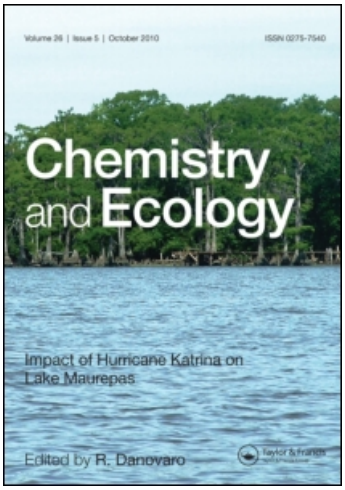

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Bioaccumulation and toxic effects of copper on growth and oxygen consumption by the postlarvae of *Penaeus indicus*

Rupa Vani Paila[a]; Prabhakara Rao Yallapragada[a]
[a] Department of Zoology, Andhra University, India

Online publication date: 13 May 2010

To cite this Article Paila, Rupa Vani and Yallapragada, Prabhakara Rao(2010) 'Bioaccumulation and toxic effects of copper on growth and oxygen consumption by the postlarvae of *Penaeus indicus*', Chemistry and Ecology, 26: 3, 209 — 221
To link to this Article: DOI: 10.1080/02757541003785809
URL: http://dx.doi.org/10.1080/02757541003785809

Chemistry and Ecology
Vol. 26, No. 3, June 2010, 209–221

Taylor & Francis
Taylor & Francis Group

Bioaccumulation and toxic effects of copper on growth and oxygen consumption by the postlarvae of *Penaeus indicus*

Rupa Vani Paila* and Prabhakara Rao Yallapragada

Department of Zoology, Andhra University, India

(*Received 7 August 2009; final version received 26 February 2010*)

The aim of this study was to assess the toxic impact of copper on postlarvae (PL) of the penaeid shrimp *Penaeus indicus*. Tolerance, growth, oxygen consumption and metal accumulation were investigated in these PL on exposure to copper. Tolerance studies were conducted for 96 h to assess the tolerance limits of *P. indicus* PL exposed to different concentrations of copper using static renewal bioassay tests. Using the Probit method, the regression equation was calculated as $Y = 0.4899 + 2.3562\,X$, with a correlation coefficient of 0.9707. The 96 h LC_{50} was 0.8204 ppm. The effect of sublethal (one-fifth of 96 h LC_{50}) copper on PL for short- and long-term exposures revealed a significant ($p < 0.05$) decrease in the rate of oxygen consumption, metabolic rate, mean length, wet and dry weight of the exposed PL over their respective controls which can be attributed to a gradual and time-dependent accumulation of the metal, as noticed in the exposed PL through accumulation studies. Overall, the data suggest that on chronic exposure even sublethal concentrations of copper can reduce the metabolic rate and growth in *P. indicus* PL. This is perhaps the first attempt to use the wild *P. indicus* PL as a bioindicator of copper toxicity.

Keywords: accumulation; copper; growth; oxygen consumption; *Penaeus indicus*; toxicity; postlarvae

1. Introduction

A great majority of the aquatic environment along the east coast of India is polluted because of rapid industrialisation, urbanisation, overexploitation of resources and human activities [1]. Aquatic organisms therefore experience toxic insult with these pollutants while using this ambient water as a source of respiratory oxygen [2] in aquaculture. Contamination of natural waters by heavy metals such as copper, zinc, lead, cadmium and iron is recognised as a serious threat to the aquatic ecosystem, showing deleterious or even toxic effects on aquatic biota, although it is also known that aquatic biota retain essential trace metals such as copper, zinc and iron in sufficient quantities in their bodies [3].

Copper sulphate ($CuSO_4.5H_2O$) is used extensively in hatcheries and aquaculture as a therapeutant to control external parasites, bacterial and fungal diseases [4,5]. Copper has an antagonistic role. Firstly, it acts as an essential trace metal required in small doses by organisms for metabolic functions [6]. Hassall and Dangerfield [7] reported that there are at least 12 major proteins that

*Corresponding author. Email: vaniprupa@yahoo.co.in

ISSN 0275-7540 print/ISSN 1029-0370 online

DOI: 10.1080/02757541003785809
http://www.informaworld.com

require copper as an integral part of their structure, including the respiratory enzyme cytochrome oxidase. Most crustaceans possess haemocyanin containing copper as their main oxygen-carrying blood protein. Secondly, it acts as a priority pollutant and is potentially a very toxic heavy metal if present in concentrations exceeding the capacity of physiological/biochemical levels [8]. Aquatic animals can thus serve as biomonitors of copper pollution [5,9,10].

Although marine organisms have an inherent ability to tackle the effects of pollutants in the environment through physiological changes, they can safeguard themselves against pollutants only up to a certain concentration, termed the 'safe level', 'tolerable level' or 'threshold level'. Because oxygen consumption is a measure of metabolism and a change in the metabolic rate indicates physiological imbalance, studies on oxygen consumption provide a useful tool in the assessment of toxicant stress on aquatic organisms. Crustacean species are well known for their biological features of concentrating heavy metals, thereby representing the pollution of an area and indicating the quality of coastal waters to be monitored. Exposure to the heavy metal copper in an aquatic environment has been reported to produce several physiological changes in shrimps, including alterations in their oxygen consumption, growth [11], metabolism [12] and survival [2].

Penaeid shrimps are economically important crustaceans and are abundant off tropical and subtropical coasts. The white prawn *Penaeus indicus* is widely distributed along the east coast of India and is renowned for its palatability and high market value, and is an important commercial crustacean for the Indian fishing industry [13]. The suitability of *P. indicus* postlarvae (PL) as a sensitive indicator of copper toxicity remains unstudied by investigators. Perusal of the available literature indicates that the acute toxicity of copper has been demonstrated in several crustaceans, e.g. marine copepod *Tigropus japonicus* [14], river prawn *Cryphiops caementarius* [15], saltwater cladoceran *Moina monogolica daday* [16], lobster *Homarus gammarus* and spider crab *Maja squinado* [17], and Palaemonoid prawn *Macrobrachium rosenbergii*, as well as a number of penaeid shrimps like *Penaeus monodon*, *P. japonicus*, *P. pencillatus*, *P. semisulcatus* and *Metapenaeus ensis* [18]. However, there is lack of toxicity data on wild *P. indicus* PL. Chinni and Yallapragada [19] conducted preliminary investigations comparing toxicity among four different heavy metals – copper, cadmium, zinc and lead – on *P. indicus* PL and determined only the lethal concentrations, concluding that copper is the most toxic, whereas lead is the least toxic. In the current investigation, an in-depth study on copper toxicity was carried out to investigate the effects of copper on the physiology of *P. indicus* PL in relation to growth and oxygen consumption, as well as bioaccumulation of the metal upon short- and long-term exposure to sublethal copper. This is perhaps first attempt to use the *P. indicus* PL as an eco-monitoring testing tool to assess the environmental hazards of copper toxicity.

