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Oxygen Consumption, Ammonia-N Excretion, and Metal Accumulation in *Penaeus indicus* Postlarvae Exposed to Lead

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Heavy metal contamination of natural waters has become inevitable because of recent industrialization and urbanization. Heavy metals such as lead, cadmium, copper and zinc are found in most of the industrial effluents. Although some trace elements (copper, zinc) are essential to sustain life, the presence of even these metals in high concentrations may have adverse effects on aquatic organisms. Several investigators have reported harmful effects of heavy metals on a variety of crustaceans (Vernberg and Vernberg 1972; Depledge 1984; Spicer and Weber 1991). However, oxygen consumption and ammonia-N excretion have been widely considered as critical factors to evaluate the physiological responses of crustaceans (Chen and Lai 1992).

The white prawn, *Penaeus indicus,* is an important commercial crustacean for the Indian fishing industry. These prawns utilize estuaries and backwaters around Visakhapatnam as their nursery grounds (Satyavathi 1999). According to Sarma *et al*. (1996), heavy metals including lead were present in surficial sediments of some of these areas. Therefore, the present investigation was initiated to study the effect of lead by exposing the postlarvae (PL) of *P. indicus* to a sublethal concentration. Their oxygen consumption, ammonia-N excretion and metal accumulation were monitored at different intervals of lead exposure up to 30 days.

MATERIALS AND METHODS

Postlarvae (PL) of *P. indicus* were collected from Gosthani estuary of Bheemunipatnam (Lat $19^0 54^1$ N and Long $83^0 28^1$ E), adjoining the harbour township of Visakhapatnam on east coast of India. Almost uniform sized active PL (1-1.2 cm) were chosen for the experiment. The plastic containers and glassware used for all these experiments were washed carefully and rinsed with metal-free water. About 20 to 25 PL were maintained in each plastic container consisting of 4L of seawater and they were continuously aerated throughout the experiment. These PL were allowed to acclimatize to the laboratory conditions (29 ± 1°C and 20 ppt) for 48 hr prior to experimentation.

Stock solution of the metal was prepared by dissolving lead acetate

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[$(CH_3COO)_2Pb.\ 3H_2O$] (analytical grade) in distilled water. Appropriate volume of stock solution was added to seawater to get the sublethal concentration (1.44 ppm) (Satyavathi 1999).

The PL were exposed to the sublethal concentration of lead for 30 days, Parallel controls were maintained along with the experiment. They were fed with commercial larval feed (Lux Water Base, Nellore, India) twice a day (10:00 and 16:00) based on 20% of body weight per day. The medium was renewed for every 24 hr. Both control and exposed samples were taken at intervals of 24 hr, 48 hr, 96 hr, 10 days and 30 days for estimation of oxygen consumption, ammonia-N excretion and metal accumulation.

Oxygen consumption was measured by using a respiratory chamber of 300 mL capacity equipped with an oxygen electrode (UC1200 DO/Temp. meter, Central Kagaco Co. Ltd.) (Villarreal *et al.* 1994). Ammonia was measured in seawater by using trione [Dichloro-S-triamine 2, 4, 6 (1H, 3H, 5H)-trione] (Grosshoff and Johnson 1972). Seawater used for all the experiments was filtered through Whatman 42 filter paper. Salinity and temperature were kept constant at 20 ppt and 29 ± 1°C respectively. Only intermoult PL were used for all experiments and they were handled carefully to avoid any damage. At each interval, 10 PL were introduced into the respiratory chamber and their oxygen consumption and ammonia-N were estimated for a period of three hours with an acclimatization of l/2 hr as explained earlier. Care was taken that ammonia excreted into the water is not toxic to PL during the period of experimentation. In addition, Oxygen : Nitrogen (O:N) ratios were also calculated as the ratio of atoms of oxygen consumed to atoms of nitrogen excreted at the above intervals.

For accumulation studies, 20 PL were sacrificed at the above intervals samples and their soft tissues were isolated and pooled. The tissues were kept in hot air oven at 80°C for a period of 48 hr and the dried material was homogenized into a fine powder. Metal analysis was carried out in the dried tissue samples of both control and exposed. A known quantity of dried powder was wet-ashed with concentrated perchloric acid (AR) in Pyrex beakers (Prabhakara Rao *et al.* 1986). The wet-ash obtained was dissolved in a known amount of 0.01 N HCl. The final clean and colourless solution was used for metal estimation with Atomic Absorption Spectrophotometer (Spectra AA20).

