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Highlighted Article

A comparison of the response of *Simocephalus mixtus* (Cladocera) and *Daphnia magna* to contaminated freshwater sediments ☆

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ABSTRACT

The southeast region of Mexico is characterized by intensive oil industry activities carried out by the national public enterprise Petróleos Mexicanos (PEMEX). The freshwater lagoon "El Limón", located in the municipality of Macuspana, state of Tabasco, Mexico, has received over 40 years discharges of untreated waste waters from the Petrochemical Complex "Ciudad PEMEX", located on the border of the lagoon. To assess the toxicity of the sediments and, hence, to obtain information on the biological effects of these contaminating discharges, the cladoceran *Simocephalus mixtus* was used as a test organism in acute (48 h) and chronic (12 d) toxicity assays. For comparison purposes, bioassays were also conducted with the reference cladoceran *Daphnia magna*. The sediments of this lagoon contain important amounts of metals and hydrocarbons that have been accumulated over time; however, the acute tests only registered reduced lethal effects on the test organisms (maxima of 10% and 17% mortality for *D. magna* and *S. mixtus*, respectively). This may be due to low bioavailability of the pollutants present in the sediments. On the other hand, partial or total inhibition and delay in the start of reproduction, reduction in clutch sizes, reduced survival, as well as reduction in the size of adults and offspring were recorded in the chronic assays. The most evident chronic effects were found in *S. mixtus*; in this species, reproduction was inhibited up to 72%, whereas *D. magna* was only affected by 24%. We determined that *S. mixtus* is a more sensitive test organism than *D. magna* to assess whole-sediment toxicity in tropical environments, and that chronic exposure bioassays are required for an integrated sediment evaluation. The sediments from "El Limón" lagoon induced chronic intoxication responses and, therefore, remediation measures are urgently needed to recover environmental conditions suitable for the development of its aquatic biota.

1. Introduction

Sediments constitute a relevant environmental sector for immobilization and accumulation, as well as for transformation and activation of many of the chemical contaminants entering aquatic ecosystems. Certain environmental contaminants, in the compartmentalization process, associate to the particulate matter in sediments establishing equilibrium relations in the water-sediments interface, in which the sorption–desorption processes determine the bioavailability of toxic substances (Viganó, 2000). The study of the biological effects of pollutants in the soluble fraction (interstitial or porewater) or of those associated to particles through sorption processes constitutes a relevant aspect of ecotoxicologic assessments (Ronco and Díaz Báez, 2005). These assessments involve exposing test organisms to sediment samples obtained from the studied site (whole-sediment tests), or by exposing them to extracts or interstitial water. Assessments with benthic organisms of the toxicity of chemical pollutants associated to sediments are fundamental in evaluating ecological risks (Moreno-Garrido et al., 2003; Dekker et al., 2006).

Acute bioassays are useful to detect the presence of toxic materials at high concentrations; however, these short-term assays do not allow for the identification of sub-lethal effects at lower concentrations, which might have relevant consequences for the development and reproduction of aquatic organisms. In the last decades, development and application of bioassays have changed from short-duration tests in which the assessed response is mortality, to longer-duration exposures in which growth and reproduction are the main endpoints, and although acute tests are still a useful tool, it is considered that chronic and subchronic assays are more sensitive to chemical pollutants exposure, as

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observed in several studies (Garric et al., 2007). Hence, they have a greater ecologic relevance and constitute useful procedures to assess the risks in scenarios of actual environmental exposure (van den Heuvel-Greve et al., 2007).

Recently, proposals have been brought about by both academic and environmental agencies leading to the development and/or adaptation of international test methods using native species; however, procedures capable of measuring a larger variety of endpoints with a larger number of species are still needed to have sufficient data to generate international sediment quality criteria or guidelines (Moreno-Garrido et al., 2003; Melo and Nipper, 2007).

The use of cladocerans in toxicity assays poses many advantages; among them are their easy handling, asexual reproduction by parthenogenesis, a short lifespan, and a great sensitivity to toxic compounds. *Daphnia magna* is the cladoceran most frequently used in ecotoxicologic assays (Martínez-Jerónimo et al., 2000; Dekker et al., 2002), despite being a planktonic species from temperate climates (Martínez-Jerónimo, 2000), and its usefulness as a test organism has been questioned when making assessments in tropical latitudes, whereas *S. mixtus* is naturally distributed in America, including water bodies of the Mexican Southeast (Elías-Gutiérrez et al., 2001), and has been used previously in aquatic bioassays (Muñoz-Mejía, 1997).

The socioeconomic development of the state of Tabasco in Mexico has relied basically on oil exploitation activities, and there are clear evidences that these activities have led to significant impacts on the aquatic environment (García-Hernández, 1994; Ramos, 2003; Callejas, 2005; Aguirre-Palavicini, 2006; Cruz-Cisneros, 2006). An example of these alterations is found in the "El Limón" lagoon, which has received for more than 40 years wastewaters from the Petrochemical Complex of Ciudad Pemex (PCO-CP) (García-Hernández, 1994). Several studies have been performed, confirming the lagoon's ecological deterioration caused by the high levels of oil derivatives, as well as by the presence of significant amounts of metals and hydrocarbons, in both the water and sediments (García-Hernández, 1994; Ramos, 2003; Callejas, 2005; Aguirre-Palavicini, 2006; Domínguez-Pérez, 2006); however, none of these studies have evaluated directly the toxic effects on the aquatic organisms.

