Effects of pH on the Uptake Pattern of Metals Copper, Cadmium and Cu: Cd Mixture in Larvae of the Penaeid Shrimp

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(Received 20th September 2006, revised 3rd March 2007)

Summary: The effect of pH on the uptake of Cu and Cd along with their mixture on larvae of *Penaeus penicillatus* and *Penaeus monodon* was studied at the pH range of 5.30 to 9.00. The uptake of Cu and Cd increased with decrease of pH, as pH increased from 5.30 upto 8.34 the accumulated concentration of Cu and Cd decreased. The lethal pH found in this study was 2.5 and 9 at which all larvae died within one hour even in control sample. The lethal pH at which minimum survival rate as well as highest accumulation as observed was 5.30.There was a marked effect of pH on the uptake process of Cu and Cd, which was found to be directly proportional to the exposed concentration in media. The minimum acceptable pH was calculated at which survival rate was reduced up to 50 % over each successive developmental stage of larvae. pH was therefore found to be an effective factor for controlling survival of shrimp larvae during shrimp rearing at culture plants.

Introduction

Although seawater is well buffered around pH 8, its chemical composition may widely alter due to pollution or any other change in marine environment. Toxicity of Cu and Cd to marine organisms is believed to be controlled by the free metal ion concentration that is highly dependent upon pH [1]. The uptake of Cd also depends upon its concentration in the seawater at different pH. Chan [2] found a linear relationship between the Cd concentration in the medium and uptake of cadmium by green mussel. Bioaccumulation studies have demonstrated that the uptake of copper is directly related to the cupric ion concentration that is determined by the total dissolved copper concen-tration and the pH of the media [3]. Stephenson and Mackie [4], found a negative correlation between pH and Cd uptake. pH might influence the uptake process in two antagonistic ways *i.e.* by altering the chemical speciation of copper in the solution and by rotation of copper binding sites, which may be present at the cell surface [5]. Additionally, changes in pH can alter the physiological condition of an organism and thus indirectly affect the uptake process [6]. Crustaceans can however, regulate internal pH to some extent through Cl^-/ HCO_3^- and NaOH exchanges via the gills [7]. Tsai [8] considered pH values below 4.8 or above 10.6 lethal to pinweeds with an optimum range. Apud [9] observed that pH of 5 or below in ponds caused mortality of penaeid larvae *i.e.* lethal values of low pH (96-h LC_{50}). Thus the above mentioned studies showed that the uptake patterns of copper and cadmium differ vastly in different aquatic organisms under a specific ecological environmental factor like pH. However, experimental work on the effect of copper on cadmium uptake (or vice versa) is very scarce especially for crustaceans [10]. It may therefore be anticipated that pH should have profound and complex effects on the uptake of copper and cadmium on penaeid shrimp. In the present study effect of pH on the uptake of Cu, Cd and their 1: 1 mixture has been investigated using various larval stages of penaeid shrimp *i.e Penaeus monodon* and *Penaeus penicillatus*. Due to similarity in the physiological properties of both these metals, it has been suggested, that these are biologically antagonistic. Hence a mixture of both has been applied in this study. The aim of this study was to estimate lethal and minimum acceptable pH values, using acidified seawater for those species that dominate the worldwide cultured shrimp production [11, 12].

Results and Discussion

The results of the uptake of Cu and Cd by *Penaeus monodon* and *Penaeus penicillatus* are shown in (Fig.1) The uptake of Cu and Cd at pH = 8.34 increased gradually along with developmental

