Toxicity of metal mixtures to the Pacific white shrimp postlarvae

M.G. Frías-Espéricueta, D. Voltolina-Lobina, I. Osuna-López, G. Izaguirre-Fierro

► To cite this version:

M.G. Frías-Espéricueta, D. Voltolina-Lobina, I. Osuna-López, G. Izaguirre-Fierro. Toxicity of metal mixtures to the Pacific white shrimp postlarvae. *Marine Environmental Research*, 2009, 68 (5), pp.223. 10.1016/j.marenvres.2009.06.011 . hal-00563088

HAL Id: hal-00563088

<https://hal.science/hal-00563088>

Submitted on 4 Feb 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Toxicity of metal mixtures to the Pacific white shrimp *Litopenaeus vannamei* postlarvae

M.G. Frías-Espéricueta, D. Voltolina-Lobina, I. Osuna-López, G. Izaguirre-Fierro

PII: S0141-1136(09)00081-6
DOI: [10.1016/j.marenvres.2009.06.011](https://doi.org/10.1016/j.marenvres.2009.06.011)
Reference: MERE 3350

To appear in: *Marine Environmental Research*

Received Date: 16 June 2008
Revised Date: 19 March 2009
Accepted Date: 9 June 2009

Please cite this article as: Frías-Espéricueta, M.G., Voltolina-Lobina, D., Osuna-López, I., Izaguirre-Fierro, G., Toxicity of metal mixtures to the Pacific white shrimp *Litopenaeus vannamei* postlarvae, *Marine Environmental Research* (2009), doi: [10.1016/j.marenvres.2009.06.011](https://doi.org/10.1016/j.marenvres.2009.06.011)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 Toxicity of metal mixtures to the Pacific white shrimp *Litopenaeus vannamei*

2 postlarvae

3 M.G. Frías-Espéricueta^a, D. Voltolina-Lobina^{b*}, I. Osuna-López^a, G. Izaguirre-Fierro^a

4

5 ^aAutonomous University of Sinaloa, Faculty of Marine Science, Environmental Studies

6 Laboratory, P.O. Box 1132, Mazatlán, Sinaloa 82000, México.

7 ^bCenter of Biological Research of the North West, UAS-CIBNOR Laboratory, P.O. Box

8 1132, Mazatlán Sinaloa, 82000, México (e-mail: voltolin04@cibnor.mx)

9

10 **Abstract**

11 According to the literature, the safe level of a toxic substance for any given organism

12 may be calculated from its median lethal concentration multiplied by a suitable

13 application factor (AF: usually 0.1 and 0.01). The medial lethal concentrations for

14 *Litopenaeus vannamei* postlarvae exposed to the mixtures in equitoxic proportions of

15 Cd-Hg, Hg-Zn and Hg-Pb were close to one order of magnitude lower than the values

16 calculated from individual toxicity tests, indicating a synergistic effect, while the

17 mixture Cd-Zn showed an antagonistic effect. Exposure to the mixture of Cu, Fe, Mn,

18 Zn, Cd, Hg and Pb caused 63.3 and 100% mortality after 21 and 13 days for 0.05 and

19 0.1 AF, showing that environmentally safe concentrations of toxicants should not be

20 calculated from individual toxicity tests.

21

22 **Keywords:** Toxicity; Safe level; Metal mixtures; *Litopenaeus vannamei*

23

24 **1. Introduction**

25 Continental waters discharge into the marine environment the different substances they
 26 receive from anthropogenic and natural sources; in most situations these discharges
 27 contain complex mixtures, which are likely to cause environmental damage because
 28 mixtures of toxicants in concentrations below their individual toxic levels may have
 29 negative effects on the growth and reproduction of aquatic organisms (Enserink et al.,
 30 1991; Barata et al., 2006).

31 There is evidence that the levels of heavy metals are increasing throughout northern
 32 Mexico (Holguín et al., 2006), especially in the coastal waters and lagoons of the
 33 Pacific NW which receive industrial, agricultural and municipal wastewaters and their
 34 accompanying contaminants. In one coastal lagoon, Soto-Jiménez et al. (2003)
 35 calculated yearly anthropogenic inputs of 350, 1020 and 970 kg of Cd, Cu and Zn, and
 36 Frías-Espéricueta et al. (2005) reported concentrations of 1.1-6 µg/g of Cd, 33.8-110 of
 37 Cu, 6.4-19.4 of Pb and 1146-2531 of Zn in aquatic organisms of the NW coast of
 38 Mexico. Therefore, these discharges may become a major threat to the ecology and to
 39 the traditional fishery of the coastal states of NW Mexico, as well as to their growing
 40 shrimp culture industry (Páez-Osuna et al., 2003).

