Pak. j. sci. ind. res., vol.39, nos. 1-4, January-April 1996

ACUTE TOXICITY OF COPPER, CADMIUM AND COPPER - CADMIUM MIXTURE TO THE LARVAE OF THE SHRIMP *PENAEUS MONODON*

A. B. MUNSHI, S. Y. QUAN* AND S. J. LI*

PSIR Laboratories Complex, Off University Road,Karachi-75280, Pakistan

(Received July 25, 1996)

Lethal concentration (LC) of copper for *Penaeus monodon* at various stages of its life cycle was observed. It was 300 µg/L for nauplii while 100% mortality was observed for zoea I, Zoea II, Zoea III and Mysist I. 95.3% and 30.0% mortality was for Mysis III and postlarval stages respectively. The lethal concentration (LC) of cadmium for nauplii, zoea I, ZII, ZIII, Mysis I, Mysis III and postlarvae were between 50-250 µg/L. In mixture these values were found to be between 110-200µg/L.

Key words: *Penaeus monodon,* Heavy metals, Acute toxicity.

Introduction

Copper is an essential trace element in all living organisms but it may become toxic at increased concentrations while cadmium has definite poisoning characteristic. Acute metal toxicities for Cu and Cd determined for zoea and megalopa of brachyuran crabs and other organisms had been reported earlier [1-2]. Theoretically mortality rat is an important population size controlling parameter of a species in nature [3]. An organism, at various stages of its life cycle, may have different susceptibility to pollution including different types of metals.

Penaeus monodon having economic importance is mostly cultured in China. Therefore, it was considered essential to determine effect of metals on this species. March-April time was found suitable for its culture considering all environmental factors (temperature, salinity). During present study the toxic effects of copper, cadmium and Cu: Cd mixture on the nauplii, zoea, mysis and postlarvae of this species have been evaluated.

Materials and Methods

All larval stages from nauplii to mysis III and postlarvae were collected from Zhangpu culture plant and were brought to the Xiamen University, where they were cultured up to juvenile. At laboratory all samples were acclimatized for one hour prior to exposure to metal concentrations. Specimens (40 individuals for nauplii, zoea, mysis and 20 postlarvae) were placed in 600 ml beaker containing 300-500ml of sand filtered (4.5 um) seawater. Into each beaker was added one of six concentrations of metals. A static culture system having 30 C and 26% salinity was used for the experiment. Metal solutions were prepared from stock (10,000 µg/L) of $CdCl_2$ and $CuSO_4$. H_2O initial concentration of 8.33 µg/L up to maximum concentration of 1000µg/L were prepared by a series of dilutions using deionized water. (Tables 1, 2 and 3).

Five replicates were made for each solution with filtered sea water, unexposed larvae were kept as control. Each experiment was continued till the next stage (nauplii 24-38h, zoeaI-III 24h, mysis I-III 30-36h and pL 24h). Metal solutions were renewed for every experiment, pH and salinity of the culture water were noted every day. Laboratory wares were washed before use with 5 mol/L HCl (AGR) then rinsed with deionized water. The series of experiments were started from an initial experiment with nauplii to assay the effects of Cu, Cd and Cu: Cd mixture then to ZI, II, III, MI-III and postlarvae gradually with slight variation. All larvae were fed the same diet as that given to the test specimens, i.e. algae for zoea i, ii, iii and 48h old *Atremia* nauplii for mysis and postlarvae.

Statistical analysis. Differences between life stages exposure for each metal were analyzed by Fisher's method [4]. Turkey's Honest Significant Difference HSD test and Chi-square test were used to determine differences between means. Mortality was calculated as the proportion of dead specimens to the total number of specimen minus total number of living specimen. In each experiment numerical result was considered significant if $P<0.5$.

Results and Discussion

Nauplii. Cadmium chloride (Cadmium) caused significant nauplii mortality rate at 200 µg/L which was significantly greater than that by copper 100% + SD vs 85.0% + SD respectively and did differ obviously from that at copper rate of nauplii and concentration of metals (Tables 1,2 and 3).

