

**THE ACUTE TOXICITY OF FOUR HEAVY METALS (Cd^{++} , Cr^{+++} , Cu^{++} , and Zn^{++})
TO THE JUVENILE SPOTTED BROWN SHRIMP (*PENAEUS BRASILIENSIS*)**

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SYNOPSIS

The static bioassays were performed to determine lethal concentration (LC_{50}) values of four heavy metals (Cd^{++} , Cr^{+++} , Cu^{++} , and Zn^{++}) of spotted brown shrimp (*Penaeus brasiliensis*). The test animals were collected from the Laguna of Restinga, Nueva Esparta, and were acclimated in the laboratory condition for 11-21 days. Temperature and salinity were 22 °C and 36 ppt, respectively. The LC_{50} values (mg/l) determined by probability-logarithm transformation were as follows-cadmium: 38 (24-h), 21 (48-h), and 12 (60-h); chromium: 40 (48-h), 23 (60-h), 13 (86-h), and 10 (96-h); copper: 24 (48-h), 19 (60-h), 16 (72-h), 14 (86-h), and 10 (144-h); and zinc: 69 (24-h), 25 (36-h), 15 (48-h) and 8 (60-h). The LC_{50} values of 48-h and 60-h indicate that the most toxic heavy metals to *P. brasiliensis* in order are zinc, cadmium, copper, and chromium.

Introduction

Most previous studies concerned with the effects of heavy metals have dealt with fishes and some invertebrates (Ball, 1967; Brown, 1968; Cairns & Scheier, 1958; Eisler, 1971; Mount, 1968; Negilski, 1976; Patrick *et al.*, 1968; Pickering & Henderson, 1966; Trama & Benoit, 1960; Wisely & Blick, 1967). Information on the lethal concentration values of the crustaceans is limited to Portman (1968), Arthur & Leonard (1970), and Eisler (1971).

The pink spotted shrimp (*Penaeus brasiliensis*) is one of the most important shrimp fisheries resources in the coastal areas of Venezuela (Ewald, 1967; Khandker, 1965). On the coast of Margarita Island, this species is the major shrimp catch resource, and 95% of shrimp collected from 50 m in depth and almost 100% of shrimp from 50-70 m are *P. brasiliensis* (Khandker & Lares, 1973). The maturity and fecundity of this species was reported by Lares (1976). However, the information is limited for the effects of heavy metals in this species.

The purpose of this study is to determine survival of *P. brasiliensis* at four heavy metals, cadmium ($CdCl_2 \cdot H_2O$), chromium (CrO_3), copper ($CuSO_4 \cdot 5H_2O$), and zinc ($ZnSO_4 \cdot 7H_2O$). The pink spotted shrimp was selected as the test animal because of its abundance in the coastal areas of Venezuela and its economical and ecological significance among fisheries resources. This report investigates the effects of potential heavy metal pollutants, which may be present in domestic wastes in concentrations above ambient, especially near the industrialized areas, on components of marine biota.

Materials and Methods

The pink spotted shrimp (*Penaeus brasiliensis*) were collected from the Laguna Restinga, Nueva Esparta State, Venezuela (Fig. 1). Average total body length (from the tip of the rostrum to the end of the telson) was 40 mm. Water temperature and salinity were 26 °C and 52 ppt, respectively. The shrimp were placed in two asbestos tanks (1-x 1-x 1-m) and transported from Margarita to Cumaná with aeration. During transportation, salinity was adjusted to 36 ppt. About 2,000 shrimp were placed in each asbestos tank and their survival was excellent (almost 100%). The shrimp were held in Turpialito Mariculture Tanks (2-x 4-x 1-m depth) for 15 days and transported from Turpialito to the physiological ecology laboratory of the University of Oriente, Cumaná. In the laboratory, animals were placed in aquaria (30-x 60-x 40-cm depth) and acclimated for 11-21 days. Acclimation temperature and salinity were 22 °C \pm 1.5 and 36 ppt, respectively.