41 Metals may affect the metabolism of penaeid shrimp: for instance, exposure to Cd or to
 42 Zn affects the gill functions of *Litopenaeus vannamei* and *L. schmitti* (Wu and Chen,
 43 2004; Barbieri, 2007), and of *Farfantepenaeus brasiliensis* challenged with sublethal
 44 levels of Hg (Barbieri et al., 2005).

45 However, there is scant information concerning the toxicity of metal mixtures for the
46 white shrimp *Litopenaeus vannamei*, which is the most important species for Mexican
47 aquaculture. The purpose of this study was to assess the effects of mixtures of some
48 essential (Cu, Fe, Mn and Zn) and non essential (Cd, Hg and Pb) metals on mortality,
49 feeding and growth of postlarvae of the Pacific white shrimp *L. vannamei*.

50

51 **2. Materials and Methods**

52 **2.1 Toxicity ratios, toxic unit and application factors calculations**

53 The safe level of a toxic substance (concentration which presumably has no sublethal or
54 chronic effect), may be predicted multiplying its median lethal concentration (usually
55 96-h LC50) by a suitable application factor (AF: usually 0.1 and 0.01) (Buikema et al.,
56 1982; Mariño-Balsa et al., 2000).

57 The 96-h LC50 of Cd, Cu, Fe, Hg, Mn, Pb and Zn for *L. vannamei* postlarvae were
58 calculated with a computer program based on Probit analysis (Finney, 1971), using the
59 results results of previous experiments (Frías-Espericueta et al., 2001, 2003). The
60 individual 96-h LC50 values were used to calculate the nominal equitoxic
61 concentrations of the experimental mixtures for the application factors 0.1, 0.05, 0.025
62 and 0.01 (Table 1).

63 The individual stock solutions were prepared in MilliQ deionized water with
64 concentrations equivalent to the 96-h LC50 of the water-soluble salts CdCl₂, CuCl₂,
65 FeCl₃•6H₂O, HgCl₂, MnCl₂•4H₂O, PbCl₂ and ZnCl₂; the nominal test concentrations of
66 each metal were obtained adding appropriate aliquots of each stock solution to the

67 shrimp culture water. These salts react with seawater (mainly with Cl^- and OH^-), and
 68 generate CdCl_2 , $\text{Cu}(\text{OH})_2$, FeOH , HgCl and PbCO_3 as the dominant chemical species for
 69 Cd, Cu, Fe, Hg and Pb, whereas the free ion is dominant in the case of Mn and Zn
 70 (Mantoura et al., 1978; Hall et al., 1995).

71 In one first experiment, which lasted one week (168 h), we used the binary mixtures Cd-
 72 Hg, Cd-Zn, Hg-Zn and Hg-Pb with a 1:1 toxicity ratio (TR), and the experiment was
 73 carried out for different toxic units (TU), calculated with the equations:

$$74 \quad \text{TR} = \text{M1-m/LC50 M1} : \text{M2-m/LC50 M2}$$

75 and

$$76 \quad \text{TU} = (\text{M1-m/LC50 M1}) + (\text{M2-m/LC50 M2})$$

77 M1-m and M2-m are the concentrations in the mixture of metals M1 and M2, and LC50
 78 M1 and LC50 M2 their respective 96-h LC50 (Logan and Wilson, 1995).

79 The effect of any given binary metal mixture was determined from the sum of toxic
 80 contributions (S) calculated as:

$$81 \quad \text{S} = ([\text{A}]/\text{LC50 M1}) + ([\text{B}]/\text{LC50 M2})$$

82 Where [A] and [B] are the concentrations of each metal in the mixture causing 50%
 83 mortality. S values <1, =1, or >1 were taken as proof of synergism, simple additivity or
 84 antagonism, respectively (Vermeulen, 1995).

85 In the second experiment the design was similar and the AFs were the same, but the
 86 organisms were exposed to the mixture of the seven metals and the exposure lasted 21
 87 days.

