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EFFECTS OF SALINITY ON ACUTE TOXICITY OF LEAD NITRATE TO LARVAL STAGES OF GIANT FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE MAN)

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ABSTRACT

An exploratory test for the acute toxicity of lead nitrate on early larval stages of Macrobrachium rosenbergii *was carried out at salinity 15 ppt in a static water system. The median lethal concentrations (LD_{50}) of lead nitrate at 24, 48, 72 and 96 h were 35, 25, 15 and 1 ppm, respectively. These median lethal concentrations were then used as starting concentrations for determining combined effects of salinity and lead nitrate on survival of 4-day-old larvae (larval stage II), 14 day-old larvae (larval stage V), and 24 day-old larvae (larval stage VIII) of* M. rosenbergii. *The combined test was run under similar conditions to the exploratory test but at different salinities (from 5 to 25 ppt). For all stages of larvae, salinity showed a strong influence on larval survival. At salinities closed to 15 ppt, the larvae were more tolerant to lead nitrate toxicity than at other salinities. Toxicity of lead nitrate increased with increasing salinity and LD_{50} varied with larval stages. The 14-day-old larvae were the most resistant to lead nitrate.*

INTRODUCTION

Lead, one of the most common of the toxic heavy metals, is introduced into the environment mainly by combustion of leaded gasoline and discharge of industrial waste, residues from mining and solid wastes from consumer items, such as wet cell batteries, paints and pesticides[1]. Industrial expansion involves an increase in production and utilization of lead, and it causes widespread lead contamination in the atmosphere and in natural water. It is particularly abundant in sediments and suspended particles, in soil, and in living organisms that can magnify it along the food chain[1]. Lead is harmful to aquatic fauna and to humans. Toxicity of lead result from changes in biological structures and systems to irreversible and inflexible conformations leading to deformity or death[2]. Lead contamination is considered to be a serious environmental problem[3].

The giant freshwater prawn, *Macrobrachium rosenbergii* (de Man), an economically important crustacean spread widely in rivers and lakes of Thailand. It has been known as a luxury food in Thailand for along time[4]. and it is commercially cultured in the central Thai provinces of Supanburi, Ayudhya, Pathumthani and Nakorn-Pathom. In the United States, prawn imports are estimated to be more than a million pounds per year[5].

Giant freshwater prawn adults live in fresh water, but larval development takes place in brackish water. After metamorphosis, juveniles migrate back to fresh water[6]. This migration may result in exposure to heavy metal contamination, especially lead, when the route passes through big cities or industrial areas.

It would be interesting to know whether salinity changes occurring during development and migration could affect lead toxicity for the giant freshwater prawn. To understand the role of salinity and lead interaction on survival of prawn larvae, the present experiments were conducted. The knowledge gained may provide data to help in the information of guidelines for lead pollution.

MATERIALS AND METHODS

M. rosenbergii larvae at age 4 days (stage II), age 14 days (stage V), and age 24 days (stage VIII, the final stage before metamorphosis) were obtained from the hatchery at the Department of Marine Science, Chulalongkorn University. The larvae were reared in 10 liter-tanks at a salinity of 15 ppt and at 28±2°C. Newly hatched *Artemia salina* nauplii were fed to the larvae twice a day. An experimental stock solution of lead was prepared from Lead nitrate, $Pb(NO_3)_2$ (99.99%, BDH Chemical Poole England [No. 1469]). The experiments were divided into exploratory and combination tests.

Exploratory test

The median lethal concentrations (LD_{50}) of lead nitrate for giant freshwater prawn larvae were determined 24, 48, 72 and 96 hours after exposure to concentrations of lead nitrate range from 1 to 75 ppm at 15 ppt salinity. Thirty larvae aged 4-6 days were used in each beaker (500 ml) and each test concentration was replicated twice. The LD_{50}és were calculated by probit analysis. The results of this experiment was used to determine the range of lead nitrate concentration to be used in test on the combined effects of salinity and lead nitrate.

Combination tests

Lead nitrate concentrations of 1, 15, 25, and 35 ppm were tested at salinities of 5, 10, 15, 20 and 25 ppt. Larvae of *M. rosenbergii* aged 4, 14 and 24 days were selected as test animals. The treatment combination was performed in a 500 ml beaker with an initial of 30 animals at ambient temperature (26-28°C). Three replications were done for each treatment combination. During operation, *Artemia salina* nauplii were fed twice a day. The experimental solutions were changed from freshly prepared solutions daily to remove waste materials and to determine survival numbers of the prawn larvae.

