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Acute Toxicity of Cadmium, Mercury, and Lead to Whiteleg Shrimp (*Litopenaeus vannamei*) Postlarvae

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Despite national policies regarding the use and management of coastal resources, pollution problems are increasing in Mexico and, at the current rates of input, metals contamination will become stressful to many ecosystems (Villanueva and Botello 1996), to the point that any level of exposure of the organisms participating in their food chains could cause severe disruptions of the normal functions of the biological systems, where the metals are accumulating.

The whiteleg shrimp *Litopenaeus vannamei* is a tropical species that is geographically distributed from Sonora, Mexico, to northern Peru. It is cultured in extensive, intensive, and semi-intensive systems and is, together with *Litopenaeus stylirostris*, the most popular shrimp for aquaculture in Mexico as well as in other Central and South America countries. In the last decade, shrimp aquaculture has rapidly increased in Mexico, where most shrimp farms are located in zones with high agricultural activities, high use of pesticides, and in which studies carried out for more than 25 years have demonstrated high concentrations of Hg, Pb, Cd, Cr, Ni and Zn (Villanueva and Botello 1996). However, information about metal toxicity for these organisms is scarce. This paper provides information on the mortality of *L. vannamei* postlarvae, exposed under laboratory conditions to individual concentrations of Cd, Hg and Pb, the three heavy metals widely recognized as the most toxic in our environment.

MATERIALS AND METHODS

Postlarvae (PL12) were obtained from a commercial facility located close to Mazatlán (Mexico), and held for three days under laboratory conditions in a 50 l stocking tank. During acclimation the postlarvae were fed with *Artemia* sp. nauplii, and seawater was changed every day (Buikema et al. 1982). The mean wet weight of postlarvae was 4.22 ± 1.35 mg. Seawater used in the bioassays was pumped from the Mazatlan Bay and was filtered through a sand and gravel bed, one cartridge system of 10, 5 and 1 μm, and finally treated with activated charcoal. Chemical characteristics of filtered seawater are given in Table 1; each value was calculated with conventional methods (APHA- AWWA-WPCF 1992).

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Metal test solutions were prepared by dissolving the required amounts of $CdCl_2$, $HgCl_2$ and $PbCl_2$ (Baker GR grade) in seawater. The concentrations of total Cd, Hg and Pb (nominal concentrations) ranged from 1 to 25, 0.5 to 3, and 60 to 350 mg L^{-1}, respectively. Throughout the experiments salinity was 34 ppt, pH ranged between 8.0 and 8.1, and water temperature was 26-27°C.

Table 1. Chemical characteristics of seawater used in bioassays

Parameter	Dissolved metals (μg L^{-1})
Total alkalinity: 2.12-2.15 meq L^{-1}	Cd: 0.3-0.4
PH: 8-8.1	Cr: 0.14-0.15
Ammonia-N: 9-13 μg L^{-1}	Cu: 8-13
Nitrite-N: 4-5.3 μg L^{-1}	Fe: 1.6-2.9
Nitrate-N: 222-284 μg L^{-1}	Mn: 1.5-2.3
Phosphate-P: 70-83 μg L^{-1}	Pb: 1.1-1.3
	Zn: 67-85

Short-term (24, 48, 72, and 96 hr) median lethal concentration (LC_{50}) toxicity tests were conducted following the methods described by APHA-AWWA-WPCF (1992), and Buikema et al. (1982). Postlarvae were sampled randomly from the stocking tank and placed in triplicate 600 ml glass beakers for each of the treatments where they were kept for three days before bioassays, with 12 hr light: 12 hr dark cycle. All glassware was acid-washed before use. Each flask containing 300 ml of test solution and 10 test postlarvae, was aerated by an airstone, and feeding was the same as during the acclimation period. Each test solution and the control seawater medium were renewed daily, in accordance with a static renewal method for toxicity tests (Buikema et al. 1982), and postlarvae were not transferred to new flasks. During the experiment, dissolved oxygen was 5.49 ± 0.34 mg L^{-1} in all containers. All observations on survival and culture conditions were carried out at 12-hr intervals. Death was assumed when postlarvae were non-motile and showed no response when touched with a glass rod. Controls were run in parallel with similar conditions.

LC_{50} values and their 95% confidence limits for total metals were calculated with a computer program based upon a method described by Finney (1971), transforming the mortality data in probit mortality. With this method, the estimated probit line and results of a chi-square test for goodness of fit were determined. A z-test for the comparison of two LC_{50} values at the 5% level of significance (APHA-AWWA-WPCF 1992) were carried out between each LC_{50} value.

A valid criticism of bioassays is that the results obtained with only one group of test organisms are considered as representative of the species in question. To avoid this problem, we obtained the LC_{50} of the three metals using 5-6 groups of

postlarvae at different times and from different broodstocks, to test the toxicity of different concentrations of each metal in each of these separate experiments.