88 **2.2 Acclimation and water characteristics**

89 For each experiment, we used postlarvae (PL12) of *L. vannamei* (1.2 ± 0.2 cm) obtained
90 at different times and of different parental origin from a local commercial hatchery. The
91 postlarvae were acclimated during three days to the experimental conditions in a
92 common 50 l container with natural seawater, previously filtered through a sand and
93 gravel bed, one cartridge system (10 to 1 μ m) and treated with 0.5- μ m mesh activated
94 charcoal. The water was renewed daily and the postlarvae, kept with a 12:12-h
95 light:dark photoperiod, were fed ad libitum freshly hatched *Artemia* sp. nauplii supplied
96 twice daily in equal rations (Frías-Espericueta et al., 2003).

97 After this first period of acclimation, triplicate groups of 10 postlarvae for each
98 experimental treatment, taken at random from the common tank, were placed in 600 ml
99 acid-washed glass beakers with 300 ml of filtered seawater aerated with an airstone,
100 where they were kept for three additional days under the same conditions.

101 Throughout this period and during the experiment, the mean background concentrations
102 of Cd, Cu, Fe, Mn, Pb and Zn, determined by atomic absorption spectrophotometry
103 (Spectra AA Varian), were 0.35, 8.0, 1.6, 1.5, 1.1 and 6.7 μ g/l, respectively.

104 Temperature was maintained nearly constant at 29 ± 0.5 °C and the mean values of
105 salinity was 35.4 ± 0.2 ppt. The ranges of the chemical characteristics of the seawater
106 used for the experiments are given in Table 2.

107 It is generally accepted that the toxicity of metals is inversely related to ambient salinity
108 (Hall et al., 1995). However, although in Mexican shrimp farms *L. vannamei* may be
109 grown at salinities ranging from <1 to 50 ppt, all commercial shrimp hatcheries of the
110 Mexican NW use full strength seawater with a salinity close to that of this study,

because they are located in the marine coastal zone where the most common salinity values range between 37 and 35 ppt (Cano-Pérez, 1991).

2.3 Exposure experiments

After this period, each group of three beakers was added the respective metal mixture; in all cases the experimental conditions were the same as during the acclimation period, but the test solutions were renewed every 48 h and the organisms were fed individual daily rations of 20 *Artemia* nauplii, supplied every 12 hours in equal portions.

In both experiments, observations on survival and molting were carried out at 12-h intervals in each container. Death was assumed in absence of response to a mechanical stimulus with a glass rod. The food consumption in each test solution during the second experiment was calculated by difference between the nauplii supplied daily to each container and those remaining in the flask 24 hours later.

3. Results

3.1 Mortality

Previous experiments with *L. vannamei* postlarvae showed that Hg and Zn are the most toxic among the metals used in this work (Frías-Espéricueta et al., 2001, 2003). This was confirmed by the results of the first experiment, since the lowest TU₅₀ was observed with the Hg-Zn binary mixture.

The TU₅₀ values of each binary mixture decreased with the time of exposure (Table 3), the three mixtures Cd-Hg, Hg-Zn and Hg-Pb caused 50% mortalities at metal concentrations close to one order of magnitude lower than their individual 96-h LC₅₀,

and the respective sums of toxic contributions were <1 ($S = 0.21, 0.16$ and 0.22 for Cd-Hg, Hg-Zn and Hg-Pb, respectively). This indicates a synergistic effect, whereas the mixture Cd-Zn showed an antagonistic effect ($S = 2.58$).

In the second experiment with the mixture of the seven metals, survival was 100% in the control cultures and in those exposed to 0.01 and 0.025 AF, whereas mortality was 63.3 and 100% after 21 and 13 days for those exposed to 0.05 and 0.1 AF, respectively (Table 4).

3.2 Food consumption, molting and growth

In the second, multi-metal exposure experiment, there was no significant correlation between instant mortality and ecdysis ($P > 0.1$ in all cases). However, we noticed a decreasing number of exuviae with increasing AF, as well as a progressive decrease of the final mean weights of the postlarvae, which were significantly ($p < 0.05$) lower with 0.025 and 0.05 AF than in the control cultures and in those exposed to 0.01 AF (one-way ANOVA and Tukey's multiple comparison tests, $\alpha = 0.05$) (Table 4).

There were no significant differences between the food consumption observed in the cultures exposed to 0.01 and 0.025 AF and those of the control. The mortality in the cultures exposed to 0.05 and 0.1 AF did not allow a meaningful comparison with the rest of the treatments.

