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TOXIC EFFECTS OF CHROMIUM ON BIO-ACCUMULATION, GROWTH, OXYGEN CONSUMPTION AND METABOLIC RATE IN POSTLARVAE OF *PENAEUS MONODON*

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Abstract

As the human population is rapidly increasing and the world has become more industrialized, the chemical wastes have increased to dangerously high levels in some areas leading to aquatic pollution. Today pollution is more threatening because many of the released pollutants degrade slowly or do not degrade at all, leading to accumulation in biota. The ocean, being so vast is constantly being used as a dumping ground which can neutralize some of these chemical wastes. Most of these effluents contain several toxic materials and among all of them, the heavy metals are considered to be more important. Chromium is one of the heavy metals reported in the industrial effluents. Although trivalent chromium (Cr III) is required in trace amounts for sugar and lipid metabolism, few cases have been reported where its complete removal from the diet has caused chromium deficiency. It is toxic in larger amounts. Hexavalent chromium (Cr VI) is toxic and carcinogenic, so that abandoned chromium production sites need environmental clean-up . Chromium levels are elevated in soil, air, water and biota in the vicinity of electroplating and metal finishing industries, municipal treatment plants, tanneries, oil drilling operations and cooling towers. There is no bio magnification of chromium in food chains but the concentrations are usually highest at the lowest trophic levels. Chromium is also reported to be present in the sediments of coastal and estuarine waters (0.8 to 2.7µg/g;[14]). As these waters are frequently used for aqua farms in coastal districts, the post larvae (PL) are prone to heavy metal toxicity, particularly chromium. Probit analysis [5] was carried out to assess the LC_{50} value of chromium for 24, 48, 72 and 96 hrs. The metal accumulation, growth, oxygen consumption and metabolic rate was chosen for further study on PL of *P. monodon* for both short term [24,48,72 and 96hrs] and long-term exposure [10,20 and 30 days]. In conclusion the PL of P. monodon are sensitive to sublethal concentration of chromium indicating increase in accumulation of chromium leads to a decrease in metabolic rate, oxygen consumption and growth.

Keywords: Heavy metal toxicity, chromium, tolerance, metal accumulation, growth, oxygen consumption, crustacean, *Penaeus monodon*

INTRODUCTION

Industrialization and urbanization can cause contamination of natural waters by heavy metals. The coastal waters are constantly prone to heavy metal pollution by the industrial effluents and domestic sewage. Most of these effluents contain toxic materials and among all of them heavy metals are considered to be more important. Heavy metals as the pollutants of the aquatic environment form a major hazard to aquatic life because of their toxicity, persistence and bio-accumulation in the food chain. Most of the industrial effluents contain heavy metals like cadmium, copper, lead, zinc etc. Heavy metals are accumulated in marine sediments where they are incorporated into biological and chemical cycles, affecting the water column and biota. Aquatic organisms experience direct water borne contact with these metal pollutants. As these heavy metals are non-biodegradable, they can lead to metal concentration phenomena in the organisms and act as a source of continuous stress. The marine biota use the ambient medium as a source of respiratory oxygen, trace metals and for various metabolic activities.

Chromium is an essential trace element in humans and some species of laboratory animals. At high environmental concentrations, chromium is a mutagen, teratogen and carcinogen. There is no report of bio-magnification of chromium in the food chains but the concentrations are usually highest at the lowest trophic levels. Chromium is used principally in metallurgy and chemical industries [10]. Major atmospheric emissions of chromium are from the chromium alloy and metal producing industries. Lesser amounts come from coal combustion, municipal incinerators; cement production and cooling towers [20]. Chromium levels are elevated in soil, air, water and biota in the vicinity of electroplating and metal finishing industries, municipal treatment plants, tanneries and oil drilling operations. In aquatic environments, the major sources of chromium include the electroplating, metal finishing industries and municipal treatment plants whereas relatively minor sources are iron and steel foundries, inorganic chemical pollutants tanneries, textile manufacturing and runoff from urban and residential areas [20]. Chromium phosphate salts used as fertilizers may be an important source of chromium in soil, water and some foods [10]. In general, elevated levels of chromium in biological samples have been positively correlated with increased industrial and other uses of the element, especially associated with plating and foundry applications, chemical manufacturing and corrosion inhibition [19]. Chromium can exist in oxidative states ranging from -2 to +6, but is most frequently found in the environment as a trivalent (+3) and hexavalent (+6) oxidation states.