2. Materials and methods

2.1. *Animals*

Uniform sized *P. indicus* PL (1.6–2.3 cm) were obtained from the wild catch of Gosthani estuary, which is clear and free of pollution, located near Bheemunipatnam (17.5 °N 83.3 °E), 30 km from Visakhapatnam of Andhra Pradesh, on the east coast of India. Because different species of PL were fished out during the wild catch, utmost care was taken to segregate the PL of select species, which were then immediately transferred into a plastic container filled with ambient estuarine water and supplied with constant aeration. The PL were then carefully transported to laboratory where they were transferred into a plastic tank of 50 L capacity. Ambient water was filtered to avoid unwanted debris. Salinity (10‰) was determined and the same salinity was maintained throughout the experiment. Constant aeration was provided to the PL and they were acclimatised

to laboratory conditions for two days before starting the experiment. PL were fed with commercial diet (EPAC-XL, Inve, The Netherlands) twice a day. Excess feed was removed daily by siphoning and the ambient water in the tank was renewed daily.

2.2. *Toxicity*

An acute toxicity bioassay was conducted in the laboratory to determine the effect of copper on the *P. indicus* PL by exposing them to different concentrations of the metal following the 'static renewable bioassay' method [20]. The tolerance response was evaluated by exposing the animals for a short time. In general, the level of tolerance in an animal to toxic substance is observed for 96 h [21].

A stock solution of copper (1%) was prepared from $CuSO_4.5H_2O$ (AR) in distilled water and required concentrations were prepared from the stock following the method of Bambang et al. [2]. Preliminary tests were conducted to ascertain the exposure range of the metal concentrations and based on these results, four concentrations, namely 0.5, 1.0, 2.0 and 4.0 ppm, were chosen for the test procedures. Selected experimental concentrations were made by adding appropriate volumes of freshly prepared stock solution to seawater.

Groups of 25 uniform-sized PL were put in each of the troughs containing 5 L of seawater (10‰) with the metal toxicant at the desired concentration. A parallel control without the toxicant was also maintained. Salinity (10‰), temperature ($29 \pm 1\,^{\circ}C$) and pH (7.8) were monitored in all control and exposed troughs. Seawater was renewed every 24 h and constant aeration was provided throughout the experiment. Each bioassay was conducted in duplicate and the experiment was repeated 15 times. Mortality was recorded every 24 h and dead animals were removed daily. Total lack of movement and response after repeated touches with a blunt needle were the criteria to consider an animal dead. All the animals in the control group survived. The PL were subjected to 96 h of exposure and the mortality data were recorded. Probit equation was used to calculate the different lethal concentrations for *P. indicus* PL on 96 h exposure along with the standard error and fiducial limits [22].

2.3. *Sublethal exposure*

A concentration of one-fifth of the 96 h LC_{50} value, i.e. 0.1641 ppm, was considered sublethal and all other experiments were carried out on exposure to this concentration. PL were exposed for 30 days in plastic tanks of 50 L capacity. Salinity (10‰), temperature ($29 \pm 1\,^{\circ}C$) and pH (7.8) were monitored throughout the experiment. The sublethal concentration was renewed every 24 h and constant aeration was provided. PL were fed a commercial diet twice a day. Excess feed was removed daily by siphoning and the tanks were washed daily. PL were not fed during or before the experiments. A parallel control was maintained without the metal toxicant. The environmental parameters and feeding regime were followed for the control as explained above, similar to the exposed *P. indicus*. The samples were collected both from control and exposed *P. indicus* at six different intervals, i.e. 24, 48 and 96 h (short-term) and 10, 20 and 30 days (long-term). The following parameters were estimated for both control and exposed PL.

2.3.1. *Metal accumulation*

At the end of each interval, 25 PL were collected from both the control and exposed tanks, washed thoroughly and pooled separately. Metal analysis was then carried out on the dried tissue samples of both control and exposed PL for all the six time intervals following dry-ash method [23]. A known quantity of dried tissue powder was dry-ashed in a muffle furnace at

800 °C for 6 h. The dry ash obtained was acid-digested in a known quantity of 2 N HNO_3. The final clear colourless solution was then used for copper analysis using graphite furnace atomic absorption spectrophotometer (Model: Perkin–Elmer No. 3110). Each sample was analysed in triplicate.

2.3.2. *Oxygen consumption and metabolic rate*

Routine oxygen consumption was measured by following the method of Villareal et al. [24]. A respiratory chamber of 300 mL 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 six time intervals at the same times of day to avoid any possible diurnal rhythms. At each time interval, a batch of 10 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 periods of 1 h. 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, the wet weight of the PL used for the experiment was used to calculate the metabolic rates. Routine oxygen consumption for 10 PL was expressed as $mgO_2 \cdot h^{-1}$ and routine metabolic rate was expressed as weight specific oxygen consumption, i.e. $mgO_2 \cdot h^{-1} \cdot g^{-1}$ wet weight.

2.3.3. *Growth*

At each of the above six different intervals, 25 PL were isolated, blot dried to remove adhered water and their total length (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 48 h and their dry weights were also taken. The total length was measured in millimetres and a digital balance of 0.1-mg sensitivity was used to determine the wet and dry weights. Daily weight gain was calculated using the respective dry weights of PL exposed to the sublethal concentration of copper by using the formulae described by Winberg [25] and Winberg et al. [26]. The mean specific rate of growth was calculated by the equation:

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

where

g = the mean specific rate of growth in PL,

W_n = the mean dry weight of PL at n,

W_{n+1} = the mean dry weight of PL at $n + 1$,

t = the number of days exposed,

G (fractional daily weight gain) $= e^{g-1}$.

