The Separate and Combined Effects of Copper and Cadmium on the Survivorship of Penaeid Shrimps

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Summary: Critical survival rate for all larval stages of penaeid shrimp was determined using static bioassay with copper, cadmium and their 1:1 mixture. The lethal concentration (24 h LC_{50}) was 200 μg/L of Cu, 250μg/L of Cd and 300 μg/L of mixture in average for all larval stages along with slight variation at every stage (Nauplii - Myses III) in *Penaous monodon* LC_{50} was 400 μg/L. In *Penaeus penicillatus* LC_{50} the values were found to be 200 mg/L of Cu, 230 μg/L of Cd and 250 μg/L for mixture in Nauplii to Myses however for postlarvae the LC_{50} was within the same as in *Penaeus monodon*. The estimation of survival rate within 24-48 h under lethal and acceptable concentration of copper and cadmium showed culturing farmers at most productive and safe condition of their ponds.

Introduction

In studying the effects of pollution on aquatic organisms, the survival rate is considered to be the best index of any metal stress, because it is the least variable. There is no comprehensive studies have been published on the effect of metal contaminated seawater on the survival rate of larval stages of penaeid shrimp. However, the concentrations of trace metals in marine environment have been widely reported [1]. Among metals the cases of cadmium and copper poisoning have been reported by workers [2].

During present study we investigated the affects of Cu, Cd and when touched with a mechanically stimulated. The organisms with same concentration and combined metal effect were determined by plotting probit transformed percent [7-8]. LC_{50} values mean ± SD for every metal on each stage was calculated. All recorded values are average of four times observations.

Results and Discussion

The lethal concentration (LC_{50}) for nauplii of *P.penicillatus* was found to be 100g/L and in *P.monodon* was almost 200μg/L after 24h. All nauplii were found dead within 6h i.e. zero percent survival rate in test medium of 400 mg/L of Cu, Cd an mixture. Fifty percent of zoaes (I,II,III) of both species were remained alive after 24 h in 200 μg/L of Cu, Cd and mixture. Fifty percent myses (I,II,III) were found alive in 200 μg/L of Cu after 24h. In medium of 400μg/L of Cu Cd and mixture only postlarvae of both species were still showing movement even after 48 h in 200 μg/L. No larvae were died in control solution. The probit survivorship as a function of time factor is shown in Fig. 1 and 2, along with their standard deviation (±. SD) each stage (Table 1 and 2).

Results of present study are showing a progressive increase in tolerance to metals with progressing larval stages. For myses III and postlarvae, the data revealed a decrease in LC_{50} value with increasing duration of exposure in both species. Exposure of larval to both metals and their mixture caused significant effects on survivorship at each concentration except 10 mg/L 1:1 mixture of both metals under laboratory conditions on the survival of two penaeid shrimp i.e. *Penaeus monodon* and *Penaeus penicillatus* (from nauplii to postlarvae). For all experiments the determined survival rate has been determined for on various periods of time intervals.

During present study reduction in the survival rate was found to watch that of Cancer magistere(s) be similar except duration of exposure, because the survival rate after 6th is quite different from that of after 48h. (Fig. 1 and 2). As per literature, penaeid