Iniciarina (Purina Co.) was fed as the rate of about 5% of total body wet weight of shrimp every day during acclimation periods. The food was not provided 24 hours prior to the experiment.

The metals used were cadmium, chromium, copper, and zinc. The amounts required to make 10 g/l stock solution for each metals are: 17.9 g ($CdCl_2 \cdot H_2O$), 19.2 g (CrO_3), 39.3 g ($CuSO_4 \cdot 5H_2O$), and 43.9 g ($ZnSO_4 \cdot 7H_2O$).

The different concentrations used in each bioassay were prepared from the respective stock solution as indicated in Table I. The sodium citrate was used as a chelating agent in the copper and zinc experiments.

Twenty animals were placed in 4 replicate flasks, 16-x 16-x 20-cm depth, (5 animals in a flask) at each lethal concentration of heavy metal. Four-hundred-eighty (480) shrimps were tested using the four heavy metal toxicants. Food was not supplied during bioassay experiments. The experiments lasted until 50% or

Table I - Concentration of heavy metals in bioassay experiments (mg/l) and hydrological data.

Metal	Concentration (mg/l)	Temperature (°C)	Salinity (PPT)	pH
Cadmium	0, 1, 5, 20, 60, 150	22.0-22.7	36	7.3-8.4
Chromium	0, 5, 10, 20, 40, 80	21.5-23.5	36	7.9-8.0
Copper	0, 5, 10, 20, 40, 80	21.7-22.5	36	7.7-7.8
Zinc	0, 5, 10, 20, 40, 80	21.3-23.2	36	7.5-7.7

more of the animals died in the weakest lethal concentration or until the animals in the controlled solution started to die.

The mortality was observed at 30-min, 1-h, 2-h, 4-h, 8-h, 12-h, 24-h, 36-h, 48-h, 60-h, 72-h, 84-h, 96-h, 120-h, and 144-h intervals. The lethal concentration value, LC_{50} (the lethal concentration of 50% mortality), was determined by straight-line graphical interpolation of probability (Y-axis) - logarithm (X-axis) transformation.

Results and Discussion

The percentages of survival of the pink spotted shrimp (*Penaeus brasiliensis*) tested at the lethal concentration of four heavy metals is presented in Figure 2. The lethal concentration values (LC_{50}) determined by probability logarithm transformation are summarized in Table II.

Table II - Lethal concentration (LC_{50}) values of four heavy metals to the juvenile pink spotted shrimp (*Penaeus brasiliensis*) acclimated at 22 °C and 36 PPT.

Metal	LC_{50} value (mg/l)							
	24-h	36-h	48-h	60-h	72-h	84-h	96-h	144-h
Cadmium	38		21	12				
Chromium			40	23		13	10	
Copper			24	19	16	14		10
Zinc	69	25	15	8				

The toxic levels of heavy metals tested were different depending upon exposure time. The percentages mortality at each lethal test concentration showed that cadmium was more toxic than zinc during 24 hours of exposure; however, zinc was more toxic to the pink spotted shrimp during 48 hours of exposure. The 48-h lethal concentration values of cadmium and copper were similar; however, the values were lower than those of chromium. The 84-h LC_{50} values of chromium and copper were similar. Also, the 96-h LC_{50} of chromium and the 144-h LC_{50} of copper were identical. The above information indicates that zinc is less toxic during short term period (24-h) but more toxic during long term period (after 24-h). The comparative results of the 48-h LC_{50} values indicate that zinc is the most toxic heavy metal to the pink spotted shrimp and chromium is the least toxic (Table II). The 60-h LC_{50} values indicate that the order of sensitivity to *P. brasiliensis* is zinc, cadmium, copper and chromium.

