The Effect of Water Salinity on the Uptake of Metals Related to Survival of Penaeid Shrimp

A. B. MUNSHI, A. S. HINA, AND T. H. USMANI
Centre for Environmental Studies,
PCSIR Laboratories Complex Karachi-75280, Pakistan

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Summary: The influence of water salinity on uptake of cadmium and copper by penaeid shrimp was studied in the laboratory experiments. It was found that higher exposed concentration of copper and cadmium results in more bioaccumulation as determined by AAS. The value of water salinity of between 20°/∞ and 30°/∞ found more suitable for maximum survival of penaeid shrimp under metal contaminated environment. Uptake of copper and cadmium was found to be inversely proportional to salinity, which causes decrease in growth rate of fauna, i.e. 20°/∞ more than that of 30°/∞. The water salinity of 40°/∞ or above was not therefore suitable for culturing penaeid shrimp because 100% mortality was observed at this particular salinity within 3hours even in control.

Introduction

Salinity is among the most important environmental factors and exerts various effects on the vitality of marine organisms. It acquires particular importance in marine areas with unstable salinity regimes, such as river estuaries, salt marshes, etc. only organisms with effective adaptations to unfavorable salinity can live in such habitats. In this case, so-called critical phase of development, which is characterized by the smallest adaptive capabilities in respect to the unfavorable environmental factors, often plays a role in the development of stable populations of a certain species and in their further survival. As a rule, in most organisms, all the earl ontogenetic phases (embryos, larvae, and young) are critical [1].

Many investigators have reported the effects of salinity on biotoxicity of cadmium [2]. It has been observed that the mortality of shrimp caused by metal contamination clearly decreases with increased salinity of water [3] and shows linear relation to the salinity of the medium [4]. Frank and Robertson [5] have reported that toxicity of Cd to blue crab decreases with an increase in salinity of water. Salinity strongly influences survival and growths of marine organisms [6] and marine invertebrates positively require specific salinity range at different developmental stages [5]. Some investigators have also implicated salinity in bioaccumulation of copper. Any factor which affects concentrations of Cu^{+2} in sea water is bound to influence its bio availability like salinity can influence copper accumulation by marine biota [7]. Wright and Zamuda [8] have observed direct relation between the water salinity and bioaccumulation of copper. It may therefore be generalized that $CuCl_2$ complexes will increase as the salinity of marine system increases and it would decrease bioaccumulation of copper. The uptake of heavy metals by marine organisms from any media or solution into the bodies of marine invertebrates at permeable surfaces is generally considered to be a passive process, depending upon various ecological factors including salinity. It has also been postulated, that salinity affects the biological activity or physiological process directly, leading to alternations in the metabolic and filtration rates and the feeding habits of invertebrates [9].

The present studies have been carried out in these laboratories In the light of existing available information on the marine organisms and metal toxicity in relation to the salinity and its objective is to determine the effects of variation in salinity of water on the uptake of copper and cadmium by penaeid shrimp.

Results and Discussion

The unexposed larvae (control group) shows an inverse relationship between survival rate and salinity up to 30 but at 40, almost all larvae died within 3 hours in both of the species (Fig 1 and Fig. 2).

At the salinity 20°/∞ survival was reduced as the concentration of metal increased. The lethal concentration in media for nauplii of *P.monodon* at

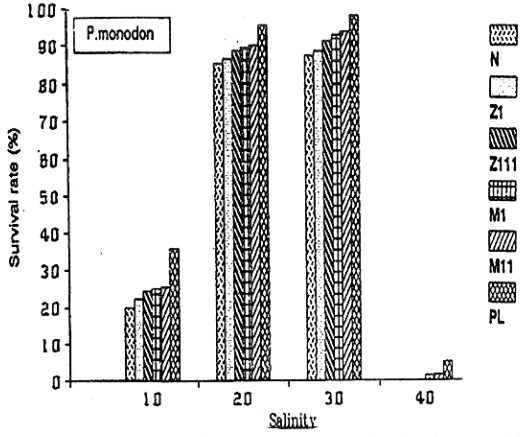

Fig. 1. Effect of salinity on survival of penaeid shrimp.