G was multiplied by the replicate dry weight to produce values of daily weight gain for the PL.

2.4. *Statistics*

The above three experiments were repeated five times. Mean values and standard deviations were calculated for each interval using standard methods [27]. The exposed values were compared with their respective controls by using Student's t-test [27].

3. Results

The regression line representing the relationship between Probit values and the concentration of copper for the *P. indicus* PL is shown in Figure 1. The linear regression equation obtained using the Probit method was $Y = 0.4899 + 2.3562\ X$ with a correlation coefficient of 0.9707. The LC_{50} value for 96 h for *P. indicus* PL was 0.8204 ppm. The other lethal concentrations calculated by using the Probit equation are given in Table 1. The calculated safe concentration, i.e. one-hundreth of the LC_{50} [17] of copper, was $8.204\ \mu g{\cdot}L^{-1}$.

Figure 2 represents tissue metal accumulation over increasing time in PL exposed to sublethal copper over their respective controls. The results indicate a significant ($p < 0.05$) accumulation of copper in the exposed PL compared with their respective controls from 24 h to 30 days of exposure. A gradual and time-dependent increase in the metal accumulation was noticed in the exposed PL over their respective controls, ranging from 161.8 to 322.2% at 24 h and 20 days of exposure, respectively. The per cent increase in the metal content of the exposed PL compared with their respective controls for other time intervals was 178.0 for 48 h, 197.1 for 96 h, 243.9 for 10 days and 239.8 for 30 days of sublethal copper exposure. The gradual increase in the metal content in the exposed PL was from $49.75\ \mu g{\cdot}g^{-1}$ dry weight at 24 h exposure to $69.67\ \mu g{\cdot}g^{-1}$ dry weight on 30 days exposure. However, the metal content in the control PL was almost the same for all intervals i.e. 19.0, 18.25, 17.5, 16.5, 15.0 and $20.5\ \mu g{\cdot}g^{-1}$ dry weight for 24, 48, 96 h, 10, 20 and 30 days, respectively.

The results of oxygen consumption and metabolic rate for control and exposed *P. indicus* PL on short- and long-term exposures to sublethal copper are detailed in Table 2. Time-dependent,

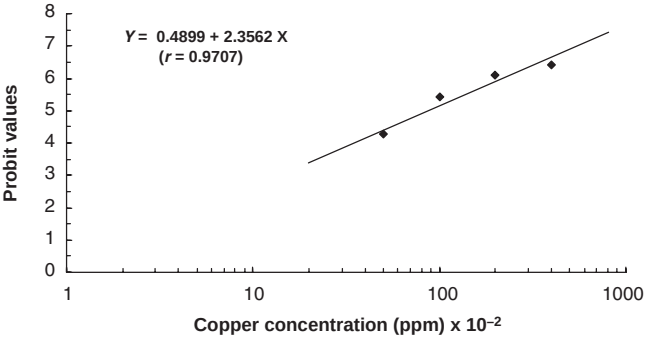

Figure 1. Regression line representing the relation between Probit values and copper concentrations in *Penaeus indicus* postlarvae.

Table 1. Lethal concentrations of copper for *Penaeus indicus* postlarvae.

Lethal concentration	Concentration (ppm) ± SE
LC_5	0.1644 ± 0.0072 (0.1502–0.1786)
LC_{10}	0.2345 ± 0.0087 (0.2175–0.2515)
LC_{25}	0.4244 ± 0.0110 (0.4029–0.4459)
LC_{50}	0.8204 ± 0.0135 (0.7939–0.8469)
LC_{75}	1.5860 ± 0.0261 (1.5349–1.6371)
LC_{90}	2.8704 ± 0.0708 (2.7316–3.0092)

Notes: Values were calculated from the regression equation $Y = 0.4899 + 2.3562$ X, correlation coefficient (r) = 0.9707. Each value represents the concentration ± standard error (SE). Values in parentheses represent 95% fiducial limits.

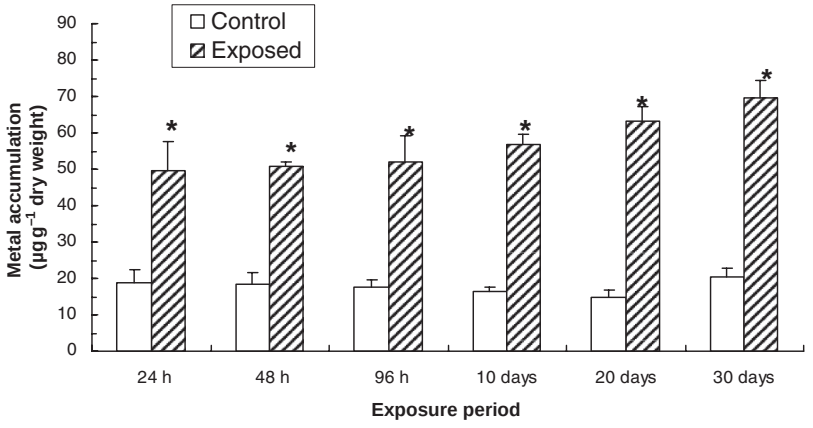

Figure 2. Metal accumulation in *Penaeus indicus* postlarvae exposed to sublethal copper (0.1641ppm). Vertical lines represent standard deviations (SD). Values are shown as mean ± SD ($n = 5$). Asterisks denote significant differences from the control at $p < 0.05$.

Table 2. Routine oxygen consumption and metabolic rate in *Penaeus indicus* postlarvae exposed to sublethal copper (0.1641 ppm).