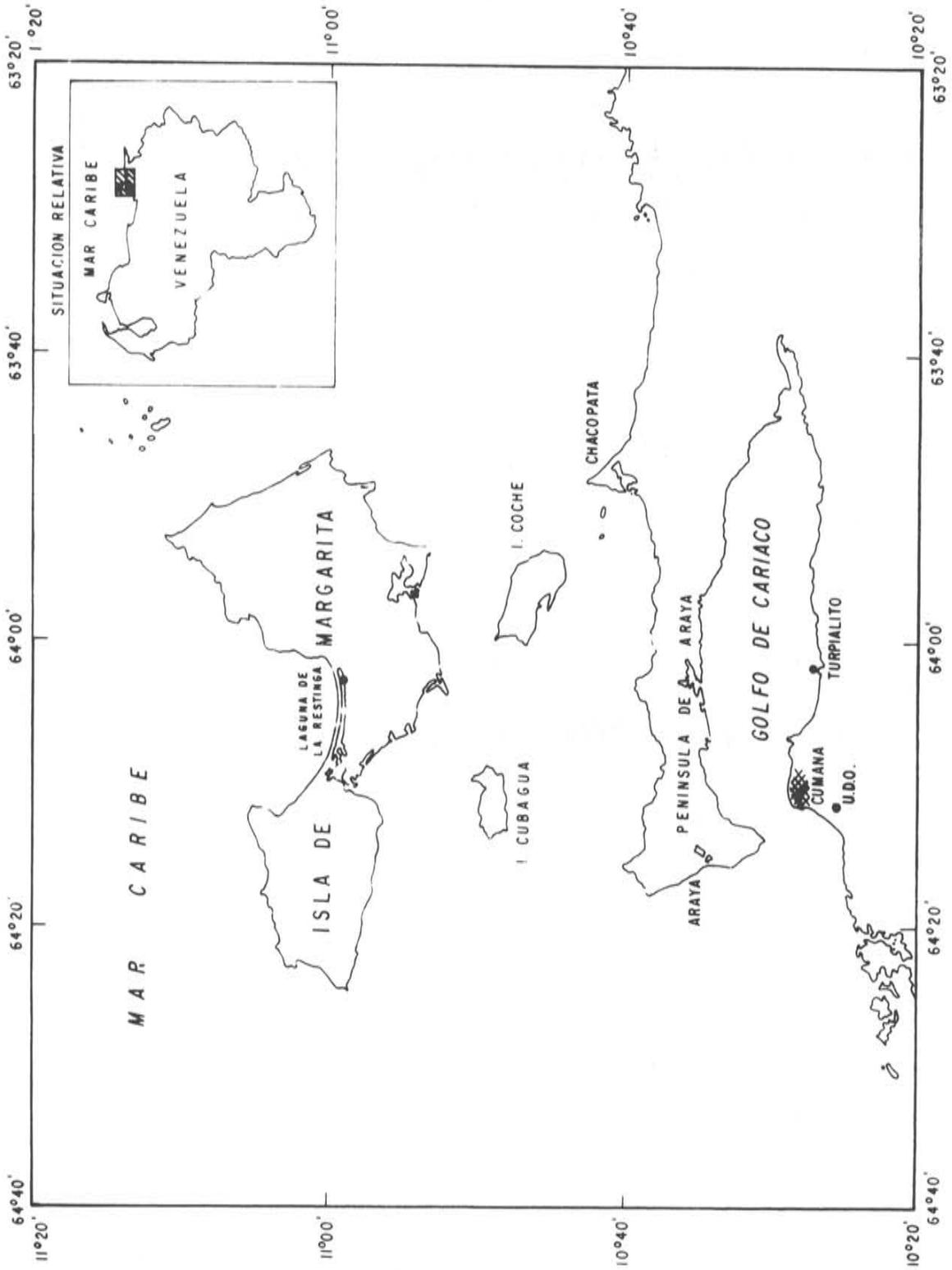


Fig. 1 - Map showing study area.