*Department of Oceanography & Institute of Subtropical Oceanography, Xiamen University, Xiamen 361005, P. R. China

Mortality of nauplii was higher than that of latter stages. At a concentration higher than of 500 µg/L 100% mortality of nauplii was observed within 24th after initial exposure.

II. Zoea. Mortality differed at different stages of Zoea I, ii and iii under the same concentration. At higher concentrations i.e. 200, 500 and 1000µg/L of cadmium the mortality rate of Zoea I, II and III was 100% that is different from those of copper (77.0% 75.0% and 72.8%) for 200 µg/L (Table 1,

TABLE. 1. MORTALITY OF *PENAEUS MONODON* LARVAE EXPOSED TO VARIOUS CONCENTRATIONS OF COPPER. DATA ARE MEAN PERCENTAGE ±SD.

Caoncentrations		Mortality (%)						
S.No.	µg/L	Nauplii	Zoea I	Zoea II	Zoea III	Mysis I	Mysis III	PL
1.	8.33	50±21.81	-	-	-	-	-	-
2.	10.00	-	4.0±2.24	3.84±8.45	5.00±19.27	3.5±2.80	2.2±27.57	-
3.	16.66	15.0±11.8	-	-	-	-	-	-
4.	20.00	-	-	14.0±8.74	14.0±10.27	-	-	-
5.	25.00	17.5±9.31	14.1±2.29	-	-	-	-	-
6.	50.00	22.5±4.31	16.6±9.89	-	15.2±9.07	7.50±12.8	-	2.00±14.1
7.	83.00	-	-	18.8±7.29	-	-	-	-
8.	100	32.5±2.56	19.8±6.69	-	8.0±16.27	10.0±10.3	6.5±23.90	10.0±6.18
9.	133	45.0±6.78	-	36.8±10.71	-	-	-	-
10.	166	49.0±2.56	41.3±7.92	39.86±6.71	-	-	-	-
11.	200	85.0±3.78	77.0±2.47	75.0±9.50	72.8±9.34	65.3±6.20	8.82±20.68	15.0±1.18
12.	300	100.0±0.0	100±0.00	93.4±5.78	92.30±3.56	87.4±4.70	40.0±10.23	20.0±3.82
13.	350	-	-	100.0±0.00	100±00.00	100±00.00	95.3±2.00	30.0±2.56
14.	400	-	-	-	-	-	100.3±0.0	78.2±9.30
15.	500	-	-	-	-	-	-	100.0±0.0
16.	1000	-	-	-	-	-	-	-
17.	Control	0.2±6.06	1.0±5.34	0.3±25.79	4.0±68.35	0.3±25.47	1.3±23.47	0.0±53.77

*=not applied.

TABLE. 2. MORTALITY OF *PENAEUS MONODON* LARVAE EXPOSED TO VARIOUS CONCENTRATIONS OF CADMIUM. DATA ARE MEAN PERCENTAGE ±SD.

Caoncentrations		Mortality (%)						
S.No.	µg/L	Nauplii	Zoea I	Zoea II	Zoea III	Mysis I	Mysis III	PL
1.	8.33	1.0±25.5	-*	-	-	-	-	-
2.	10.00	-	4.9±23.63	4.71±22.68	3.90±22.71	7.51±23.36	4.0±3.75	-
3.	16.66	7.0±19.5	-	-	-	-	-	-
4.	20.00	-	16.3±21.53	15.9±11.48	15.0±11.61	15.0±15.86	-	-
5.	25.00	9.9±16.69	-	-	-	-	-	-
6.	50.00	-	17.9±18.63	17.0±10.38	16.9±9.70	25.0±13.86	-	3.00±16.16
7.	83.00	24.9±1.64	-	-	-	-	-	-
8.	100	50.5±2.56	21.0±7.53	19.9±7.48	19.8±6.81	-	17.9±9.85	10.0±21.18
9.	133	40.0±13.4	-	-	-	-	-	-
10.	166	98.0±7.46	40.8±12.27	-	-	-	-	-
11.	200	100±0.00	-	38.8±11.42	36.8±10.19	37.5±10.96	21.0±6.75	17.0±2.16
12.	300	100±0.0	96.86±8.27	94.46±7.04	91.96±5.29	40.0±9.14	40.8±13.05	29.0±9.84
13.	350	-	-	-	-	90.05±19.14	60.2±4.56	-
14.	400	-	-	-	-	95.6±8.69	90.6±10.25	
15.	500	100±0.00	-	-	-	100±0.00	100.±0.00	55.0±5.84
16.	1000	-	-	-	-	-	100±0.00	89.9±5.14
17.	Control	4.9±21.5	1.9±26.54	0.99±24.39	1.99±24.62	0.99±29.86	0.99±3.90	0.99±18.17

