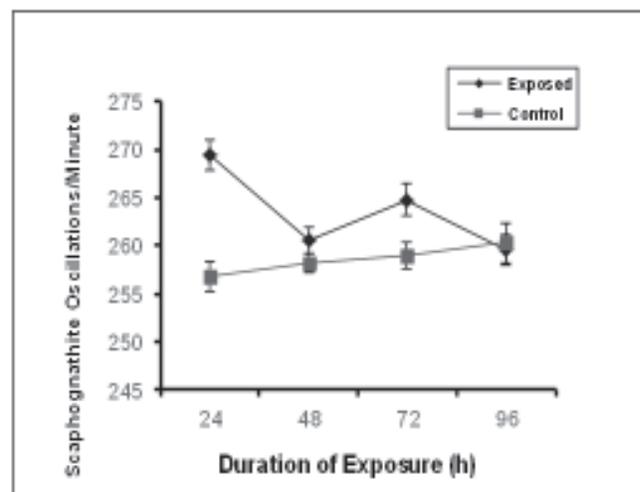


Fig. 6 : Scaphognathite oscillations/min of female *M. dayanum* after acute exposure of  $\text{CdCl}_2$ .

prawns than the controls ( $189.50 \pm 1.91/\text{min}$ ). At 48 h, a declining trend in the response was observed in the experimental animals but remained higher ( $198.40 \pm 2.77/\text{min}$ ) than the controls ( $192.9 \pm 1.93/\text{min}$ ), the difference between control and test values being insignificant ( $P > 0.05$ ). The heart beat rate at 72 h of exposure remained higher ( $204.20 \pm 4.19/\text{min}$ ) in experimental prawns than the controls ( $196.70 \pm 2.53/\text{min}$ ), both the values being statistically insignificant ( $P > 0.05$ ). The response in test animals increased ( $208.90 \pm 2.68/\text{min}$ ) significantly ( $P < 0.001$ ) than controls ( $195.80 \pm 1.38/\text{min}$ ) after 96 h of exposure. The results clearly indicated a highly significant ( $F = 4.65$ ;  $P < 0.05$ ) change in heart beat

**Table 1 : Alterations in behaviour of *Macrobrachium dayanum* after acute exposure of CdCl<sub>2</sub>.**

| Exposure (h) | Surfacing |   | Swimming |   | Colour fading |    | Loss of balance |     | Mucous secretion |     | Fighting |   |
|--------------|-----------|---|----------|---|---------------|----|-----------------|-----|------------------|-----|----------|---|
|              | M         | F | M        | F | M             | F  | M               | F   | M                | F   | M        | F |
| 24           | ++        | + | ++       | + | +             | +  | +               | +   | ++               | ++  | ++       | + |
| 48           | +         | - | +        | - | +             | +  | +               | +   | ++               | ++  | +        | - |
| 72           | -         | - | -        | - | +             | +  | ++              | ++  | ++               | ++  | -        | - |
| 96           | -         | - | -        | - | ++            | ++ | +++             | +++ | +++              | +++ | -        | - |

M, male; F, Female; - (nil), + (less), ++ (more), +++ (prominent).

**Table 2 : Alterations in behaviour of *Macrobrachium dayanum* after sub-acute exposure of CdCl<sub>2</sub>.**

| Exposure (days) | Surfacing |   | Swimming |    | Colour fading |    | Loss of balance |     | Mucous secretion |     | Fighting |   |
|-----------------|-----------|---|----------|----|---------------|----|-----------------|-----|------------------|-----|----------|---|
|                 | M         | F | M        | F  | M             | F  | M               | F   | M                | F   | M        | F |
| 10              | +++       | + | +++      | ++ | +             | +  | +               | +   | +                | +   | +++      | + |
| 20              | -         | - | ++       | -  | ++            | +  | ++              | ++  | ++               | ++  | +++      | - |
| 30              | -         | - | -        | -  | +++           | ++ | +++             | +++ | +++              | +++ | +        | - |

M, male; F, Female; - (nil), + (less), ++ (more), +++ (prominent).

rate of male prawns due to acute exposure of CdCl<sub>2</sub> whereas changes in the control animals were insignificant ( $F = 3.69$ ;  $P < 0.05$ ).