Although trivalent chromium (Cr III) is required in trace amounts for sugar and lipid metabolism, few cases have been reported where its complete removal from the diet has caused chromium deficiency. It is toxic in larger amounts. Hexavalent chromium (Cr VI) is toxic and carcinogenic. Satyavathi [8] reported lead toxicity in postlarvae of *Penaeus indicus*. Guven [8] studied acute lethal toxicity of copper to *Gammarus pulex* (L.). Chinni [2] studied the toxicity of copper, cadmium, zinc and lead to *Penaeus indicus* postlarvae. The toxic effect of copper on *Penaeus*

monodon was reported by Durga Bhavani [4]. Chinni [3] observed the acute toxicity of lead on tolerance, oxygen consumption, ammonia-N excretion and metal accumulation in *Penaeus indicus* postlarvae. While studying the bioaccumulation of copper, Reddy [15] reported the sublethal levels of copper for postlarvae and juveniles of freshwater prawn *Macrobrachium rosenbergii*. Neelima [12] reported the toxicological studies on postlarvae of *Penaeus monodon* on exposure to cadmium. Srinivasa Rao [18] studied the effect of copper on postlarvae of freshwater shrimp *Macrobrachium rosenbergii.* Kiran kumar [9] reported the effect of lead on postlarvae of *Penaus monodon*.

Materials and methods:

The postlarvae (PL) of *Penaeus monodon* were collected from a local hatchery and they were transported to the laboratory in plastic bags. The PL were acclimatized to the laboratory conditions for about 48hrs in plastic troughs containing seawater and proper aeration was provided in order to maintain the optimal level of dissolved oxygen throughout the experimental period. The salinity of the seawater was maintained at 10ppt throughout the experiment. The pH and temperature were maintained at 8 and 29 ± 1°C respectively. The larvae were maintained in the troughs without crowding. Uniform sized PL (0.9 -1.0cm) were chosen for the experiment. The PL were fed with commercial diet (Highashi 3000 started B, Highashimanu Co. Ltd., Japan) twice a day. Excess feed and excretory wastes were removed everyday by siphoning. To determine the effect of chromium on *P. monodon* PL, the animals were exposed to different concentrations of chromium for a period of 96hrs to know the nature and response of PL to the toxicant. The toxicant was prepared by dissolving appropriate amounts of potassium chromate (AR) in distilled water to prepare 1% stock solution. Appropriate amounts of stock solution were added to 3L of seawater in each trough in order to get the final desired concentrations of 2.5, 5.0, 10 and 20ppm of chromium. The seawater in each trough was renewed after every 24hrs with respective concentrations of toxicant. At the same time, the troughs were cleaned with soap water and rinsed several times with tap water. Parallel controls were maintained without the metal toxicant. The mortality was recorded for every 24hrs up to a period of 96hrs. Dead PL were confirmed by response to touch of the blunt end of the needle and were removed. The experiment was repeated five times and the average mortality rates were considered for statistical evaluation. Probit method [5] was adopted to calculate LC_{50} value for 24, 48, 72 and 96hrs by using average mortality rates. The lethal concentrations together with the standard error and fiducial limits were calculated.

Plastic troughs of 20L capacity were used and 15L of 10ppt seawater was taken in each trough. During the experimentation, the PL were exposed to the 1.2056ppm of chromium which represents 1/5th of 96hr LC_{50} value, for a period of 30days. A control was maintained simultaneously without the metal toxicant. Appropriate amounts of stock solution were added to 15L of seawater to get the final desired concentration. Samples were taken from both the control and exposed at intervals of 24hrs, 48hrs, 96hrs, 10days, 20days and 30days. At the end of each interval 30 PL were collected both from the control and exposed tanks, washed thoroughly and pooled up separately.

The samples were dried in hot air oven at 60ºC for 48hrs. The dried material was then homogenized into a fine powder with mortar and pestle. The powder was stored in glass vials and preserved in desiccators prior to analysis.

Metal accumulation analysis was then carried out with the dried tissue samples of both the control and exposed PL for all the six-time intervals following dry-ash method [25]. A known quantity of dried tissue powder was dry-ashed in a muffle furnace at 800ºC for 6hrs. The dry ash obtained from the above process was dissolved in a known quantity of 2N HNO_3. The final clear and colourless solution was then used for chromium analysis using graphite furnace atomic absorption spectrophotometer (Model: Perkin Elmer No. 3110). Each sample was analysed in triplicate.