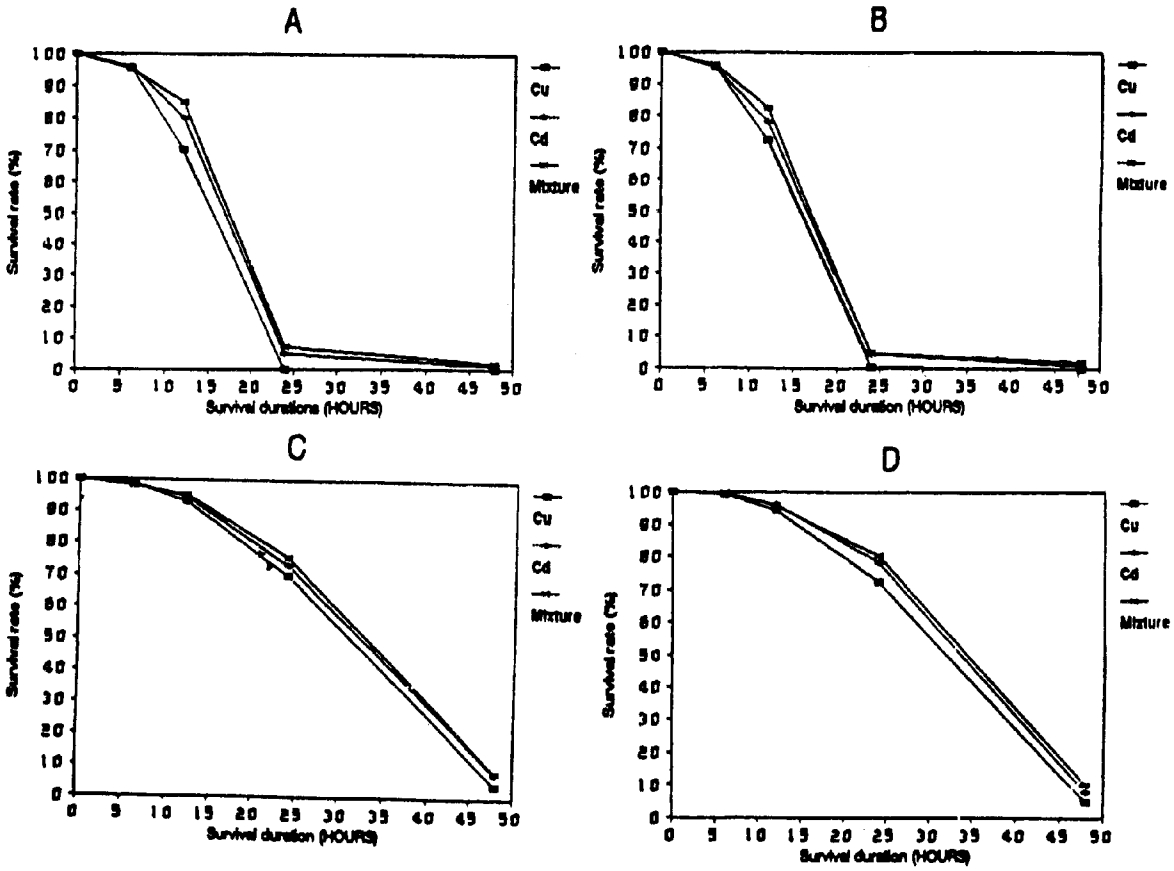

Fig. 1 *P.penicillatus*: Relationship between probit of (A) Zoea (B), Mysis (C) and postlarvae (D) for 48 hours at 100 μg/L concentration.

shrimp larvae usually require lesser than 4 days to develop to each successive stage in the laboratory (experimental condition) like in the hatchery [10]. During present study our aim was only to determine survival rate for short period (48h) therefore, we could not obtained LC_{50} for all specimen for a period of 96h. However, a safe level for maximum survival of shrimp can be obtained by multiplying an application factor and the (96) LC_{50} value for the stage in which species is most sensitive to the pollutant particularly copper and cadmium (II). Lower concentration of copper and cadmium (i.e. 10 and 50 μg/L) were found more toxic to nuplii, whereas Myses (I,II,III) shown lower mortality at same concentrations.

However, for postlarvae these play no significant role on survival of penaeid shrimp. Similar findings have been reported from studies on crab larvae, echinoderm and other molluscs [12, 5].

At lower concentration of copper, cadmium and their 1:1 mixture have significant effect on the survival on Nauplii, thus having found lowest tolerance to copper as compared to cadmium and mixture. In case of both species copper reacted antagonistically to cadmium when applied in mixture. For every metal postlarvae shown maximum survival rate during (48) of exposure.

The influence time of larval survival varied with each metal and concentration tested. Almost all