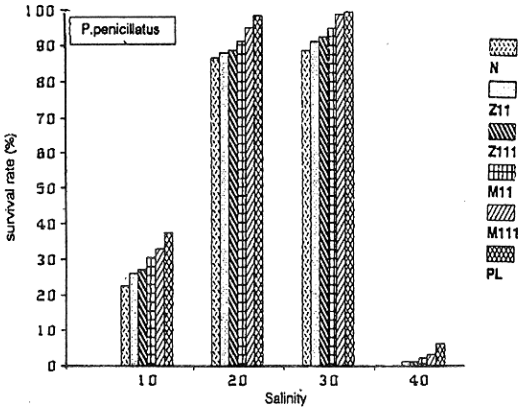

Fig. 2. Effect of salinity on survival of penaeid shrimp.

10 °/∞ was found 50 µg/L at which 50% nauplii and at the same salinity 50% zoea (I, II, III) were died at a concentration of 100 µg/L after 6h of exposure. The exposed concentration found to be lethal (LC_{50}) for mysis (I, II, III) and post larvae were 200 µg/L and 400 µg/L respectively. In *Penaeus penicillatus* the LC_{50} values for nauplii and zoea I was found to be 50 ug/L after 3 hours, where as that of zoea II and zoea III at 10 salinity is the same as that of *P. monodon* after 6hours .The LC_{50} for mysis (I, II, III) and post larvae of *P. penicillatus* was found to be 200 µg/L after 6h exposure. At salinity 20 °/∞ the average lethal concentration of copper for all stages of larvae was 250 µg/L, cadmium 300 µg/L and mixture was 350 µg/L after 8h in both species (Fig 3-8). The maximum survival was observed at salinity

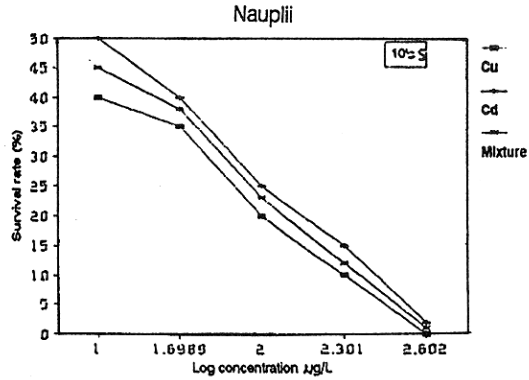

Fig. 3. *P. monodon*: Effect of exposed metal concentration on survival rate under various salinity and larval stage.

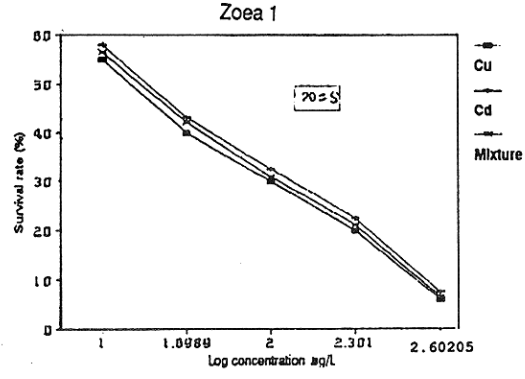

Fig. 4 *P. monodon*: Effect of exposed metal concentration on survival rate under various salinity and larval stage.

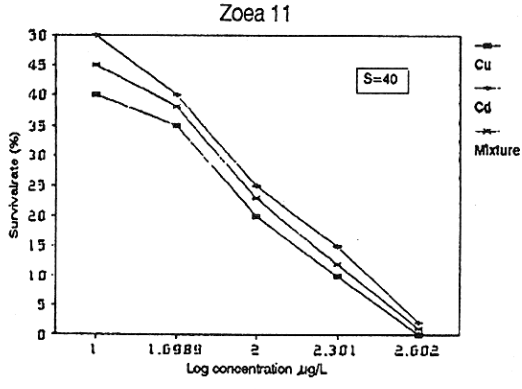

Fig. 5: *P. monodon* Effect of exposed metal concentration on survival rate under various salinity and larval stage.