Routine oxygen consumption was measured by following the method of Villarreal [21]. A respiratory chamber of 300ml capacity equipped with an oxygen electrode (Elico Ltd. Hyderabad, INDIA) was used to determine the dissolved oxygen. Seawater used for all these experiments was filtered through Whatman (No.42) filter paper. Measurements were carried out for all the six time intervals at the same period of the day to avoid any possible diurnal rhythms. At each time interval, a batch of 20 healthy and active PL were randomly collected from the rearing tanks and introduced into the respiratory chamber. The amount of oxygen consumed was estimated for a period of 1hr. Triplicates were maintained for both the control and exposed samples at each interval. Care was taken not to allow the oxygen levels in the respiratory chambers to fall below the critical levels during experimentation. At the end of the experiment, wet weight of the PL used for the experiment was taken to calculate the metabolic rates. Routine metabolic rate was expressed as weight specific oxygen consumption i.e. mgO_2/hr/gm wet weight.

Samples were taken from both the control and exposed at intervals of 24hrs, 48hrs, 96hrs, 10days, 20days and 30days. At each interval, 30 PL were isolated from control and exposed PL and their total lengths (from anterior tip of the rostrum to the posterior tip of the telson) and wet weights were taken. The PL were then dried individually in an oven at 60°C for 48hrs and their dry weights were also noted down. The total length was measured with milli meter (mm) scale and, their wet and dry weights were determined by using a digital balance of milligram (mg) sensitivity. Blotting papers were used to blot the water adhering to PL before determining their individual wet weights. The daily weight gain was calculated using the respective dry weights of control and exposed PL by using the formulae described by Winberg [22] and Winberg [23]. The mean specific rate of growth was calculated by the equation

$g = (\ln W_{n+1} - \ln W_n)/t$

where

g = the mean specific rate of growth in PL

W_n = the mean dry weight of PL at n i.e., Control

W_{n+1} = the mean dry weight of PL at n+1 i.e., at each interval

t = the number of days exposed

$G = e^{g} - 1$

Where G is the fractional daily weight gain

G was multiplied by the replicate dry weights to calculate the values of daily weight gain (WG) for the PL.

The daily weight gain was calculated for both control and exposed PL at 24hrs, 48hrs, 96hrs, 10days, 20days and 30days.

Statistical evaluation: The average mortality rates of PL were calculated from all five experiments. The mortality rates were processed by using the Probit method [5]. The lethal concentrations along with the standard errors and fiducial limits were calculated [5].

Metal accumulation, oxygen consumption and growth experiments were repeated five times. The mean values (n=5) and standard deviations were calculated for each interval using standard methods. The exposed values were then compared with their respective controls by using Student's t-test [19] and significant differences were calculated at $P<0.05$.

Results:

The mean percent mortality rates in PL of *P. monodon* against different concentrations of chromium are given in Figure1. The linear regression equation obtained for log concentrations of exposure and probit values of percent mortality was Y = 2.3108 + 3.4467 X with a correlation coefficient of 0.9753. In Figure 2, the regression line representing the relationship between probit values and concentrations of chromium for PL of *P. monodon* is presented. The above data indicate that the mortality rates in PL of *P.monodon* were increasing with increasing concentrations of chromium. At a lower concentration of 2.5ppm, the mortality rate was found to be 14.4%. At higher concentration i.e., 20ppm, the mortality rate was 96.8%. The LC_{50} value for 96hrs was calculated to be 6.0280ppm for PL of *P.monodon*.

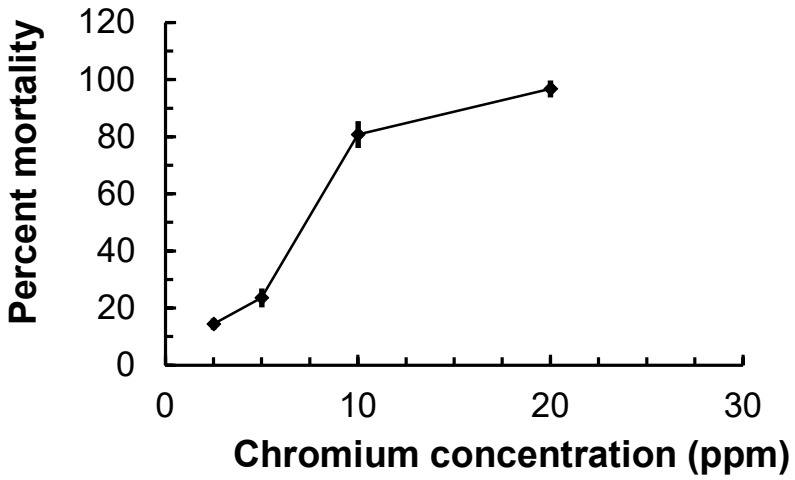

Fig. 1. Mortality rates of *P. monodon* PL exposed to different concentrations of chromium at 96hrs.Vertical lines represent standard deviation