Combined effects of lead nitrate concentration and salinity on survival of each larval stage were determined by multiple regression analysis by using a model called a two-factor second order polynomial or a response surface analysis method[7,8]. The model was described:

$$Y = b_0x_0 + b_1x_1 + b_{11}x_1^2 + b_2x_2 + b_{22}x_2^2 + b_{12}x_1x_2$$

where Y = the estimated response (% survival); x_0 = intercept; x_1 and x_2 = the linear effect of salinity (Sal) and lead nitrate concentration (Conc), respectively; x_1^2 and x_2^2 = the quadratic effect of salinity (Sal^2) and lead nitrate concentration (Conc^2), respectively; x_1x_2 = the linear*linear interaction between salinity and lead nitrate concentration (Sal*Conc); and $b_0, b_1,..$, etc. = the regression coefficients. All data were interpreted by the Statistical Analysis System (SAS).

RESULTS

Exploratory test

The mortality caused by various lead nitrate concentrations is shown in Table 1. These values were used to calculate LD_{50} by probit analysis. The LD_{50}'s of lead nitrate were 35, 25, 15 and 1 ppm, respectively, at 24, 48, 72 and 96 hours and at a salinity of 15 ppt.

Combined test with 4-day-old larvae

Without lead nitrate, prawn larvae showed a maximal survival at salinity 15 ppt. The combined effects of salinity and lead nitrate on survival results are shown in Table 2.

After 24 hours, the highest percent survival was found in treatments where the lead nitrate concentration was less than 15 ppm and the salinity was between 10 and 25 ppt. Higher larval mortality occurred at high lead nitrate concentrations and high salinity. Response surface analysis of survival data produced the following second order polynomial expression:

$$Y = 75.1559 + 0.5480Sal + 0.015Sal^2 - 0.0527Conc + 0.0051Conc^2 - 0.0607Sal*Conc, \quad R^2 = 0.4804.$$

The equation was used to generate a contour of the response as shown in Figure 1a.

After 48 hours, the highest percent survival was found where the lead nitrate concentration was less than 15 ppm and the salinity was between 10 and 20 ppt. Higher larval mortality occurred at high lead nitrate concentration and higher salinity. Response surface analysis of survival data produced the following second order polynomial expression:

$$Y = 73.6427 + 1.4132Sal - 0.0429Sal^2 + 0.2805Conc - 0.0168Conc^2 - 0.0658Sal*Conc, \quad R^2 = 0.622.$$

The equation was used to generate a contour of the response as shown in Figure 1b.

After 72 hours, the highest percent survival was found where the lead nitrate concentration was 1 ppm and the salinity was 15 ppt. Higher larval mortality occurred at high lead nitrate concentration and high salinity. Response surface analysis of survival data produced the following second order polynomial expression:

$$Y = 43.4902 + 4.2198Sal - 0.1419Sal^2 - 0.6271Conc + 0.0165Conc^2 - 0.0655Sal*Conc, \quad R^2=0.422.$$

The equation was used to generate a contour of the response as shown in Figure 1c.

After 96 hours, the highest percent survival was found where the lead nitrate concentration was 1 ppm and the salinity was 15 ppt. Moreover, larval mortality increased with increasing lead nitrate concentration and salinity. Response surface analysis of survival data produced the following second order polynomial expression:

$$Y = 43.3900 + 3.6476Sal - 0.1257Sal^2 - 2.1491Conc + 0.0439Conc^2 - 0.0392Sal*Conc, \quad R^2=0.3717.$$

The equation was used to generate a contour of the response as shown in Figure 1d.

During the experiment, the sick larvae showed a syndrome of less movement, swam with no direction, tried to reach surface of the solution for respiration, sank to bottom of the container and died finally.

Combined tests with 14-day larvae

The survival of 14-day larvae (larval stage V) on combined effects of salinity and lead nitrate was different from that of the 4-day larvae. The larvae were more resistant to toxicity of lead nitrate. The combined effects of salinity and lead nitrate on survival results are shown in Table 3.