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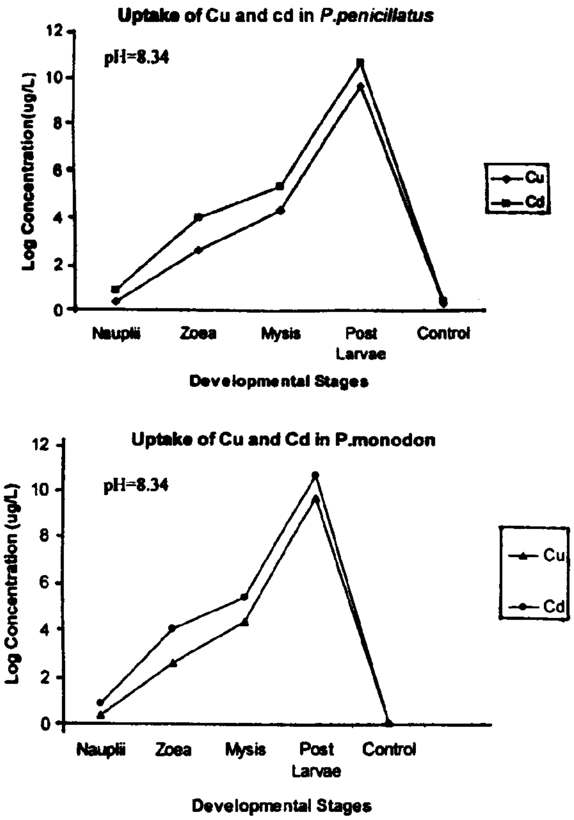

Fig. 1: Uptake of Copper and Cadmium various stages when exposed to concentration of 200 mg/ L values are means of 3 replicates with ± SD.

stage of larvae. In *P. monodon*, more copper accumulated than that in the *P. penicillatus* at concentration of 200 μg/ L in the media and uptake of Cd was also lower as compared to copper (Fig. 1). Effect of pH on uptake of copper and cadmium at different concentrations for each specimen was noted at pH = 8.34 (Figs. 2 and 3). It is the optimum pH at which lowest mortality was observed during all experiments. As pH increased, the survival rate also increased upto pH = 8.34, however it decreased afterwards. No alive nauplii, zoea, mysis and post larvae were observed at pH = 9.0. Nauplii were found to have lower survival rate at pH = 8.34 as compared to mysis and postlarvae. Two percent post larvae died at pH = 6-7.4, however all larvae died within 3h at pH = 2.5. The 48 h - LC_{50} value was 6.5 (95 % confidence limit) and the effect of pH on survival was not significant ($p > 0.05$) for *P. monodon* and *P. penicillatus*. The comparison of results at different pH and uptake of Cu and Cd, revealed, that at similar

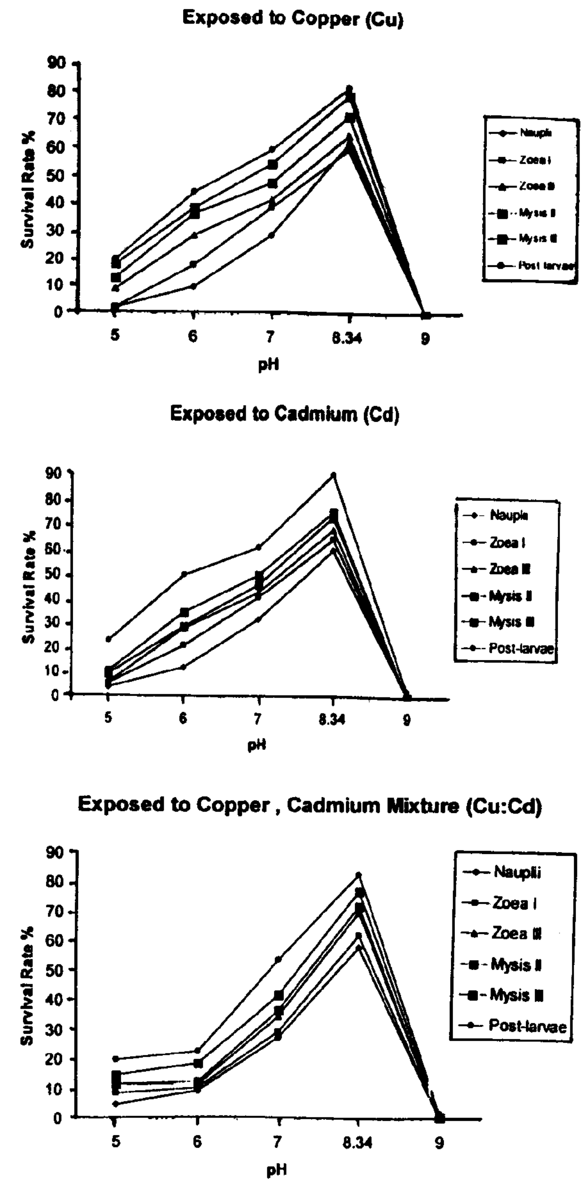

Fig. 2: Effect of pH on survival of *Penaeus monodon* over each development stage exposed to Cu, Cd and their mixture at concentration of 100 μg/ L. Results are slopes with 95 % confidence limits of least square fite, survival rate increased linearity with pH up to 8.34 then at pH = 9.0 survival rate decreased up to zero.