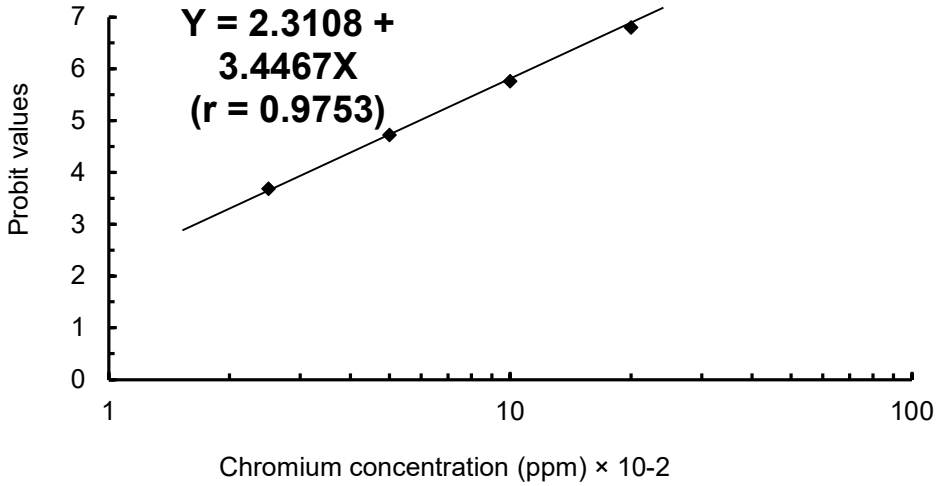

Fig. 2 Regression line representing the relation between probit values and chromium concentration in *P. monodon* PL at 96hrs.

The results of metal accumulation in the PL of *Penaeus monodon* exposed to sublethal chromium at six different time-intervals are presented in Figure 3. The data clearly showed a significant ($P<0.05$) accumulation of chromium in the exposed PL compared to their respective controls from 24hrs onwards till 30days of exposure. However, the metal content in the control PL for all the six exposure periods remained almost the same.

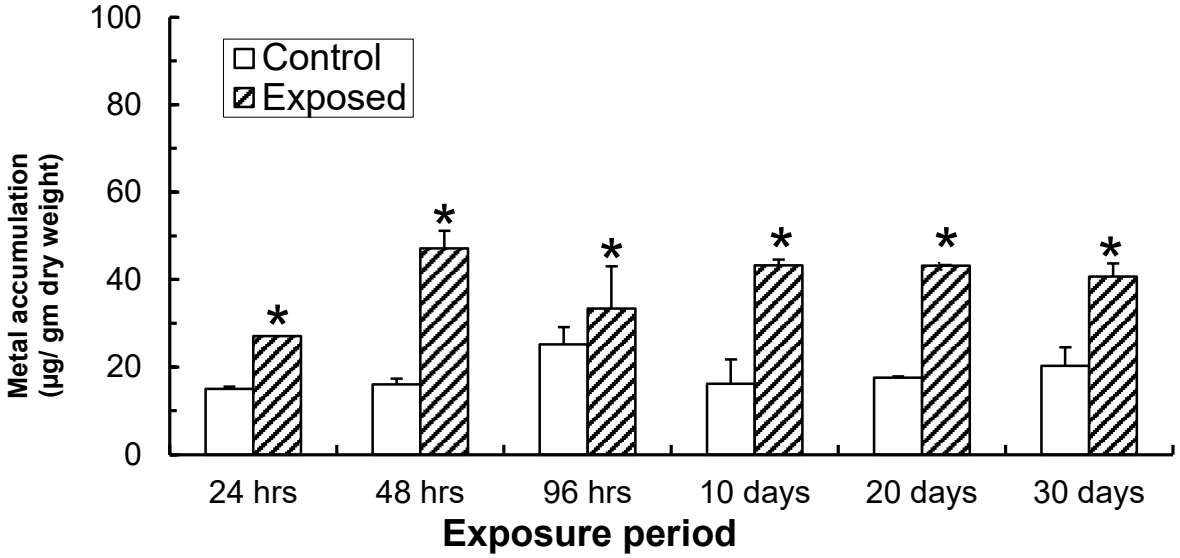

Fig. 3 Metal accumulation in *P. monodon PL* exposed to sublethal chromium. Vertical lines represent standard deviation. *Significantly different from their respective controls at $P < 0.05$