Alterations in heart beat rate of the control and experimental female *M. dayanum* exposed to CdCl<sub>2</sub> have been depicted in Fig. 8. The experimental females recorded highly significant ( $P < 0.001$ ) increase ( $197.9 \pm 1.43/\text{min}$ ) in heart beat rate at 24 h than the controls ( $181.7 \pm 1.23/\text{min}$ ). However, a declining trend in the response ( $188.2 \pm 2.34/\text{min}$ ) was observed at 48 h of exposure but remained higher than the control ( $183.5 \pm 2.19/\text{min}$ ), both the values being statistically insignificant ( $P > 0.05$ ). At 72 h, significant ( $P < 0.05$ ) increase in heart beat rate was observed in exposed prawns. The response further increased ( $194.3 \pm 1.48/\text{min}$ ) significantly ( $P < 0.001$ ) in the test prawns than controls ( $184.9 \pm 2.15/\text{min}$ ) at 96 h of exposure. Overall, the heart beat rate of female prawns varied significantly with duration in experimental animals ( $F = 1.27$ ;  $P > 0.05$ ) but it was not significant in the controls ( $F = 25.37$ ;  $P < 0.001$ ).

#### Sub-acute exposure

For sub-acute experiments, *M. dayanum* were

exposed to 25% 96 h LC<sub>50</sub> concentration of CdCl<sub>2</sub> - 0.0375 mg/l for males and 0.040 mg/l for females and alterations in the behavioural activities in both the sexes of prawns during the experimental period have been summarized in Table 2. On day 10 of exposure, the prawns exhibited enhanced surfacing, swimming and fighting tendencies, the responses being more pronounced among males as compared to the females. Secretion of mucous in the gills, carapace, abdominal and telson region were observed which was more prominent in the abdominal region. Feeding was reduced while blackening was noticed in abdominal and gill region with slight decrease in body colouration. Frequent cleaning was also observed and prawns lied in groups clinging to the walls of the aquaria. On day 20, all the activities (except fighting in males) reduced in the exposed prawns in comparison to control group. Mucous secretion increased in the cephalothorax, gill, abdominal region in males and females and telson region in males. Fighting increased in the males and they appeared highly irritated. The loss of balance in swimming and crawling was noticed. Blackening appeared first on the abdominal region in males and in females on gills, carapace and appendages. A further increase in the body

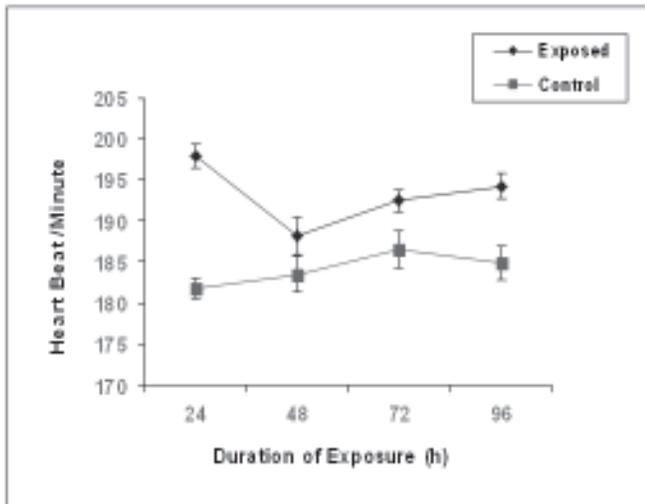


Fig. 7: Heart beat/min of male *M. dayanum* after acute exposure of CdCl<sub>2</sub>.