In the present study, the acute and chronic toxicity of sediment samples from the "El Limón" were assessed. A regionally distributed, littoral cladoceran species was used as test organism; responses were compared with those obtained with the reference cladoceran *D. magna*, a planktonic species used to determine toxicity in water samples in Mexico (SECOFI, 1995), but also suggested for whole-sediment assessments (ASTM, 2005).

2. Materials and methods

2.1. Study area

The "El Limón" lagoon is located in the municipality of Macuspana, state of Tabasco, in southeastern Mexico. Five sampling sites were established in this lagoon (Fig. 1); their determination was based on previous works performed by the Mexican Institute of Petroleum (Instituto Mexicano del Petróleo, 1994; García-Hernández, 1994) taking into account the direction of currents during the rainy season (south–north), the discharging site of the PCO-CP, and the inputs from its tributaries, such as the mouthing of the "Los Monos" stream. It must be pointed out that station 1 is the closest to the PCO-CP discharge point, whereas station 2 is the farthest.

Approximately 500 g of sediment were sampled at each site by means of a Van Veen dredge (2000 ml capacity) and placed into wide-mouth plastic containers. The samples were maintained at low temperature (4 °C) during their transport to the laboratory, where they were used immediately for toxicity analyses. Samples were identified according to their sampling site as: S1, S2, S3, S4, and S5.

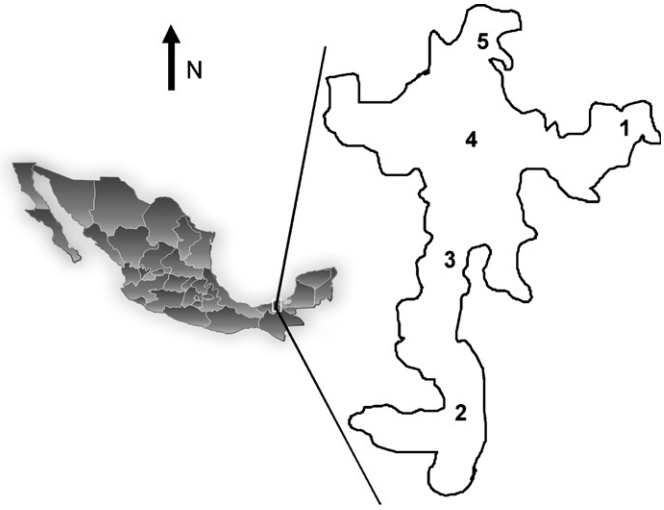

Fig. 1. Map of the "El Limón" lagoon showing the location of the sampling points.

2.2. Test organisms and culture conditions

The cladoceran *Simocephalus mixtus* was used to assess the toxicity of "El Limón" lagoon sediments, through acute and chronic bioassays. *S. mixtus* was considered an adequate species for this type of assessments, as it is naturally distributed in the littoral zone of fresh lacustrine water bodies, associated to the rhizosphere of the anchored, emerging, and floating aquatic vegetation, aside from being closely related with sediments, where it spends most of its time; but, it is also able to swim in the water column.

For comparison purposes, we also conducted bioassays with the cladoceran *D. magna*, which is the species most widely used as an international reference organism and is also suggested to be used to assess freshwater sediments (ASTM, 2005). Both cladocerans were obtained from the Cladoceran Live Collection of the Experimental Hydrobiology Laboratory (National School of Biological Sciences, National Polytechnic Institute, Mexico), where they have been maintained for more than 10 years in monospecific cultures.

Neonates (age <24 h) of both species were used as test organisms. To obtain them, we established batches of adult parthenogenetic females of known age for each cladoceran, maintained individually in 150-ml containers, at a temperature of 23 ± 2 °C, and a 16:8 h (light:darkness) photoperiod, using as culture medium reconstituted hard water (hardness = 160–180 mg l^{-1} as $CaCO_3$; US EPA, 2002); the batches contained at least 25 adult females in order to obtain enough neonates for the tests. Adult females were fed the microalga *Pseudokirchneriella subcapitata* (formerly, *Selenastrum capricornutum*) at a constant concentration of 1×10^6 cells/ml.

2.3. Acute toxicity tests

Acute (48 h) toxicity of sediments was assessed according to the protocol established by ASTM (2005). Assessments were made in 150-ml Pyrex® flasks, containing 20 ml homogenized sediment plus 80 ml of reconstituted hard water, carefully added to each container to avoid resuspending the sediments. After two hours of stabilizing the samples, 10 neonates of each species were placed in each test container, making three replicates per treatment, aside from the control series that contained only the diluting water. Bioassays were performed at 25 °C and a 16:8 h (light:darkness) photoperiod. Observations were made after 24 and 48 h, recording the number of dead individuals and taking notes of any visible effects on the mobility of surviving organisms.

2.4. Chronic toxicity tests

Short-duration chronic exposure assays were performed according to the ASTM (2005) protocol, slightly modified to detect better any sub-lethal effects. Briefly, the original protocol for *D. magna* indicates that tests must be started with 5-day-old juveniles, and that exposure must last 7 days, a period during which three reproductions could occur at the recommended temperature (25 °C). However, in our case the test was started with neonates (age <24 h), therefore its total duration was extended to 12 days. We performed this modification to the protocol because we feel that probable sub-lethal toxic effects on development, particularly on the reproductive process, could start at early developmental stages. At the age of 5 days, gonadal maturation might already be advanced and quite likely would not be susceptible to modification by the presence of toxic materials, which could lead to underestimation of the toxicity.