After 24 hours, both salinity and lead nitrate showed less effect to larval mortality. Response surface analysis of percent survival produced the following second order polynomial expression:

$$Y = 95.3994 + 0.4076Sal - 0.0181Sal^2 - 0.5737Conc + 0.0094Conc^2 \; 0.0023Sal*Conc, \quad R^2=0.1667.$$

The equation was used to generate a contour of the response as shown in Figure 2a.

After 48 hours, the highest percent survival was found at salinity between 10 and 15 ppt. The lead nitrate concentration range in the present experiment showed less effect to larval mortality. Response surface analysis of survival data produced the following second order polynomial expression:

$$Y = 81.0470 + 2.5981Sal - 0.1029Sal^2 - 0.7411Conc + 0.0079Conc^2 + 0.0110Sal*Conc, \quad R^2=0.1888.$$

The equation was used to generate a contour of the response as shown in Figure 2b.

After 72 hours, the highest percent survival was found where the lead concentration was less than 15 ppm and the salinity was between 10 and 15 ppt. Higher larval mortality was found in the treatments of high lead nitrate concentration and high salinity. Response surface analysis of survival data produced second order polynomial expression:

$$Y = 81.4595 + 2.2576Sal - 0.0981Sal^2 - 0.2717Conc - 0.0088Conc^2 - 0.0054Sal*Conc, \quad R^2=0.2758.$$

The equation was used to generate a contour of the response as shown in Figure 2c.

After 96 hours, the highest percent survival was found in a low lead nitrate concentration at the salinity of 15 ppt. It was quite clear that salinity between 10 and 15 ppt was an optimal condition for the prawn larvae. At different lead nitrate concentrations the larvae showed more survival at these salinities. Response surface analysis of survival data produced the following second order polynomial expression:

$$Y = 60.7379 + 4.4752Sal - .01571Sal^2 - 0.4575Conc - 0.0032Conc^2 - 0.0198Sal*Conc, \quad R^2=0.3026.$$

The equation was used to generate a contour of the response as shown in Figure 2d.

Combined tested with 24-day larvae

The prawn larvae aged 24 days showed higher survival rate at low salinity than at high salinity. The combined effects of salinity and lead nitrate on survival results are shown in Table 4.

After 24 hours, the highest percent survival was 100 where the lead nitrate concentration was 1 ppm and the salinity was 10 ppt. Higher larval mortality occurred at high salinity with less effect of lead nitrate. Response surface analysis of survival data produced the following second order polynomial expression:

$$Y = 61.7050 + 7.7660Sal - 0.3200Sal^2 - 0.2157Conc + 0.0272Conc^2 - 0.0934Sal*Conc, \quad R^2=0.8016.$$

The equation was used to generate a contour of the response as shown in Figure 3a.

After 48 hours, the highest percent survival of larvae (100%) was found where the lead nitrate concentration lower than 25 ppm and the salinity was between 10 and 15 ppt. Furthermore, high larval mortality was found at high salinity and high lead nitrate concentration. Response surface analysis of survival data produced the following second order polynomial expression:

$$Y = 56.9159 + 8.9676Sal - 0.3895Sal^2 + 1.7451Conc - 0.0394Conc^2 - 0.0848Sal*Conc, \quad R^2=0.2733.$$

The equation was used to generate a contour of the response as shown in Figure 3b.

After 72 hours, the highest percent survival was found where the lead nitrate concentration was 1 ppm and the salinity was 10 ppt. Moreover, at high salinity larval mortality was higher than at low salinity. The effect of lead nitrate was no important. Response surface analysis of survival data produced the following second order polynomial expression:

$$Y = 66.5158 + 5.3071Sal - 0.2400Sal^2 - 0.9991Conc + 0.0466Conc^2 - 0.0992Sal*Conc, \quad R^2=0.7058.$$

The equation was used to generate a contour of the response as shown in Figure 3c.

After 96 hours, the highest percent survival was found where the lead nitrate concentration was 1 ppm and the salinity was between 5 and 15 ppt. Furthermore, high larval mortality was found at higher salinity. However, the effect of lead nitrate concentration showed no important to larval mortality. Response surface analysis of survival data produced the following second order polynomial expression:

$$Y = 75.9000 + 2,8684Sal - 0.1619Sal^2 - 1.8576Conc + 0.0523Conc^2 - 0.0639Sal*Conc, \quad R^2=0.6129.$$

The equation was used to generate a contour of the response as shown in Figure 3d.

