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Toxicity of Metals and Pesticides Using the Sperm Cell Bioassay with the Sea Urchin *Arbacia spatuligera*

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The need to implement toxicity bioassays to protect early life stages of aquatic organisms has encouraged the development of bioassays using gametes and larvae as test organisms. Among these, the sea urchin sperm cell bioassay has the convenient properties of being rapid and sensitive to a wide range of toxicants (Mc Gibbon & Moldan, 1986) and more sensitive to complex effluents than are tests with adult animals. The sperm cell toxicity test requires only 60 minutes of toxicant exposure, whereas other marine embryo and larval development tests require 2 to 4 days of exposure, both having similar sensitivities (Dinnel, 1995). The U.S. EPA (1994) has included this test, using laboratory protocols for *Arbacia punctulata,* in its Complex Effluent Assessment Program. The bioassay using different species of sea urchins has been intensively used to assess toxicity of specific compounds (Neiheisel & Young, 1992; Huffman, 1992; Burgess *et al.,* 1993; Cekolin *et al.,* 1993, Morell & Adams, 1993; Nipper *et al.,* 1993; Gaete *et al.* 1996; Warnau *et al.,* 1996, Mwatibo & Green, 1997) and of whole water samples of effluents and receiving waters (Sibley *et al.,* 1993; Krause, 1994; Zuñiga *et al.,* 1995; Riveros *et al.,* 1996 a, b).

The purpose of this study is to present information about the sensitivity of the sperm cell tests with *Arbacia spatuligera* to organic and inorganic compounds found in some Chilean marine ecosystems (Ahumada, 1992; Castilla & Correa, 1997). We report the median effective concentration (EC50) to Cu, Cr, Cd and Zn and the pesticides pentachlorophenol and 2,4-D. We also describe the protocol implemented for this species, present along most of the south-eastern Pacific coastline (Larrain, 1975).

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MATERIALS AND METHODS

Adult sea urchins were obtained by SCUBA diving from unpolluted sites at Coliumo Bay (3°32'S; 72°56'W), central Chile. Individuals were maintained in aquaria with constant aeration, and with complete water replacement every fourth day. Temperature was kept at 13±2°C in a thermoregulated room. Diet was composed of *ad libitum* quantities of mollusks and fishes.

Spawning was elicited by either a mild (12 V) electric shock (when the gonads were fully developed during the reproductive season) or an injection of 2 ml of KCl 0.5 M into the coelomic cavity (when the gonads were receding) (Bay-Schmith, 1981). The eggs spawned by every female sea urchin were tested for viability by inducing fertilization and immediately checking the formation of the fertilization membrane. Only females whose eggs showed a 90% fertilization success or more contributed to the pool of eggs for each bioassay. At least 4 females were used to complete an egg stock. Eggs were washed 3 times in 300 ml of seawater before using them in the bioassays. Sperm viability was assessed by observing their mobility in seawater. At least 4 males were used to yield a "dry" sperm stock for each bioassay, which was maintained on ice until its use in the experiment. For the bioassays, the egg stock was diluted to 2000 eggs/ml. Counting was done using a Sedgwick-Rafter chamber. Sperm stock was diluted to $7x10^7$ sperms/ml using a Neubauer chamber (U.S. EPA, 1994).

We used 4 replicates per treatment in every experiment, each consisting of a new glass test tube with 5 ml of testwater. The seawater (33‰ ± 2) used for dilution and control water, obtained from Coliumo Bay, was previously filtered with 0.5 µm cellulose filters. The toxicants tested were Cu ($CuSO_4 x\ 5\ H_2O$), Cd ($CdSO_4 x\ 8/3\ H_2O$), Cr ($K_2Cr_2O_7$), Zn ($ZnCl_2$), pentachlorophenol (PCP), and 2,4-D. In the pentachlorophenol bioassays, acetone was used as solvent, and a control for acetone (0.1 ml/l) was also included.

In the bioassays, 100 µl of the sperm suspension were added to the test tubes with the toxicant dilution. After 60 minutes, 1 ml of the egg suspension was added to each test tube. An ongoing monitoring of the control test tube showed that sometime after 10 to 20 minutes, 80% fertilization was reached. At that

moment, the tests were stopped by adding 2 ml of a 10% solution of formaldehyde in seawater. Finally, 100 eggs were observed for each test tube, and the number of fertilized and unfertilized eggs, as judged from the presence or absence of the fertilization membrane was recorded. The 50% effective concentrations (EC50) were estimated for each bioassay using the PROBIT method (U.S. EPA, 1988).