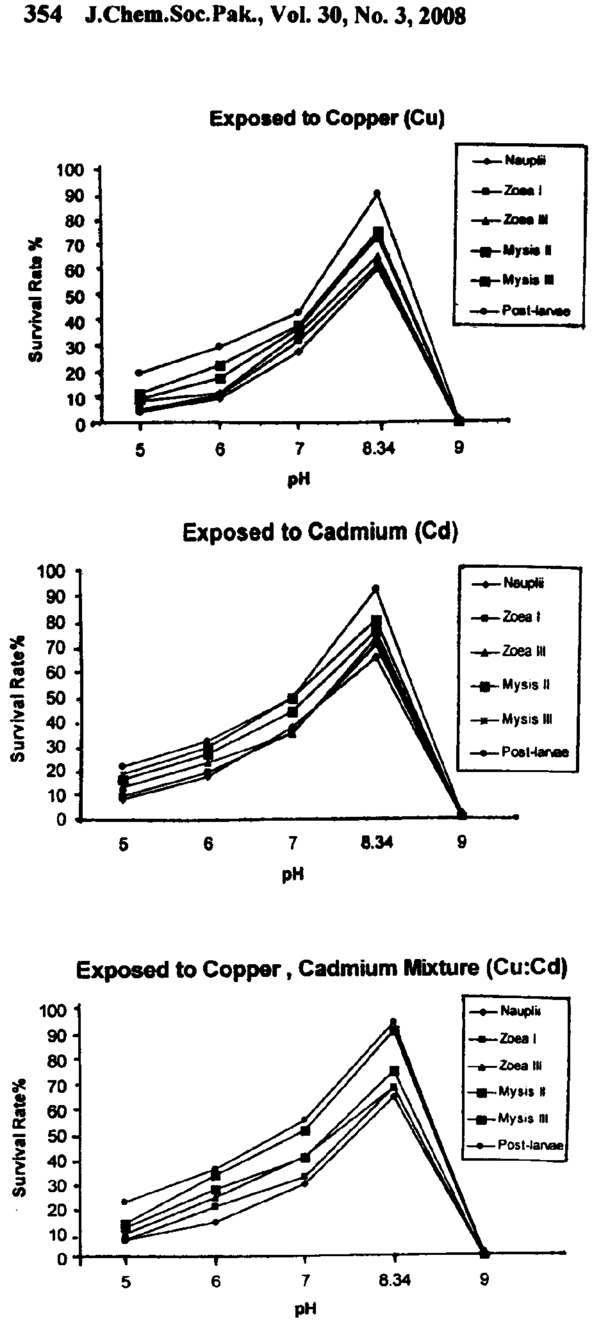

Fig. 3 Effect of the pH on survival of *Penaeus penicillatus* each development stage exposed to Cu, Cd and their mixture at concentration of 100 μg/L. Results are solpes with 95 % confidence limits of least square fits. Survival rate increased linearity with pH up to 8.34 then at pH = 9.0 survival are become decreased up to zero.

exposed concentration, lower amount of metals was determined in nauplii (Tables 1 and 2). The calculated regression slopes of metal content against each larval stage suggested that amount of Cd and Cu was affected by developmental stage of larvae.

Table-1: Penaeus monodon-Experimental groups of larvae concentrations of exposed metals and uptake of metals determined by AAS.

