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

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Lethal concentration (LC) of copper for *Penaeus monodon* at various stages of its life cycle was observed. It was 300 μ g/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 μ g/L. In mixture these values were found to be between 110-200 μ g/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.

*Department of Oceanography & Institute of Subtropical Oceanography, Xiamen University, Xiamen 361005, P. R. China Metal solutions were prepared from stock (10,000 μ g/L) of CdCl₂ and CuSO₄. H₂O initial concentration of 8.33 μ g/L up to maximum concentration of 1000 μ g/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 Chisquare 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 μ g/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).

Mortality of nauplii was higher than that of latter stages. At a concentration higher than of 500 μ g/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 $1000\mu g/L$ 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 $\mu g/L$ (Table 1,

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

PERCENTAGE ±	SD.
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Caoncentrations					Mortality (%)			
S.No.	µg/L	Nauplii	Zoea I	Zoea II	Zoea III	Mysis I	Mysis III	PL
1.	8.33	50±21.81	-		-	EX	REO.CT 14	20 - 2
2.	10.00		4.0±2.24	3.84±8.45	5.00±19.27	3.5±2.80	2.2±27.57	6
3.	16.66	15.0±11.8				- R	<u>10. 21.954</u>	18.8 - S
4.	20.00	128.01 82	23 - 7.5x M	14.0±8.74	14.0±10.27	8-5.61	-	
5.	25.00	17.5±9.31	14.1±2.29	-	1	R.	8402.11	EF I
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	LADRIG M	P#2.14- 0.0	18.8±7.29	8541 M	CHALON.	-	- 200
8.	100	32.5±2.56	19.8±6.69	ALL 19. 19	8.0±16.27	10.0±10.3	6.5±23.90	10.0±6.18
9.	133	45.0±6.78	Landara in t	36.8±10.71	-	1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 -	1.1	
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	(*):()- 34	12:48 0- 03	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	A	Self-un-tion rea	in all -	A Prest Three of	ieinoge-ischeit	idis datem visit	100.0±0.0
16.	1000	and a stand sta	and bring the month	arter stering	hos minute of its	di medi-aveni t	attinte-of row	vilon-e //
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	_*	warete contractor	HOT FORM	they were 17.9	sugarb-lien. S	and her negret	
2.	10.00	ate and the second	4.9±23.63	4.71±22.68	3.90±22.71	7.51±23.36	4.0±3.75	A CONTRACTOR	
3.	16.66	7.0±19.5	an anti-	in culture in	OC noise	and aryon be	taspiti-que al	in all all all he	
4.	20.00	Anna - Anna -	16.3±21.53	15.9±11.48	15.0±11.61	15.0±15.86	a dalardi . mire	e societa-officia	
5.	25.00	9.9±16.69	staats of an index	wind only eA	com Passail	ant had-theting	and all Artheorem	LadeT_,dated	
6.	50.00	Annual To be to	17.9±18.63	17.0±10.38	16.9±9.70	25.0±13.86	adulte 1-1 6700	3.00±16.16	
7.	83.00	24.9±1.64	nin-um main mi	and the	spess tails of his	lugar - compan	ie for - endefi	linguages serve	
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	and 157 To me tall	f main no	alles Bosta of	ent of a - instance	Breathing of p	active discovery	
10.	166	98.0±7.46	40.8±12.27	There are	Autor incom	ningte bie-tra s fra	ettion (-Tha Dib	an 10 20 m	
11.	200	100±0.00	in Burn and her	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	Distant	hat off-to Bost	COV LVT OR	-	90.05±19.14	60.2±4.56	and will filmer	
14.	400		Dest with a d	And Inter The	animation from	95.6±8.69	90.6±10.25		
15.	500	100±0.00	and the Transit	and the law	carrier an	100±0.00	100.±0.00	55.0±5.84	
16.	1000	- Antrat dealers	the selection of the	Rent/T-brie	wood?- (b) at	tion to this with	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.

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and a state of				PERCENTAGE	± SD.			
Caoncentrations			Mortality (%)					nas updaren ya
S.No.	µg/L	Nauplii	Zoea I	Zoea II	Zoea III	Mysis I	Mysis III	PL
1. 10	8.33	5.63±21.20	007.12* 200	i territa V pri com	and the particular	notine a state of	o mansoli .	a.mat
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	(<u>1</u>) 1996	aloha	1.49±10.5		And	-
4.	20.00	they I a	15.3±12.41	14.9±16.53	14.1±11.3	1.18±21.35	iqueri _ d	- 00 S
5.	25.00	17.0±9.83	-	-	-	13	17497 - 1	5.8 - S.
6.	50.00	Contro 68	17.0±10.71	AS -5.00+19.	2-28-5 - 1	- 1.0i.2.2	00	- S.
7.	83.00	21.9 ± 4.93				- 8.1	1:46).21	24 - E
8.	100	14 - 14 - 14 - 14 - 14 - 14 - 14 - 14 -	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	-	-	- (4	Sel 14- 11	10-11- 00	. K 251
10.	166	85	r jaz 17- 18	1941 El -	- ()	(enabl- 11	3022.5±4.	6 30
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	1000 10 100	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	-		- 40	17.2-0C-	88.60±5.51	69.03±3.63	ERT 1989
14.	400		S	- 15	8	ECAR IN- M	79.81±4.75	51.0±9.32
15.	500	100.0±00.00	ALE TO	1 e-2 ft - 12	Aug 75.046	100 ± 00.00	100.0 ± 00.00	87.0±5.32
16.	1000	000005 00	N#4.797	1 and 10- 10	2 98 des	Diffection - (1)	100.0±00.00	000 - 300
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

TABLE. 3. MORTALITY OF PENAEUS MONODON LARVAE EXPOSED TO VARIOUS CONCENTRATION OF CU: Cd MIXTURE DATA ARE MEAN

*= 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 $10\mu g/L$ copper and mixture showed no toxic effect and considerable mortality occurred from concentration of 16 $\mu g/L$. 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 200 $\mu g/L$ of copper. 16% of ZI observed at concentration of 50 $\mu g/L$ 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 $500\mu g/L$ 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

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 μ g/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.

In the present study, otherent transmittations were manufactured by using four different stabilizers and by varying their disers. Powdet traffic offers more convenience than fluid mills because at directly gives standardized mills [4], therefore it was decided to use strunget wilk powder (SMP).

ing direct scatte injection Ultra Flight Temperature (UHT technique. UHT treated products are storile and casily digestable, besides having long-life, even if not refrigerated Blanc et al. [7] reported that dread UHT milk was preferred in all organoloptic assessments to the indirect UHT milk and the was generally talkininguishable in flavour from panterter and generally talkininguishable in flavour from panterter atter storage in flavour determined

In the present study, an autompt has brees made to study whether frust milk drinks have acceptinglity or not because the general observation is that mile studies are popular beterages of the summer season in Pateriam and that the people of Pateriam relieb such boverages. If this observation is conmined then one of the 25 dairy plants of Pakistan could be persuaded to take instantive in markeong the frust-tails here orages.

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The study (was divised into two phases 1a the first phase) to formulations (Table 1) were manufactured by varying levels of apple puts, stabilizers and their dozes. This exercise was conducted in the laboratories of the Department of Tored Tachauloc. University of Agriculture, Fulsalabad, in the same

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