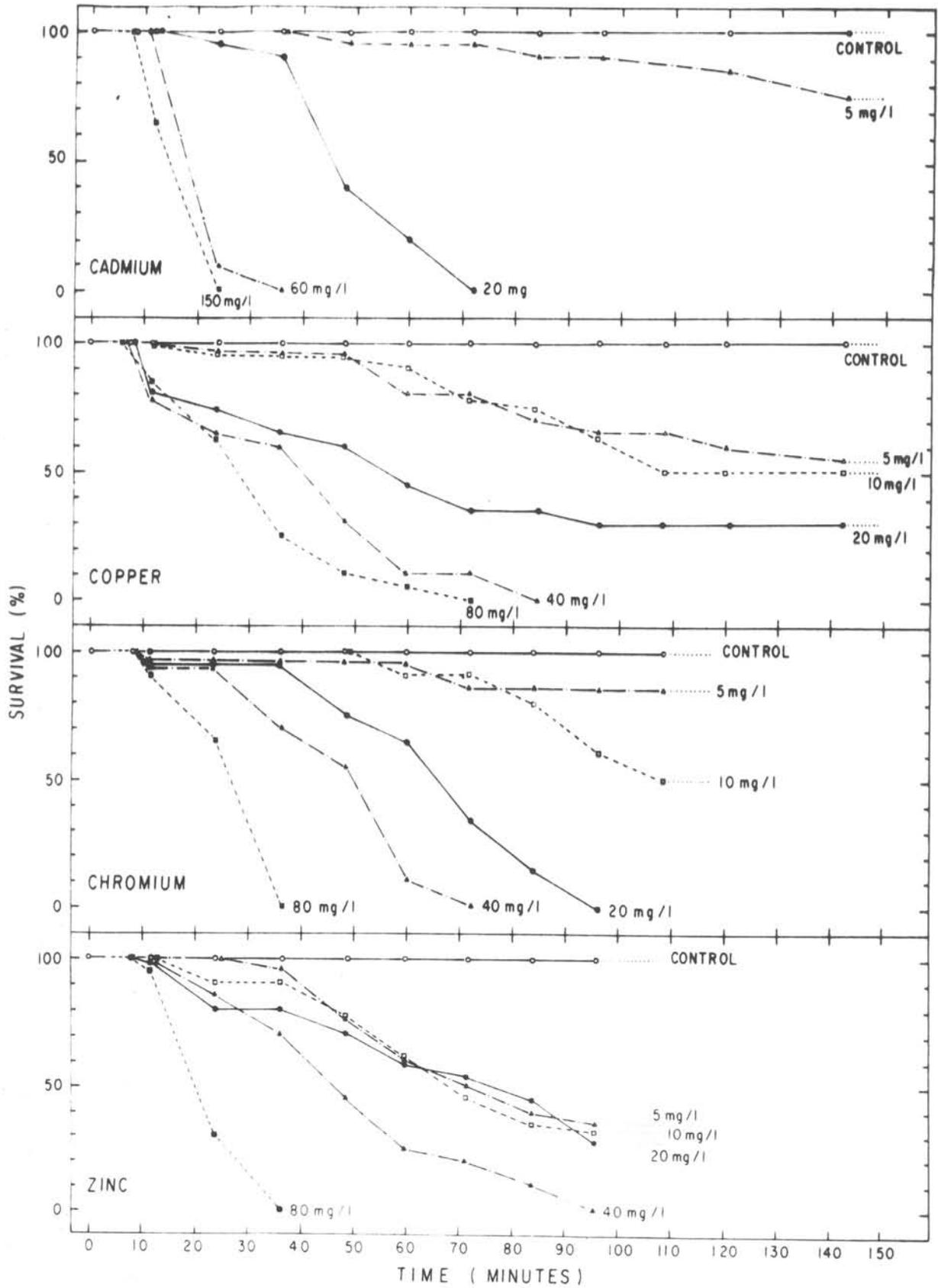


Fig. 2 - The percentages of survival of the pink spotted shrimp (*Penaeus brasiliensis*) acclimated at 22°C and 36‰ and tested at the lethal concentration of four heavy metals.