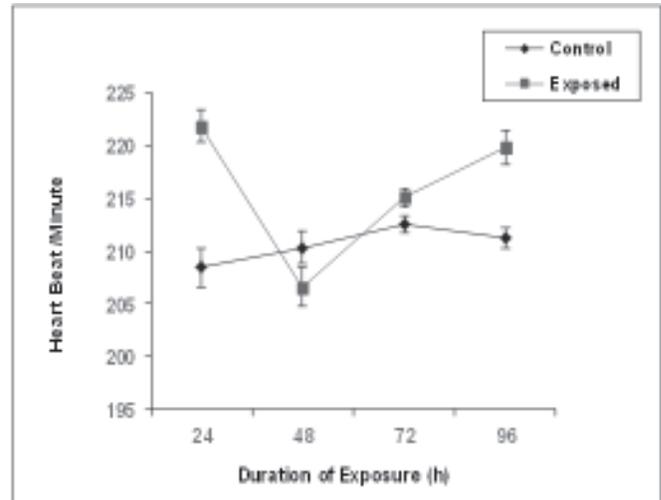


Fig. 8: Heart beat/min of female *M. dayanum* after acute exposure of CdCl<sub>2</sub>.

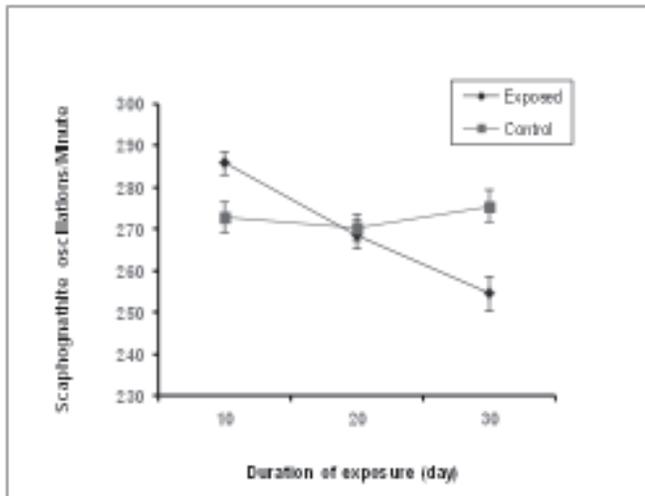


Fig. 9: Scaphognathite oscillations/min of male *M. dayanum* after sub-acute exposure of CdCl<sub>2</sub>.

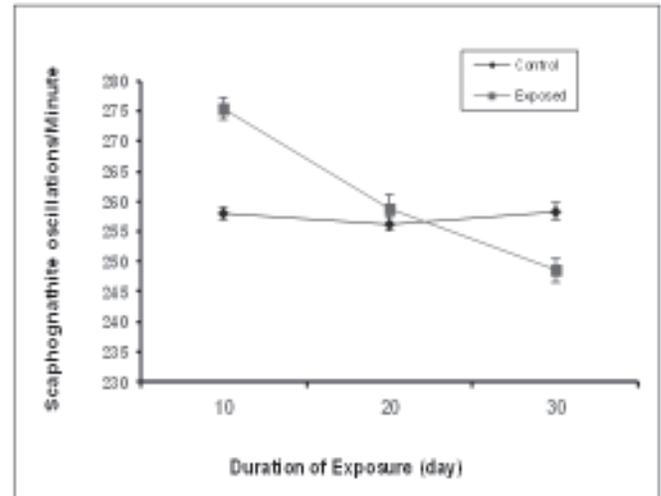


Fig. 10: Scaphognathite oscillations/min of female *M. dayanum* after sub-acute exposure of CdCl<sub>2</sub>.

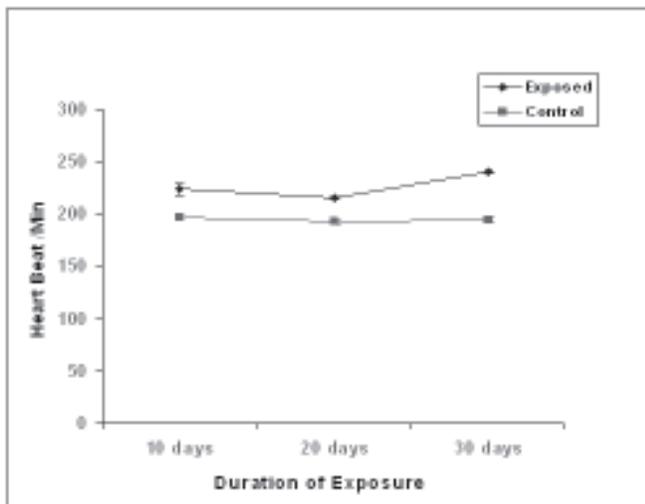


Fig. 11: Heart beat /min of male *M. dayanum* after sub-acute exposure of CdCl<sub>2</sub>.