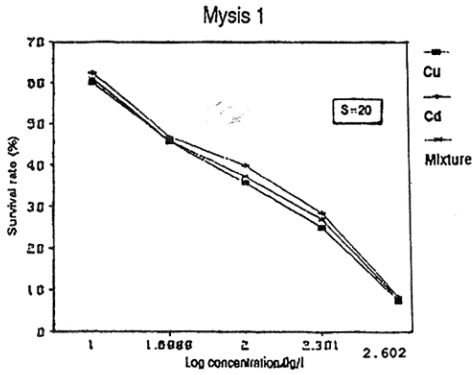

Fig. 6: *P. monodon*: Effect of exposed metal concentration on survival rate under various salinity and larval stage.

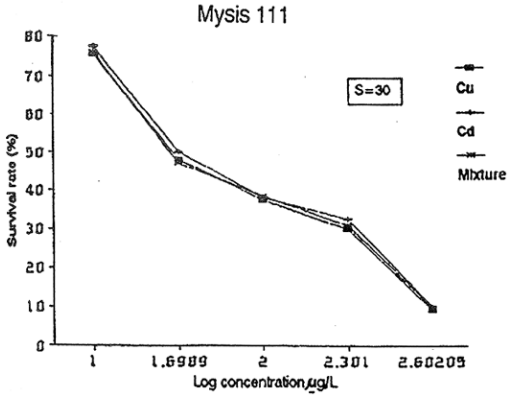

Fig. 7 *P. monodon*: Effect of exposed metal concentration on survival rate under various salinity and larval stage.

30°/₀₀ in mixture at 200µg/L after 8 hours. The LC_{50} of copper for nauplii and zoea I of *P. monodon* after 6hours, and for zoea II, III and mysis I was 200µg/L, mysis. 50% mysis II, III and post larvae at salinity 30 were died ,when exposed to 400 µg/L after 5hours. Almost same was observed for *P. penicillatus* except post larvae for them LC_{50} was 300 µg/L for 12 hour exposure.

At salinity 40°/₀₀, all larvae up to mysis III of both species died within 3 hours. However, post larvae of *P. monodon* and *P. penicillatus* remained alive at Salinity 40°/₀₀ up to 5 hours, then mortality was initiated first in postlarvae of *P. monodon* then of *P. penicillatus*. Copper was found to be more toxic

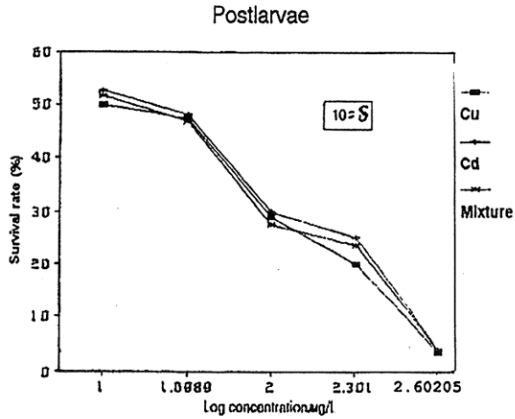

Fig. 8 *P. monodon*: Effect of exposed metal concentration on survival rate under various salinity and larval stage.

than cadmium at every level of salinity (Fig. 3-8) and Fig. 4 (9-12).

It was also observed that survival rate of both the species at decreased every stage as salinity increased after 30 which was faster in napulii as compared to postlarvae. The exposed concentration of copper and cadmium as determined by AAS, in samples of *P. monodon* and *P. penicillatus,* on dry weight basis are given in Table-1. In control, cadmium concentration at salinity 10 and 20°/₀₀ was found to be much higher than that of copper, but lower than that of at salinity 30 and 40. All of the concentrations were calculated on dry weight basis and plotted after taking their log versus different salinities at every larval stage (Fig. 4 -7) and (Fig. 9-12).