RESULTS AND DISCUSSION

No shrimp died in the controls run in parallel with the bioassays of postlarvae exposed to different concentrations of total Cd, Hg, and Pb. With regard to Cd, no shrimp died in 1 mg L^{-1} during the first 48 hr of exposure. In 1, 2.5, 5, and 10 mg L^{-1} total Cd, mortalities of 20, 56.6, 60, and 93.3% were recorded, respectively, after 96 hr exposure and a 100% mortality occurred in 15, 20, and 25 mg L^{-1} at 96, 72, and 12 hr of exposure, respectively. According to Landis and Yu (1999) the main biochemical effects of Cd are: (a) it can bind with SH-containing ligands in the membrane and other cell constituents, causing structural and functional disruptions, and (b) it can uncouple oxidative phosphorylation and impair the cell energy metabolism.

Mortalities in 0.5, 1, 1.25, and 1.5 mg L^{-1} total Hg were 0, 6.6, 46.6, and 96.6%, respectively; while in 2, 2.5, and 3 mg L^{-1}, 100% mortality occurred at 72, 48, and 24 hr, in the order. Hg is considered highly toxic to all living organisms, and its toxicity is due to (a) inhibition of a high variety of enzyme systems (with thiol ligands) and (b) because it inhibits active transport mechanisms through dissipation of normal cation gradient and destroys mitochondrial apparatus. In addition it causes swelling of cells leading to lysis (Landis and Yu 1999).

Regarding total Pb, mortalities of 10, 23.3, 33.3, 36.6, 50, 66.6, and 90% were noted in 60, 80, 100, 125, 150, 200, and 250 mg L^{-1}, respectively; while 100% mortality was observed in 300 and 350 mg L^{-1} at 48 and 24 hr of toxic exposure, respectively. Landis and Yu (1999) pointed out that (a) Pb has a high affinity for the sulfhydryl group (SH) and therefore some enzymes are inhibited; (b) Pb is similar in many aspects to Ca and may perform a competitive action on mitochondrial respiration and neurological function and (c) Pb can interact with nucleic acids, leading to either decreased or increased protein synthesis.

Body concentrations of non-essential metals as Cd or Hg do not appear to be regulated by any crustacean, decapod or otherwise. Therefore, these metals accumulate to high levels in the tissues (Zanders and Rojas 1992) causing death of the test organisms.

During late proecdysis and ecdysis shrimps take water to increase their size, resulting in hydration of their tissues and an increase in the blood volume (Dall et al. 1990). This water influx would favour metal accumulation in postlarvae, possibly causing the lethal effect. According to Zanders and Rojas (1992), the physiology and the metabolic responses of crustaceans to heavy metal exposure appear to be strongly affected by the molting cycle. In our study, some postlarvae

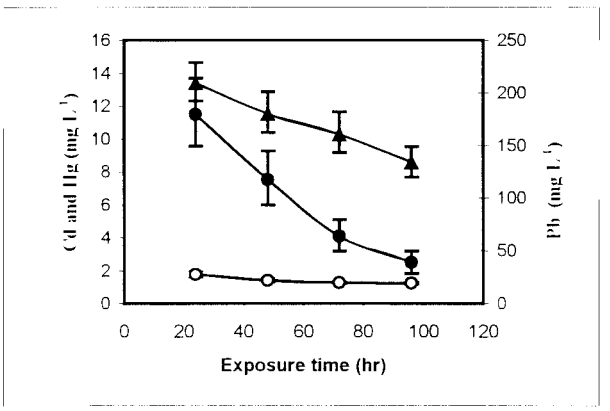

Figure 1. LC_{50} values and 95% confidence limits for total Cd (●), Hg (○) and Pb (▲) in *L. vannamei* postlarvae (PL18) exposed from 24 to 96 hr

reached the ecdysis phase, and no relation was observed between mortality and ecdysis in the case of the Cd and Pb bioassays. In Hg bioassay, no mortality was found in 0.5 mg L^{-1}, in spite of the fact that 20% reached the ecdysis stage; indicating the existence of defense mechanisms, such as metallothioneins, which tend to bind actively with Hg (Landis and Yu 1999) and decrease its toxicity in the test organisms. However, in 1.25 mg L^{-1}, only the individuals that died during the first 60 hr of exposure, were in ecdysis stage. Zanders and Rojas (1992) observed that most of the amphipod *E. rapax* which died during the first 24-48 hr of Cd exposure were in the ecdysis phase.

LC_{50} values and their 95% confidence limits (error bars) for total Cd, Hg and Pb are shown in the Fig. 1 and table 2. A comparison between the LC_{50} (APHA-AWWA-WPCF 1992) for each bioassay at different exposure times, was carried out. This analysis revealed no significant difference ($P<0.05$) between 72-hr and 96-hr for total Hg, and 48-hr and 72-hr for total Pb. Significant differences ($P<0.05$) were observed between all Cd values and the others comparisons for total Hg and Pb.

