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Acute Toxicity and Synergism of Cadmium and Zinc in White Shrimp, *Penaeus setiferus,* Juveniles

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Toxic effects of individual heavy metals on decapod crustaceans have been reported frequently, but little information exists concerning interactions. Among the non-essential heavy metals, cadmium is one of the most hazardous elements in the aquatic environment (UNEP 1985); on the other hand, zinc is an essential element, but toxic when present in greater than trace amounts. Biological effects of cadmium in aquatic organisms are complex due to the interactions with both environmental variables and other toxic agents. In decapod crustaceans, the toxicity of cadmium and zinc is modified by salinity, temperature, hypoxia, calcium ion concentrations and life-cycle stage (Thurberg et al. 1973; Vemberg et al. 1974; Rosenberg and Costlow 1976; Johnson 1988; Bjerregaard and Depledge 1994).

In recent years heavy metal pollution has increased in coastal waters of the Gulf of Mexico, mainly in lagunar-estuarine ecosystems (Botello et al. 1992). These systems are essential environments for shrimp production. White shrimp (Penaeus setiferus) supports one of the most important fisheries in the Campeche Sound (Gracia and Soto 1990). Despite the economic importance of the resource, studies concerning the toxic effects of heavy metals on these organisms are scarce. The present study examined the toxicity of cadmium and zinc to white shrimp juveniles. Additionally, the interaction of these metals was examined.

MATERIALS AND METHODS

Juvenile Penaeus setiferus (4.10 ± 0.29 g, wet weight) were captured during the winter months at Términos Lagoon, Campeche, Mexico. Shrimp were placed in 500-L containers fit with biological filters and were held for five days in 31.4% sea water (11 ± 1 ppt) at 23 ± l°C. To obtain the desired salinity, dechlorinated tap water was mixed with full-strength filtered sea water. Shrimp were fed daily with Camaronina commercial pellets (35% protein content; Purina Ralston Co.) at 10% body weight. A 12:12 hr light: dark cycle was maintained.

After acclimation, shrimp were exposed to cadmium and/or zinc for 96 hr; acute

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static toxicity tests were carried out according to UNEP (1987); water remained unchanged during the test and thus metals were not replenished. Test solutions were prepared from concentrated $CdCl_2$.2% H_2O and $ZnCl_2$ (Baker; 99%) stock solutions added to 11 ppt aquarium seawater. The actual metal concentrations tested were 0 (control), 0.43, 0.76, 2.58, 4.46, 4.80 and 5.80 mg Cd^{2+}/L and 0 (control), 1.40, 8.50, 11.60, 28.40, 62.50 and 105.10 mg Zn^{2+}/L. One replicate was used for each condition.

Groups of ten intermolt shrimp were transferred randomly from the holding tanks to 20-L glass aquaria filled with 11 ppt seawater, Animals were not fed 24 hr prior to the addition of the metals nor during the exposure period. Water temperature was maintained at 23 ± l°C, pH at 8.3 ± 0.05 and dissolved oxygen at 6.9 ± 0.05 mg/L. Aquaria were covered with plastic to reduce evaporative loss. Metal concentrations were verified at the beginning and at the end of the test experiments; in all experimental conditions, cadmium and zinc levels were not modified along the exposure time. Metal analyses were performed using an atomic absorption spectrophotometer (Shimadzu AA-630-12) according to Ponce-Vélez and Botello (1991). Shrimp mortality was recorded at 1, 6, 12, 48, 72 and 96 hr after the addition of toxicants. The absence of response to gentle mechanical stimulus was the criterion for death.

After the median lethal concentration (96-hr LC50) for each metal was obtained, two toxicity series for cadmium-zinc mixtures were conducted at toxicity ratios (TR) of 1:1 and 2.3:1. Experiments were carried out in duplicate with five test solutions with the toxicity units (TU) presented in Table 1. The metal concentrations in the mixtures are also shown,

Toxicity ratios and toxicity units were calculated according to Sprague and Ramsay (1965) as follows:

$$TR = Cd\text{-}m / Cd\text{-}t : Zn\text{-}m / Zn\text{-}t \; ; \; TU = (Cd\text{-}m / Cd\text{-}t) + (Zn\text{-}m / Zn\text{-}t)$$

where <u>m</u> refers to the metal concentrations (mg/L) in the mixture and <u>t</u> refers to their 96-hr LC50 (mg/L) values. The treatment of the animals and mortality observations were as mentioned above,

Table 1. Concentrations of cadmium and zinc (mg/L) in the mixtures at different toxicity ratios (TR) and toxicity units (TU).

TR	TU	Cd (mg/L)	Zn (mg/L)	TR	TU	Cd (mg/L)	Zn (mg/L)
	0.10	0.05	2.07		0.07	0.05	1.05
1:1	0.15	0.07	3.25	2.3:1	0.20	0.14	2.68
	0.28	0.14	6.15		0.26	0.18	3.38
	0.38	0.19	8.31		0.41	0.28	5.39
	0.55	0.27	12.06		0.59	0.41	7.85

The median lethal concentrations of cadmium and zinc (48, 72 and 96-hr LC50) and the toxicity units of the mixture of metals at both TR (1:1; 2.3:1) where 50 % of the animals died (48, 72 and 96-hr TU50), were estimated by the probit-log model using the DORES computational program (Ramírez 1989).