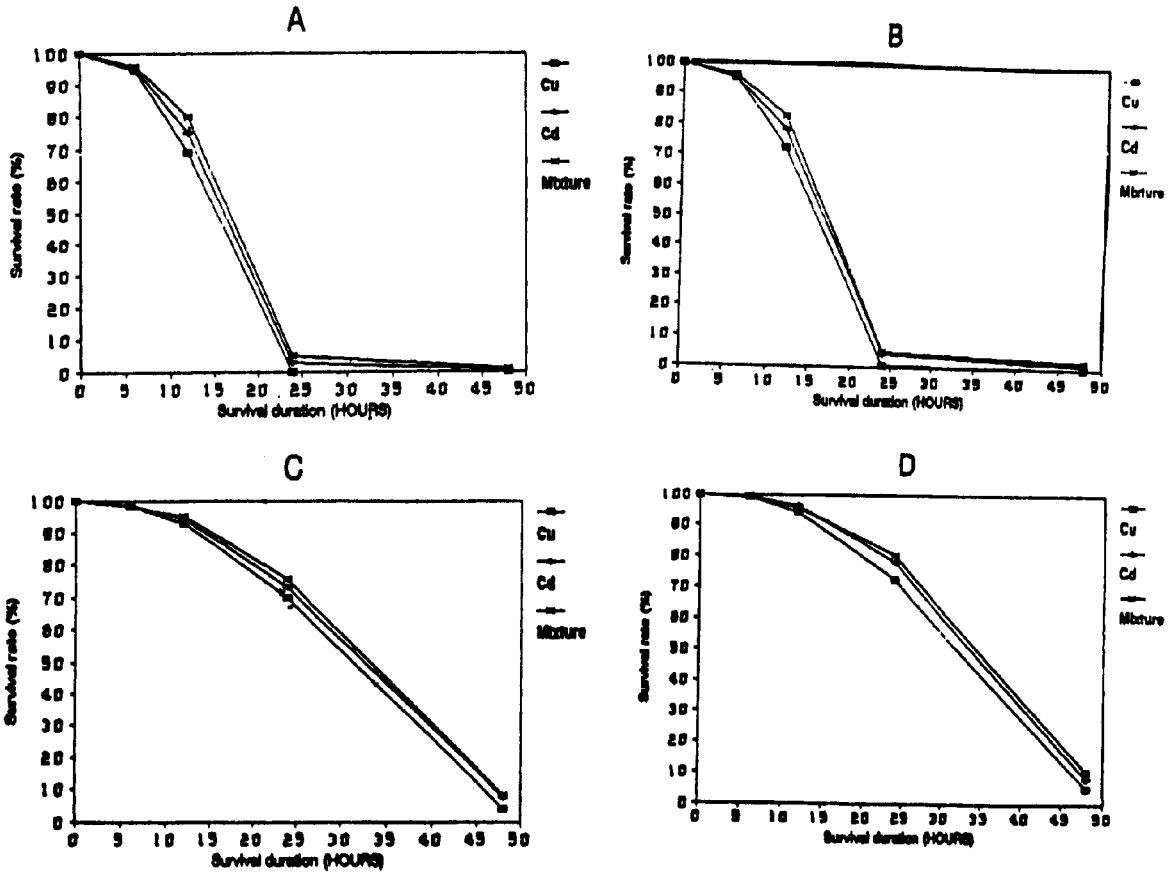

Fig. 2: *P. monodon*, Relationship between probit of average survival (%) time period (H) for Nauplii (A), Zoea (B), Mysis (C) and Postlarvae (D) for 48 hours at 100 μg/L concentrations.

larval stage species for each metal reduced survival rate up to zero percent at 400 mg/L concentration as early as 24h after initial exposure for postlarvae survival rate at 10 mg/L reduced only up to 5% with the exception of mixture after 24 h exposure.

It seems to determine indicators of a sub lethal toxic stress, because their sensitivity is found to be higher than have lethal.

Toxicity indices

The lethal concentration of all metals to marine organisms varies considerably with test species, life history stages and experimental conditions but the reported values are generally above 50 ppb for cu 10 μg/L for Cd when 100% of Cancer Anthony dead [5].

According to our knowledge there is no previous study considering the effects of metals on every larval developmental stages of penaeid shrimps in comparison of two species i.e. *Penaeus penicillatus* and *Penaeus monodon*. The main idea of present study generated from the concept that whenever larvae exposed to essential and non-essential metals results in a biochemical introduction of products due to metal binding [13]. Although metal binding proteins immobilize metals and effectively protect host organisms against toxicity

Table-1: Survivorship of different *P. penicilatus* larval stages (48 hours) exposed to various concentrations of metals. Data in mean percentage ± SD.