A gradual and time-dependent increase in the metal accumulation was noticed in the exposed PL ranging from 80.44% to 194.68%. The percent increase in the metal content of the exposed PL compared to their respective controls for different time intervals was 80.44% for 24hrs,194.68% for 48hrs, 32.60% for 96hrs, 167.80% for 10days and 145.86% for 20days and 100.32% for 30days of sublethal chromium exposure. The increase in the metal content in the exposed PL was from 27.066µg/gm dry weight at 24hrs, 47.150µg/gm dry weight at 48hrs, 33.350µg/gm dry weight at 96hrs, 43.250µg/gm dry weight at 10days, 43.150µg/gm dry weight at 20days and 40.667µg/gm dry weight at 30days of exposure. However, the metal content in the control PL was almost the same for all intervals i.e. 15.0, 16.0, 25.15, 16.15, 17.55 and 20.30µg/gm dry weight for 24hrs, 48hrs, 96hrs, 10days, 20days and 30days respectively.

The results of routine oxygen consumption for control and exposed PL of *Penaeus monodon* on short and long-term exposures to sub-lethal chromium are detailed in Figure 4.

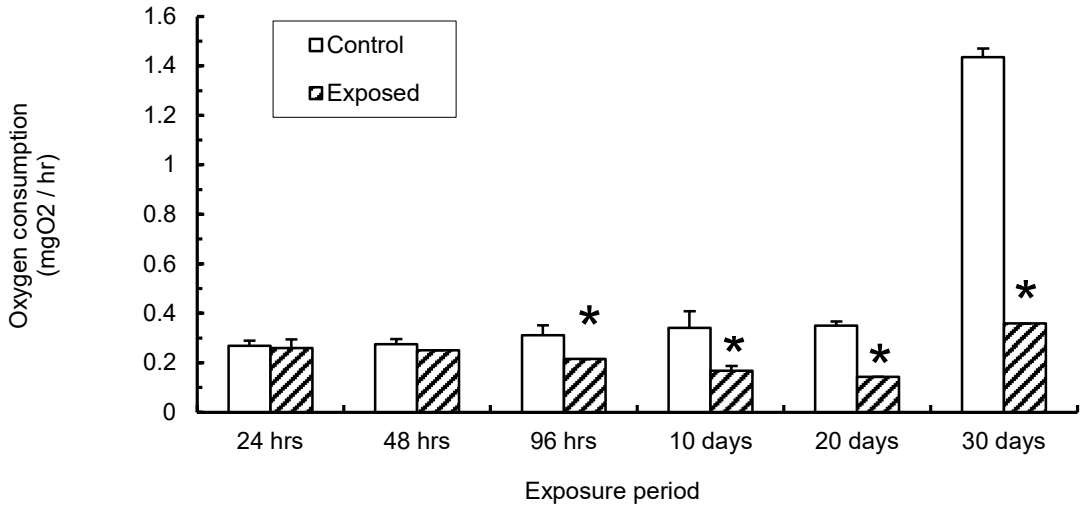

Fig. 4 Oxygen consumption in *P. monodon* PL exposed to sublethal chromium Vertical lines represent standard deviation. *Significantly different from their respectively controls at P < 0.05

Time-dependent and gradual decrease in oxygen consumption rates have been noticed in the exposed PL over their respective controls at all intervals. A maximum decrease of 75% on 30days exposure and a minimum decrease of 3.34% for 24hrs exposure were observed in the exposed PL. The percent decrease in oxygen consumption rates in the exposed PL over their respective controls was 3.34, 8.69, 30.74, 50.88, 58.97 and 75 at 24hrs, 48hrs, 96hrs, 10days, 20days and 30days respectively.

A significant ($P<0.05$) decrease in routine oxygen consumption was observed from 96hrs onwards till 30days on sublethal exposure of chromium. However, there was a gradual increase in routine oxygen consumption of control and exposed PL with increasing time period i.e. with increase in size of PL (Figure 4).

Similarly, there was a significant (P<0.05) decrease in metabolic rates of the exposed PL over their respective controls from 24hrs onwards as shown in Figure 5. A maximum decrease of 25.28% was observed in metabolic rate of the exposed PL over its control on 30days exposure. The percent decrease in metabolic rates in the exposed PL over their respective controls was 1.15, 11.89, 14.84, .29, 9.23 and 25.28% at 24hrs, 48hrs, 96hrs, 10days, 20days, and 30days respectively.