*=no change observd.

TABLE. 3. MORTALITY OF *PENAEUS MONODON* LARVAE EXPOSED TO VARIOUS CONCENTRATION OF Cu: Cd MIXTURE DATA ARE MEAN PERCENTAGE ± SD.

S.No.	Caoncentrations µg/L	Mortality (%) Nauplii	Zoea I	Zoea II	Zoea III	Mysis I	Mysis III	PL
1.	8.33	5.63±21.20	-*	-	-	-	-	-
2.	10.00	-	6.88±21.83	4.00±27.43	3.41±22.0	-	3.0±29.66	-
3.	16.66	14.9±1.93	-	-	1.49±10.5	-	-	-
4.	20.00	-	15.3±12.41	14.9±16.53	14.1±11.3	1.18±21.35	-	-
5.	25.00	17.0±9.83	-	-	-	-	-	-
6.	50.00	-	17.0±10.71	-	-	-	-	-
7.	83.00	21.9±4.93	-	-	-	-	-	-
8.	100	-	19.0±8.71	19.0±12.43	17.2±8.23	7.5±14.98	16.8±15.86	10.5±21.18
9.	133	31.56±8.50	-	-	-	-	-	-
10.	166	-	-	-	-	-	-	-
11.	200	-	40.1±12.39	57.1±25.66	36.1±10.6	12.5±9.98	19.90±12.76	15.9±15.78
12.	300	-	94.86±7.09	93.4±6.97	91.3±6.87	25.0±2.51	36.80±7.14	24.8±6.88
13.	350	-	-	-	-	88.60±5.51	69.03±3.63	-
14.	400	-	-	-	-	-	79.81±4.75	51.0±9.32
15.	500	100.0±00.00	-	-	-	100±00.00	100.0±00.00	87.0±5.32
16.	1000	-	-	-	-	-	100.0±00.00	-
17.	Control	1.9±24.93	0.9±26.81	0.2±21.23	1.02±8.20	0.8±21.18	0.3±32.8	0.0±31.68

*= not change observed.

2 and 3). In mixture metals showed antagonistic effect. Therefore, mortality was found either lower than that in copper and cadmium or equivalent to that in copper (Table 3).

The concentration of 10µg/L copper and mixture showed no toxic effect and considerable mortality occurred from concentration of 16 µg/L. Larval mortality at stages of Zoea II and III was not the same as that of Zoea I. In ZII it was found to be 75.0% and ZIII 72.80% at concentration of 200µg/L of copper. 16% of ZI observed at concentration of 50 µg/L for copper but for Cd and mixture they were 17.90% and 17.0% respectively.

Mysis. In the experiment 40 mysis Near present in 500 ml filtered sea water, treated with different concentrations of metals, (Table 1-3). At the concentration of the 500µg/L mortality rate was 100% for cadmium, copper and mixture, that means susceptible for metal effluent as compared to that zoea and nauplii.

Postlarvae. Exposure of postlarvae to three metal solutions (Cu, Cd and Cu:Cd mixture) caused signific ant mortality under higher concentration. However, larval mortality caused by copper and cadmium was significantly lower than nauplii for that were exposed to these metals.

