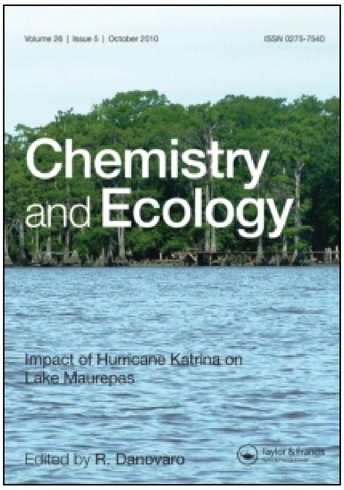

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EFFECT OF WATER VARIABLES ON LEAD TOLERANCE IN POSTLARVAE OF *PENAEUS INDICUS* (H. MILNE EDWARDS)

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Lead tolerance in *Penaeus indicus* post-larvae (PL) was studied in relation to the water variables: salinity, temperature and pH. The LC_{50} for 96 hrs was 7.22 ppm at ambient conditions of salinity (20‰), temperature (29°C) and pH (7.2). The post-larvae were found to be sensitive to salinity variations with a significant ($P < 0.05$) low LC_{50} values at lower (2‰) and higher (29‰) ranges. Though a decrease in LC_{50} value was observed both at higher (45°C) and lower (10°C) temperatures, it was significantly ($P < 0.05$) low only at higher temperature indicating more toxicity. The LC_{50} values also showed a significant ($P < 0.05$) decrease in acidic (pH 2.8) and alkaline (pH 11.0) conditions. The data indicate that lead toxicity increases in the PL of *P. indicus* with variations in the water variables.

Keywords: Prawn (*Penaeus indicus*); lead; tolerance; salinity; temperature; pH

INTRODUCTION

The commercially important Indian white prawn, *Penaeus indicus*, spends its early stages in brackish water areas where they are subjected to several pollutants (Thomas and Noble, 1993) and environmental fluctuations (Vernberg and Vernberg, 1972). Although organisms show some resistance to toxicants, such mechanisms are impaired on

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exposure to environmental stress (Thurberg *et al.*, 1973; Jones, 1975). Determination of lethal concentrations (LC) for toxicants is an essential prerequisite in all toxicological investigations and several investigations have been carried out on heavy metal toxicity in prawns (Rodriguez and Establier, 1983; Diaz, 1995; Bombang *et al.*, 1995; Gao and Zou, 1995; Chen *et al.*, 1996). The environmental variables such as salinity, temperature and pH play a significant role in the assessment of toxicity levels in prawns (Bombang *et al.*, 1995; Manikumar, 1986). Moreover the larval stages of prawns are very sensitive to both toxicants and environmental factors (Thomas and Noble, 1993).

Interestingly, the post-larvae (PL) of *P. indicus*, of Visakhapatnam (India) coast, experience wide fluctuations in environmental conditions (salinity 14 to 28‰; temperature 22 to 31°C; pH 7.2 to 12.01) and heavy metal concentrations particularly lead (7 to 22 $\mu g l^{-1}$) (Satyavathi, 1999). Hence, investigations were carried out to study the effect of salinity, temperature and pH on tolerance of *P. indicus* PL exposed to different concentrations of lead.

MATERIALS AND METHODS

Collection

Post-larvae (PL) of *P. indicus* were collected from Gosthani estuary (Latitude 18°19'N and Longitude 82°57'E),Visakhapatnam, on east coast of India. The PL were collected with a scoop net, immediately transferred into plastic containers containing sea water and transported immediately to the laboratory. The PL were handled with utmost care to avoid damage. Then they were maintained in the laboratory in plastic troughs containing with filtered sea water with aeration. Crowding was avoided during maintenance of the larvae in laboratory. The PL were maintained under ambient environmental conditions (salinity 20‰; temperature 29°C, pH 7.2) in the laboratory 48 hours before using for different experiments. The PL were fed with commercial larval feed (Lux Water Base, Nellore, India) twice a day (10.00 and 16.00 hours) based on 20% of total body weight per day.

Metal Toxicant

The metal salt, lead acetate [($CH_3COO)_2$ Pb, $3H_2O$)](AR) was used and a stock solution was prepared by dissolving it in distilled water. Appropriate amount of this stock solution was added to sea water to get the final desired concentrations of lead.