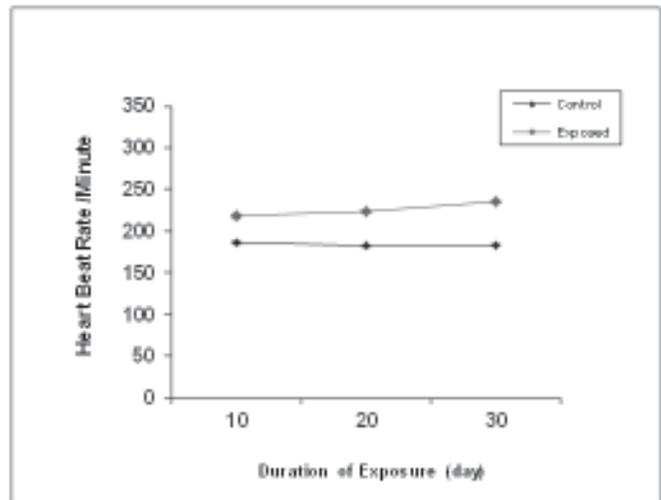


Fig. 12: Heart beat /min of female *M. dayanum* after sub-acute exposure of CdCl<sub>2</sub>.

discolouration was observed in males but not noticeable in case of females. Cleaning of gills, antennae and chelipede legs became rapid. On day 30 of exposure, the prawns gathered near the aerator. Their body and even the cephalothorax became soft, slime secretion increased in the abdominal region followed by gill, lateral abdominal and in the cephalothorax regions. Prawns with broken rostrum and chelipede legs were seen and exhibited rapid backward movement with rapid pleopod movement. They rubbed their body against each other. A prominent decrease in colouration was observed, feeding almost stopped and no horizontal or vertical movement seen. Fighting and other activities in the exposed prawns decreased significantly and they did not respond to gentle paddling. Blackening became prominent and in males it appeared first on the abdominal region and in females on gills, carapace and appendages (Fig. 3, 4). Further, loss of capability of food detection, capturing and consumption were also observed in the CdCl<sub>2</sub> exposed *M. dayanum*.

Changes in the scaphognathite oscillations of *M. dayanum* males exposed to sub-acute CdCl<sub>2</sub> toxicity for 30 days have been given in Fig. 9. The response was significantly higher ( $P < 0.01$ ) in exposed prawns on day 10 ( $285.9 \pm 2.74/\text{min}$ ) as compared to the controls ( $272.9 \pm 3.53/\text{min}$ ). On day 20 of exposure, scaphognathite oscillations decreased in the exposed prawns ( $268.6 \pm 3.43/\text{min}$ ) below than the controls ( $270.3 \pm 3.26/\text{min}$ ), difference between the two values was insignificant ( $P > 0.05$ ). After day 30, there was a progressive decline in the response in exposed prawns ( $254.5 \pm 4.19/\text{min}$ ) than the controls ( $275.5 \pm 3.79/\text{min}$ ), the difference was highly significant ( $P < 0.005$ ). Overall, the values varied significantly in experimental prawns ( $F = 20.12$ ;  $P < 0.001$ ) while fluctuations were insignificant in the controls ( $F = 0.54$ ;  $P > 0.05$ ).