4. Discussion

The antagonistic effect of Cd and Zn coincides with the protective effect of Zn noticed in the mysid *Praunus flexuosus* challenged with a mixture of metals (McLusky and Hagerman, 1987), and with the results of Barata et al. (2006) who pointed out that,

since Zn, Cu and Cd are inducers of metallothionein synthesis, there is a high probability of competition for binding sites, and that for this reason their combined effects may be expected to be antagonistic.

According to Brzóska and Moniuszko-Jakoniuk (2001), since many toxic effects of Cd result from interactions with essential metals, including Zn, the availability of Zn is an important factor with respect to Cd toxicity. While exposure to Cd interferes with the Zn biological functions, dietary Zn intake affects Cd absorption and accumulation, and prevents or at least reduces its adverse effects.

A similar antagonistic effect of Cu and Zn was observed in *L. vannamei* and *Daphnia magna* by Frías-Espéricueta et al. (2003) and by Cañizares-Villanueva et al. (2000), although Negilski et al. (1981) and Vanegas et al. (1997) found a synergistic effect in the crustaceans *Callinassa australiensis* and *Litopenaeus setiferus* exposed to a mixture Cd-Zn, which could be explained by genetic differences or by different chemical characteristics of the respective aquatic environments.

The synergism of the mixture Cd and Hg was observed in the mussel *Perna viridis* by Mohan et al. (1986). Both metals have a high affinity for protein binding sites, which might explain their increased toxicity when they occur at relatively high concentrations in the environment (Barata et al., 2006). A similar explanation is likely for the increased toxicity of the mixtures Hg-Zn and Hg-Pb, although we could not trace other studies on the interactive effect of these metals.

The mortalities of *L. vannamei* observed in the mixture of the seven metals agree with those observed by Chu and Chow (2002) and Verslycke et al. (2003), when the

nematode *Caenorhabditis elegans* and the crustacean *Neomysis integer* were exposed to combinations of metals in concentrations deemed to be individually safe. These results confirm that metal interactions must not be neglected, since even if they are present in minute amounts, their interactions may generate a severe environmental stress.

When *L. vannamei* are exposed to metals, these are accumulated in gill tissues (Wu and Chen, 2004) and cause cytological and histochemical damage to the gill epithelium. The resulting alterations in gill functions and inhibition of oxygen consumption (Soegianto et al., 1999a,b; Barbieri, 2009) could explain the mortalities observed in this study.

Wong et al. (1993) found that metal exposure of *Metapenaeus ensis* postlarvae affects their rate of predation and consumption of *Artemia* nauplii, and Barata and Baird (2000) observed that Cd and Cu strongly impair feeding of *Daphnia magna*. Although our results do not confirm that food consumption of *L. vannamei* postlarvae is a good indicator of metal stress, their reduced growth coincides with that observed in *Ceriodaphnia dubia* and *Daphnia magna* exposed to metal mixtures, and in this case it could be due to a greater energy allocation to protect cells from metal accumulation and toxic effects (Spehar and Fiandt, 1986; Enserink et al., 1991).

The presence of metals in Mexican coastal waters should be of concern for commercial hatcheries, because it may result in alterations of different metabolic functions, such as those observed in different penaeid shrimps exposed to metals alone (Soegianto et al. 1999a,b; Barbieri, 2007, 2009) or in mixtures (Wu and Chen, 2004). It should also be of environmental concern, because metals cause increased susceptibility to other stressors in aquatic organisms (Liao et al., 2006).

Some authors have pointed out that it is difficult to extrapolate laboratory results to the natural environment, because other variables (pH, alkalinity, natural chelators, temperature and salinity, among others) may affect the response of aquatic organisms modifying the availability as well as the toxicity of metals (Wong et al., 1993; Wu and Chen, 2004).

The results of several authors indicate that *L. vannamei* is a good test organisms for laboratory experiments on the effects of heavy metals, but there is insufficient evidence that it could be used for the in situ assessment of water pollution in field conditions. Although our results indicate that 0.01 AF is a safe level for the survival of shrimp postlarvae, those of other authors indicate metabolic dysfunctions when metals are present at sublethal levels, which might have a severe effect in the long term. Therefore, we feel that additional observations on the histological and biochemical effects of sublethal concentrations of metals should be used to validate our result.

We also feel that the allowable concentrations of toxic substances for regulatory use should not be based on untested hypothesis and that, since different species may show different responses (Spehar and Fiandt, 1986), any allowable limit may be too permissive to protect some species when other toxicants are also present.