S. No.	Dry weight (g)	L/stage	Metal	Exposed Conc. Ug/L	Uptake of metals determined By ASS (ug/g)		
					pH	Cu	Cd
1.	0.0021	N	Cu	10	8.34	0.13151	0.1000
2	0.0031	N	Cd	100	7.00	0.3469	0.4365
3.	0.0103	ZI	Cd	200	8.34	2.3100	8.2310
4.	0.0137	ZI	Mix	50	5.00	3.102	2.9800
5.	0.0132	ZII	Cu	10	6.00	0.3666	0.4545
6.	0.0060	ZII	Cu	400	7.00	28.000	0.3330
7.	0.0330	ZII	Cu	200	8.34	19.878	1.0909
8.	0.1010	ZIII	Cd	10	7.25	3.4653	0.6336
9.	0.0162	ZIII	Cu	200	8.34	19.753	4.1358
10.	0.1020	ZIII	Cu	10	8.34	0.5392	0.2156
11.	0.0249	ZIII	Cu	100	8.34	1.5261	0.4819
12.	0.0140	ZIII	Mix	100	8.34	2.3570	0.8571
13.	0.0210	MI	Cd	100	7.00	13.8095	5.1904
14.	0.0189	MI	Mix	200	8.34	6.6666	0.5820
15.	0.0200	MI	Cu	200	8.34	6.6500	4.5500
16.	0.0260	MI	Cu	10	5.00	1.8920	1.0071
17.	0.0137	MI	Cd	400	8.34	0.2510	10.6714
18.	0.0310	MI	Cu	400	8.34	15.9624	1.2001
19.	0.0320	MI	Cd	200	7.00	2.4100	5.6702
20.	0.0289	MI	Mix	400	6.00	8.7412	7.8971
21.	0.3000	MIII	Cu	400	8.34	15.1674	0.1340
22.	0.0394	MIII	Cd	200	7.00	1.2001	6.2410
23.	0.0356	MIII	Mix	400	6.00	7.8210	8.0120
24.	0.1015	PL	Cd	100	8.34	1.2101	5.4236
25.	0.9830	PL	Cd	100	8.34	1.2410	5.0181
26.	0.0667	PL	Mix	200	5.00	4.2400	4.0010
27.	0.1200	PL	Cd	400	6.00	3.1031	12.1896
28.	0.1201	PL	Mix	10	6.00	1.2101	1.2013
29.	0.100	PL	Cd	50	5.00	1.0102	2.6780

A significant relation between pH and uptake of metal was observed. On exposure of mixture of both Cu and Cd, an increase in uptake of both metals was observed. Maximum uptake was observed at pH-5 during all the exposure experiments and minimum was observed at pH = 8.34. At pH = 9, all larvae died within 1 hour, after exposure to 100 μg/ L of Cu and Cd. The accumulation rate declined with increasing hydrogen ion concentration, but there were marked differences in accumulation during Cu, Cd and Cu: Cd exposure experiments, which may be explained by different mode of action of each metal during uptake process of metals.

Various studies have so far focused on the effect of pH, related to toxicity of copper and cadmium, on a variety of marine and mostly fresh water organisms. However, few have studied the effect of pH on the uptake or accumulation of copper

Table-2: Penaeus penicillatus - Experimental groups of larvae, concentrations of exposed metals and uptake of metals determined by AAS.

S. No.	Dry weight (g)	L/stage	Metal	Exposed Conc. ug/ L	pH	Uptake amount determined by AAS (ug/ g) Cu	Cd
1.	0.0025	N/ 50	Cd	100	7.00	0.3467	0.4365
2.	0.0040	N/ 50	Cd	10	6.00	0.0134	0.1300
3.	0.O130	Z1	Cu	100	7.12	9.2300	1.9230
4.	0.0120	Z1	Cu	400	7.50	19.333	2.9166
5.	0.0321	Z11	Cd	100	5.00	2.1010	7.8140
6.	0.0123	Z11	Mix	100	6.00	5.4340	6.0100
7.	0.0358	Z111	Mix	50	6.43	4.2458	1.4525
8.	0.0232	M1	Cu	100	6.00	7.3706	2.5431
9.	0.0179	M1	Cd	10	5.00	0.0134	0.1300
10.	0.0215	M1	Cu	400	7.00	19.467	3.0130
11.	0.0310	M1	Mix	100	6.00	6.2140	5.8920
12.	0.0242	M11	Cu	10	5.00	1.6780	1.0105
13.	0.0251	M11	Cd	100	6.00	1.2160	7.2910
14.	0.0200	M11	Mix	200	7.00	6.9867	1.5821
15.	0.0310	M111	Cu	10	5.00	0.8290	0.0140
16.	0.0156	M11	Cd	400	6.00	0.4501	8.4520
17.	0.1298	M11	Mix	200	7.00	5.2410	6.0018
18.	0.1001	PL	Cd	-	7.04	0.2997	0.1498
19.	0.0983	PL	Mix	10	7.00	1.0885	0.3867
20.	0.0567	PL	Cd	10	7.00	33.192	0.4585
21.	0.0314	PL	Cd	10	7.50	1.4285	1.8226
22.	0.8870	PL	Mix	100	8.34	4.2101	5.1012
23.	0.7100	PL	Cu	10	5.00	1.0056	0.1400
24.	0.1005	PL	Cd	100	5.00	0.1456	2.4301
25.	0.0435	PL	Cu	100	6.00	0.1012	2.8710
26.	0.0978	PL	Cu	100	6.00	4.2160	2.2106
27.	0.0988	PL	Mix	100	5.00	4.2456	3.9830
28.	0.0598	PL	Cu	400	5.00	16.871	1.2101
29.	0.0899	PL	Cd	10	8.00	0.9810	1.3102