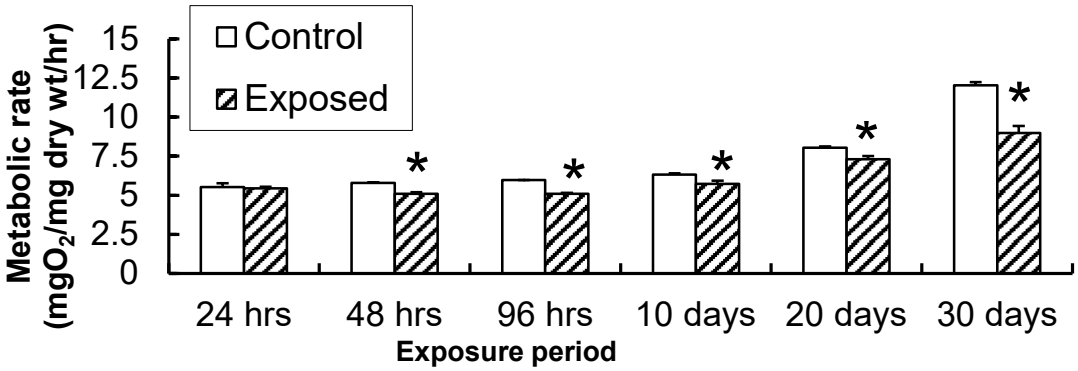

Fig.5 Routine metabolic rate in *P. monodon* PL exposed to sublethal chromium. Vertical lines represent standard deviation. *Significantly different from their respective controls at P < 0.05

The toxic effect of chromium on growth parameters such as mean total length, wet weight, dry weight and weight gain of exposed PL of *P. monodon* against the control PL is presented in Figures 6.1 to 6.4 respectively. The mean total length of the exposed PL of *P. monodon* showed a gradual decrease with increasing period of exposure but a significant decrease (P < 0.05) was observed from 96hrs to 30days on sublethal exposure to chromium (Figure 6.1). The percent decrease in total length was found to be 1.14, 0.63, 1.34, 6.80, 10.57 and 7.47 at 24hrs, 48hrs, 96hrs, 10days, 20days and 30days of exposure respectively.

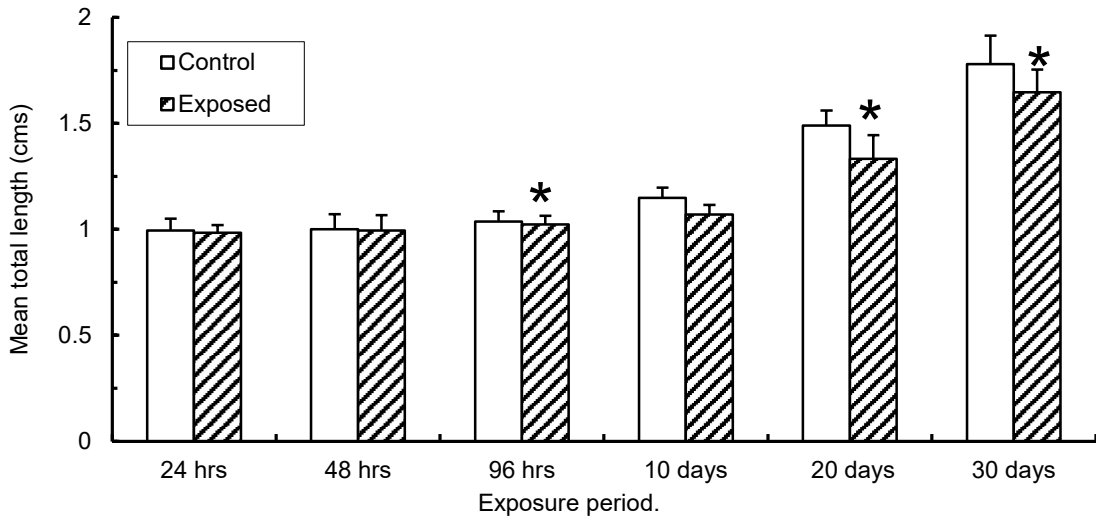

Fig. 6.1 Mean total length in *P. monodon PL* exposed to sublethal chromium. Vertical lines represent standard deviation. *Significantly different from their respective controls at P < 0.05

Similarly, the mean wet weight of the exposed PL of *P. monodon* was also found to decrease gradually on sublethal exposure to chromium. A significant decrease ($P < 0.05$) was noticed from 96hrs to 30days exposure (Figure 6.2). The percent decrease over their respective controls was 2.16, 3.69, 1.74, 17.64, 15.22, and 25.26 at 24hrs, 48hrs, 96hrs, 10days, 20days and 30days respectively.