	Routine oxygen consumption ± SD (mg $O_2 \cdot h^{-1} \cdot 10\,PL^{-1}$)		Routine metabolic rate ± SD (mg $O_2 \cdot h^{-1} \cdot g$ wet weight)	
Length of exposure	Control	Exposed	Control	Exposed
24 h	2.250 ± 0.150	1.950 ± 0.141 (13.3)*	5.887 ± 0.454	5.094 ± 0.273 (13.5)*
48 h	2.387 ± 0.093	2.017 ± 0.177 (15.5)*	6.161 ± 0.367	5.336 ± 0.278 (13.4)*
96 h	2.780 ± 0.172	2.315 ± 0.150 (16.7)*	6.346 ± 0.432	5.446 ± 0.299 (14.2)*
10 days	3.900 ± 0.158	3.025 ± 0.228 (22.4)*	6.522 ± 0.445	5.809 ± 0.296 (10.9)*
20 days	4.975 ± 0.228	3.5 ± 0.360 (29.6)*	6.311 ± 0.260	5.604 ± 0.717 (11.2)*
30 days	6.464 ± 0.730	4.25 ± 0.453 (34.2)*	6.121 ± 0.652	5.226 ± 0.465 (14.6)*

Notes: Each value represents the mean ± standard deviation (SD) ($n = 10$). The value in the parentheses represents the percent decrease over its respective control. *Significantly different from its respective control at $p < 0.05$.

gradual and significant ($p < 0.05$) decreases in oxygen consumption rates have been noticed in the exposed PL over their respective controls at all intervals. The per cent decrease in the oxygen consumption of exposed PL over their respective control was 13.3, 15.5, 16.7, 22.4, 29.6 and 34.2 at 24, 48, 96 h, 10, 20, 30 days, respectively. A maximum decrease of 34.25% was observed on 30 days' exposure to sublethal copper and a minimum decrease of 13.33% was observed for 24 h exposures. Similarly, there was a significant ($p < 0.05$) decrease in metabolic rates of exposed PL over their respective controls from 24 h onwards. The per cent decrease was 13.5, 13.4, 14.2, 10.9, 11.2 and 14.6 at 24, 48, 96 h, 10, 20, 30 days, respectively. A maximum decrease of 14.62% was observed on 30 days' exposure and a minimum decrease of 10.92% was observed for 10 days' exposure to sublethal copper.

The effect of sublethal copper (0.1641 ppm) on growth parameters such as mean total length, wet weight and dry weight of exposed *P. indicus* PL against their control is shown in Table 3. The results indicate a significant decrease ($p < 0.05$) in all the growth parameters from 96 h onwards in the exposed PL compared with their respective controls. The decrease was, however, not significant for 24 and 48 h exposure periods. The decrease in the mean length, wet weight and dry weight was 3.6, 7.2 and 6.0% respectively for 96 h exposed PL over their respective controls. A maximum decrease of 16.1, 30.9 and 30.7% in mean length, wet weight and dry weight was observed in 30 day exposed PL over their respective controls (Table 3). A significant decrease

Table 3. Mean total length, wet weight and dry weight in *Penaeus indicus* PL exposed to sublethal copper (0.1641 ppm).

Length of exposure	Mean length ± SD (mm)		Mean wet weight ± SD (mg)		Mean dry weight ± SD (mg)	
	Control	Exposed	Control	Exposed	Control	Exposed
24 h	20.950 ± 0.820	20.875 ± 1.435 (0.3)	38.345 ± 5.409	38.137 ± 4.926 (0.5)	8.318 ± 1.103	8.286 ± 0.995 (0.4)
48 h	21.068 ± 1.602	20.986 ± 1.660 (0.4)	38.907 ± 5.834	38.423 ± 4.606 (1.2)	8.950 ± 0.762	8.790 ± 0.953 (1.8)
96 h	21.823 ± 1.152	21.028 ± 1.164 (3.6)*	42.125 ± 4.434	39.075 ± 4.916 (7.2)*	10.467 ± 1.087	9.841 ± 1.087 (6.0)*
10 days	23.221 ± 0.975	21.850 ± 1.195 (5.9)*	57.161 ± 5.756	48.778 ± 5.112 (14.7)*	13.807 ± 1.583	11.277 ± 1.086 (18.3)*
20 days	26.540 ± 1.951	23.547 ± 1.496 (11.3)*	78.571 ± 6.149	61.844 ± 5.512 (21.3)*	21.085 ± 1.966	15.809 ± 1.803 (25.0)*
30 days	29.813 ± 1.971	25.0 ± 1.497 (16.1)*	118.973 ± 11.621	82.154 ± 7.183 (30.9)*	30.064 ± 3.198	20.842 ± 2.109 (30.7)*

Notes: Each value represents mean ± standard deviation (SD) ($n = 75$). Values in the parentheses represent the percent decrease over its respective control. *Significantly different from its respective control at $p < 0.05$.

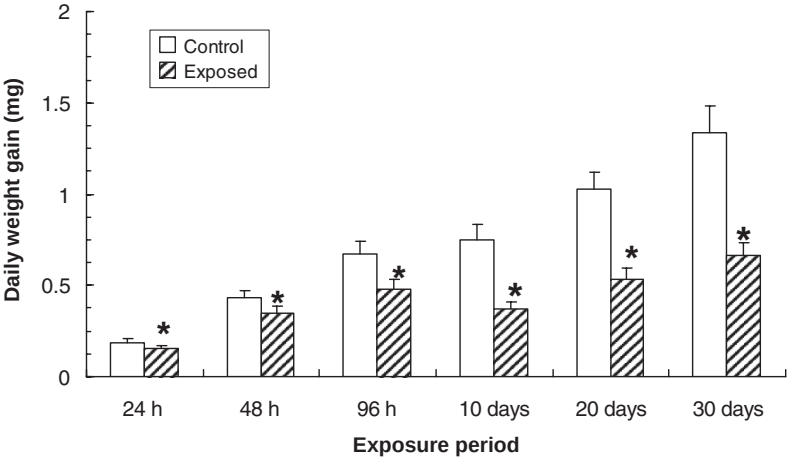

Figure 3. Daily weight gain in *Penaeus indicus* postlarvae exposed to sublethal copper (0.1641ppm). Vertical lines represent standard deviations (SD). Values are shown as mean ± SD ($n = 65$). Asterisks denote significant differences from the control at $p < 0.05$.

($p < 0.05$) in the daily weight gain in the exposed PL over their respective controls was noticed from 24 h onwards. There is a gradual decrease from 13.3% at 24 h exposure to a maximum of 50.3% on 30 days exposure. The decrease was 20.8% for 48 h, 29.1% for 96 h, 50.1% for 10 days and 48.1% for 20 days (Figure 3).