Like *Penaeus penicillatus* dissolved copper and cadmium were found to be more toxic for nauplii than for postlarvae [5]. It was perhaps due to smaller size of nauplii [6]. These results were found to be similar to the findings reported for echinoderm [7].

The acute toxicity for 24-48h are only short-term experiments which can provide only basic information about effect of metals on shrimp larvae. The exact mechanism behind the mortality can not be discussed without research up to the molecular level. However, it is well documented that marine organism can accumulate metals like cadmium in high concentration [8], during exposure experiment. All nauplii were dead in 1000 µg/L of Cu and Cd were dead after 24hr. all zoea in 500µg/L of Cd and mysis after 36hr. at the same concentration of copper. Postlarvae (PL5) were dead after 10hr. in 250µg/L. Chi square analysis showed the probit of mortality had a positive linear regression with metals concentration and that all values were found to be satisfactory. As the larval shrimps developed, they showed a progressive increase tolerance to Cu and Cd. Therefore, for mysis and postlarvae, the data revealed a decrease in LC50 value as compared to nauplii and zoea with increasing duration of exposure. Lang *et al.*[3] reported that the 24hr. LC50 value for stage II cypris of *Balanus improvisusme* were 88 µg/LC at 15 salinity and 200 µg/L at 30 salinity. Connor [1] reported LC50 48hr.and 96hr.for decapod larvae *(Homarus gammarus)* as 100-300 µg/L of Cu and *Paragrapus quadridentatus* as 170 µgL of Cu in 48hr. Lethal concentration of Cu and Cd to yellow crab *Cancer anthonyi* has been reported as 100µg/L and 10µg/L respectively at which 100% mortality was observed [2]. Additional information on the influence of Cu and Cd on another larval crustacean and copepoda have been

provided by Sullivan *et al.* [9] and Moraitou Aposto-lopoulou and Verriopoulos [10]. The LC value obtained during present studies are little lower than reported values, which have been described above.

Since *Penaeus monodon* larvae require fewer than 4 days to develop to each successive stage in the hatchery [11], we could not obtained the 96hr. LC for the nauplii, zoea and mysis stages but for the postlarvae it was found to be 10 and 20 µg/L for Cu and Cd respectively. For nauplii, zoea and mysis a 'safe level' can be provided by multiplying an application factor and 96h LC50 value for the stage in which the species is the most sensitive *et al.* [12]. In conclusion LC50 for 24hr. value indicated that nauplii had the lowest tolerance to Cu and cadmium among all the four larval stages Cu is found to be more toxic as compared to cadmium. In mixture effect of both metals may be antagonistic.

Acknowledgement. Authors thank to Mr. Chen Jinidi Vice Professor at Oceanography Dept. for providing specimen for culture plant at Xiamen University.

References

1. P. M. Connor, Mar. Pollut. Bull., **3,** 190 (1972).
2. J. M. Macdonald, J. D. Shields and R. K. Zimmer-Faust, Mar. Biol., **98,** 201 (1988).
3. W. H. Long, Mar. Biol., **58,** 139 (1980).
4. P. R. Sokal and F. J. Rohlf, *Biometry,* (W. H. Freeman & Co. San Francisco, 1981), 2nd ed., pp.1-135.
5. A. B. Munshi, Y. S. Quan and S. J. Li., Chin. Ocean. Limnol., **2,** 23 (1996).
6. M. R. Reeve, M. A. Walter, K. Darcy, T. Ikeda, Bull. Mar. Sci., **27,** 105 (1977).
7. H. A. Lee and Xu, Mar. Pollut. Bull., **15,** 18 (1984).
8. S. Ray, Experimentia, **40,** 14 (1984).
9. B. K. Sullivan, E. Buskey, D. C. Miller and P. J. Ritacco, P. J., Mar. Biol., **77,** 299 (1983).
10. M. Moraitou-Apostolopoulou and G. Verriopoulos, Mar. Pollut. Bull., **10,** 88 (1979).
11. I. C. Liao, Tungkang Marine Laboratory, Tungkang, Taiwan **55,** (1977).
12. J. B. Sprague, Water Res., **5,** 245 (1971).