It was therefore observed, that logarithmic transformations of both concentrations and salinities make the relationship more linear. All the values of metals uptake determined by AAS, seem to be directly proportional to the exposed concentrations. (Table-1 and 2)

Overall uptake of copper, cadmium and their mixture varied with salinity of water medium. The optimum salinity at 100 µg/L of copper and cadmium for zoea I, II, III was 20°/₀₀ and for mysis I, II, III was 30°/₀₀. In all of the experiments uptake of both of the metals in the body of specimen was approximately linear in developmental stage with passage of time. Higher uptake of copper and cadmium were found at

Table-1: The Uptake of Metals at each Larval Stage for *P.monodon* under different Experimental Conditions

S.#	Larval Stage	Salinity ‰	Exposed metal concentration µg/L		Uptake Amount Determined by AAS Cu (µg/L)	Cd (µg/L)
1	Nauplii	10	10	Cu	4.57	36.0
2	"	30	200	Cd	2.33	5.16
3	"	20	Control		3.48	1.17
4	ZI	30	50	Cu	1.810	0.20
5	"	10	200	Cu	10.41	1.322
6	"	10	400	Cd	6.136	8.045
7	"	40	50	Mix	2.803	0.1515
8	"	10	Control		1.741	4.333
9	ZII	20	10	Cu	0.921	0.392
10	"	30	30	Cu	02.971	1.855
11	"	30	200	Mix	15.23	5.285
12	"	40	400	Cd	03.665	15.100
13	"	20	50	Cd	04.750	5.000
14	"	20	Control		01.000	0.042
15	Z III	30	50	Cu	0.241	1.352
16	"	10	10	Mix	0.475	0.495
17	"	30	200	Cd	9.928	12.142
18	"	30	Control		2.314	1.261
19	MI	30	400	Cd	1.208	17.73
20	"	20	200	Cu	9.928	12.142
21	"	40	100	Mix	2.471	0.149
22	"	30	Control		1.346	0.930
23	MII	30	10	Cd	2.590	0.551
24	"	40	Control		0.356	0.432
25	MIII	20	100	Cu	2.590	5.368
26	"	10	200	Cd	12.88	3.185
27	"	40	50	Mix	1.670	2.342
28	"	30	Control		1.670	2.342
29	PL	30	50	Cu	5.320	6.740
30	"	30	10	Cd	4.609	3.687
31	"	40	400	Mix	6.782	8.787
32	"	10	400	Mix	12.45	8.585
33	"	20	50	Cu	2.935	4.428
34	"	30	10	Mix	1.028	0.280
35	"	40	100	Cd	6.111	2.098
36	"	20	Control		1.004	0.980

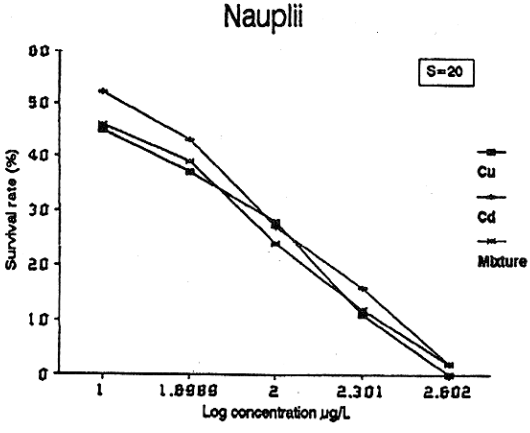

Fig. 9 *P. penicillatus*: Effect of Metal Uptake on survival rate under various salinity and larval stage.

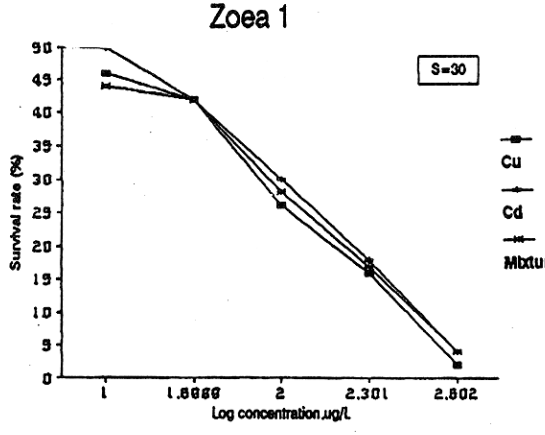

Fig. 10 *P. penicillatus*: Effect of Metal Uptake on survival rate under various salinity and larval stage.