RESULTS AND DISCUSSION

The concentration-response curves and the EC50 values for the different toxicants tested in each bioassay are shown in Figure 1. The order of sensitivity of the bioassay to the toxicants is as follows: Cu>Zn>PCP>Cr>Cd>2,4-D.

Average EC50 values of each of the the toxicants on *Arbacia spatuligera* fertilization are shown in Table 1, compared to results of bioassays with other sea urchin species, obtained from the literature, over 60 minutes exposure of sperms. These results are similar to previous findings indicating that in general the sea urchin fertilization test is especially sensitive to metals, due most likely to the effect of excess cations on sperm mobility (Nacci *et al.*, 1991). It must be noted however, that PCP is more toxic than both Cr^{VI} and Cd. It is observed that the order of sensitivity to the toxicants for *Arbacia spatuligera* is similar to those reported for other sea urchins. In fact, all species in Table 1 show a high sensitivity to Cu and Zn and smaller sensitivity to the other toxicants.

The protocol described in this paper for sperm cell toxicity test with *Arbacia spatuligera* followed the guidelines previously developed by the U.S. EPA (1994) with *A. punctulata*. Modifications introduced were a reduction in temperature from 20°C to 13°C, to better approximate average ambient temperatures at which natural populations of sea urchins are exposed, and increased sperm/egg ratio from 2500 to 3500, as a result of calibration tests that showed that this higher ratio allowed over a 90% fertilization of eggs in control seawater.

A. spatuligera has a seasonal cycle of maturity (Bay-Schmith, 1981). Therefore, availability of mature individuals is a major concern for the applicability of the bioassay. Unpublished results from our laboratory show that *A. spatuligera* can be maintained with mature gonads year-round if a rich protein diet

Table 1. Comparative EC50 (mg/L) values (mean ± St. Dev. or confidence interval) of pesticides and heavy metals of sperm cell test with *Arbacia spatuligera* and other echinoid species.

HEAVY METALS

SPECIES	Copper	Chromium	Cadmium	Zinc
A. spatuligera (a)	0.018 ± 0.01	20.7 ± 8.7	140.9 ± 88.1	0.116 ± 0.061
(b)	0.008 (0.007-0.01)			
E. mathaei (c)	0.014		>0.1	
L. variegatus (d)				0.068 ± 0.033
A. punctulata (e)	0.012 (0.011-0.120)		38.0 (34.3-42.5)	0.121 (0.110-0.113)
(f)	0.011 ± 0.006			
(g)		341.8 ± 53.01		
S. droebachiensis (h)	0.059 (0.051-0.068)		26 (21-23)	0.383 (0.302-0.491)
S. purpuratus (h)	0.025 (0.012-0.06)		18 (15-23)	0.262 (0.21-0.31)
S. franciscanus (h)	0.019 (0.016-0.025)		12 (8-20)	0.313 (0.261-0.378)

PESTICIDES

	PCP	2,4-D
A. spatuligera (a)	4.23 ± 1.97	205.4 ± 42.4
A. punctulata (e)	0.9 (0.8-1.0)	

(a) This study, *Arbacia spatuligera,* (b) *Arbacia spatuligera* (Zuñiga *et al.,* 1995), (c) *Echinometra mathaei* (Neiheisel & Young, 1992), (d) *Lytechinus variegatus* (Morrell & Adams, 1993), (e) *Arbacia punctulata* (Burgess *et al.,* 1993), (f) *Arbacia punctulata* (Nacci *et al.,* 1986), (g) *Arbacia punctulata* (Adams & Slaughter-Williams, 1988), (h) *Strongylocentrotus droebachiensis, Strongylocentrotus purpuratus, Strongylocentrotus franciscanus* (Jop, 1989).