The 100% survival times (Figs. 3-6) at each lethal concentration were as follows:

- Cadmium: 36-h (5 mg/l), 12-h (20-60 mg/l), and 8-h (150 mg/l)
 Chromium: 48-h (5 mg/l), 36-h (10 mg/l), 24-h (20 mg/l), 12-h (40 mg/l), and 8-h (80 mg/l)
 Copper: 60-h (5 mg/l), 24-h (10 mg/l), and 8-h (20-80 mg/l)
 Zinc: 24-h (5 mg/l), 12-h (10-40 mg/l), and 8-h (80 mg/l)

The survival times at lethal concentration (5 mg/l) indicate that the most toxic heavy metals to *P. brasiliensis* in order are zinc, cadmium, chromium and copper.

The incipient lethal level (threshold, Figs 3-6) of four heavy metals tested were as follows:

- Cadmium: 5 mg/l (48-h), 20 mg/l (24-h), and 60 mg/l (12-h).
 Chromium: 5 mg/l (72-h), 10 mg/l (48-h), 20 mg/l (36-h), and 40 mg/l (24-h).
 Copper: 5 mg/l (144-h), 10 mg/l (60-h), and 20 mg/l (12-h).
 Zinc: 5 mg/l (36-h) and 40 mg/l (24-h).

The threshold information, also, indicates that the order of sensitivity of the pink spotted shrimp is zinc, cadmium, chromium and copper.

Comparisons with former work are difficult because of variations in the experimental periods and the differences in the species of crustaceans employed.

The 96-h LC₅₀ of the pink spotted shrimp for cadmium was not determined: however, its value should be greater than 5 mg/l because 75% of animals survived for 144 hours at lethal concentration of 5 mg/l. This indicates that *P. brasiliensis* has a greater resistance to cadmium pollutants than *Crangon septemspinosa*, *Pagurus longicarpus*, *Palaemonetes vulgaris*, and *Carcinus maenus* (Table III).

The 48-h LC₅₀ value of *P. brasiliensis* for copper was higher than that of *Gammarus pseudolimnaeus* and *Pandalus montagui* but lower than that of *Crangon crangon* and *C. maenus* (Table III). This indicates that the order of sensitivity to copper would be *G. pseudolimnaeus*, *P. montagui*, *P. brasiliensis*, *C. crangon*, and *C. maenus* among the crustaceans reported.

The 48-h LC₅₀ value of *P. brasiliensis* for zinc was a little higher than that of *P. montagui* and *C. maenus*; however, lower than that of *C. crangon*. This indicates that *C. crangon* is the most

Toxicant	Species	LC ₅₀ mg/l	Water Quality Condition		Source
			Temperature (°C)	Salinity (‰)	
Cu	<i>Gammarus pseudolimnaeus</i>	0.02 (96-h)	-	-	Arthur & Leonard (1970)
Cu	<i>Pandalus montagui</i>	0.2 (48-h)	15	-	Portman (1968)
Cu	<i>Crangon crangon</i>	30 (48-h)	15	-	"
Cu	<i>Carcinus maenus</i>	100 (48-h)	15	-	"
Cd	<i>Crangon septemspinosa</i>	0.32 (96-h)	20	20	Eisler (1971)
Cd	<i>Pagurus longicarpus</i>	0.32 (96-h)	20	20	"
Cd	<i>Palaemonetes vulgaris</i>	0.42 (96-h)	20	20	"
Cd	<i>Carcinus maenus</i>	4.1 (96-h)	20	20	"
Zn	<i>Pandalus montagui</i>	10 (48-h)	15	-	Portman (1968)
Zn	<i>Crangon crangon</i>	100 (48-h)	15	-	"
Zn	<i>Carcinus maenus</i>	12 (48-h)	15	-	"

Table III - LC₅₀ values and experimental conditions from crustaceans exposed to copper, cadmium, zinc.

resistant to zinc toxicity and the other 3 species have similar sensitivity (Table III).

Overall information indicates that *P. brasiliensis* is much more resistant to heavy metal pollutants than primitive crustaceans; however, more sensitive than brachyran decapods.