Acknowledgments.

Supported by CONACYT and CIBNOR projects 32501-T and AC1.4. The authors thank M.A. Herrera-Vega, G. Barrón-Gallardo and H. Bojórquez-Leyva for technical assistance.

References

- Barata, C., Baird, D.J., 2000. Determining the ecotoxicological mode of actions of toxicants from measurements on individuals: results from short duration chronic test with *Daphnia magna* Straus. *Aquatic Toxicology* 48, 195-209.
- Barata, C., Baird, D.J., Nogueira, A.J.A., Soares, A.M.V.M., Riva, M.C., 2006. Toxicity of binary mixtures of metals and pyrethroid insecticides to *Daphnia magna* Straus. Implications for multi-substance risks assessment. *Aquatic Toxicology* 78, 1-14.
- Barbieri, E., 2007. Use of oxygen consumption and ammonium excretion to evaluate the sublethal toxicity of cadmium and zinc on *Litopenaeus schmitti* (Burkenroad, 1936, Crustacea). *Water Environment Research* 79, 641-646.
- Barbieri, E., 2009. Effects of zinc and cadmium on oxygen consumption and ammonium excretion in pink shrimp (*Farfantepenaeus paulensis*, Pérez-Farfante, 1967, Crustacea). *Ecotoxicology* 18, 312-318.
- Barbieri, E., Passos, E.A., García, C.A.B., 2005. Use of metabolism to evaluate the sublethal toxicity of mercury on *Farfantepenaeus brasiliensis* larvae (Latreille 1817, Crustacean). *Journal of Shellfish Research* 24, 1229-1233.

- Brzóška, M.M., Moniuszko-Jakoniuk, J., 2001. Interactions between cadmium and zinc in the organism. *Food Chemistry and Toxicology* 39, 967-980.
- Buikema, A.L., Niederlehner, B.R., Cairns, J.Jr., 1982. Biological monitoring. Part IV. Toxicity treating. *Water Research* 16, 239-262.
- Cano-Pérez, F.A. 1991. Golfo de California. Oceanografía física, in: De la Lanza-Espino, G., Oceanografía de los Mares Mexicanos, AGT Editor, Mexico, D.F. pp. 453-514.
- Cañizares-Villanueva, R.O., Martínez-Córdova, F., Espinoza-Chavez, F., 2000. Acute toxicity to *Daphnia magna* of effluents containing Cd, Zn and a mixture Cd-Zn after metal removal by *C. vulgaris*. *Environmental Toxicology* 15, 160-164.
- Chu, K.W., Chow, K.L., 2002. Synergistic toxicity of multiple heavy metals is revealed by a biological assay using a nematode and its transgenic derivate. *Aquatic Toxicology* 61, 53-64.
- Enserink, E.L., Mass-Diepeveen, J.L., Van Leeuwen, C.J., 1991. Combined effects of metals: an ecotoxicological evaluation. *Water Research* 25, 679-687.
- Finney, D.J., 1971. Probit Analysis. 3rd edition. Cambridge University Press, London.

- 265 Frías-Espericueta, M.G., Voltolina, D., Osuna-López, J.I., 2001. Acute toxicity of Cd, Hg
266 and Pb to whiteleg shrimp *Litopenaeus vannamei* postlarvae. Bulletin of Environmental
267 Contamination and Toxicology 67, 580-586.
- 268
- 269 Frías-Espericueta, M.G., Voltolina, D., Osuna-López, J.I., 2003. Acute toxicity of copper,
270 zinc, iron and manganese and of the mixtures copper-zinc and iron-manganese to whiteleg
271 shrimp *Litopenaeus vannamei* postlarvae. Bulletin of Environmental Contamination and
272 Toxicology 71, 68-74.
- 273
- 274 Frías-Espericueta, M.G., Osuna-López, J.I., Estrada-Toledo, F.J., López-López, G.,
275 Izaguirre-Fierro, G., 2005. Heavy metals in the edible muscle of shrimps from several
276 coastal lagoons located in the NW of Mexico. Bulletin of Environmental Contamination
277 and Toxicology 74, 1098-1104.
- 278
- 279 Hall, Jr.L.W., Ziegenfuss, M.C., Anderson, R.D., Lewis, B.L., 1995. The effect of
280 salinity on the acute toxicity of total and free cadmium to a Chesapeake Bay copepod
281 and fish. Marine Pollution Bulletin 30, 376-384.
- 282
- 283 Holguín, C., Rubio, H., Olave, M.E., Saucedo, R., Gutiérrez, M., Bautista, R., 2006.
284 Calidad del agua del Río Conchos en la región de Ojinaga, Chihuahua: parámetros
285 fisicoquímicos, metales y metaloides. Universidad y Ciencia 22, 51-63.
- 286