4. Discussion

Lloyd [28] suggested that acute toxicity tests and LC_{50} values are very useful for assessing the safe level of toxicity and future monitoring of the environment. In the current study, tolerance experiments conducted on *P. indicus* PL exposed to different concentrations of copper for 96 h at 10‰ salinity revealed that the mortality rate increases with increasing metal concentration. An LC_{50} value of 0.8204 ppm for 96 h was obtained in this investigation. Several investigators have reported similar results on exposure to copper and other toxicants in the postlarvae of various shrimps. A comparison of LC_{50} data of various shrimps indicate that the 96 h LC_{50} values for the postlarvae of *P. monodon*, *P. japonicus*, *P. semisulcatus*, *P. pencillatus*, *Metapenaeus ensis* and *M. rosenbergii* exposed to copper sulphate were 0.73, 1.18, 3.2, 0.3, 3.73 and 1.14 $mg{\cdot}L^{-1}$, respectively [18]. Because our investigation revealed the LC_{50} value for copper in *P. indicus* PL as 0.8204 $mg{\cdot}L^{-1}$, it can be concluded that the sensitivity of different penaeid species for copper is in the order *P. pencillatus* > *P. monodon* > *P. indicus* > *P. japonicus* > *P. semisulcatus*. However, varying experimental conditions for different bioassays restrict the comparison of absolute values of LC_{50} between species; nevertheless, the toxicity ranges of different metals can be compared. Because the LC_{50} values vary with temperature, salinity, dissolved oxygen, water pH, animal age and other such extrinsic or intrinsic factors, the conditions at which the bioassays are conducted is very important. In our investigation, the obtained LC_{50} value for copper toxicity in *P. indicus* PL holds good under the experimental conditions followed. Other reported 96 h LC_{50} values for copper were 1.4 $mg{\cdot}L^{-1}$ for *Farfanpenaeus paulensis* PL [11], 1.03 $mg{\cdot}L^{-1}$ for *Callianassa australiensis* juveniles [29], 0.900 $mg{\cdot}L^{-1}$ (36‰) for *P. merguensis* juveniles [30] and 0.546 $mg{\cdot}L^{-1}$ for river prawn *Cryphiops caementarius* PL [15]. Thus, a comparison of the available LC_{50} data on PL of various marine shrimps exposed to copper toxicity indicate that *P. indicus* PL are sensitive indicators of copper toxicity as revealed from their lower LC_{50} value than PL of most of the

other marine shrimps. A comparison of 96 h LC_{50} values of *P. indicus* PL with other heavy metal toxicants reveal that copper is more toxic than lead, zinc and cadmium, although it is less toxic than mercury. The reported LC_{50} values for 96 h on *P. indicus* PL are 7.223 mg·L^{-1} (20‰) for lead, 6.2231 mg·L^{-1} (20‰) for zinc, 3.1191 mg·L^{-1} (20‰) for cadmium [19] and 0.042 mg·L^{-1} for mercury [31].

Our investigation resulted in a 96 h LC_{50} value of 0.8204 ppm for *P. indicus* PL at 10‰ salinity. Comparing these results with those of Chinni and Yallapragada [19] who reported a 96 h LC_{50} value of 2.535 ppm at 20‰ in the same species of the same stage, it should be stated that low salinity depressed the LC_{50} value by 67.64%. This indicates that copper toxicity increases with decreasing salinity. Low salinity is associated with increased uptake of ions that might increase metal accumulation [32]. Many authors have similarly reported a relatively high toxicity of metals at low salinity [2,30]. Daly et al. [33] reported that crustaceans, in particular, are more sensitive to metals at the time of moulting because ecdysis involves the formation of a new cuticle with an increased uptake of water and ions (including Cu^{2+}) to expand the body volume [34]. It is therefore possible that the stress of moult and higher copper toxicity at low salinity become synergistic [33] and contribute to higher toxicity.

Several investigators have reported the presence of heavy metal copper as a pollutant along the coast of Visakhapatnam [35,36]. The use of copper compounds in controlling bacterial, fungal, protozoan and other infectious diseases in hatcheries has been reported by Reddy et al. [5]. Thus, there is also a chance of copper contamination from hatcheries located along this coast, because these hatcheries release contaminated water into the coastal region. Our investigations revealed the presence of copper along the coastal waters of Visakhapatnam and the levels range from 0.975 to 3.45 μg·L^{-1} in the surface water and from 0.875 to 4.325 μg·L^{-1} in bottom waters. Sarma et al. [36] reported copper levels between 30 and 89 μg·g^{-1} in the surficial sediments of Visakhapatnam waters. Although the coastal waters of Visakhapatnam were found to be contaminated by the heavy metal copper, the backwaters of Bheemunipatnam, which was the site of collection of the *P. indicus* PL, showed nondetectable levels of copper. Our investigation resulted in a safe level of 8.204 μg·L^{-1} for *P. indicus* PL at low salinity (10‰), as derived from the formula given by Marino-Balsa et al. [17], to obtain the maximum permissible concentration (MPC) with a protection factor of 100 according to the formula $MPC = LC_{50}/100$.

Because aquatic organisms absorb heavy metals from contaminated water and the food chain which are then deposited into their bodies in a process called 'bio-accumulation' [37], they are good indicators for environmental pollution. Our investigation revealed a gradual accumulation of copper in the tissues of exposed PL with increasing length of exposure. However, the metal content in the control PL remained almost same at all time intervals, varying between 15.0 to 20 μg·g^{-1} dry weight. This is probably the amount of copper present in hemocyanin, the respiratory pigment of crustaceans. Significantly high copper accumulation in exposed PL from 24 h onwards may be attributed to the moulting of shrimps in the PL stage. During ecdysis, shrimps' water intake increases to increase in their size, resulting in hydration of their tissues and favouring metal accumulation in PL [2]. Furthermore, dissolved metals are considered more toxic because they are more easily absorbed by aquatic organisms than the particulate fraction [38]. A similar increase in the accumulation of copper with increasing exposure times have been reported by several investigators in other shrimps like *P. orientalis* [39], *Palaemon elegans* [40], *Callianassa australiensis* [29], *P. monodon* [41] and *M. rosenbergii* [5].