Experimental Procedures

Static bioassays were conducted with 24 hours renewal of the medium (FAO, 1977). Initially, experiments were carried out to determine the LC_{50} values at optimum conditions of salinity, temperature and pH. A separate control was maintained. At each concentration 10 PL were exposed in a plastic trough containing 4 litres of test solution. The medium was renewed for every 24 hours up to 96 hours and the mortality rates were recorded. All the troughs were aerated continuously and the environmental parameters were kept constant as above.

The above static bioassay experiments with 24 hours renewal of medium were conducted at different salinities, temperatures and pH. Preliminary experiments were carried out to determine the exposure concentrations as well as the lower and higher ranges of water variables. The low salinity water was prepared by adding distilled water to normal sea water (32‰). Sea water was mixed with rock salt to obtain the high salinities, then the water was filtered and the salinity was adjusted. The salinity was determined by titration with silver nitrite and the values obtained using Knudsen's hydrographic table. The low and high temperatures were maintained in a B.O.D incubator. The low pH was prepared by adding hydrochloric acid to the normal sea water (7.2). A solution of 1N sodium hydroxide was added to sea water for obtaining high pH. A pH meter with 0.01 sensitivity was used to adjust pH.

A range of lead concentrations (1, 5, 10 and 20 ppm) was used for the different experiments. Respective controls for low and high ranges of the parameters were maintained without metal toxicant for all the experiments. The environmental parameters were kept constant unless otherwise mentioned. Dead PL were removed at each observation (24 hours), the criterion for death was failure to respond to mechanical stimulation.

Statistics

All the experiments were repeated five times and the average mortality rates were calculated for control and different exposure parameters. LC_{50} values were determined by adopting the Probit method of Finney (1977). In this method the obtained mortality rates were converted into probit values and they were used for the calculation of LC_{50} values and for plotting the regression line. However, the derived probit equations were provided in the respective figures. Standard errors and fiducial limits were also calculated using the method of Finney (1977) for control and exposed individuals. Student's '*t*' test (Snedecor and Cochran, 1967) was used to compare the lethal concentrations of control with those of exposed individuals.

RESULTS

The average percent mortality rates for PL of *P. indicus* after 96 hours of exposure to different concentrations of lead at different environmental conditions are given in Figure 1. The data indicate that the mortality rates were increasing with increasing concentration of lead in PL of *P. indicus* at all the environmental parameters studied. At 1 ppm the average mortality was found to be 23% and a mortality rate of 70% was observed at a higher concentration (20 ppm) at optimum conditions. The linear regression equation obtained for log concentration of exposure and probit values of percent mortality was $Y = 4.1638 + 0.9738\,X$ with a correlation coefficient (r) of 0.9613. The different lethal concentrations calculated at ambient conditions were given in Table I and the LC_{50} value for 96 hours was 7.22 ppm. The safe concentration was calculated according to Kameswara Rao (1974) and this was about 1/100th of LC_{50} (96 hours) which was 72.23 $\mu g\ l^{-1}$ for PL of *P. indicus* (Tab. I).

Salinity – Dependent Toxicity

The slope of regression lines established to obtain the LC_{50} values for 96 hours of exposure at high (29‰) and low (2‰) salinities together with ambient (20‰) were given in Figure 2. At different salinity ranges, the mortality rates increased with increasing concentration of