- 287 Liao, C.M., Chang, C.F., Yeh, C.H., Chen, S.C., Chiang, K.C., Chio, C.P., Chou,
288 B.Y.H., Jou, L.J., Lien, G.W., Lin, C.M., Shen, H.H., Wu, G.D., 2006. Metal stresses
289 affect the population dynamics of disease transmission in aquaculture species.
290 Aquaculture 257, 321-332.
- 291
- 292 Logan, D.T., Wilson, H.T., 1995. An ecological risk assessment method for species
293 exposed to contaminant mixtures. Environmental Toxicology and Chemistry 14, 51-
294 359.
- 295
- 296 Mantoura, R.F.C., Dickson, A., Riley, J.P., 1978. The complexation of metals with
297 humic materials in natural waters. Estuarine, Coastal and Marine Science 6, 378-408.
- 298
- 299 Mariño-Balsa, J.C., Poza, E., Vazquez, E., Berras, E., 2000. Comparative toxicity of
300 dissolved metals to early larval stages of *Palaemon serratus*, *Maja squinado* and
301 *Homarus gammarus* (Crustacea: Decapoda). Archives of Environmental Contamination
302 and Toxicology 39, 345-351.
- 303
- 304 McLusky, D.S., Hagerman, L., 1987. The toxicity of chromium, nickel and zinc: effects
305 of salinity and temperature, and the osmoregulatory consequences in the mysid *Praunus*
306 *flexuosus*. Aquatic Toxicology 10, 225-238.
- 307

- 308 Mohan, C.V., Gupta, T.R.C., Shelly, H.P.C., Menon, M., 1986. Combined toxicity of Hg
309 and Cd to the mussel *Perna viridis*. Diseases of Aquatic Organisms 2, 65-72.
310
- 311 Negilski, D.S., Ahsanullah, M., Mobley, M.C., 1981. Toxicity of Zn, Cd and Cu to the
312 shrimp *Callinassa australiensis*. Marine Biology 64, 305-309.
313
- 314 Páez-Osuna, F., Gracia, A., Flores-Verdugo, F., Lyle-Fritch, L.P., Alonso-Rodríguez,
315 R., Roque, A., Ruiz-Fernández, A.C., 2003. Shrimp aquaculture development and the
316 environment in the Gulf of California ecoregion. Marine Pollution Bulletin 46, 806-
317 815.
318
- 319 Soegianto, A., Charmantier-Daures, M., Trilles, J.P., Charmantier, G., 1999a. Impact of
320 cadmium on the structure of gills and epipodites of the shrimp *Penaeus japonicus*
321 (Crustacea:Decapoda). Aquatic Living Resources 12, 57-70.
322
- 323 Soegianto, A., Charmantier-Daures, M., Trilles, J.P., Charmantier, G., 1999b. Impact of
324 copper on the structure of gills and epipodites of the shrimp *Penaeus japonicus*. Journal
325 of Crustacean Biology 19, 209-223.
326
- 327 Soto-Jiménez, M.F., Páez-Osuna, F., Ruiz-Fernández, A.C., 2003. Geochemical
328 evidences of the anthropogenic alteration of trace metal concentrations of the sediments
329 of Chiricaúeto marsh (SE Gulf of California). Environmental Pollution 125, 423-432.

330

331 Spehar, R.L., Fiandt, J.T., 1986. Acute and chronic effects of water quality criteria-
332 based metal mixtures on three aquatic species. *Environmental Toxicology and*
333 *Chemistry* 5, 917-931.

334

335 Vanegas, C., Espina, S., Botello, A.V., Villanueva, S., 1997. Acute toxicity and
336 synergism of Cd and Zn in the white shrimp *Penaeus setiferus* juveniles. *Bulletin of*
337 *Environmental Contamination and Toxicology* 58, 87-92.

338

339 Vermeulen, A.C., 1995. Elaborating chironomid deformities as bioindicator of toxic
340 sediments stress: the potential application of mixture toxicity concepts. *Annales*
341 *Zoologici Fennici* 32, 265-285.