TABLE 1 Percent mortality of *M. rosenbergii* larvae at various lead nitrate concentrations.

Lead nitrate concentration (ppm)	number of larvae in experiment	Replication	Percentage of mortality larvae											
			24 hours			48 hours			72 hours			96 hours		
			No. of mortality	Death (%)	Death average (%)	No. of mortality	Death (%)	Death average (%)	No. of mortality	Death (%)	Death average (%)	No. of mortality	Death (%)	Death average (%)
0	30	1	0	0.00	0.00	2	6.67	5.00	1	10.00	6.67	1	13.35	8.33
	30	2	0	0.00		1	3.33		0	3.33		0	3 33	
1	30	1	3	10.00	15.00	4	23.33	33.33	3	33.33	41.67	2	40.00	50.00
	30	2	6	20.00		7	43.33		2	50.00		3	60 00	
15	30	1	13	43.33	33.33	3	53.33	43.33	2	60.00	53.33	5	76.67	73.34
	30	2	7	23.33		3	33.33		4	46.67		7	70.00	
25	30	1	11	36 67	38.33	5	53.33	53.33	6	73.33	70.00	5	90 00	86.67
	30	2	12	40.00		4	53.33		4	66 67		5	83.33	
35	30	1	13	43.33	50.00	10	76.67	80.00	2	83.33	86.67	2	80.00	91 67
	30	2	17	56 67		8	83.33		2	90.00		1	83 33	

TABLE 2 Mean percent survival of *M. rosenbergii* larvae age 4 days under a range of salinities and lead nitrate concentrations observed at 24, 48, 72 and 96 hours. Each value is the mean of three replicates.

HOUR	SALINITY (ppt)	CONCENTRATION (ppm)				
		0	1	15	25	35
24	5	90.00	63.33	58.33	71.67	78.33
	10	85.00	81.67	81.67	73.33	66.67
	15	100.00	81.67	86.67	58.33	56.67
	20	95.00	65.00	76.67	46.67	40.00
	25	100.00	90.00	75.00	66.67	55.00
48	5	90.00	70.00	75.00	60.00	45.00
	10	85.00	80.00	81.67	85.00	61.67
	15	100.00	71.67	71.67	51.67	41.67
	20	90.00	61.67	48.33	53.33	18.33
	25	100.00	75.00	66.67	31.67	18.33
72	5	90.00	23.33	51.67	53.33	36.67
	10	85.00	56.67	55.00	68.33	51.67
	15	100.00	58.33	51.67	40.00	40.00
	20	90.00	45.00	26.67	41.67	11.67
	25	95.00	20.00	41.67	15.00	5.00
96	5	90.00	23.33	33.33	15.00	23.33
	10	85.00	41.67	43.33	63.33	41.67
	15	100.00	48.33	40.00	5.00	38.33
	20	90.00	28.33	20.00	18.33	1.67
	25	95.00	5.00	45.00	8.33	0.00

TABLE 3 Mean percent survival of *M. rosenbergii* larvae age 14 days under a range of salinities and lead nitrate concentrations observed at 24, 48, 72 and 96 hours. Each value is the mean of three replicates.

HOUR	SALINITY (ppt)	CONCENTRATION (ppm)				
		0	1	15	25	35
24	5	100.00	96.67	91.67	93.33	80.00
	10	95.00	93.33	90.00	90.00	98.33
	15	100.00	93.33	90.00	93.33	88.33
	20	100.00	93.33	90.00	95.00	83.33
	25	100.00	93.33	78.33	90.00	90.00
48	5	95.00	93.33	86.67	83.33	71.67
	10	95.00	81.67	86.67	83.33	88.33
	15	100.00	93.33	96.67	85.00	83.33
	20	100.00	88.33	90.00	91.67	81.67
	25	100.00	65.00	66.67	70.00	80.00
72	5	95.00	88.33	90.00	73.33	63.33
	10	95.00	88.33	85.00	78.33	83.33
	15	95.00	86.67	95.00	75.00	66.67
	20	100.00	70.00	86.67	66.57	68.33
	25	100.00	55.00	70.00	58.33	50.00
96	5	95.00	61.67	83.33	56.33	53.33
	10	95.00	78.33	73.33	73.33	76.67
	15	95.00	83.33	90.00	68.33	51.67
	20	100.00	83.33	80.00	55.00	56.67
	25	100.00	40.00	63.33	46.67	40.00

TABLE 4 Mean percent survival of *M. rosenbergii* larvae age 24 days under a range of salinities and lead nitrate concentrations observed at 24, 48, 72 and 96 hours. Each value is the mean of three replicates.