Table-2: The Uptake of Metals at each Larval Stage for *P.penicillantus under different Experimental* Conditions

S.#	Larval Stage	Salinity	Exposed metal concentration		Uptake Amount Determined by AAS	
		‰	µg/L		Cu (µg/L)	Cd (µg/L)
1	Nauplii	40	10	Cu	0.35	0.166
2	"	30	50	Cd	5.166	6.33
3	"	10	Control		2.010	1.567
4	ZI	20	200	Cu	0.065	0.227
5	"	10	50	Cu	3.100	2.80
6	"	10	10	Cd	3.582	1.813
7	"	20	100	Mix	1.192	1.230
8		10	Control		2.320	1.398
9	ZII	40	10	Cu	2.178	3.928
10	"	20	100	Cu	2.971	1.855
11	"	40	50	Mix	5.844	5.064
12	"	20	100	Cd	2.345	9.938
13	"	30	200	Cd	8.314	7.349
14	"	20	Control		1.231	2.042
15	Z III	20	50	Cu	0.197	0.093
16		nd	nd		nd	nd
17	MI	10	10	Cu	2.588	6.965
18		nd	200	Cu	nd	nd
19		20	400	Mix	6.374	8.290
20		30	Control		1.714	5.428
21	MII	20	10	Cu	2.504	0.551
22		40	Control		0.256	0.332
23	MIII	20	10	Cu	1.800	0.768
24		nd		nd	nd	nd
25		nd		nd	nd	nd
26		30	Control		0.670	0.342
27	PL	20	100	Cu	6.778	9.539
28		40	200	Cu	2.940	2.602
29		10	200	Cd	2.057	1.865
30		10	400	Cd	2.998	1.138
31		20	100	Mix	7.073	3.897
32		10	200	Mix	2.023	1.680
33		20	Control		0.119	0.189

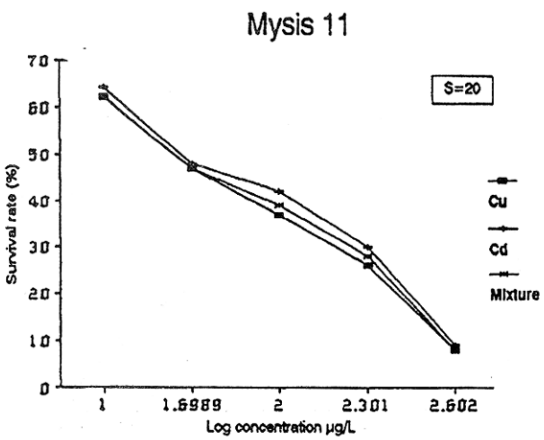

Fig. 11 *P. penicillatus*: Effect of Metal Uptake on survival rate under various salinity and larval stage.

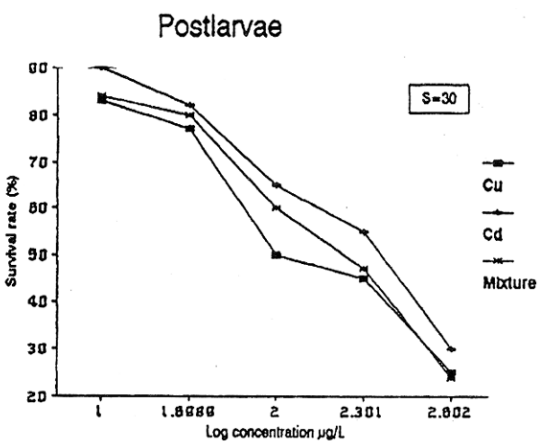

Fig. 12 *P. penicillatus*: Effect of Metal Uptake on survival rate under various salinity and larval stage.

lower salinities of water. Analysis of variance shows that there is no significant difference between regression lines for salinity 20 and 30‰ but for salinity 10 and salinity 40‰ are significantly different. The uptake of copper was found to be greater than that of cadmium, whereas the uptake on exposure in the form of mixture was lower than that of copper and cadmium exposed individually on the same value of salinity. The increased uptake of copper or cadmium represents a non-steady state of concentration of increase in concentration of media. Total development time from nauplii to the postlarvae displayed much variation depending upon salinity of water, being shortest at water salinity of 30‰, and longer at higher salinity, even at lower concentration of copper and cadmium.