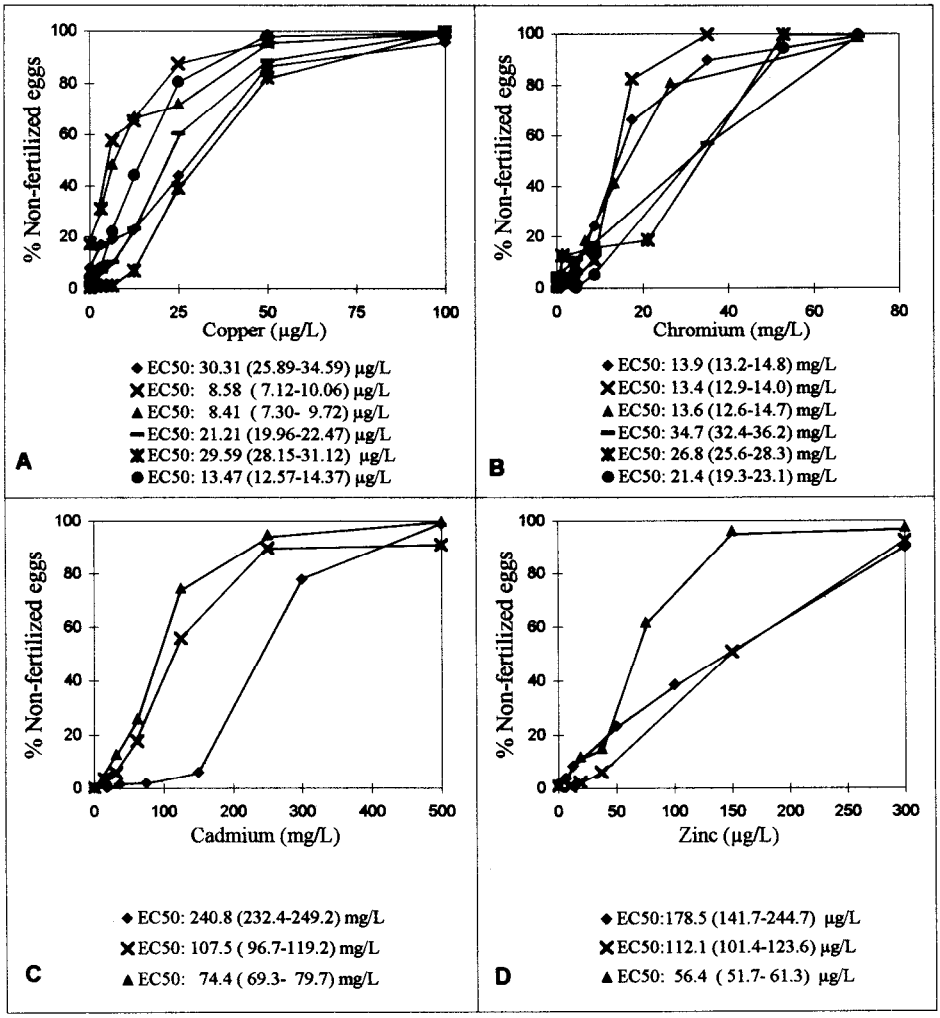

Figure 1. Percentage of non-fertilized eggs following 60 minutes exposure of *Arbacia spatuligera* sperm to differents toxicants. The points correspond to the means of the replicates from each concentration. The EC50 values and the confidence intervals, obtained from the Probit method, are shown for each bioassay. (It continues in following page).

is fed to mature individuals of both sexes. A similar finding was reported by Lawrence *et al.* (1992) for *Paracentrotus lividus* with a diet based on fish meal pellets.

The sea urchin sperm cell test with *Arbacia spatuligera*, set up this manner, has several important advantages that make it an

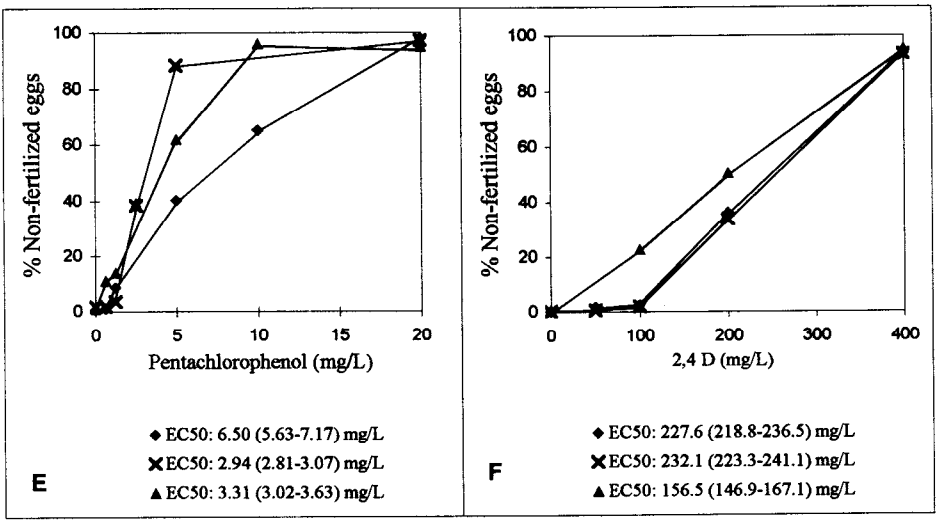

Figure 1. (Continuation).

extremely useful tool in the environmental monitoring of Chilean coastal habitats: it is a short duration test, sensitive to several toxicants, requires small amounts of test water, can be used year-round when a stock of sea urchins properly fed is available in the laboratory and is based on a species present along most of the Chilean coastline.

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