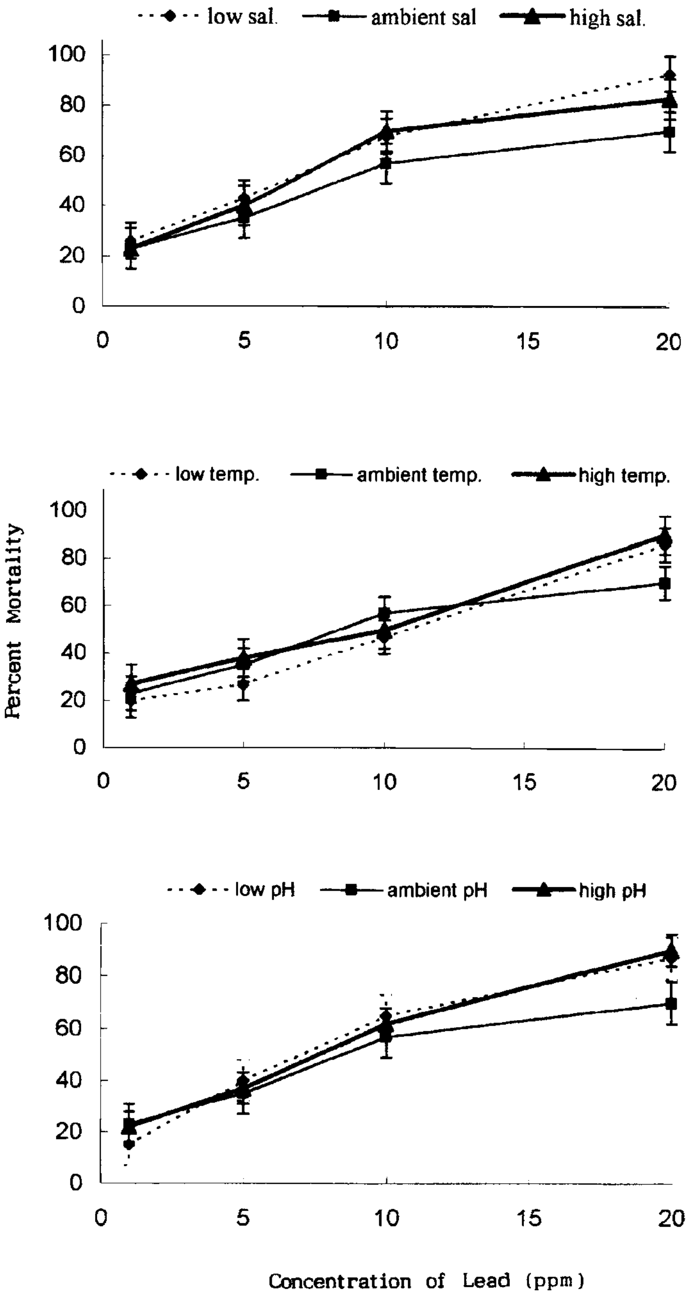

FIGURE 1 The percent mortality in *P. indicus* PL exposed to different concentrations of lead at different environmental variables (salinity, temperature, pH).

TABLE I Lethal concentrations of lead for *P. indicus* PL at different water variables. The details of calculations were given in materials and methods. Each value represents concentration ± standard error. The values in the parentheses represent 95% fiducial limits

Water variables	*Lethal concentration ± SE (ppm)*	*Safe concentrations* ($\mu g\,l^{-1}$)
Ambient		
(salinity 20‰, temp. 29°C, pH 7.2)		
LC_5	0.15 ± 0.01(0.08–0.21)	
LC_{25}	1.47 ± 0.09 (0.16–0.36)	72.23
LC_{50}	7.22 ± 1.59 (10.3–5.69)	
Salinity		
High salinity (29‰)		
LC_5	0.23 ± 0.04 (0.15–0.32)	
LC_{25}	1.50 ± 0.06 (1.38–1.61)	54.51
LC_{50}	5.45 ± 0.08 (5.30–5.60)	
Low salinity (2‰)		
LC_5	0.35 ± 0.06 (0.47–0.23)	
LC_{25}	1.51 ± 0.04 (1.58–1.46)	41.07
LC_{50}	4.11 ± 0.28 (4.65–3.56)	
Temperature		
High temperature (45°C)		
LC_5	0.24 ± 0.10 (0.04–0.45)	
LC_{25}	1.41 ± 0.41 (0.60–2.21)	47.74
LC_{50}	4.77 ± 0.84 (3.13–6.40)	
Low temperature (10°C)		
LC_5	0.39 ± 0.19 (0.23–0.77)	
LC_{25}	2.14 ± 0.52 (1.12–3.15)	68.36
LC_{50}	6.84 ± 1.13 (4.62–9.04)	
pH		
High pH (11)		
LC_5	0.36 ± 0.17 (0.28–0.69)	
LC_{25}	1.64 ± 0.52 (0.62–2.65)	46.47
LC_{50}	4.65 ± 0.72 (3.93–5.34)	
Low pH (2.8)		
LC_5	0.51 ± 0.19 (0.13–0.88)	
LC_{25}	2.01 ± 0.52 (0.98–3.33)	51.83
LC_{50}	5.18 ± 0.73 (3.75–6.61)	

lead, but lead toxicity was more at low and high salinities than ambient. The mortality was maximum (93%) at low salinity followed by 83% at high salinity on exposure to 20 ppm of lead and these rates were higher than at ambient conditions (Fig. 1). The LC_{50} values of