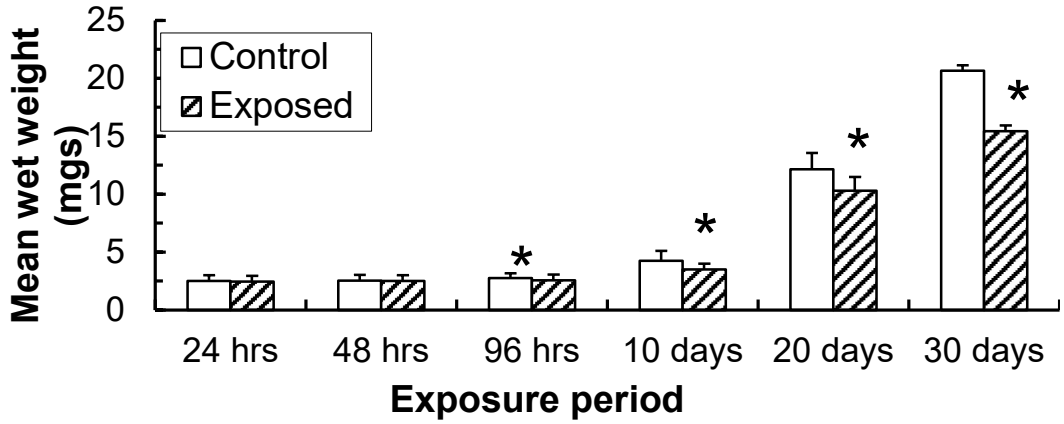

Fig. 6.2 Mean wet weight in *P. monodon PL* exposed to sublethal chromium. Vertical lines represent standard deviation. *Significantly different from their respective controls at P < 0.05

The mean dry weight was almost the same in both control and exposed (Figure 6.3) upto 96hrs. At 10days, 20days, and 30days of exposure, there was a decrease in dry weight and was significant ($P < 0.05$) from their respective controls (Figures 6.3). A percent decrease of 4.62, 3.69, 1.74,

8.69, 15.0 and 36.17 was observed over their respective controls at 24hrs, 48hrs, 96hrs, 10days, 20days and 30days.

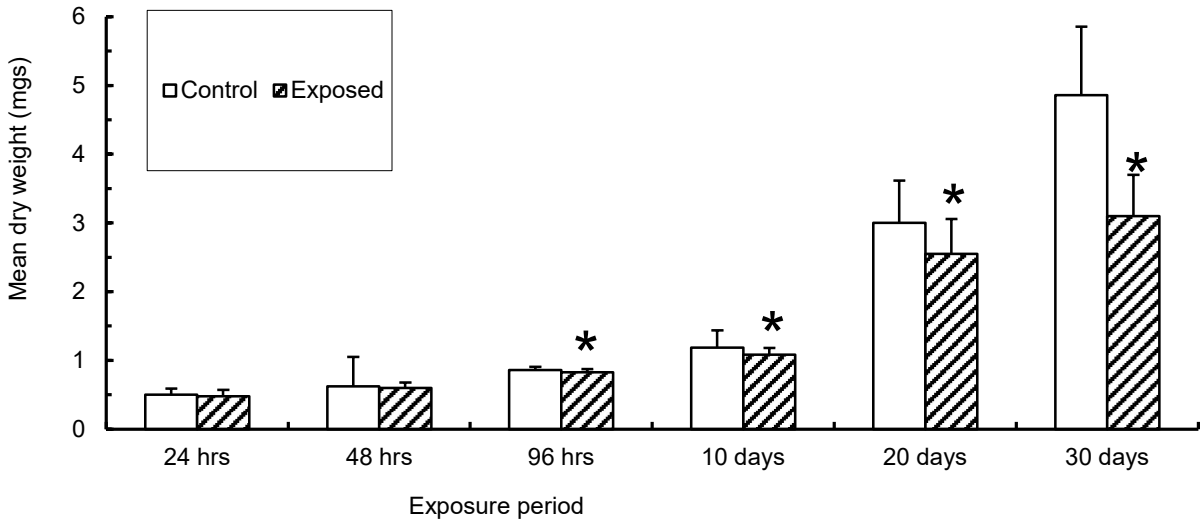

Fig. 6.3 Mean dry weight in *P. monodon PL* exposed to sublethal chromium. Vertical lines represent standard deviation. *Significantly different from their respective controls at P < 0.05

The values of weight gain in these PL exposed to sub lethal concentration of chromium was found to show a change from 24hrs onwards both in control and exposed. At 96hrs, the decrease was very less when compared to other values, but significant decrease was observed from 48hrs to 30days (Figure 6.4). The percent decrease values were 12.98, 13.83, 2.05, 18.39, 23.05 and 42.07 for 24hrs, 48hrs, 96hrs, 10days, 20days and 30days respectively.