larval stage	Metals	Concentration (µg/L) 0	10	50	100	200	400	500
Nauplii	Cu	100	97.0 ± 1.33	70.0 ± 2.34	50.0 ± 2.78	0.00	0.00	-
	Cd	"	nd	nd	nd	nd	nd	-
	Mix.	"	97.8 ± 0.21	70.0 ± 9.33	10.7 ± 3.67	20.45 ± 2.70	0.00	-
Zoeal	Cu	100	98.7 ± 0.46	80.0 ± 2.34	70.0 ± 4.89	30.0 ± 5.89	0.00	-
	Cd	"	98.0 ± 7.80	82.5 ± 3.50	75.4 ± 3.39	35.3 ± 3.67	0.00	-
	Mix.	"	98.5 ± .09	85.8 ± 3.60	77.5 ± 2.09	34.2 ± 1.89	0.00	-
ZoealI	Cu	100	98.5 ± 1.89	87.7 ± 7.45	80.3 ± 2.78	40.6 ± 3.56	1.23 ± .00	-
	Cd	"	nd	nd	nd	nd	nd	-
	Mix	"	98.5 ± 2.45	87.2 ± 4.12	80.11 ± 2.45	44.2 ± 1.78	1.00 ± 2.89	-
ZoealII	Cu	100	99.3 ± 2.56	90.8 ± 4.78	82.9 ± 3.00	47.7 ± 4.56	1.00 ± 1.23	-
	Cd	"	99.1 ± 2.67	91.3 ± 1.67	83.1 ± 7.00	48.3 ± 3.78	2.00 ± 1.45	-
	Mix	"	99.0 ± 1.98	91.0 ± 4.7	85.9 ± 2.89	49.2 ± 3.67	2.00 ± 0.98	-
Mysis I	Cu	100	nd	nd	nd	nd	nd	-
	Cd	"	99.0 ± 1.20	92.4 ± 2.00	87.1 ± 2.56	50.7 ±2.80	8.50 ± 2.56	-
	Mix	"	99.0 ± 1.02	93.7 ± 00.	88.7 ± 2.89	51.5 ± 4.60	9.2 ± 3.90	-
Mysis II	Cu	100	99.1 ± 2.33	94.3 ± 3.00	89.6 ± 3.65	56.9 ± 7.50	5.89 ± 5.90	-
	Cd	"	99.2 ± 0.00	95.0 ± 6.10	89.8 ± 1.65	57. ± .80	4.80 ± 2.56	-
	Mix	"	99.3 ± 0.00	98.5 ± 2.09	90.0 ± 2.56	56.6 ± 3.70	.45 ± 4.66	-
Mysis III	Cu	100	99.0 ± 2.70	96.7 ± 3.70	91.0 ± 1.67	60.6 ± 4.67	5.90 ± 2.34	-
	Cd	"	99.2 ± 4.10	98.2 ± 3.07	91.5 ± 2.20	61.2 ± 3.20	6.00 ± 1.10	-
	Mix	"	99.0 ± 2.35	98.0 ± 1.78	92.1 ± 3.20	60.3 ± 2.70	6.70 ± 1.09	-
Postlarvae	Cu	100	100 ± 0.00	98.5 ± 3.67	96.2 ± 2.78	62.5 ± 6.67	7.8 ± 3.09	1.0 ± 0.2
	Ca	"	100 ± 0.00	99.1 ± 2.40	97.2 ± 5.40	63.7 ± 3.56	10.1 ± 2.70	1.5 ± 1.2
	Mix.	"	100 ± 0.00	99.2 ± 3.67	98.4 ± 3.80	62.23 ± 4.70	15.0 ± 2.80	2.3 ± 2.0

*Mix=mixture of Cd:Cu(1:1)
nd=not determined
O=Concentration of control
Exposure time=48 h

Table-2: Survivorship of different *P. monodon* larval stages (48 hours) exposed to various concentrations of metals. Data in mean percentage ± SD.