HOUR	SALINITY (ppt)	CONCENTRATION (ppm)				
		0	1	15	25	35
24	5	100.00	98.33	93.33	96.67	96.67
	10	100.00	95.00	91.67	88.33	95.00
	15	100.00	93.33	93.33	93.33	86.67
	20	95.00	90.00	85.00	51.67	55.00
	25	90.00	28.33	3.33	1.67	1.67
48	5	100.00	83.33	90.00	85.00	83.33
	10	100.00	86.67	86.67	76.67	90.00
	15	100.00	88.33	86.67	86.67	86.67
	20	95.00	70.00	53.33	33.33	28.33
	25	90.00	15.00	1.67	0.00	0.00
72	5	100.00	70.00	83.33	85.00	76.67
	10	100.00	81.67	75.00	68.33	85.00
	15	100.00	78.33	80.00	85.00	85.00
	20	95.00	55.00	33.33	3.33	0.00
	25	90.00	11.67	0.00	0.00	0.00
96	5	100.00	66.67	73.33	66.67	63.33
	10	100.00	66.67	66.67	53.33	68.33
	15	100.00	63.33	71.67	53.33	65.00
	20	90.00	35.00	18.33	15.00	0.00
	25	90.00	8.33	0.00	0.00	0.00

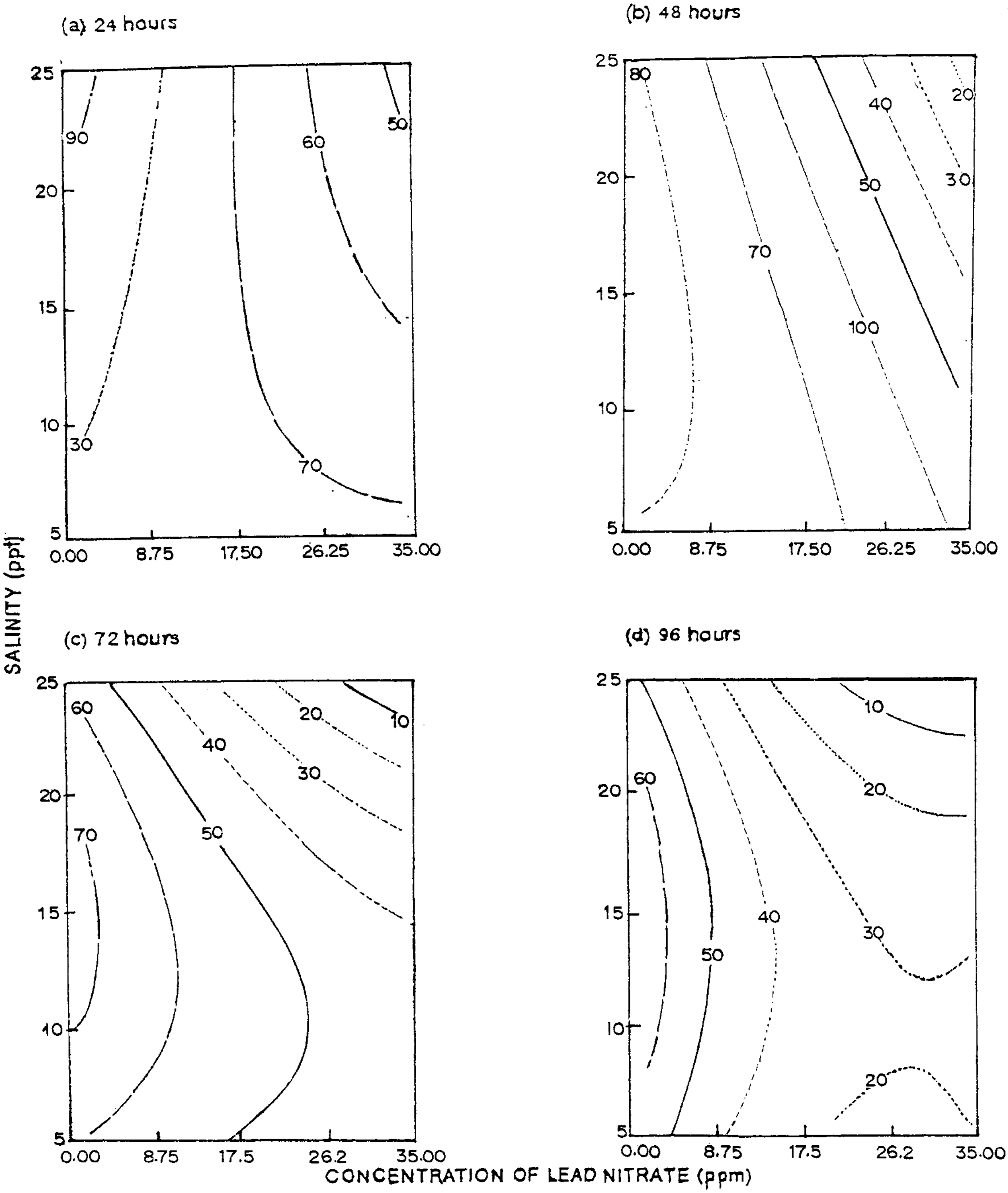

Fig.1 The combined effects of salinity and lead nitrate concentration on survival rate (%) of 4-day old *M. rosenbergii* larvae; (a) 24 hours, (b) 48 hours, (c) 72 hours, and (d) 96 hours.