RESULTS AND DISCUSSION

It is known that environmental variables modify heavy metal toxicity (Bjerregaard and Depledge 1994). Thus, to avoid interferences, salinity was maintained at 11 ppt which is the optimum for juvenile P. setiferus corresponding to their blood osmotic equilibrium with the environment at 23 ± 1°C (Vanegas 1996). The increment in heavy metal toxicity at salinities below the optimum for various organisms has been reported in several crustacean species as in Gammarus duebeni exposed to zinc (Johnson and Jones 1990) and in Callinectes similis exposed to cadmium (Frank and Robertson 1979). Similarly, in Carcinus maenas and Cancer irroratus copper and cadmium exerted a synergistic toxic effect at salinities below the species' optimum ones (Thurberg et al. 1973).

The median lethal concentrations (96-hr LC50) for cadmium and zinc were 0.99 and 43.87 mg/L, respectively (Table 2). As expected, white shrimp juveniles were considerably more sensitive to cadmium than to zinc; cadmium toxicity was 44 times greater than that for zinc. There were no significant differences in LC50 values ($P > 0.05$) as time of exposure increased from 48 to 96 hr, although a decrease in concentration was observed. In aquatic organisms, sensitivity to heavy metals is related to the metal biological activity. In this context, the greater toxicity of cadmium might be expected since zinc is an essential metal that is regulated by decapod crustaceans, whereas cadmium has no known biological function (Rainbow and White 1989).

Juvenile P. setiferus were less sensitive to cadmium than other shrimps such as Crangon septemspinosa and Palaemonetes vulgaris and the isopod Elasmopus rapax whose 96-hr LC50 values were estimated between 0.32 to 0.42 mg/L (Zanders and Rojas 1992). However, the shrimp were more sensitive to cadmium than Callinectes sapidus and C. similis for which 96-hr LC50 values were 4.7 mg/L and 6.35 to 11.6 mg/L, respectively (Frank and Robertson 1979; Ramírez et al. 1989). On the other hand, white shrimp were less susceptible to the toxic effects of zinc than Daphnia magna and Ceriodauhnia dubia, whose 48-hr LC50 values were 1.22 and 0.32 mg/L, respectively (Magliette et al. 1995). Studies concerning the acute effect of zinc on aquatic crustaceans are scarce; thus, comparison of zinc toxicity in P. setiferus with other estuarine and marine shrimps was not possible.

For shrimp exposed to both heavy metals simultaneously, the toxic action of mixtures was synergistic (Table 2) since the TU values were less than unity at all times of exposure (Sprague and Ramsay 1965). At 1:1 TR, the TU calculated was reduced significantly ($P < 0.05$) between 48 and 96 hr (60%); whereas in the

Table 2. Metal concentrations (mg/L) in mixtures at different toxicity ratios (TR) and their toxicity units (TU) which caused death of 50% of the juvenile P. setiferus exposed for 48 to 96 hr.

		Mixtures			LC50	
TR	Time hr	TU	Cd (mg/L)	Zn (mg/L)	Cd (mg/L)	Zn (mg/L)
	48	0.78 (0.34)	0.39 (0.17)	16.15 (7.04)	1.35 (0.21)	78.86 (35.77)
1:1	72	0.40 (0.07)	0.20 (0.04)	8.28 (2.07)	0.99 (0.20)	51.64 (17.94)
	96	0.31 (0.04)	0.16 (0.02)	6.42 (0.83)	0.99 (0.20)	43.87 (21.36)
	48	0.55 (0.14)	0.39 (0.10)	8.25 (2.10)		
2.3:1	72	0.34 (0.05)	0.24 (0.04)	5.10 (0.75)		
	96	0.28 (0.03)	0.20 (0.02)	4.20 (0.45)		

mean values (± SEM)

mixture in which cadmium was present in higher concentration (2.3:1 TR), TU decreased significantly ($P < 0.05$) at both 48 to 72 hr (38%) and 48 to 96 hr (49%). Thus, the toxicity of both metals increased with time of exposure but differences were observed between TR of mixtures; zinc toxicity increased 35% in the 2.3:1 TR mixture whereas cadmium toxicity remained similar to that of the 1:1 TR mixture.

There was a great increase in toxicity of metals when both were present in mixtures, as compared with their individual LC50 values (Table 2). At similar exposure times, cadmium in combination with zinc increased in toxicity 4 to 6 times as the TR was raised; similarly, zinc toxicity in a mixture at 1:1 TR was 5 to 7 times greater than its LC50 but 10 times greater than this when cadmium was raised to a 2.3:1 TR. In relation to the metal concentrations in mixtures that produced 50% mortality, the values were lower than the LC20 obtained in their single toxicity tests. These results reflect the complexity of the metals' interaction. Thus, the co-exposure to cadmium and zinc had an effect on juvenile P. setiferus that cannot be explained by simple addition of single metal effects; furthermore, the toxicity was related to the ratio of the metals in the mixtures.

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