Changes in the scaphognathite oscillations observed in *M. dayanum* females during sub-acute exposure of CdCl<sub>2</sub> have been summarized in Fig. 10. The response increased significantly ( $P < 0.0001$ ) in the test prawns ( $275.5 \pm 1.77/\text{min}$ ) on day 10 in comparison to the controls ( $257.9 \pm 1.07/\text{min}$ ). On day 20, scaphognathite oscillations decreased in the exposed animals ( $258.7 \pm 2.31/\text{min}$ ) but remained above the controls ( $256.3 \pm 1.06/\text{min}$ ), the difference between the two values was insignificant ( $P > 0.05$ ). There was declining trend in scaphognathite oscillations of exposed prawns ( $248.5 \pm 1.88/\text{min}$ ) on day 30 than the controls ( $258.3 \pm 1.47/\text{min}$ ), the decrease was highly significant ( $P < 0.001$ ). Overall, the values of the experimental prawns varied significantly ( $F = 2.92$ ;  $P < 0.05$ ) while the control values did not ( $F = 0.84$ ;  $P > 0.05$ ).

Variations in heart beat rate of *M. dayanum* males exposed to long-term CdCl<sub>2</sub> toxicity have been given in Fig. 11. The response was significantly ( $P < 0.001$ ) higher ( $224.3 \pm 5.74/\text{min}$ ) in the exposed prawns on day 10 than the controls ( $197.2 \pm 2.22/\text{min}$ ). However, the heart beat rate declined in the treated animals ( $215.6 \pm 1.51/\text{min}$ ) on day 20 than the controls ( $193.4 \pm 2.32/\text{min}$ ), the difference between the two values was significantly higher ( $P < 0.0001$ ). After day 30 of exposure, the response increased further ( $P < 0.001$ ). The heart beat rate was higher in the test animals than controls throughout the experiments. Overall, the values of experimental prawns varied significantly ( $F = 12.95$ ;  $P < 0.001$ ) whereas variations were insignificant in control groups ( $F = 0.84$ ;  $P > 0.05$ ).

Alterations in heart beat rate of the females *M. dayanum* exposed to long-term CdCl<sub>2</sub> toxicity have been summarized in Fig. 12. The response was significantly higher ( $P < 0.001$ ) in exposed females ( $218.2 \pm 2.44/\text{min}$ ) on day 10 as compared to the control ( $186.3 \pm 2.15/\text{min}$ ). Heart beat rate increased further on day 20 in exposed animals ( $223.4 \pm 1.32/\text{min}$ ) than the controls ( $181.8 \pm 1.47/\text{min}$ ), the differences between the two values were highly significant ( $P < 0.001$ ). On day 30, the heart beat further increased ( $234.5 \pm 1.01/\text{min}$ ) significantly ( $P < 0.001$ ) in exposed animals in comparison to the controls ( $183.3 \pm 1.03/\text{min}$ ). Overall, there were significant changes in the heart beat rate in experimental animals ( $F = 23.80$ ;  $P < 0.001$ ) than in controls ( $F = 1.37$ ;  $P > 0.05$ ).

In general, crustaceans are more sensitive to metals and employed as better bio-indicator of freshwater reservoirs. Since behaviour of any living animal is the complex of observable, recordable and measurable activities including its integrated movements, it is a sensitive indicator of toxicity in the aquatic organisms (Sharma and Shukla, 1990; Buskey, 1998; Roast *et al*, 2001). The behavioural changes after exposure to heavy metals and pesticides have been reported in fish (*Heteropneustes fossilis*-Rajan and Banerjee, 1991, Khan *et al*, 2006; *Cyprinus carpio*-Thatheyus, 1992; *Channa punctatus*-Sornaraj *et al*, 1994) and shellfish (*M. malcolmsonii*-Vijayakumaran and Geraldine, 1996a, b; Vijayaraman *et al*, 1999, mysid *Neomysis integer*-Roast *et al*, 2001; crayfish *Procambarus clarkii* and *Orconectes australis packardii*-Bierbower and Cooper, 2009) and crab *Neohelice granulata*-Medesani *et al*, 2011). The initial hyperactivity observed in both the sexes of *M. dayanum* exposed to CdCl<sub>2</sub> may be the avoidance reaction of the animal to the toxicant. Similar avoidance behaviour due to exposure to detergent and heavy metals has also been reported in *Macrobrachium lamarrei*

(Sharma and Shukla, 1990, 2006). Heavy metal (Pb, Cu, Ni, Zn, Hg and Cr) exposures also induced avoidance response in the rainbow trout, *Oncorhynchus mykiss* (Svecevičius, 2001, 2005). The cause of such reaction may be due to alterations in sensitivity of chemoreceptors and olfaction. It is known that sublethal concentration of trace metals can block the function of chemoreceptor within few seconds after exposure (Hara, 1992).