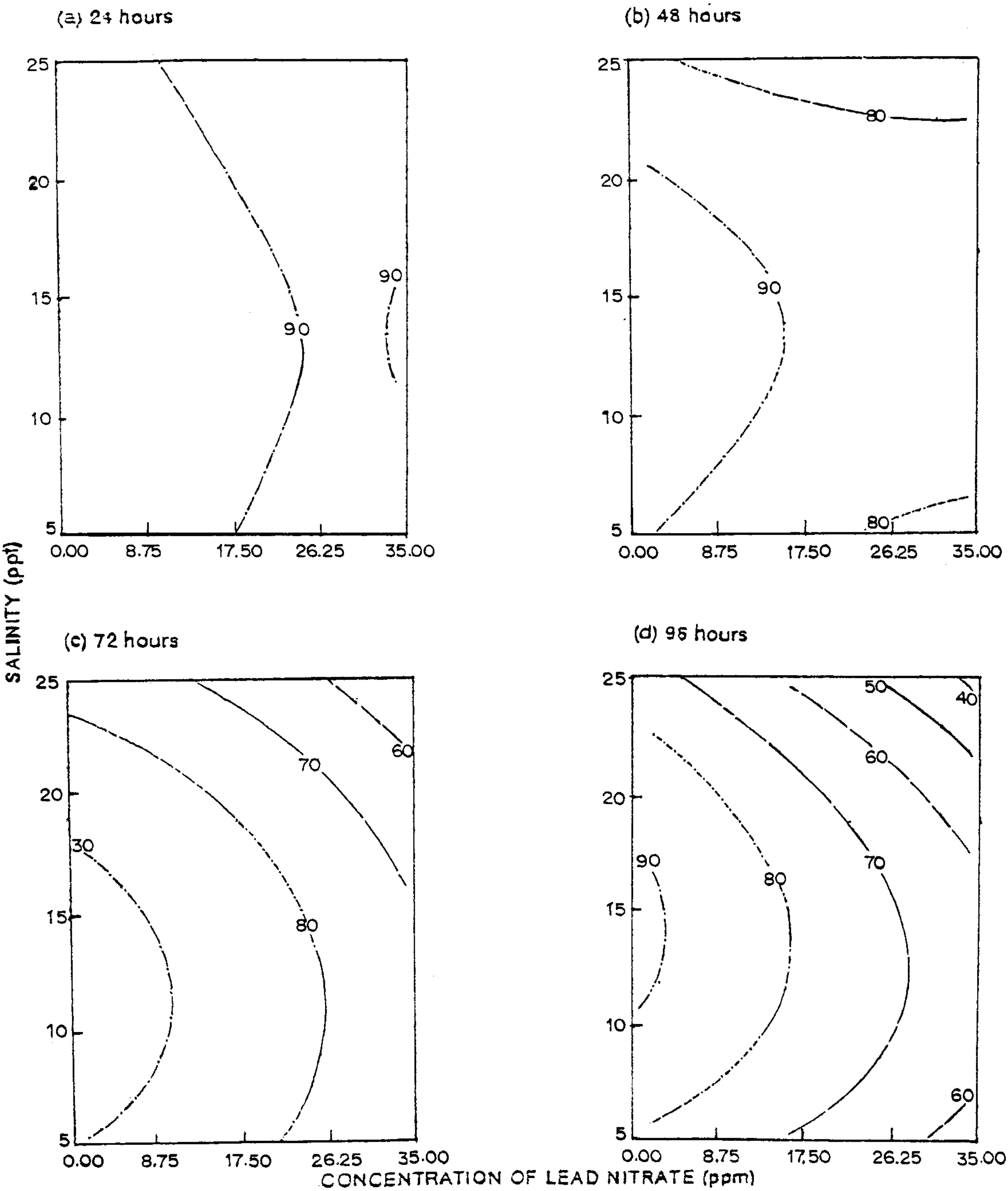

Fig.2 The combined effects of salinity and lead nitrate concentration on survival rate (%) of 14-day old *M. rosenbergii* larvae; (a) 24 hours, (b) 48 hours, (c) 72 hours, and (d) 96 hours.