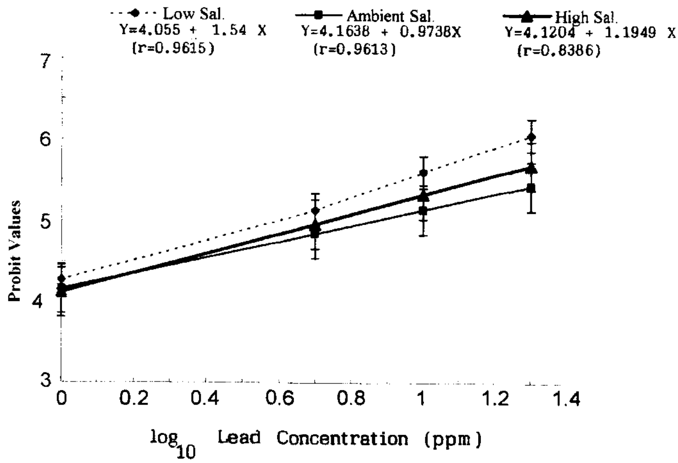

FIGURE 2 Comparison of dose-mortality regression line at low, high and ambient salinities for PL of *P. indicus* exposed to lead.

lead for PL of *P. indicus* were 4.11 and 5.45 ppm at low and high salinities respectively and these values were significantly ($P < 0.05$) low when compared with ambient salinity indicating more sensitivity (Fig. 2). At different salinities the order of lead toxicity was low salinity > high salinity > ambient salinity. The safe concentrations of lead for PL of *P. indicus* at low and high salinities. were 41.1 and 54.5 μg l^{-1} respectively (Tab. I).

Temperature – Dependent Toxicity

Figure 3 represents a comparison of the regression lines obtained at low (10°C) and high (45°C) temperatures with that of ambient (29°C) on exposure to different concentrations of lead. The percentage mortality rates obtained were high (86% and 90%) for both temperatures on exposure to lead (20 ppm) than at ambient (Fig. 1). However, at both the temperatures, the toxicity increased with increasing concentration of metal. The LC_{50} values for 96 hours (Tab. I) were 6.84 and 4.77 ppm for low and high temperatures respectively. A comparison of LC_{50} values indicate that the value at high temperature was significantly ($P < 0.05$) lower than the ambient, indicating more toxicity. The safe concentrations of lead for PL of *P. indicus* at low and high temperatures were 68.3 and 47.4 μg l^{-1}.

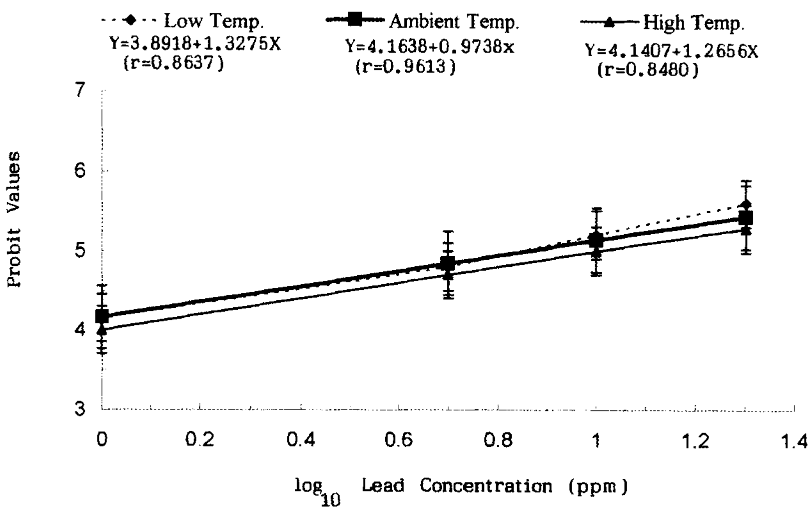

FIGURE 3 Comparison of dose-mortality regression line at low, high and ambient temperatures for PL of *P. indicus* exposed to lead.