Studies carried out earlier have indicated, that environmental factors may affect the rates of uptake of heavy metals in marine bivalves [9]. In the present study. The effect of one of the parameter of water medium i.e. is salinity on the uptake of cadmium, copper and their mixture. The present study clearly indicates the influence of copper and cadmium under conditions of storage in water of different salinities on the development of two penaeid shrimp i.e. *P. monodon* and *P. penicilatus*. It has been observed that *P. monodon* is an efficient osmoregulator in the salinity range of 15 ~30 and has an is osmotic concentration of salinity 23 – 25 ‰ [6]. It is close to mid way between the two salinities tested in the present study. Gurney [10] considered that, different physiological rates have various tolerance limits, therefore, two species of penaeid shrimps were selected for the present study. It has been reported, that the filtration rate decreases at low salinities [3] and produces increasing survival, because there will be less metal accumulated from particulates or food.

Salinity not only affects the uptake of metal but also creates notable effects on the development of penaeid larvae. In the laboratory experiments, at lower salinity of 10‰, nauplii were found to be incapable of even completing development as well as at highest salinity of 40‰, nauplii were found immobile within one hour. However, mysis and postlarvae have possibly a wider tolerance than nauplii and zoea, enabling them to withstand and survive in extremely saline conditions.

The results obtained in the study indicate that salinity should be taken intoconsideration as an important factor, in estimating the survival and development of penaeid shrimp and uptake of metals. However, in many copepods from coastal waters, such as Acartia clausi and A.steuri [11], salinity has been shown to have very little influence on developmental rates. It is therefore apparent from these results, that salinity interacts with the uptake of copper and cadmium to produce the greatest part of decline in survival rates in metal polluted sea water. Lower salinity results in higher uptake of copper and cadmium, which also reduces natural pond productivity, presumably by redcucing the availability of nutrients [2].

The aim and objective of this study is to provide data to assist with the management of fresh-sea water combination shrimp farming pond by the adjustment of salinity. However, results from laboratory experiments were directly applicable to hatchery situations as found in case of *Metapenaeus macleayi* [12].

In conclusion it may be stated, that lower salinity results in more uptake of copper and cadmium from the media creating more toxicity. The survival rate of penaeid shrimp was found too be directly proportional to the uptake of metals and inversely proportional to the salinity. Every stage responds differently to various salinities as well as towards each metals, resulting in different rates of survival.

Experimental

Specimen (from nauplii till postlarvae) of *Penaeus monodon* and *Penaeus penicillatus* were collected from a hatchery. All specimens were maintained in sea water having salinity 27.65, and allowed to acclimatize for at least 2 hours prior to an experiment. Metal solutions were prepared in deionized water from $CuSO_4.7H_2O$ and $CdCl_2$. All of the experiments were carried out with 50 nauplii, 40 individual of Zoea and Mysis and 20 specimens of postlarvae in a 600ml beaker, containing 500 ml of aerated, filtered sea water with metal solutions of copper and cadmium of concentrations varying between 10µg/L up to 400 µg/L and their 1:1 mixture at different salinities of 10, 20, 30 and 40 .Lower salinities were obtained by dilution with fresh water and higher salinity was achieved by adding sodium chloride. After completion of each successive larval stage, individuals were removed, blotted and dry weights obtained by oven drying at 80°C for a period of one hour in tarred glass counting vials.

Sample Preparation for AAS

All specimens were digested in closed glass ampoules, using extra pure nitric acid, perchloric acid and hydrogen fluoride (HNO_3: $HClO_3$: HF) in 2:1:2 ratio. These ampoules were placed in a sand bath and heated initially at 90°C for 2 hours and then at 140°C for 4 hours. Cooled digests were then diluted with deionized (Milli- Q) water to prepare solutions of about 3N HNO_3 for analysis of copper and cadmium by atomic absorption spectrophotometer (AAS). The cadmium was analyzed by graphite furnace AAS, and copper by flame AAS. Blanks and standard reference materials were run along with samples. In addition to the determination of metal concentrations by AAS in specimens of penaeid shrimp, their dry and wet weights, and effect of salinity of water medium on their survival was also investigated.

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