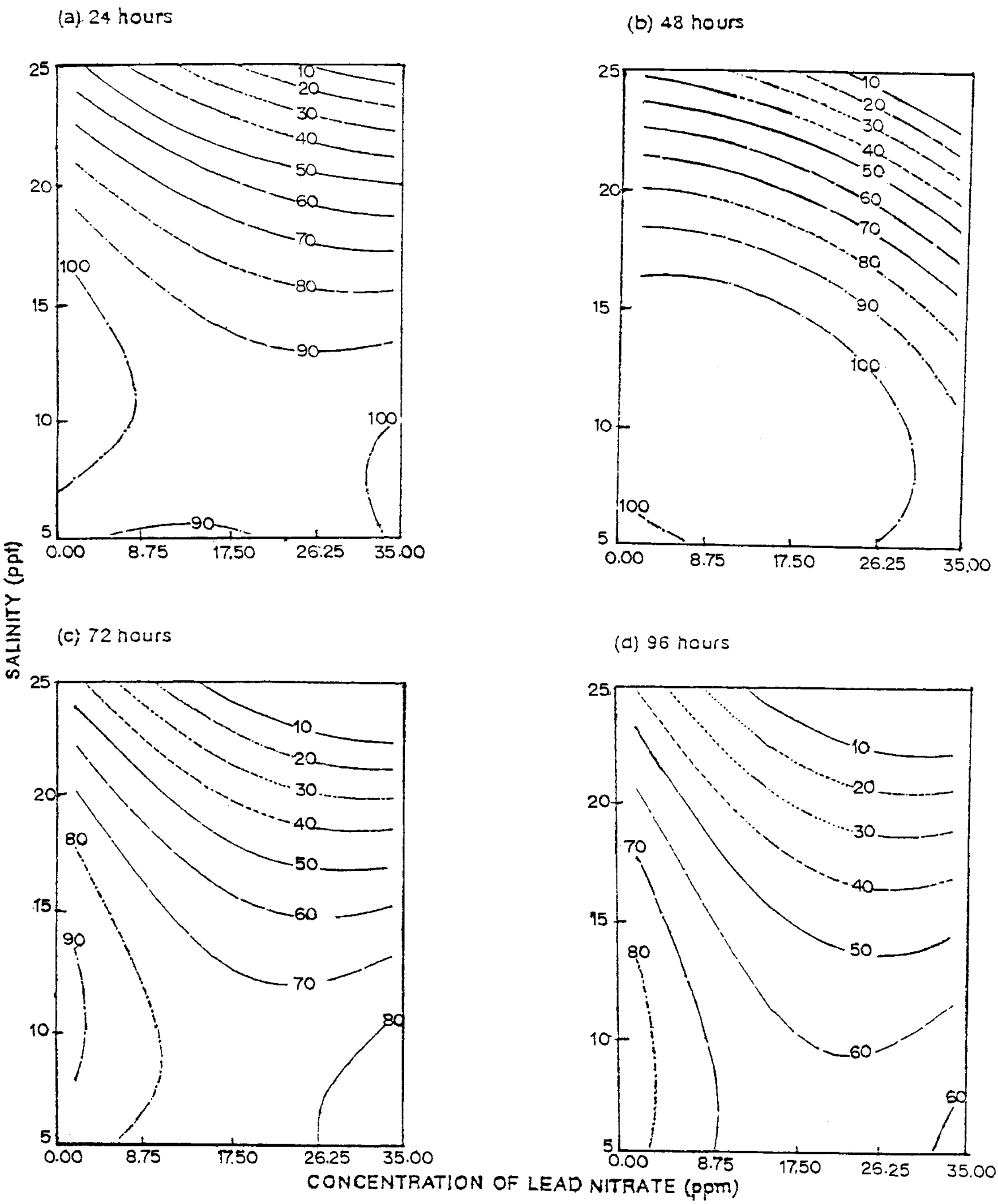

Fig.3 The combined effects of salinity and lead nitrate concentration on survival rate (%) of 24-day old *M. rosenbergii* larvae; (a) 24 hours, (b) 48 hours, (c) 72 hours, and (d) 96 hours.

DISCUSSION

The larvae of *M. rosenbergii* survive and develop in an estuarine environment where many environmental parameters are not stable. The water may be contaminated by toxicants from human activities[9] and salinity can vary abruptly depending upon local conditions. Prawn larvae can tolerate a broad range of salinity, but Ling and Merican[10] and Fujimura[11] have reported that the optimum salinity for *M. rosenbergii* larvae is 12-14 ppt, and that the optimum salinity changes during larval development[12]. This quite agrees with our results, in which the highest percent survival of 4, 14 and 24 days old larvae corresponded with salinity 15, 10-15, and 10 ppt, respectively. The median lethal concentration of lead nitrate at 96 hours (1 ppm) provides strong information that giant freshwater prawn larvae are very sensitive to lead nitrate toxicity. This compares with Kalayanamitr[13] results which showed that median lethal doses of lead nitrate for *M. rosenbergii* juveniles were 290.8, 259.5, 243.7 and 233.4 ppm at 24, 48, 72 and 96 hours exposure, respectively.

One difficulty encountered in tests with high concentration of heavy metals in solution is that the effective concentration can be reduced by absorption to the container wall, precipitation, volatilization, and uptake by organisms[14]. For example, in our tests only a period of 24 hours at a salinity of 25 ppt with a lead nitrate concentration of 35 ppm, some amount of lead nitrate precipitated at the bottom of the container. We attempted to compensate for this by every 24 hours replacing the test solution with freshly prepared solution.

