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Use of sodium dodecyl sulfate and zinc sulfate as reference substances for toxicity tests with the mussel *Perna perna* (Linnaeus, 1758) (Mollusca: Bivalvia)

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Abstract

Effects of anthropogenic pollution have been observed at different trophic levels in the oceans, and toxicity tests constitute one way of monitoring these alterations. The present assay proposes the use of two reference substances, sodium dodecyl sulfate (SDS) and zinc sulfate, for *Perna perna* larvae. This common mussel on the Brazilian coast is used as a bioindicator and is of economic interest. The chronic static embryo–larval test of short duration (48 h) was employed to determine the NOEC, LOEC, and IC_{50} for SDS and zinc sulfate, as well as the coefficient of variation. Salinity, pH and un-ionized ammonia (NH_3) and dissolved oxygen (DO) concentrations were measured to monitor water quality. The results demonstrated that the main alterations in veliger larvae are the development of only one shell, protruded mantle, malformed shell, formation of only part of a valve, clipped edges, uneven sizes and presence of a concave or convex hinge. NOEC values were lower than 0.25 mg L^{-1} for zinc sulfate and 0.68 mg L^{-1} for SDS. The coefficient of variation was 17.63% and 2.50% for zinc sulfate and SDS, respectively.

Keywords: Toxicity test; Mussel; Larvae; *Perna perna*; Sodium dodecyl sulfate; Zinc

1. Introduction

Pollution caused by effluent emissions could be responsible for pathogenic microorganisms and contamination by chemical compounds that might jeopardize economic activities such as fishing and aquaculture. Toxicity tests using reference substances are one way of monitoring the environment in these situations and of understanding the effects of xenobiotics on aquatic organisms.

Toxicity tests are widely used to monitor effluent discharges because they provide a direct measurement of the combined effects of effluent constituents on a representative aquatic organism, and are able to detect the presence of toxic compounds in small amounts and thus help to protect species that inhabit waters receiving such effluents. Further, they provide information on the antagonistic, synergistic, or additive effects of the effluents, characterized as complex mixtures (Hunt and Anderson, 1989; Hunt et al., 1997). Toxicity tests using mollusk species are essential for any monitoring program; because of the natural distribution of these species in coastal areas, mollusks suffer the greatest impact of different types of effluents.

The first larval stages of bivalves have been shown to be highly sensitive to pollutants, particularly heavy metals, but also pesticides, detergents, and antifouling paints (Beiras and His, 1994, 1995). Toxicity tests have been used to evaluate the abnormal development of

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bivalve shells and as indicators of chronic toxicity over short periods (48 h generally) (Conroy et al., 1996). These tests are quick and reliable biological methods for testing seawater quality and monitoring marine pollution (Beiras and His, 1994, 1995).

Reference substances are used to make comparisons between tests (whether of long or short duration), to make interlaboratory calibration possible, and to compare methods that use different organisms (Hunt and Anderson, 1989). Furthermore, they can reveal differences in the sensitivity of different batches of test organisms in periods of acclimation, disease, density, or handling stress and may also be used to evaluate reproducibility and validate tests (Ong and Din, 2001). The ideal reference substance must be toxic in low concentrations, quickly lethal, stable, nonselective, detectable through known analytical techniques, and, further, able to furnish consistent laboratory results (Ong and Din, 2001). Sodium dodecyl sulfate (SDS) is used as a reference substance in toxicity tests evaluating sediment quality and is commonly found in domestic sewage (Carr et al., 1996; Langdon et al., 1996). Zinc sulfate is also used as a reference substance in toxicity tests because of its stability in solution. It can easily be chemically analyzed, poses little danger, and is frequently found in high concentrations in effluents (Hunt and Anderson, 1989). Zinc sulfate was used because it is a nontoxic anion, which in small quantities does not change the ionic balance of seawater (Marchán et al., 1999).

The aims of this study were to verify, through toxicity tests, the possibility of using mussel larvae (*Perna perna*) as a biological model in environmental monitoring, and to indicate the use of an organic compound (SDS) and a metal (zinc sulfate) as reference substances for toxicity tests with the early life stages of the mussel.

2. Material and methods

2.1. Collection, preparation of adult animals, and gamete elimination

Mussels were collected in a free dive on the rocky Taubate shore (23°54.34′S and 45°27.56′W), off the southern point of São Sebastião Island, São Paulo, Brazil. Foul matter was removed, the byssus was cut off, and the animals were washed in fresh water. They were then maintained out of the water at 17 °C (±2.0 °C) for about 15 h. Gamete liberation was induced by various stimuli—removal of foul matter, exposure to air, thermal shock, temperature alternation, exposure to filtered and UV-sterilized seawater—and indirectly by the presence of spontaneously liberated gametes.

2.2. Gamete treatment and fertilization

Gametes were separated by sex after elimination. More than three adults had their gametes eliminated; the eggs were washed first and then the spermatozoids. Different mesh sizes (100, 80, 70, 60, 50, 30, and 22 μm) were used in sequence in the washing of gametes. The gametes were then resuspended in a beaker (1000 mL) containing sterilized (UV) and filtered seawater. Spermatozoids were collected in a 50-mL beaker placed in an ice bath. Then they were filtered through 100- and 80-μm meshes into a 60 mL beaker containing similarly sterilized and filtered seawater.

The egg solution was stirred with a plunger; an aliquot was separated for counting in a Sedgewick–Rafter chamber by optical microscopy. The mean number of eggs per milliliter was determined by three counts. Fertilization occurred when a suspension of fresh sperm was added to the egg suspension and gently stirred. The suspension of sperm was calculated to represent 10% (v/v) of the eggs present in the aliquot. For example: to 600 eggs in solution, 60 μL of sperm solution, representing 10% (v/v), must be added. The solution was kept at constant temperature (24±0.5 °C) for an hour, after which it was stirred gently every 30 min until used.

2.3. Test type and characteristics

The test employed was the chronic static embryo–-larval test of short duration. This test was 48 h long. During this period, the glass containers were covered with transparent plastic film and kept in a chamber at constant temperature (24±0.5 °C) on a 12 h light/12 h dark photoperiod. As recommended by the American Society for Testing and Materials (ASTM, 1992) 4-h-old embryos (maximum time after fertilization) were used for the test. Five concentrations with five replicates each and five control flasks were used for each test. For physical–chemical analyses two extra replicates were prepared so that readings could be taken at the beginning and the end of the experiment. The volume of the test solutions was established at 15 mL, each test flask receiving an aliquot of egg solution containing a maximum of 30 eggs/mL (ASTM, 1992).

Solutions were prepared immediately before use. The nominal concentrations of zinc sulfate ranged from 0.25 to 0.96 mg L^{-1}, the dilution factor was 1.4. For SDS, the dilution factor was 1.1, and nominal concentration ranged from 0.68 to 0.99 mg L^{-1}.

2.4. Test conclusion

Embryo–larval development was stopped after 48 h with 0.5 mL of buffered saline formaldehyde 4%. Counting was carried out in a Sedgewick–Rafter chamber, under an optical microscope with 100 ×