All these experiments were repeated five times with independent samples and each sample was analyzed in triplicate. The mean value and standard deviation were calculated at each interval. The mean values were compared by using Student's 't' test (Sokal and Rohlf 1995) and a value of $P < 0.05$ was accepted as statistically significant.

RESULTS AND DISCUSSION

The results on rates of oxygen consumption and ammonia-N excretion for control and exposed PL were presented in Table 1. Though there was a decrease in weight specific oxygen consumption in both control and exposed PL, the decrease was significant ($P < 0.05$) in exposed PL in relation to time. A maximum decrease of 40.5% was observed after 30 days of exposure and a minimum decrease (19.4%) on 24 hr exposure to lead (Table 1). The decrease in oxygen consumption was significant ($P < 0.05$) from 24 hr up to 30 days exposure. In control PL, the rate of ammonia-N excretion increased with increasing time but the increase was not much in exposed PL. The percent decrease in ammonia-N excretion over their respective controls was from 35.9 to 70.3 over 30 days of exposure (Table 1). However, the decrease in ammonia-N excretion observed in exposed PL over their respective controls was significant ($P < 0.05$) at all time periods.

Table 1. Effect of sublethal exposure of lead on oxygen consumption and ammonia-N excretion of *P. indicus* PL. Each value represents the mean ± standard deviation of five observations. The values in the parentheses represent percent decrease over their respective controls. *Significantly different from their respective controls at $P < 0.05$.

Exposure period	24 hr	48 hr	96 hr	10 days	30 days
Oxygen consumption rate (mgO2/gm/min)					
Control	0.01162 ± 0.00236	0.01125 ± 0.00063	0.01120 ± 0.00124	0.00920 ± 0.00088	0.00749 ± 0.00110
Exposed	0.00937 ± 0.00140* (19.4)	0.00832 ± 0.00168* (26.0)	0.00735 ± 0.00059* (34.3)	0.00445 ± 0.00035* (35.5)	0.00445 ± 0.00012* (40.5)
Ammonia-N excretion rate (µg/gm/min)					
Control	0.02259 ± 0.00201	0.02469 ± 0.00470	0.02632 ± 0.00053	0.04166 ± 0.00128	0.09295 ± 0.00521
Exposed	0.01446 ± 0.00047* (35.9)	0.01492 ± 0.00075* (39.6)	0.01546 ± 0.00043* (41.3)	0.01765 ± 0.00074* (57.6)	0.01925 ± 0.00071* (79.3)

The data indicated that lead inhibited both oxygen consumption and ammonia-N excretion in *P. indicus* PL. A similar decline (29%) of oxygen uptake was reported in shrimp *Caridina rajadhari* by Chinnayya (1971) on exposure to lead nitrate for 10 days. Mercury was also found to inhibit respiration in the grass shrimp *Palaemonetes pugio* (Anderson *et al.* 1973). But Green *et al. (1976)* reported that the respiratory rates did not change in postlarvae of white shrimp *Penaeus setiferus* when exposed to 0.5 and 1.0 ppb of mercury. While studying the toxicity of cadmium and mercury ions on common prawn *Palaenzon serratus,* Papathanassiou (1983) reported an inhibition in respiratory potentials, Inhibition in oxygen consumption during lead exposure was also observed in molluscs *(Nerita albicilla)* (Anuprasanna and Prabhakara Rao 1993). According to Spicer and Weber (1991) cytological damage occurs in crab and shrimp species on heavy metal exposure and it may be in the form of thickening of branchial epithelium and profound changes in haemolymph pattern in the gill with concomitant increase in vacuolization and reduced haemolymph spaces causing perfusion stagnation. This kind of change might have occurred in PL of *P. indicus* on exposure to lead. However, some investigators reported that during exposure. to sublethal concentration of copper and zinc, the above condition was reversed after 18 days in *Carcinus maenas* (Boitel 1990) and 30 days in *Cancer pagurus* (Spicer and Weber 1991) even in the continued presence of toxicant, A similar pattern of recovery has also been demonstrated for the sabellid polychaete, *Eudistylia vancouveri* (Young and Roesijadi 1993). In the present investigation, *P. indicus* PL did not show any recovery during 30 days exposure.