and cadmium at each developmental stage of penaeid shrimp larvae [6,13,14].

Earlier results have shown that there is no relationship between toxicity of metals and pH or pH is less important factor for controlling toxicity of metals in marine organisms [15,16]. Another study had reported, that pH is one of the most important factors for controlling the survival of penaeid shrimp during their culturing in ponds [14]. According to literature survey cited, there is no study using standard toxicological methodology, which describes lethal or minimum acceptable values of pH for uptake of metals in penaeid shrimp. It has been demonstrated in this study that uptake of Cu and Cd by penaeid shrimp larvae may be altered by variation of pH, further causing their developmental delay and mortality. Since increased accumulation was determined at lower pH that further enhanced their bioaccumulation as the uptake of Cu and Cd is interrelated to the survival. Maximum mortality was observed at pH = 2.5 and pH = 9. Since at pH = 9 all larvae were found dead within one hour, therefore it is difficult to say that mortality was due to toxicity of metal or due to highly alkaline media, as considerable amount, of NaOH was used to maintain pH = 9. Survival was not significantly decreased ($p > .005$) at or above pH = 6, although there was a significant correlation between exposed concentration of metals, pH range and accumulated amount of metal. Many investigators have however observed a linear relationship between the uptake of Cd and Cu concentrations in the medium [2,17,18] and it may be justified due to regularity mechanism in marine organisms. Decapods crustaceans for example regulate body concentrations of the essential metals like zinc, copper and manganese, balancing metals uptake rates by varied rates of metal excretion [19]. During present study, a linear relationship was observed between size of specimen (Nauplii to post larvae) and collective dry weight of each larval stage. The relationship observed between metal concentration and size (weight) gives an idea about the biological mechanism of accumulated metals in marine organisms. The uptake of copper and cadmium by nauplii was not significantly related to the exposed concentration and at the similar exposed concentration (at pH = 5.0), the determined amount was the same as that was in post larvae. It indicates that a significant proportion of the metal content is surface adsorbed [13]. Smaller specimen having smaller surface area to volume ratio. It was observed that all larvae exposed to lower pH i.e. 4.5 and 6 also had lower ($p < 0.001$) dry matter contents (Tables 1 and 2). This decrease recorded during the present study may have been due to concentration of exposed metal, either copper or cadmium, as has been recorded for other crustaceans [20]. It seems to be very difficult to correlate the effect of pH, exposed concentration of metals and survival of penaeid shrimp larvae, as it has been found that at lowest pH (2.5) and highest pH (9), all larvae died before completion of successive developmental stage and the uptake amount determined by AAS cannot accurately justify the specific stage of accumulation at specified pH. Thus there are several biological factors such as development stage, which may contribute to the magnitude of Cu toxicity, as juvenile amphipods were 4-5 times sensitive to Cu concentrations than adults [21]. Similarly, several ecological factors also affect biotoxicity of Cd including pH.