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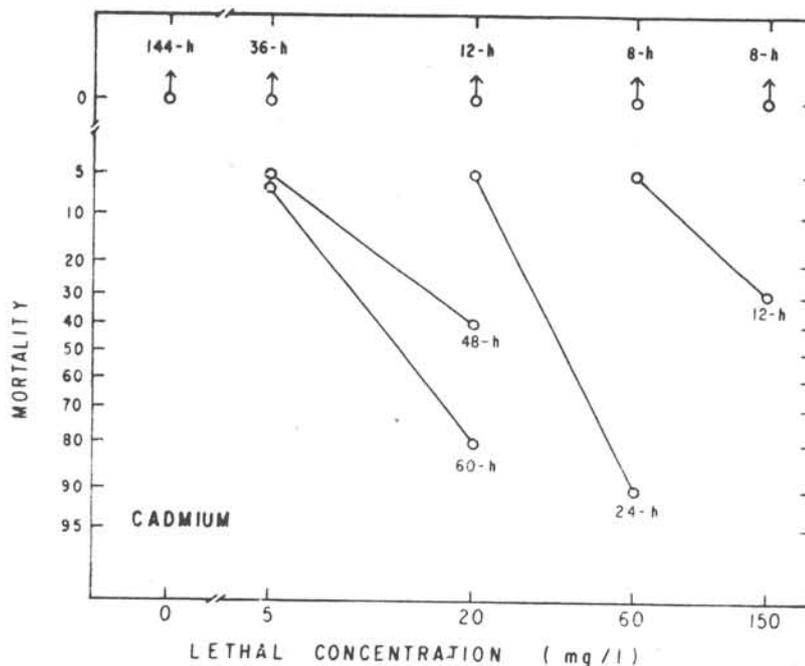


Fig. 3 - The percentage of mortality of *Penaeus brasiliensis* at various lethal concentrations of cadmium during 144 hours of exposure. ♂ indicates 100% survival time at the test concentration.

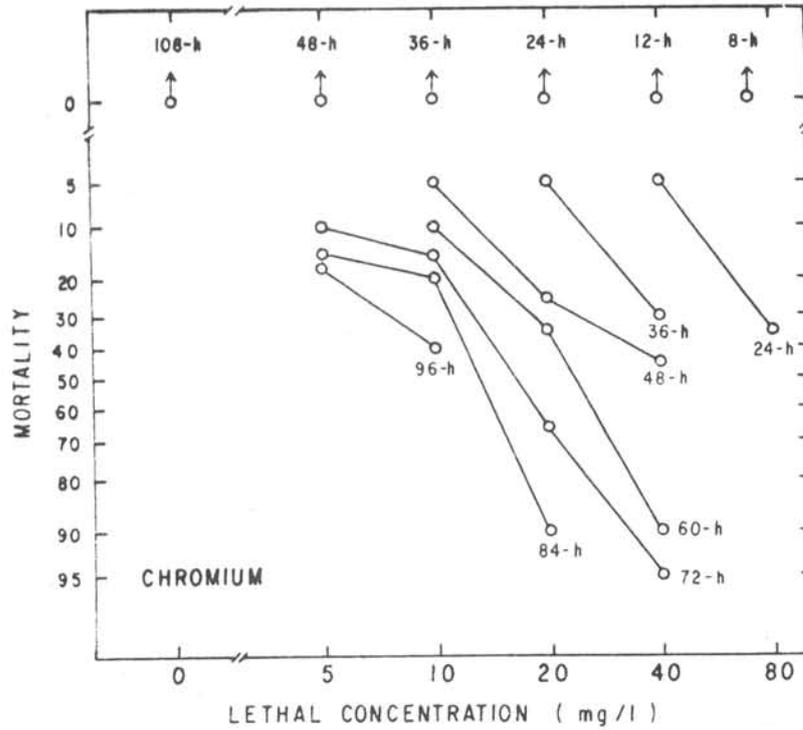


Fig. 4 - The percentage of mortality of *Penaeus brasiliensis* at various lethal concentrations of chromium during 108 hours of exposure. ♂ indicates 100% survival time at the test concentration.