It is reported that heavy metals are known to depress the respiratory rate, possibly by direct metabolic inhibition [11,42]. Spicer and Weber [43] suggested that lethal and sublethal concentrations of the essential metals Cu and Zn act on the respiratory system primarily by disrupting gill function in crustaceans and molluscs. Reduced oxygen consumption rates can thus be attributed to copper being more concentrated in gills, as reported by Paez-Osuna and Tron [44], resulting in structural changes in gills and gill damage [45,46] leading to restriction of respiratory gas

exchange [47]. However, Soegianto et al. [45], while studying copper toxicity in *P. japonicus*, reported an increased number of nephrocytes in gill filaments, a blackened appearance of the gills, necrosis of gill cells resulting in narrowed or obstructed hemolymphatic vessels, the appearance of a space between the cuticle and the epithelial cells, which contain black electron-dense material, and even fragmentation of nuclei within gill cells. Similarly, Frias-Espericeuta et al. [46] reported severe time- and dose-dependent structural damage, such as necrosis, loss of regular structure and infiltration of haemocytes in the gill tissues, as well as atrophy, necrosis and irregular tubular structure in the hepatopancreas of juvenile *Litopenaeus vannamei* exposed to different copper concentrations. A decrease in haemocyanin–oxygen binding affinity in the shore crab *Carcinus maenas* caused by copper intoxication has been reported by Truchot and Boitel [48]. Similar metal-related respiratory impairment in the brine shrimp *Artemia faranciscana* was reported by Spicer [49] on exposure to copper. Thus, the above reports suggest that the possible cause of impaired ability of copper-exposed PL to extract dissolved oxygen may be structural and physiological changes in the respiratory organs. A significant ($p < 0.05$) increase in copper accumulation in the exposed PL therefore resulted in significant retardation of routine oxygen consumption rates of exposed PL over their respective controls from 13.33% on 24 h exposure to 34.25% on 30 days exposure, and consequently decreased routine metabolic rates in *P. indicus* PL from 24 h to 30 days. The changes in the physiological activity of the exposed PL might also be caused by the interference of the toxicant with the mitochondrial membranes as reported by Webb [50].

The results obtained from our growth experiments clearly confirm that growth was inhibited significantly in *P. indicus* PL on exposure to sublethal copper for a prolonged period. A reduction in total length, wet weight, dry weight and weight gain in exposed PL over their respective controls can be attributed to an increase in metal accumulation in exposed PL over time and a simultaneous decrease in oxygen consumption and metabolic rates. These energetic imbalances might have influenced the net growth efficiency of the PL which ultimately inhibited the growth significantly from 96 h onwards. Retardation in growth of exposed *P. indicus* PL might be due to the interaction of copper with various metabolic pathways. It has a pronounced effect on many metabolic processes such as mitochondrial respiration, collagen and elastin cross-linking, antioxidant protection, lipid and lipoprotein as well as carbohydrate metabolism [51]. A higher concentration of copper was reported to significantly alter the activities of metabolic enzymes; lactate dehydrogenase and succinate dehydrogenase of carbohydrate metabolism in the hepatopancreas of crab *Sesarma quadratum* [52], and acid phosphatase, alkaline phosphatase, glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase in the hepatopancreas of freshwater prawn *M. rosenbergii* [12,53]. Acid phosphatase plays an important part in cellular metabolism, catalysing the hydrolysis of phosphoproteins and the transfer of phosphate groups. By contrast, alkaline phosphatase, a type of phosphomonoesterase, exerts its important physiological functions by catalysing the transfer of phosphate groups. Both glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase are intimately involved in detoxification. The effects of copper on growth inhibition have been reported by several investigators in various shrimps like *M. rosenbergii* [54], *Farfantepenaeus paulensis* [11], *Artemia franciscana* [49] and *P. orientalis* [55]. A similar decrease in growth rate in *P. indicus* on exposure to sublethal lead has been reported by Chinni et al. [56].

5. Conclusion

Our results suggest that *P. indicus* PL can serve as a much better sensitive indicator of copper pollution than the PL of any other marine shrimps examined to date. Moreover, accumulating

levels of copper in *P. indicus* PL grown in copper-exposed water clearly show its negative impact on physiology through the overall reduction in growth, as well as reduced oxygen consumption and metabolic rates. Our future work will focus on histopathological examination of the gills and conduct regular specific staining for metals to identify gill as well as hepatopancreas accumulation. Environmental hazards can thus be evaluated through physiological response and bioaccumulation studies using *P. indicus* PL as a biomonitor for metal pollution which is a threat to the multi-crore fishing industry. Our results prove that *P. indicus* is a good test organism for the assessment of water pollution. Therefore, these investigations provide a baseline data on tolerance levels of *P. indicus* PL in copper-polluted environments and therefore constitute a reference for future studies on the much unexplored shrimp species *P. indicus* PL from wild catch as an eco-toxicological testing scheme for hazard assessment.

Acknowledgements

This work was supported by a fellowship grant from Council for Scientific and Industrial Research (CSIR), New Delhi, India. The authors are grateful to the Department of Zoology, Andhra University for providing the research facilities.