Experimental

One hour prior to exposure to various concentrations of metals, specimens (40 individuals of nauplii, zoea, mysis each and 20 of post larvae) were placed in a beaker containing 300-500 ml of sand (4.5 μm) filtered seawater. Into each beaker one of six concentrations of metal was added. A 30 °C static culture system was used for the experiment to avoid precocious development of shrimp nauplii as observed in flow-through system. Metal solutions were prepared form stock (1000 μg/ L) of $CdCl_2$ and $CuSO_4 . 5H_2O$ at 10 μg/ L – 500 μg/ L (0.5 μg/ L by a series of dilutions using deionized water. Five replicates were run for each solution with filtered seawater as control. Each experiment continued till the next stage (nauplii 24 – 38 h, Zeoa I–III 24 h, mysis I–III 30-60 h and PL 24 h). Metal solutions were renewed for every experiment [12]. The pH of test 2 solution was adjusted by addition of HCl and NaOH and determined by pH meter and confirmed with the inert biological buffers 2-(N-morpholino) ethanesulphonic acid, MES (pKa = 6.10) and N-2-hydroxyethylpiperzine-N-2-ethanesulphonic acid, HEPES (pKa = 7.48) were used to control the pH between 5.3 to 6.8 and 6.8 to 9.0 respectively.

Each experiment was continued till next successive stage, and after completion of experiments, all samples were prepared for atomic absorption spectrometry (AAS). Given compendium of method (AAS) and instrument used for estimation of Cu and Cd.

Conclusions

It has thereafter been concluded from this study, that the minimum and maximum uptake of Cu and Cd were observed at pH = 8.34 and .5 respectively under the maximum exposed concentration of 400 μg/ L. Corresponding survival rate was found 95 % at pH = 8.34 and 70 % at pH = 5.3, under exposed concentration of 100μg/ L.

References

1. P. G. C. Campbell, and P. M. Stokes, *J. Fish. Aquat. Sci*, **42**, 2034 (1985).
2. H. M. Chan, *Mar. Ecol. Prog*, **48**, 298 (1988).
3. R. W. Zuehlke, and D. R. Kester, Copper Speciation in Marine Waters. In: C. S. Wrong, Boyle, K. W. Bruland, J. D. Burton, E. Goldberg, D(eds)., Plenum Press, New York, 773 (1983).
4. M. Stephenson and G. L. Mackie, *Can. J. Fish. Aquat. Sci.*,**45**, 1705 (1988).
5. R. J. P. Williams, *Phil. Trans. R. Soc. Lond.*, **294**, 57 (1981).
6. Knutzen, *J. Mar. Pollut. Bull.* **12**, 25 (1981).
7. Wicken, *J. F, Aquaculture*, **41**, 37 (1984).
8. C. K. Tsai, Farm Management Workshop, American Soybean Association Singapore, 56 (1990).
9. F. D. Apud, J. H. Primavera and P. L. Torres, Jr., 3rd ed, *South. East Asian Fish. Dev. Cent.*, Tigbauan, Iloilo, Philippines, 66 (1985).
10. S. D. Ray , W. Mcleese, B. A. Waiwood and D. Pezzack, *J. Envirl. Qual*, **5**, 193 (1980).
11. R. Rosenberry, *World Shrimp Farming. Aquaculture Digest*, San Diego. CA, 3 1(1991).
12. A. B. Munshi, SU Yong-quan, *Chinese Journal of Oceanology and Limnology*, **14**, 170 (1996).
13. S. L. White and P. S. Rainbow, *Mar. Ecol. Prog. Ser.*, **37**, 147 (1987).
14. G. L. Allan and G. B. Maguire, *Aquaculture*, **107**, 33 (1992).
15. S. W. Shanar, and A. W. Knight, *Comp. Biochem. Physiol.*, **82**, 273 (1985).
16. D. J. Laurine, and D. G. Mcdonld, *Can. J. Fish. Aqut. Sci.***43**, 1488 (1986).
17. S. A. Dressing, R. P. Mass and C. M. Weiss, *Bull. Environ. Contam. Toxicol.*, **28**, 172(1982).
18. D. Cossa. *Mar. Environ. Res.*, **26**, 265 (1988).
19. P. S. A. J. Rainbow, and Ed., A. A. Balkema, Rotterdam, 405 (1987).
20. D. O. Morgen and B. R. Mcmohan, *J. Exp. Biol.*, 97, 241 (1982).
21. M. Ahsanullah and T. M. Florence, *J. Mar. Biol.* **84**, 41(1984).