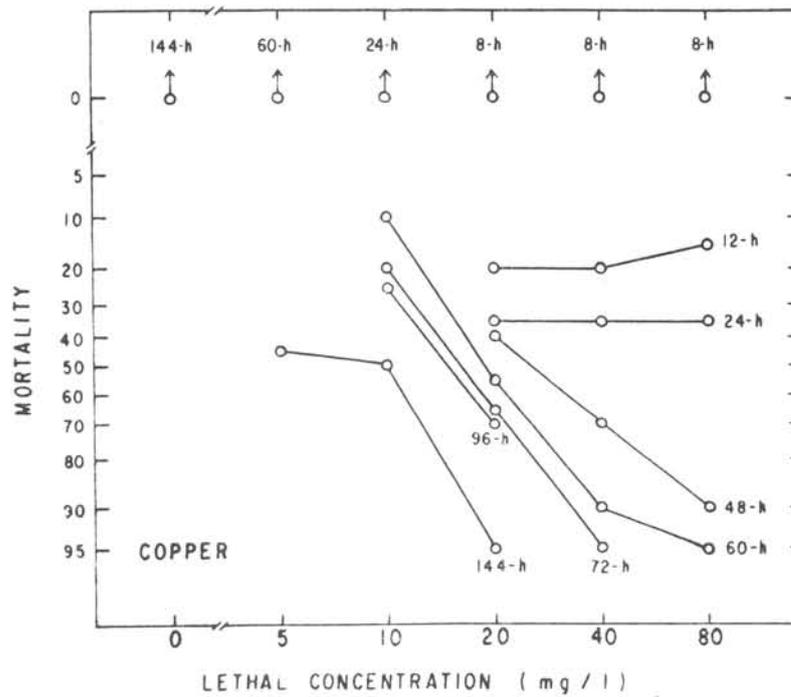


Fig. 5 - The percentage of mortality of *Penaeus brasiliensis* at various lethal concentrations of copper during 144 hours of exposure. ♂ indicates 100% survival time at the test concentration.

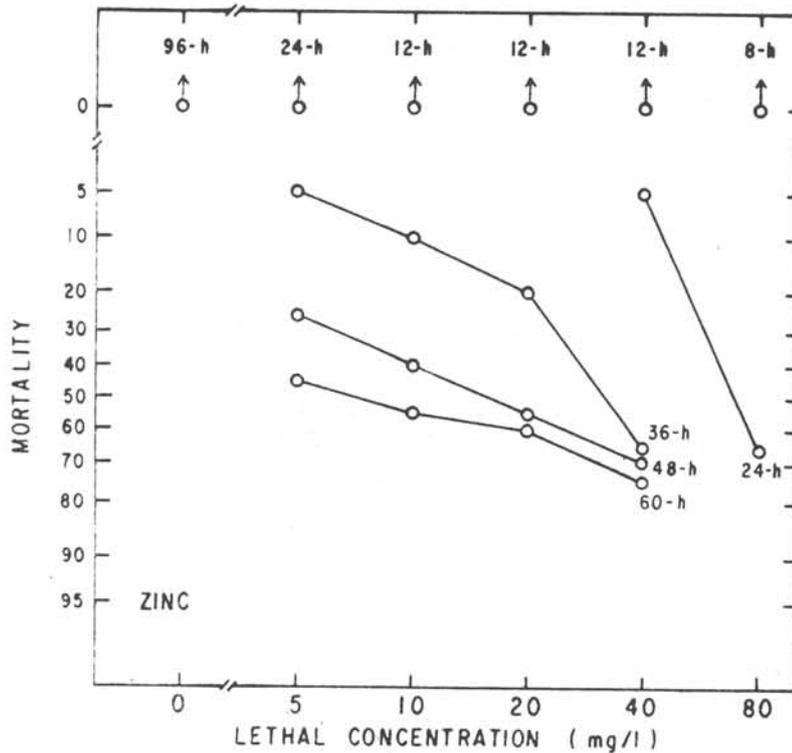


Fig. 6 - The percentage of mortality of *Penaeus brasiliensis* at various lethal concentrations of zinc during 96 hours of exposure. ♂ indicates 100% survival time at the test concentration.

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