Statistical analysis showed that the probit of mortality had a positive linear relationship with each metal, and that all values of the chi-square test were less than the tabulated critical value for $P<0.05$, suggesting that the distribution of every mortality value (probit of mortality) was close to the estimated probit lines (Finney 1971).

During the Pb assay we observed the presence of particulates at the bottom of the experimental vessels. For the quantities added of 50, 100, 150, 200, 250, 300, and 350 mg L^{-1} of total lead, dissolved lead determined by atomic absorption spectrophotometry, was 0.44, 1.72, 3.09, 7.7, 11.6, 20.2, and 57.2 mg L^{-1},

respectively. Although dissolved metals are considered more toxic since they are more easily absorbed by aquatic organisms than the particulate fraction, we decided report our results as a function of total lead; rather than dissolved Pb concentrations since, due to benthic behavior, our test organisms were always in contact with the particulate, as well as with the dissolved lead and were in fact observed browsing among the precipitates present at the bottom of the experimental containers.

Table 2. 96-hr LC_{50} values (mg L^{-1}) of total Cd, Hg, and Pb for several crustacean species

Species	Cd	Hg	Pb	Author
Palaemon serratus (larvae)*	1.68	0.074		Mariño-Balsa et al. (2000)
Maja squinado (larvae)*	0.158	0.072		Mariño-Balsa et al. (2000)
Paragrapsus quadridentatus (larvae)	0.49			Ahsanullah and Arnott (1978)
Cancer irroratus (larvae)	0.25			Benijts-Claus and Benijts (1975)
Rhithropanopeus harrisii (larvae)	3.37			Thorpe and Costlow (1989)
Palaemonetes pugio (larvae)	1.88			Thorpe and Costlow (1989)
Penaeus japonicus (larvae)	0.03			Bambang et al. (1994)
Homarus americanus (larvae)	0.078	0.02		Johnson and Gentile (1979)
Palaemonetes pugio (larvae)		2.6		Kraus and Kraus (1986)
Penaeus setiferus (postlarvae)		0.017		Green et al. (1976)
Procambarus clarkii (juvenile)			751	Naqvy and Howell (1993)
Gammarus pseudolimnaeus (adult)			0.124	Spehar et al. (1978)
Palaemon elegans (juvenile)			167	Lorenzon et al. (2000)
Cancer magister (larvae)	0.247	0.082	0.575	Martin et al. (1981)
Scylla serrata (adult)	18	0.68	>370	Krishnaja et al. (1987)
L. vannamei (postlarvae)	2.49	1.23	134	This study

* 72-hr LC_{50} values

Our 96-hr LC_{50} value for Cd was 2.49 mg L^{-1}, well within the interval reported. Postlarvae of *L. vannamei* were more tolerant than most of larval crustaceans, but less tolerant than the adults of *Scylla serrata*, probably because adult crustaceans are more tolerant to heavy metals than their larval/postlarval (Mariño-Balsa et al. 2000).

Regarding Hg, Bianchini and Gilles (1996) pointed out that 1 mg L^{-1} Hg was lethal for the crabs *Eriocheir sinensis* and *Carcinus maenas*, and Mance (1987) reported LC_{50} of 0.1 mg L^{-1} for marine crustaceans adult and larvae exposed to Hg. This study agrees with several previous investigations, which show that marine species are much more susceptible to Hg than Cd and Pb and other metals.

According to Krishnaja et al. (1987) the Pb tolerance of decapods is high, since they may accumulate high concentrations of Pb without any lethal effects. This explains why the LC_{50} values for Pb are the highest found in this work.

Toxicity data of this kind provide biological criteria to establish quality standards that protect resources of the coastal environment. In fact, it has been suggested that they may be used to obtain the maximum permissible concentrations (MPC), with a protection factor of 100 according to the formula: MPC= $LC_{50}/100$ (Mariño-Balsa et al. 2000). In this case, the MPC for rearing of *L. vannamei* would be 0.012, 0.024 and 1.3 mg L^{-1}, for Hg, Cd and Pb, respectively. However, the first physiological response of organisms to sublethal concentrations might occur at molecular level (Lorenzon et al. 2000), and this must be investigated in *L. vannamei* to validate the MPC calculated from these acute toxicity tests.

Osuna-López et al. (1989), found in the Northwest coast of Mexico an interval for Cd and Pb of 0.02-3 and 1.1-9.3 µg L^{-1}, respectively. In this context, the MPC obtained here has important implications for shrimp pond management, especially in the grow-out units of the Northwest coast of Mexico, where shrimp culture is increasing. Therefore, the interaction between metals and shrimp is an important consideration, and the synergism of these metals must be object of further studies.

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