larval stage	Metals	Concentration (µg/L) 0	10	50	100	200	400	500
Nauplii	Cu	100	90.6 ± 2.13	86.6 ± 1.44	73.3 ± 3.78	55.33 ± 2.90	0.00	-
	Cd	"	98.6± 2.43	93.3 ± 2.33	74.0 ± 0.33	56.0 ± 2.33	0.00	-
	Mix.	"	93.0 ± 1.31	70.0 ± 9.33	74.4 ± 1.67	54.05 ± 1.70	0.00	-
Zoeal	Cu	100	100.0 ± 00	87.5 ± 3.04	80.0 ± 3.09	62.5 ± 4.69	0.00 ± 0.0	-
	Cd	"	97.5 ± 2.80	93.5 ± 4.10	91.1 ± 2.49	76.2 ± 2.97	3.00±	-
	Mix.	"	98.5 ± 3.09	91.0 ± 4.60	96.2 ± 1.89	78.5 ± 2.09	6.25±	-
ZoealI	Cu	100	95.0 ± 2.39	92.5 ± 2.45	72.5 ± 3.08	52.5 ± 4.56	0.00 ± 0.00	-
	Cd	"	85.0 ± 2.63	80.0 ± 1.33	75.0 ± 3.13	62.5 ± 3.23	4.00 ± 1.23	-
	Mix	"	95.3 ± 3.05	75.0 ± 4.12	72.5 ± 4.05	62.5 ± 2.68	7.00 ± 09	-
ZoealII	Cu	100	96.4± 1.56	94.3 ± 3.78	74.3 ± 2.40	55.6 ± 3.06	1.10 ± 2.93	-
	Cd	"	98.3 ± 3.67	97.4 ± 2.57	75.7 ± 8.20	65.8 ± 2.08	5.40 ± 3.35	-
	Mix	"	97.1 ± 0.98	96.3 ± 3.28	75.0 ± 4.69	63.8 ± 1.07	6.10 ± 1.08	-
Mysis I	Cu	100	97.5± 2.53	95.6 ± 3.03	75.2 ± 2.13	62.1 ± 2.53	2.00 ± 0.33	-
	Cd	"	99.0 ± 1.20	92.4 ± 2.00	87.1 ± 2.56	50.7 ± 2.80	4.50 ± 1.56	-
	Mix	"	99.0 ± 1.02	93.7 ± 0.00	88.7 ± 2.89	51.5 ± 4.60	6.6 ±2.40	-
Mysis II	Cu	100	98.2 ± 3.43	96.2 ± 2.45	78.1 ± 2.55	65.3 ± 8.20	3.00 ± 1.30	-
	Cd	"	98.5 ± 6.20	97.2 ± 3.40	80.2 ± 3.55	67.2± 1.80	3.50 ± 3.46	-
	Mix	"	98.0 ± 1.00	97.0 ± 3.19	82.5 ± 3.46	70.6 ± 5.60	3.05 ± 2.06	-
Mysis III	Cu	100	90.0 ± 3.70	nd	88.7 ±2.07	88.7 ± 3.27	85.0 ± 1.34	7.5 ± 2.3
	Cd	"	98.7 ± 5.10	nd	93.7 ± 3.60	88.7 ± 4.20	81.8 ± 2.40	6.2 ± 3.0
	Mix	"	92.2 ± 3.35	97.0 ± 1.48	88.7 ±8.10	89.5 ± 5.10	90.0 ± 2.19	8.2 ± 2.5
Postlarvae	Cu	100	100. ± 0.00	98.5 ± 2.57	95.0 ± 3.48	70.0 ± 4.07	50.1 ± 4.45	1.0 ± 0.2
	Ca	"	100 ± 0.00	98.7 ± 3.34	97.2 ± 4.30	75.2 ± 4.26	60.1 ± 3.70	1.5 ± 1.2
	Mix.	"	100 ± 0.00	98.2 ± 4.07	96.2 ± 5.60	72.83 ± 3.80	55.2 ± 4.80	2.3 ± 2.0

*Mix=mixture of Cd:Cu(1:1)
nd=not determined
O=Concentration of control
Exposure time=48 h

causing no notable effect on survival rate up to concentration of 50 μg/L of each metal but metal may be passed at high concentrations including human [14].

Larval stages, like other planktonic animals due to their small size are particularly sensitive to pollutants and therefore, appeared as good organism to use in bioassays [3]. Our findings suggest that penaeid shrimp larvae at every stage of development are highly sensitive to copper and cadmium, a property that can be exploited in field experiments.

Experimental

Larvae of penaeid shrimp were brought from hatchery located at Xiamen University to the laboratory and acclimatized for 2 hours of all larval stages from nauplii to postlarvae were tested.

Test solutions were prepared by dissolving required amount of $CuSO_4.5H_2O$ for copper and $CdCl_2$ for cadmium in demonized water. A series of concentrations (10 μg/L, 20/μg/L, 50μg/L, 100μg/L 100 μg/L and 400 μg/L) were selected during this study. The seawater without metal (10 μg/L concentration) taken as a control. In each beaker, 50 Naupii, 40 for each stage of zoae, 40 myses and postlarve were exposed to different concentrations in 500 ml sea water. The experiments were run for each concentration in duplicate. Sort term (6h, 12h, 24h and 48h) median lethal concentration (LC_{50}) toxicity tests were carried out. Larvae were counted after every size hour during exposure period. During whole experiments nauplii were not fed [4]. Zoae were fed with *skeletonema* and myses till to postlarvae were fed with freshly hatched *Artemia* nauplii. Experimental conditions were kept constant along with temperature 26-29°C. Salinity 27.65% and pH + 8.23 [5]. Deaths were assumed when larvae were immobile and showed no response of copper and cadmium where only negligible decrease in survivorship was observed. Particularly impressive was the larval response to copper, survivorship of postlarvae after exposure for 48h was nearly identical to the survivorship of larvae in control (Table 1 and 2).

A longer exposure of copper, cadmium and their 1:1 mixture to nauplii, zoea and myses at low conçentration have a significant impact survival rate of lavae. Nauplii showed lowest tolerance to copper as compared to cadmium and 1:1 mixture. In case of both species copper reacted antagonistically to cadmium when applied in mixture. For every metal postlarvae shown maximum rate during 48h.

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