References

[1] A.K. Ray, S.C. Tripathy, S. Patra, and V.V. Sarma, *Assessment of Godavari estuarine ecosystem through trace metal studies*, Environ. Int. 32 (2006), pp. 219–223.
[2] Y. Bambang, P. Thuet, M. Charmantier-Daures, J.P. Trilles, and G. Charmantier, *Effect of copper on survival and osmoregulation of various developmental stages of the shrimp* Penaeus japonicus *Bate (Crustacea: Decapoda)*, Aquat. Toxicol. 33 (1995), pp. 125–139.
[3] A. Mohapatra, T.R. Rautray, V. Vijayan, R.K. Mohanty, and S.K. Dey, *Trace elemental characterization of some food crustacean tissue samples by EDXRF technique*, Aquaculture 270 (2007), pp. 552–558.
[4] M.F. Chen, J.A. Apperson, G.D. Marty, and Y.W. Cheng, *Copper sulfate treatment decreases hatchery mortality of larval white seabass* Atractoscion nobilis, Aquaculture 254 (2006), pp. 102–114.
[5] R. Reddy, B.R. Pillai, and S. Adhikari, *Bioaccumulation of copper in postlarvae and juveniles of freshwater prawn* Macrobrachium rosenbergii *(de Man) exposed to sub-lethal levels of copper sulphate*, Aquaculture 252 (2006), pp. 356–360.
[6] D.G. Barceloux, *Copper*, J. Toxicol. Clin. Toxicol. 37 (1999), pp. 217–230.
[7] M. Hassall and J.M. Dangerfield, *Density-dependent process in the population dynamics of* Armadillidium vulgare *(Isopoda: Oniscidae)*, J. Anim. Ecol. 59 (1990), pp. 941–958.
[8] US Environmental Protection Agency, *Updated Aquatic Life Copper Criteria*, US Environmental Protection Agency, Washington, DC, 2007.
[9] N. Nesto, S. Romano, V. Moschino, M. Mauri, and L. Da Ros, *Bioaccumulation and biomarker responses of trace metals and micro-organic pollutants in mussels and fish from the Lagoon of Venice, Italy*, Mar. Poll. Bull. 55 (2007), pp. 469–484.
[10] K. Jung and G.P. Zauke, *Bioaccumulation of trace metals in the brown shrimp* Cragon cragon *(Linnaeus, 1758) from the German Wadden sea*, Aquat. Toxicol. 88 (2008), pp. 243–249.
[11] M.H.S. Santos, N.T. Da-Cunha, and A. Bianchini, *Effects of copper and zinc on growth, feeding and oxygen consumption of* Farfantepenaeus paulensis *post larvae*, J. Exp. Mar. Biol. 247 (2000), pp. 233–242.
[12] N. Li, Y. Zhao, and J. Yang, *Effects of water-borne copper on digestive and metabolic enzymes of the giant freshwater prawn*, Macrobrachium rosenbergii, Arch. Environ. Contam. Toxicol. 55 (2008), pp 86–93.
[13] K. Gopakumar and V.N. Pillai, *Prawns and Prawn Fisheries of India*, 5th ed., Vedams, New Delhi, India, 2002.
[14] K.W.H. Kwok, K.M.Y. Leung, V.W.W. Bao, and J.S. Lee, *Copper toxicity in the marine copepod* Tigropus japonicus*: low variability and high reproducibility of repeated acute and life-cycle tests*, Mar. Pollut. Bull. 57 (2008), pp. 632–636.
[15] R. Mendoza-Rodriguez, *Acute toxicity of copper* (Cu^{2+}) *on postlarvae of river prawn* Cryphiops caementarius *(Natantia, Palaemonidae)*, Rev. Peruana Biol. 14 (2007), pp. 53–54.
[16] W. Wang, H. Kong, and D. Wu, *Acute and chronic copper toxicity to a saltwater cladoceran* Moina monogolica daday, Arch. Environ. Contamin. Toxicol. 53 (2007), pp. 50–56.
[17] J.C. Marino-Balsa, E. Poza, E. Vazquez, and R. Deiras, *Comparative toxicity of dissolved metals of early larval stages of* Palaemon serratus,Maja squinado *and* Homarus gammarus, Arch. Environ. Contam. Toxicol. 39 (2000), pp. 345–351.
[18] I.C. Liao and J.J. Guo, *Studies on the tolerance of postlarvae of* Penaeus monodon, P. japonicus, P. semisulcatus, P. penicillatus, Metapenaeus ensis *and* Macrobrachium rosenbergii *to copper sulfate, potassium permanganate and malachite green*, Coast. Fish. Ser. 24 (1990), pp. 90–94.