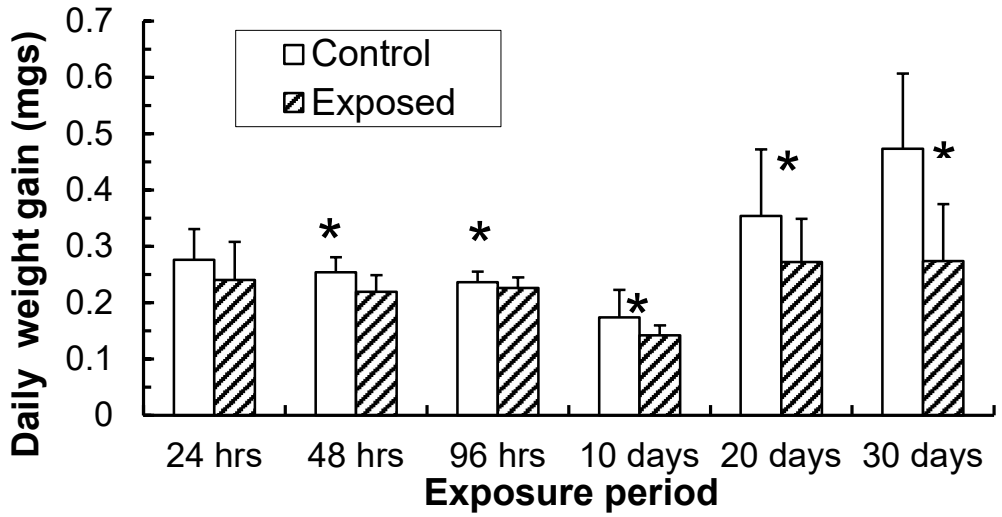

Fig. 6.4 Daily weight gain in *P. monodon PL* exposed to sublethal chromium. Vertical lines represent standard deviation. *Significantly different from their respective controls at P < 0.05

DISCUSSION:

The present investigation revealed a gradual accumulation of chromium in the tissues of the exposed PL with increasing exposure period. However, the metal content in the control PL almost remained same at all the time intervals. During ecdysis, shrimps intake of water increases owing to increase in their size, resulting in hydration of their tissues and favouring metal accumulation in PL [1]. this may be the probable reason for metal accumulation in the tissues of the PL where ecdysis occurs frequently.

In crustacean species, it is also thought that there is a relationship between metal permeability, regulation and accumulation. If the metal uptake rate in organisms is higher than the excretion rate, then metal accumulation occurs. An ideal bioindicator should be a net accumulator of the trace metal [8]. The toxicity test conducted on PL of *P. monodon* exposed to different concentrations of chromium for 96hrs indicated an increase in mortality rates with increasing time and metal concentrations. The toxicity experiments on PL of *P. monodon* exposed to chromium has revealed an LC_{50} value of 6.028ppm i.e. 6.028mg/L. Similar experiments were conducted by many investigators and reported LC_{50} values. However, studies on chromium toxicity are very much limited in crustaceans and that too on adults *Macrobrachium rosenbergii* for which the 96hrs LC_{50} value is 1250µg/L [13] but not on larval stages. Therefore comparision was made with the tolerance levels of other metals and toxicants in crustaceans.

The present investigation revealed a gradual accumulation of chromium in the tissues of the exposed PL with increasing exposure period. However, the metal content in the control PL almost remained same at all the time intervals. Further, the dissolved metals are considered more toxic since they are more easily absorbed by aquatic organisms than the particulate fraction [7]. This may be the probable reason for metal accumulation in the tissues of the PL where ecdysis occurs frequently. Chinni [2] observed a time-dependent increase in the metal concentration of *Peneaus indicus* post larvae exposed to sublethal lead. Rupa Vani [16] observed a gradual increase in accumulation of copper in post larvae of *Penaeus indicus.* Frias-Espericueta [6] reported time-and dose-dependent structural damages in the gill tissues of juvenile *Litopenaeus vannamei* exposed to different copper concentrations. Li [11] reported copper-induced structural damage to gills in the juvenile *Macrobrachium rosenbergii*.

CONCLUSION:

In the present investigation, laboratory experiments were conducted to evaluate chromium toxicity in post larvae of *Penaeus monodon* collected from local hatcheries of Visakhapatnam, reveals the marginal levels of safety for chromium i.e., 60.28µg/L. The PL of *P.monodon* are sensitive to sublethal concentration of chromium indicating an increase in the metal accumulation which in

turn leads to reduction in growth, metabolic rate and oxygen consumption in the exposed PL is very much essential for future monitoring studies of metal contamination.

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