342

343 Verslycke, T., Vangheluwe, M., Heijerick, D., De Schampelaere, K., Van Sprang, P.,
344 Janssen, C.R., 2003. The toxicity of metal mixtures to the estuarine mysid *Neomysis*
345 *integer* (Crustacea: Mysidacea) under changing salinity. *Aquatic Toxicology* 64, 307-
346 315.

347

348 Wong, C.K., Chu, K.H., Tang, K.W., Tam, T.W., Wong, L.J., 1993. Effects of
349 chromium, copper and nickel on survival and feeding behavior of *Metapenaeus ensis*
350 larvae and postlarvae (Decapoda: Penaeidae). *Marine Environmental Research* 36, 63-
351 78.

352

353 Wu, J.P., Chen, H.C., 2004. Effects of cadmium and zinc on oxygen consumption,
354 ammonium excretion, and osmoregulation on white shrimp (*Litopenaeus vannamei*).
355 Chemosphere 57, 1591-1598.

356

357

358

ACCEPTED MANUSCRIPT

Table 1. Median lethal concentrations (96-h LC50) and respective nominal equitoxic concentrations for each application factor (AF). All values in $\mu\text{g/l}$.

Metal	96-h LC50*	AF			
		0.01	0.025	0.05	0.1
Cd	2 490	24.9	62.25	124.5	249
Cu	37 300	373	932.5	865	3 730
Fe	44 200	442	1 105	2 210	4 420
Hg	1 230	12.3	30.75	61.5	123
Mn	130 000	1 300	3 250	6 500	13 000
Pb	134 000	1 340	3 350	6 700	13 400
Zn	2 080	20.8	52	104	208

*Frias-Espicueta et al. (2001, 2003)

377 Table 2. Chemical characteristics (ranges) of the seawater used in experiments.

Variable	Dissolved metals ($\mu\text{g/l}$)
Temperature: 28-30 °C	Cd: 0.3-0.4
Salinity: 34-36 ppt	Cu: 6.0-8.0
pH: 8.0-8.1	Fe: 1.5-2.6
Oxygen: 5.2-5.8 mg/l	Mn: 1.3-2.2
Total alkalinity: 2.10-2.13 meq/l	Pb: 1.0-1.1
Ammonia-N: 9.0 -13.0	Zn: 1.9-2.9
Nitrite-N: 3.9-5.2 $\mu\text{g/l}$	
Nitrate-N: 220-280 $\mu\text{g/l}$	
Phosphate-P: 68-80 $\mu\text{g/l}$	

378

379

380

381

382

383

384

385

386

387

388

389

390

391

392

Table 3. TU50 values (in parenthesis, 95% confidence limits) of binary metal mixtures for *Litopenaeus vannamei* postlarvae.

Exposure time (h)	TU50 of mixtures		Individual 96-h LC50		
	TU50	Equivalent concentrations in the mixture	(mg/l)*		
		Cd	Hg	Cd	Hg
96	0.22 (0.19, 0.26)	0.27	0.13	2.49	1.23
168	0.19 (0.16, 0.22)	0.23	0.11		
		Cd	Zn	Cd	Zn
96	2.60 (2.40, 2.80)	3.23	2.70	2.49	2.08
168	2.12 (1.88, 2.33)	2.63	2.20		
		Hg	Zn	Hg	Zn
96	0.17 (0.15, 0.19)	0.10	0.17	1.23	2.08
168	0.14 (0.12, 0.15)	0.08	0.14		
		Hg	Pb	Hg	Pb
96	0.23 (0.19, 0.27)	0.14	15.40	1.23	134.2
168	0.20 (0.17, 0.23)	0.12	13.40		

*Frias-Espicueta et al. (2001, 2003)

Table 4. Survival (%), number of exuviae, final weight (W, in mg) and food consumption (FC in % of control) of *L. vannamei* postlarvae exposed to different application factors. Different letters indicate significant difference ($\alpha = 0.05$).

AF	Survival %	Exuviae (N)	W	FC (%)
control	100	98	18.2 ± 5.3^a	100
0.01	100	88	13.1 ± 4.9^a	100
0.025	100	47	8.3 ± 1.7^b	97.6
0.05	37	57	4.0 ± 1.1^c	--
0.1	0*	17*		--

* 100% mortality after 13 days of exposure