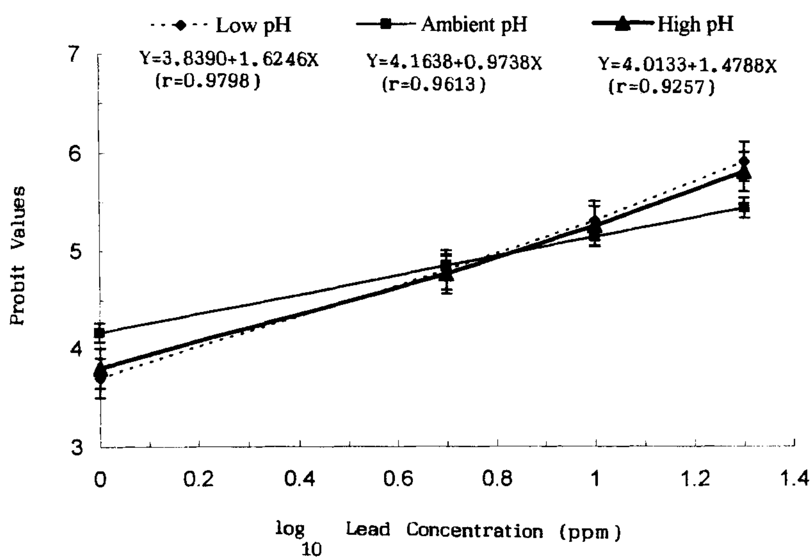

FIGURE 4 Comparison of dose-mortality regression line at low, high and ambient pH for PL of *P. indicus* exposed to lead.

pH Dependent Toxicity

A comparison of regression lines for both pH conditions namely pH 2.8 and pH 11 with that of ambient (pH 7.2) was represented in Figure 4. A change in the LC_{50} values for 96 hours was observed in relation to pH variation (Tab. I). A significant decrease ($P < 0.05$) was noticed in all lethal concentrations of high and low pH when compared with that of ambient pH. The LC_{50} values and safe concentration for PL of *P. indicus* were 5.18 ppm and 51.8 μg l^{-1} respectively for low pH (2.8) and 4.65 ppm and 46.4 μg l^{-1} at high pH. At different pH conditions, the lead toxicity was in the order of high pH > low pH > ambient pH.

DISCUSSION

The mortality rates observed in PL of *P. indicus* increased with increasing concentration as well as exposure time. Similar increase in mortality rate with an increase in metal concentration and exposure time was reported in PL of shrimps (Diaz, 1995; Gao and Zou, 1995; Ostrensky and Wasidesky, 1995; Carmel *et al.*, 1983; Green *et al.*, 1976), adults and juveniles (Eisler, 1971; Nimmo and Bahner, 1976; Ahsanullah *et al.*, 1981; Lin and Tin, 1993; Bombang *et al.*, 1995).

A comparison of LC_{50} values of different metals (Cd, Cu, Hg, Zn) with shrimps of different life stages is presented in Table II. It is evident that larval stages of shrimps in general, are more sensitive to toxicants when compared to juveniles and adults. This might be due to high surface volume ratio for the PL than juvenile and adult. However, toxicity ranges of different metals can be compared but it is not possible to compare the absolute values of LC_{50} because of the varying experimental conditions for different bioassays. The LC_{50} values vary with salinity, temperature and other extrinsic factors such as dissolved oxygen, pH of water and intrinsic factors like the age of animals (Bombang *et al.*, 1995); hence the conditions at which the bioassay is conducted, is important. The acute toxicity test and LC_{50} values are very much used in the assessment of safe level of toxicants and future monitoring of the environment (Lloyd, 1977). In the