It is also evident that ammonia-N excretion decreased in *P. indicus* PL on exposure to sublethal concentration of lead. Kinne (1976) suggested three routes for loss of metabolic ammonia from fish and crustaceans. They are diffusion of ammonia from blood to water, exchange and transport of NH_4^+ (ionized ammonia) with Na^+ and conversion to non-toxic compound like urea. Diffusion of NH_3 from blood to water is the main route for loss of metabolic ammonia in crustaceans since the blood levels of ammonia are normally much higher than the ambient concentration. This kind of mechanism for ammonia-N excretion might have occurred in these PL. The decrease in ammonia-N excretion by PL in the presence of toxicant can be attributed to the reduction of oxygen consumption or interaction of lead with pathways for the production of ammonia-N. Cheung and Cheung (1995) also reported a significant decrease of oxygen consumption and ammonia excretion in *Perna viridis,* exposed to heavy metals.

The O:N ratios for control and exposed PL were presented in Figure 1. In control PL, the O:N ratios showed a decreasing trend with time indicating a shift from greater utilization of lipid substrates to more protein usage as has been observed in *Homarus americanus* (Capuzzo and Lancaster 1979), *Hyas araneus* (Anger 1986) and *Palaemonetes pugio* (McKenney and Celestial 1993). A similar trend was noticed in exposed PL but the ratios were found to be significantly ($P < 0.05$) high when compared with their respective controls at all intervals. This might be due to theinteraction of lead with protein metabolism (Satyavathi 1999).

Figure 2 represents the tissue metal concentrations during sublethal exposure to lead. The results indicate that there was a gradual increase in the accumulation of lead in the exposed PL whereas in control PL, the metal content was almost constant at all time intervals (Fig. 2). But the increase was significant at all time intervals over their respective controls. However, the increase in accumulation

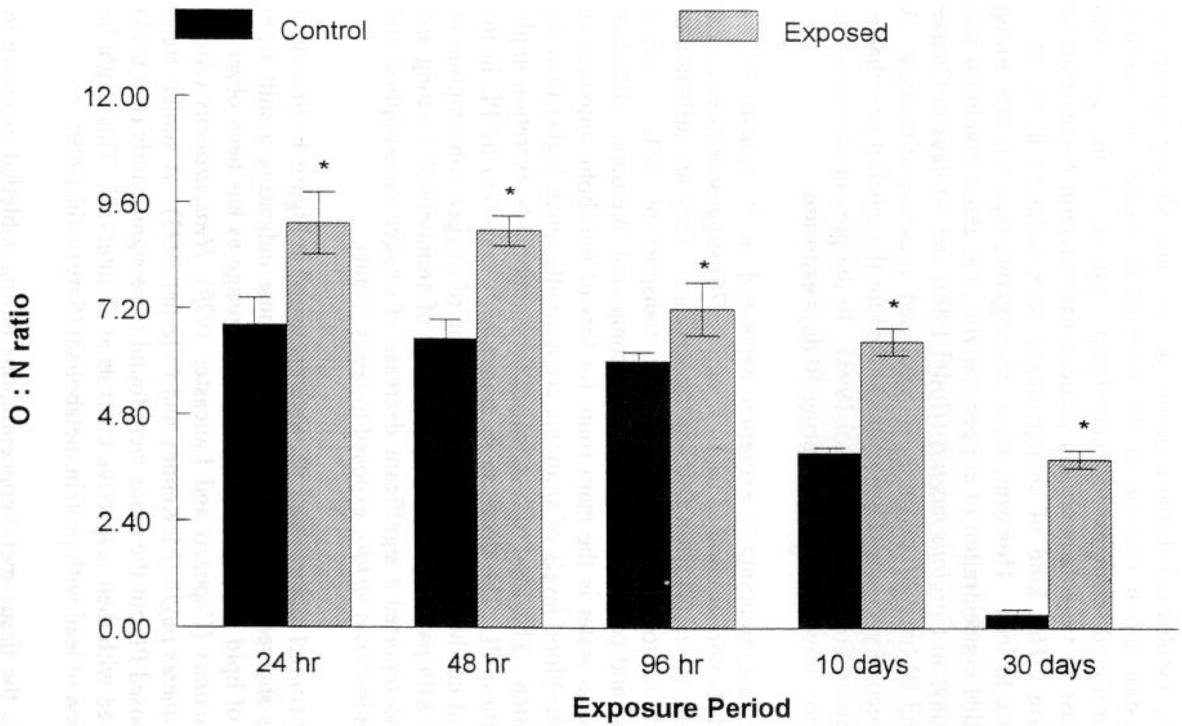

Figure 1. Oxygen : Nitrogen ratios in control and exposed PL to sublethal concentration of lead. The vertical lines represent standard deviation. *Significantly different from their respective controls at $P < 0.05$.