Increased mucous secretion in the gills, carapace, abdominal region and almost whole of the body surface after cadmium exposure are the general response of *M. dayanum* to the toxicant which may be a protective device for inhibiting uptake of the heavy metal through gills and other epithelial lining. Heavy metals can be trapped in mucous layer which is made up of barrier glycoproteins (Roberts, 2001). Similar profuse mucous secretion has also been reported in *Channa punctatus* and *Liza parsia* subjected to heavy metal pollution (Agarwal, 1991; Pandey, 1994; Pandey *et al.*, 1997). Acute cadmium toxicity induced decreased swimming, scrapping of antennae by chelepede and surfacing may be due to altered carbohydrate metabolism and changes in muscle fibres operating scaphognathite. Loss of balance and erratic swimming observed in *M. dayanum* may be due to some neurological impairments and damage of the receptors. Heavy metal pollution also causes changes in swimming performance, orientation, coordination of movement, aggregation and subsequent lethargy (Weber and Spicler, 1994). Loss of capability of food detection, capturing and consumption in *M. dayanum* due to prolonged exposure of CdCl<sub>2</sub> is similar to those reported for fishes - *Coregonus clupeaformis* (McNicol and Scherer, 1991), *Ctenopharyngodon idella* and *Cyprinus carpio* (Jeziarska *et al.*, 2002; Drasticova *et al.*, 2004) and shessfishes- *Penaeus japonicus*, *Penaeus penicillatus*, *Penaeus chinensis*, *Macrobrachium assamensis assamensis*, *Macrobrachium lamarrei*, *Mytilus edulis planulatus*, *Metapenaeus ensis*, *Mysis mixta* and *Neomysis integer* due to heavy metals and pesticide toxicity (Liao and Chien, 1990; Wong *et al.*, 1993; Pal *et al.*, 1995; Maruthanayagam *et al.*, 1996; Roast *et al.*, 2000, 2001, 2002; Engstrom *et al.*, 2001) which may be due to damaged chemoreception (Hara, 1992).

Similar to the blackening in gills, abdomen, carapace and appendages observed in *M. dayanum* have also been recorded in *Penaeus duroranum*, *Palaemonetes pugio* and *Palaemonetes vulgaris* after CdCl<sub>2</sub>, *Caradina rajadhari* after CuSO<sub>4</sub>, *Palaemonetes pugio* after chromium VI, *Caradina rajadhari* and *M. kistenensis* after Hg and *M. dayanum* as well as *M. lamarrei* after Hg, Cd, Pb, Ni and Cu exposures (Sharma and Shukla,

2006; Verma *et al.*, 2005; Lodhi *et al.*, 2006; Tiwari, 2009; Verma, 2012). Gill blackening might be due to death of cells in gill tissue and melanin deposition. In crustaceans, gill blackening may also be due to metallic sulphides. after Hg and Cd exposures.

Cadmium induced marked aggression in *M. dayanum* after sub-acute exposure, the intensity of aggression being more pronounced among males than in females. Similar findings have also been reported in *M. dayanum* (Verma *et al.*, 2005) and *M. lamarrei* (Sharma and Shukla, 2006) after Cd and Hg exposure. The heavy metal toxicity induces various physiological and metabolic changes that enhanced aggressiveness. Alteration in chemical synaptic transmission increases aggressive behaviour and fighting encounters. Heavy metals like Cd, Pb, Hg, Al and Ni affect chemical synaptic transmission in brain and peripheral system of the animals (Goyer, 1993; Casdorff and Walker, 1995).