TABLE II LC_{50} values of shrimps at different water variables

S.no.	Species	Stage	Metal	Salinity (ppt)	Temperature (°C)	LC_{50}	Exposure period (hours)	Reference
1.	*Penaeus monodon*	PL	Cd	34	24 ± 2	2.28 mg/liter	96	Diaz (1995)
2.	*P. japonicus*	Nauplii	Cd	34	*	1 µg/liter	48	Bombang *et al.* (1995)
3.	*P. japonicus*	Zoea	Cd	34	*	16.3 µg/liter	48	Bombang *et al.* (1995)
4.	*P. japonicus*	PL	Cd	34	*	735 µg/liter	48	Bombang *et al.* (1995)
5.	*P. japonicus*	Juvenile	Cd	34	*	2050 µg/liter	48	Bombang *et al.* (1995)
6.	*P. pencillatus*	PL	Cd	32	28 ± 0.5	3.025 ppm	96	Gao and Zou (1995)
7.	*P. pencillatus*	Juvenile	Cd	30 ± 2	27 ± 2	1.90 mg/liter	48	Lin and Tin (1993)
8.	*P. pencillatus*	Juvenile	Cd	30 ± 2	27 ± 2	3.40 mg/liter	24	Lin and Tin (1993)
9.	*P. pencillatus*	Juvenile	Cu	30 ± 2	27 ± 2	3.26 mg/liter	24	Lin and Tin (1993)
10.	*P. pencillatus*	Juvenile	Cu	30 ± 2	27 ± 2	2.20 mg/liter	48	Lin and Tin (1993)
11.	*P. pencillatus*	Juvenile	Hg	30 ± 2	27 ± 2	0.14	24	Lin and Tin (1993)
12.	*P. pencillatus*	Juvenile	Hg	30 ± 2	27 ± 2	0.06 mg/liter	48	Lin and Tin (1993)
13.	*P. pencillatus*	Juvenile	Zn	30 ± 2	27 ± 2	4.25 mg/liter	24	Lin and Tin (1993)
14.	*P. pencillatus*	Juvenile	Zn	30 ± 2	27 ± 2	2.09 mg/liter	48	Lin and Tin (1993)

* Data not available.

present investigation, the calculated safe levels at different salinities, temperatures and pH are well below the level of metal concentrations reported in the habitat. Therefore, these studies are helpful to monitor the lead pollution in the coastal waters.

Salinity levels, which vary with the changing tidal action, are important in metal toxicity for the animals. The results of toxicity of PL of *P. indicus* on exposure to lead varied significantly depending on the salinity of the sea water used in the experiment. However, the effects of salinity on heavy metals toxicity might be different for different organisms and may be species specific (Bombang *et al.*, 1995; Carmel *et al.*, 1983; Denton and Burdon Jones, 1982; O'Hara, 1973). The increase in the toxicity of lead in PL of *P. indicus* at low and high salinities might be due to osmoregulatory impairments in estuarine organisms following heavy metal toxicity (Thurberg *et al.*, 1973; Jones, 1975). However the comparison of regression lines for low, ambient and high salinities (Fig. 2) shows that the salinity effect was not much at low concentrations of lead but these were differences in the mortality rates with increasing concentration of lead. This might be an adaptation of PL which experience low salinities more often than higher. It is clear from the results that the toxicity increases both at high (45°C) and low (10°C) temperatures. Similar effects were noticed in most of the animals in relation to toxicants (Vernberg and Vernberg, 1972; O'Hara, 1973; Denton and Burdon Jones, 1982; Kulkarni, 1983). In the present investigation, a comparison of LC_{50} values of *P. indicus* PL show that the effect was more at high temperature than at low and ambient levels. The comparison of regression values (Fig. 3) shows that the variation in temperature effect was more at a higher concentration of lead. The increase in toxicity of lead with increase in temperature in *P. indicus* PL might be due to an increase in the metabolism with increasing temperature. A decrease in temperatures results in low metabolic rate which has a profound effect on the reduction of toxicity (Manikumar, 1986). Increase in the toxicity of heavy metals at high temperatures can also be attributed to the rates of biochemical and metabolic processes, diffusion and active transport of the toxic materials across the membranes (MacInnes and Calabrese, 1979). The higher the metabolic rate of toxicant that reaches the gills which are the major sites of uptake (Lloyd and Jordon, 1963). However, according to Cairns and Scheier (1964), it is difficult to generalize the temperature effect of the toxicity of pollutants.

The LC_{50} values of both low and high pH were less indicating more toxicity than the ambient. Similar effects of pH on toxicity were reported in postlarvae and juveniles of freshwater shrimp, *Macrobrachium rosenbergii* (Straus *et al.*, 1991); *Macrobrachium rosenbergii* larvae (Armstrong *et al.*, 1978) on exposure to ionized ammonia and un-ionized ammonia.

However in the habitat of PL, the environmental parameters (salinity, temperature and pH) are influenced by tidal cycles as well as season. According to Satyavathi (1999), the water salinity, temperature and pH fluctuate throughout the season. Therefore, the present study suggests that toxicity of lead depends on the fluctuations of water variables in the habitat.

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