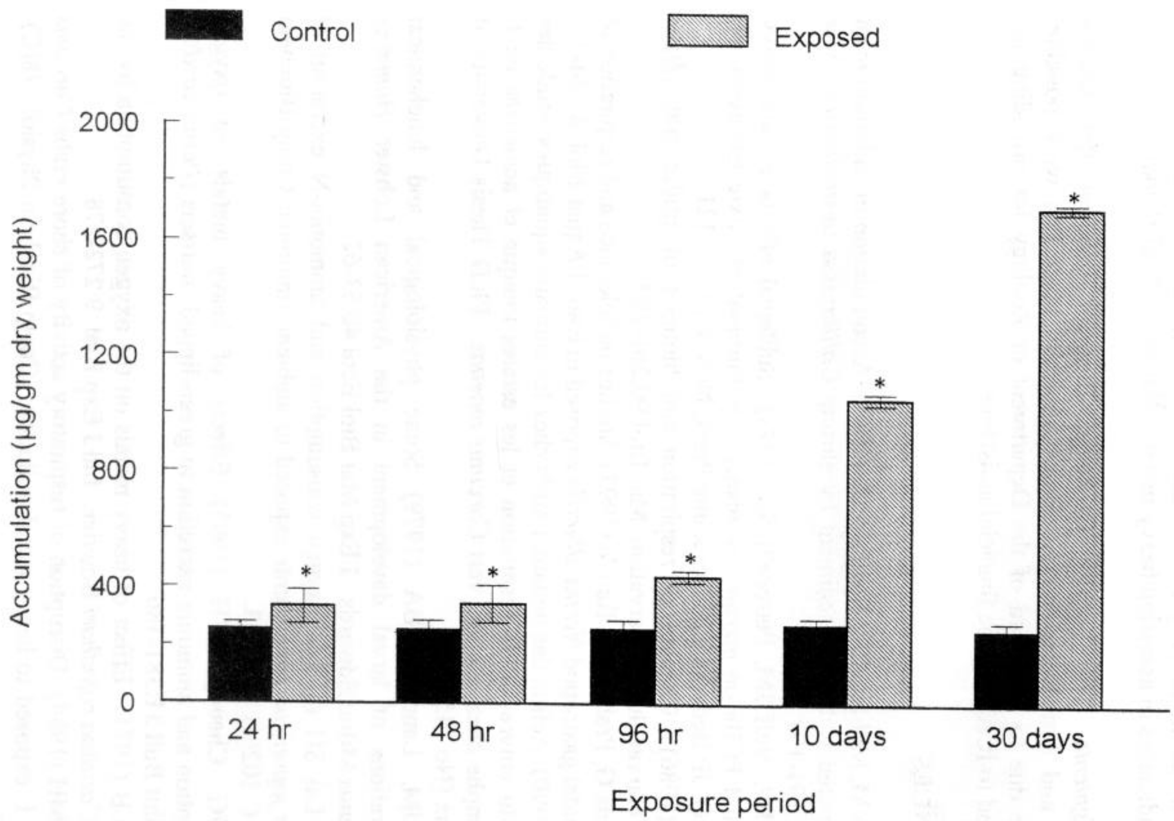

Figure 2. Accumulation of lead in PL of ***P. indicus*** on exposure to sublethal concentration. The vertical lines represent standard deviation. *Significantly different from their respective controls at $P < 0.05$.

was almost 6.6 times of the control on 30 days exposure. Similar results were noticed in shrimps by Liu *et al.* (1988) and Ahsanullah *et al.* (1984).

It appears from the study that lead accumulated in the tissues of PL might have interacted with the mitochondrial membranes decreasing the metabolic rate and ammonia-N excretion. Further studies indicated a significant decrease in mitochondrial ATPases of lead-exposed PL (Satyavathi 1999). Therefore, oxygen consumption, ammonia-N excretion and metal accumulation can be used as sensitive indicators in assessing heavy metal pollution in PL of *P. indicus.*

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REFERENCES

Ahsanullah M, Mobley MC, Negiliski DJ (1984) Accumulation of cadmium from contaminated water and sediment by shrimp *Callianassa australiensis*. Mar Biol 182:191-197.