Our results strongly indicated that 14-day old larvae were more tolerant to lead nitrate than other larval stages. Consequently, its LD_{50}-96 hours of lead nitrate was higher than 35 ppm comparing to 4 and 24-day old larvae's LD_{50}-96 hours which were much lower than 35 ppm (Figure 1, 2 and 3). It can be explained that 14-day old larvae (stage V) are full planktonic stage and has longer moulting duration, comparing to 4-day old larvae (stage II) with moulting duration only one or two days. All crustaceans, moulting is very important for their survival. During moulting or early post-moulting, the prawn are extremely weak to environmental changes. This may be a reason why early larval stage of the giant freshwater prawn are less tolerant to toxicity of lead nitrate. Hence, our experiment clearly indicated that the higher salinity, the more lead nitrate sensitivity can be detected in the giant freshwater prawn larvae. Connor's[15] report encourages our experimental results in that larvae of marine crustaceans are more sensitive to heavy metal toxicity than the adult. Vernberg and Vernberg[16] clearly reviewed that larval stages of marine crustaceans are extremely sensitive to toxicity and environmental changes which also supports our results. In addition, the combined effect of salinity and lead nitrate toxicity in this study verified that at higher salinity the toxicity of lead is magnified. Hence, Lead nitrate is much more toxic to the prawn larvae at high salinity.

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