[19] S. Chinni and P.R. Yallapragada, *Toxicity of copper, cadmium, zinc and lead to* Penaeus indicus *postlarvae: effects of individual metals*, J. Environ. Biol. 21 (2000), pp. 255–258.
[20] American Public Health Association (APHA), *Standard Methods for the Examination of Water and Wastewater*, 20th ed., American Water Works Association and Water Pollution Control Federation, Washington DC, 2000.
[21] R.E. Warner, *Bioassays for micro chemical environmental contaminant*. Bull. WHO 36 (1967), pp. 181–207.
[22] D.J. Finney, *Probit Analysis*, 3rd ed., Cambridge University Press, London, 1971.
[23] G.A. Zachariadis, J.A. Stratis, I. Kaniou, and G. Kalligas, *Critical comparison of wet and dry digestion procedures for trace metal analysis of meat and fish tissues*, Microchim. Acta 119 (1995), pp. 3–4.
[24] H. Villareal, P. Hinjosa, and J. Naranjo, *Effect of temperature and salinity on the oxygen consumption of laboratory produced* Penaeus vennamei, Comp. Biochem. Physiol. 108 (1994), pp. 331–336.
[25] G.C. Winberg, *Methods for the Estimation of Production of Aquatic Animals*, Academic Press, New York, 1971.
[26] G. Winberg, G.K. Patalas, J.C. Wright, A. Hillbrecht-Ilkowska, W.E. Cooper, and K.H. Mann, *Methods for calculating productivity*, in *Secondary Productivity in Freshwaters*, W.T. Edmondson and G.C. Winberg, eds., Blackwell Scientific, Oxford, 1971, pp. 296–316.
[27] G.W. Snedecor and W.G. Cochran, *Statistical Methods*, Iowa State University Press, Ames, IA, 1967, pp. 296–316.
[28] R. Lloyd, *Are short term toxicity tests a dead end?* Proc. Assoc. Adv. Sci. Aston (1977), pp. 102–109.
[29] M. Ahsanullah, D.S. Negilski, and M.C. Mobley, *Toxicity of zinc, cadmium and copper to the shrimp* Callianassa australiensis *(L): effects of individual metals*, Mar. Biol. (Berl.) 64 (1981), pp. 299–304.
[30] G.R.W. Denton and C. Burdon-Jones, *The influence of temperature and salinity upon the acute toxicity of heavy metals to the banana prawn (*Penaeus merguensis *de man)*, Chem. Ecol. 1 (1982), pp. 131–143.
[31] S. Das, S.K. Patro, and B.K. Sahu, *Variation of residual mercury in Penaeid prawns from Rushikulya estuary, east coast of India*, Ind. J. Mar. Sci. 30 (2002), pp. 33–37.
[32] D.Nugegoda and P.S. Rainbow, *Salinity, osmolality and zinc uptake in* Palaemon elegans *(Crustacea:Decapoda)*, Mar. Ecol. Prog. Ser. 55 (1989), pp. 149–157.
[33] H.R. Daly, B.T. Hart, and I.C. Campbell, *Copper toxicity to* Paratya australiensis, *IV. Relation with ecdysis*, Environ. Toxicol. Chem. 11 (1992), pp. 881–883.
[34] A.P.M. Lockwood and C.B.E. Inman, *Changes in the apparent permeability to water at moult in the amphipod* Gammarus duebeni *and the isopod,* Idotea linearis, Comp. Biochem. Physiol. 44A (1973), pp. 943–952.
[35] D. Satyanarayana, I.M. Rao, and B.R. Prasada Reddy, *Chemical oceanography of harbour and coastal environment of Visakhapatnam (Bay of Bengal) part I. Trace metals in water and particulate water*, Ind. J. Mar. Sci. 14 (1985), pp. 139–146.
[36] V.V. Sarma, S.J.D. Varaprasad, G.V.M. Gupta, and V. Sudhakar, *Petroleum hydrocarbons and trace metals in Visakhapatnam harbour and Kakinada bay, east coast of India*, Ind. J. Mar. Sci. 25 (1996), pp. 148–150.
[37] R.E. Menzer and J.O. Nelson, *Water and soil pollutants*, in *Toxicology: The Basic Science of Poisons*, 2nd ed., J. Doull, C.D. Klaassen, and M.O. Amdur, eds., Macmillan, New York, 1980, p. 636.
[38] M.G. Frias-Espericueta, D. Voltolina, and J.I. Osuna-Lopez, *Acute toxicity of cadmium, mercury and lead to white leg shrimp (*Litonepaeus vannamei*) postlarvae*, Bull. Environ. Contam. Toxicol. 67 (2001), pp. 580–586.
[39] K. Liu, Y. Wu, H. Zhao, L. Hou, and F. Sun, *Accumulation and toxicity of copper in prawn* Penaeus orientalis, Oceanol. Limnol. Sin. Haiyang yu Hyzhaw 19 (1988), pp. 133–139.
[40] S.L. White and P.S. Rainbow, *Regulation and accumulation of copper, zinc and copper by the shrimp* Palaemon elegans, Mar. Ecol. Prog. 8 (1982), pp. 95–101.
[41] G. Vogt and E.T. Quinitio, *Accumulation and excretion of metal granules in the prawn*, Penaeus monodon, *exposed to water borne copper, lead, iron and calcium*, Aquat. Toxicol. 28 (1994), pp. 223–241.
[42] J.P. Wu and H.C. Chen, *Effects of cadmium and zinc on oxygen consumption, ammonium excretion and osmoregulation of white shrimp (*Litopenaeus vannamei), Chemosphere 57 (2004), pp. 1591–1598.
[43] J.I. Spicer and R.E. Weber, *Respiratory impairment of crustaceans and molluscs due to exposure of heavy metals*, Comp. Biochem. Physiol. 100 (1991), pp. 339–342.
[44] F. Paez-Osuna and M.L. Tron, *Concentration and distribution of heavy metals in tissues of wild and farmed shrimp,* Penaeus vannamei *from the northwest coast of Mexico*, Environ. Int. 22 (1996), pp. 443–450.
[45] A. Soegianto, M. Charmanitier-Daures, J.P. Trilles, and G. Charmaniter, *Impact of copper on the structure of gills and epipodites of the shrimp* Penaeus japonicus, J. Crust. Biol. 19 (1999), pp. 209–223.
[46] M.G. Frias-Espericueta, R. Castro-Longoria, G.J. Barron-Gallardo, J.I. Osuna-Lopez, S.M. Abad-Rosales, F. Paez-Osuna, and D. Voltolina, *Histological changes and survival of* Litopenaeus vannamei *juveniles with different copper concentrations*, Aquaculture 278 (2008), pp. 97–100.
[47] L. Nonnotte, F. Boitel, and J.P. Truchot, *Waterborne copper causes gill damage and haemolymph hypoxia in the shore crab* Carcinus maenus, Can. J. Zool. Rev. 71 (1993), pp. 1569–1576.
[48] J.P. Truchot and F. Boitel, In vitro *and* in vivo *effects of copper on haemocyanin–O_2 binding in the shore crab*, Carcinus maenus, Comp. Biochem. Physiol., C Comp. Pharmacol. 103 (1992), pp. 339–343.
[49] J.I. Spicer, *Effect of water borne copper on respiratory and cardiac function during the early ortogeny of the brine shrimp,* Artemia franciscana *Kellogg 1908 (Branchiopoda: Anostraca)*, J. Comp. Physiol., B 165 (1995), pp. 490–495.
[50] M.E. Webb, *Interaction of cadmium with cellular components*, in *The Chemistry, Biochemistry and Biology of Cadmium*, M. Webb, ed., Elsevier, Amsterdam, 1979, pp. 285–294.
[51] G. Lutosławska and A. Fornal-Urban, *Copper and the regulation of lipid and carbohydrate metabolism, cardiovascular system function and physical performance*, Med. Sport. 13 (2009), pp 138–145.

[52] S.Valarmathi and J. Azariah, *Effect of copper chloride on the enzyme activities of the crab* Sesarma quadratum *(Fabricius)*, Turk. J. Zool. 27 (2003), pp. 253–256.
[53] X. Li, X. Hou, Q. Mao, Y. Zhao, Y. Cheng, and Q.Wang, *Toxic effects of copper on antioxidative and metabolic enzymes of the marine Gastropod*, Onchidium struma, Arch. Environ. Contam. Toxicol. 56 (2009), pp. 776–784.
[54] I. Liao and C. Hsieh, *Toxicity of three heavy metals to* Macrobrachium rosenbergii, in R. Hirano and I. Hanyu, eds., *Proceedings of the Second Asian Fisheries Forum*, Asian Fisheries Society, Manila, Philippines, 1990, pp. 923–926.
[55] F. Zhao, C. Xia, J. Zhou and F. Li, *Effect of copper and copper–methionine chelate compound on growth of* Penaeus orientalis, Shandong Fish Qilu Yuye. 2 (1993), pp. 32–35.
[56] S. Chinni, R.N. Khan, and P.R. Yallapragada, *Oxygen consumption, ammonia-N excretion and metal accumulation in* Penaeus indicus *postlarvae exposed to lead*, Bull. Environ. Contam. Toxicol. 64 (2000), pp. 144–151.