Heavy metals effect function of the brain and the cellular calcium levels, which in turn, alters body function in two ways. The calcium dependent neurotransmitter releases resulting in depression in levels of serotonin, norepinephrine and acetylcholine affecting mood and motivation (Devinsky *et al.*, 1992; Goyer, 1997). Serotonin is the main physiological regulator associated with disorders, impulsiveness and violent behaviour as well as controls the agnostic behaviour from ants to human beings and crustaceans too (Sazler, 1996; Master *et al.*, 1998; Lesch and Merschdorf, 2000; Walsh *et al.*, 2004). The aggressive encounters observed in *M. dayanum* exposed to CdCl<sub>2</sub> might be due to the alterations in serotonin levels as well as cellular permeability thereby increasing aggression. There exist no standardized data for behavioural responses needed to establish for the earliest and easiest detection of toxic effects of various pollutants, particularly heavy metals in crustaceans. As scaphognathite and heart beat activity are affected by environmental conditions including the toxicants in the aquatic environment, it may be used as a bio-monitoring techniques for the degrading water bodies (Varghese *et al.*, 1992; Varadhanan and Radhakrishnan, 2002). The initial steep decrease in scaphognathite oscillations in *M. dayanum* at 48 h followed by further decline at 96 h on exposure to CdCl<sub>2</sub> might be due to initial shock followed by an increased response at 72 h towards hypoxia probably to metal induced coagulation of mucous on gill surface. During sub-acute exposure, a general decrease was noted in scaphognathite activity which may be due to alteration in muscle fibres operating the same. Since the scaphognathite activity is under the control of neurons in the sub-oesophageal ganglion, the damage caused by

the toxicant to the ganglia and the gills might affected scaphognathite activity. The gills are damaged by the toxicant and the oxygen intake decreases impairing the functions of neurons. Present observations are in accordance with the reports of changes in opercular beats in different fishes due to exposure of various chemicals (Drasticova *et al*, 2004; Sindal *et al*, 2004) and in crustaceans (Radhakrishnaiah *et al*, 1991).

Heart beat rate in crustaceans varies with ecological condition like photoperiod, pH, temperature, size and sex. Changes in heart rates of mammals and aquatic vertebrates are indicators of mis-adjustments in the environments (Hughes *et al*, 1994). Heart beat rate of *M. dayanum* increased significantly throughout acute and sub-acute exposure to CdCl<sub>2</sub>. The initial increase of heart beat rate has also been reported in fishes (Jeziarska *et al*, 2002) and crustaceans due to HgCl<sub>2</sub>, CuSO<sub>4</sub> and PbNO<sub>3</sub> exposures (Varghese *et al*, 1990). Tachycardia in crab (*Osipode platytarsis*) after mercuric chloride exposure (Prakasam *et al.*, 1991) as well as tachycardia after acute exposure and bradycardia after sub-acute exposure of HgCl<sub>2</sub> in crab (*Gaetice depressus*) have been observed (Aagaard *et al.*, 2000). Almost similar kind of effects were also noticed in heart of dogfish (shark *Squalus acanthias*) after cadmium and copper exposure (Wang *et al*, 1999). Severe cardiovascular effects including hypertension has been reported in various classes of animals after exposure to heavy metals and pesticides (Anand *et al*, 1991). Almost similar change in heart beat rate of freshwater crab (*Barytelphusa guerini*) was reported after HgCl<sub>2</sub> exposure (Jadhav *et al*, 2007). Tachycardia has been observed in *M. dayanum* after exposure to CdCl<sub>2</sub>. It has been suggested that toxicant-induced stress increases metabolic rate that coincides with increased rate of heart beat of the crustaceans. Variations in these parameters may probably be due to the impairment in functioning of central nervous system (CNS) due to accumulation of the heavy metal in exposed prawns (Devi and Fingerman, 1997). The increase in heart beat rate in *M. dayanum* appears to be a good bio-marker, particularly in terms of sub-lethal effects for metal exposure which are common in aquatic bodies.

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