Anderson JW, Neff JM, Petrocelli SR (1973) Sublethal effects of oils, heavy metals and PCBs on marine organisms. In: Survival in Toxic Environment, Khan MQ, JP Bederka eds, Academic Press, New York, 83-121

Anger K (1986) Changes of respiration and biomass of spider crab *(Hyas araneus)* larvae during starvation. Mar Biol 90:261-269.

Anuprasanna G, Prabhakara Rao Y (1993) Studies on tolerance and respiration of an intertidal gastropod *Nerita albicilla* exposed to lead. J Aquat Biol 8 : 34-37.

Boitel F (1990) Action des metaux lourds chez les animaux aquatiques: etude des effects du cuivre sur la respiration et les estates ionique et acidebase de 1' hemolymphe chez le crabe vert *Carcinus maenas*. Ph.D Thesis University of Bordeaux (No. 459).

Capuzzo JM, Lancaster BA (1979) Some physiological and biochemical considerations of larval development in the American Lobster *Homarus americanus* Milne Edwards. J Exp Mar Biol Ecol 40:53-62.

Chen JC, Lai SH (1992) Oxygen consumption and ammonia-N excretion of *Penaeus japonicus* adolescents exposed to ambient ammonia. Comp Biochem Physiol C 102(4): 129-133.

Cheung SG, Cheung RYH (1995) Effects of heavy metals on oxygen consumption and ammonia excretion in green-lipped mussels *(Perna viridis).* Mar Pollut Bull 31:381-386.

Chinnayya B (1971) Effect of heavy metals on the oxygen consumption by the shrimp *Caridina rajadhari* Bouvier. Ind J Exp Biol 9:272-278.

Depledge MH (1984) Disruption of respiratory activity of shore crabs *Carcinus maenas* L exposed to heavy metal pollution. Comp Biochem Physiol 78(C): 445-459.

Green FA, Anderson JW, Petroulli S R, Presley B J, Sims R (1976) Effect of mercury on the survival, respiration and growth of postlarval white shrimp *Penaeus setiferus.* Mar Biol 37:75-81.

Grosshoff K and Johnson H (1972) Methods of sea water analysis. Verlagchemie, Weinheum, l-260.
Kinne O (1976) Cultivation of marine organisms: Water quality management and technology In: Kinne O (ed) Marine ecology III Cultivation Part I. Wiley Chichester 79-300.
Liu K, Wu Y, Zhao H, Hou L, Sun F (1988) Accumulation and toxicity of copper in prawn *Penaeus orientalis.* Oceanol Limnol Sin Haiyang Yu Hyzhao 19: 133-139.
McKenney CL, Celestial DM (1993) Variations in larval growth and metabolism of an estuarine shrimp *Palaemonetes pugio* during toxicosis by an insect growth regulator. Comp Biochem Physiol 105C:239-245.
Papathanassiou E (1983) Effects of cadmium and mercury ions on the respiration and survival of the common prawn *Palaemon serratus.* Rev Oceanogr Med 70:21-26.
Prabhakara Rao Y, Uma Devi V, Prasada Rao DGV (1986) Copper toxicity in tropical barnacle, *Balanus amphitrite amphitrite* and *Balanus tintinnabulum tintinnabulum.* Water Air Soil Pollut 27: 109- 115.
Sarma VV, Varaprasad SJD, Gupta GVM, Sudhakara V (1996) Petroleum hydrocarbons and trace metals in Visakhapatnam harbour and Kakinada Bay, east coast of India. Ind J Mar Sci 25: 148-150.
Satyavathi C (1999) Studies on lead toxcity in postlarvae of *Penaeus indicus* (H. Milne Edwards). Ph.D thesis Andhra University, India.
Sokal RR, Rohlf FJ (1995) Biometry : The principles and practical of statistics in biological research. W.H. Freeman & Co New York, 887 pp.
Spicer JI, Weber RE (1991) Respiratory impairment in crustaceans and molluscs due to exposure to heavy metals. Comp Biochem Physiol 100C:339-342.
Vernberg WB, Vernberg J (1972) The synergestic effects of temperature, salinity, mercury on survival and metabolism of adult fiddler crabs, *Uca pugilator.* Fish Bull 17:415-420.
Villarreal H, Hinjosa P, Naranjo J (1994) Effect of temperature and salinity on the oxygen consumption of laboratory produced *Penaeus vannamei* postlarvae, Comp Biochem Physiol 108A: 33l-336.
Young JS, Roesijadi G (1983) Respiratory adaptation to copper induced injury and occurrence of copper binding protein in the polychaete, *Eudistylia vancouveri.* Mar Pollut Bull 14: 30-32.