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# TOXICITY OF CADMIUM, ZINC AND COPPER ON SPERM CELL FERTILIZATION OF SEA URCHIN, *DIADEMA SETOSUM*

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## ABSTRACT

*The sperm cell fertilization tests using sea urchin, Diadema setosum were conducted to determine the toxicity of cadmium, zinc and copper. Five concentrations for cadmium ranging from 0.32 - 32 mg Cd/L, for zinc ranging from 0.032 - 3.2 mg Zn/L, and for copper ranging from 0.001 - 0.1 mg Cu/L, plus a control were used to investigate the effects of these metals on sperm viability as indicated by a reduction in fertilization success. This test used a short exposure period, 10 minutes sperm exposure, followed by 10 minutes egg fertilization. The experiments were repeated three times for each metal. The results indicated that copper was the most toxic to D. setosum, followed by zinc and cadmium, respectively, with toxicity values (EC50s) of 0.017 ± 0.013 mg Cu/L, 0.38 ± 0.19 mg Zn/L, and 6.28 ± 1.32 mg Cd/L, respectively. These values were calculated based on actual concentrations for cadmium and zinc, and on nominal concentrations for copper.*

## INTRODUCTION

The problem of heavy metal pollution in coastal waters and its effects on aquatic organisms is drawing increasing attention. Information concerning toxicities of heavy metals on aquatic organisms is still limited in the ASEAN region. The ASEAN-Canada Cooperative Programme on Marine Science - Phase II (CPMS-II) focuses on the need to formulate marine water quality criteria for the ASEAN region, to manage the marine environment, and to develop and nurture resources in that environment. The first step is to develop "interim" marine water quality criteria for 15 priority parameters including chemical, physical and biological parameters. The results of toxicity testing with appropriately sensitive ASEAN species will be used to validate the proposed criteria to suitably protect sensitive tropical marine habitats[1]. The bioassay to measure marine pollution using sea urchin eggs has such advantages as simplicity, easiness, speed, high sensitivity, clearness of indicatory features, uniformity, and higher accuracy and moreover different sea urchin species react to pollutants very similarly and this makes the same method available in any season of the year[2,3,4,5,6]. Toxicity tests with sea urchin are now widely adopted in England, Italy, Japan, Norway, South Africa and the USA[7]. However, such studies are rather few in Thailand. Therefore, this present study was conducted to establish baseline data on toxicity of cadmium, zinc and copper on sperm cell fertilization of blue-spot sea urchin, *Diadema setosum*, which is widely distributed over the tropical and subtropical parts of the Indo-Pacific[8]. In addition, this species can be exploited as a valuable training tool to assess and demonstrate environmental impacts in the East Asian region using survival and developmental success as indicators[9]. Cadmium, zinc and copper were selected for this study because they were among the ten most poisonous heavy metals to marine life[10].

## MATERIALS AND METHODS

The sperm cell fertilization tests were conducted according to the procedures described in Dinnel *et al.*[11]and CPMS-II[12] using the sea urchin, *Diadema setosum*. The test involves

spawning adult sea urchins, exposing the sperm to test material for 10 minutes, then eggs are added and fertilization is allowed to occur for a further 10 minutes. Samples are preserved and the number of fertilized and unfertilized eggs in each replicate is counted to determine treatment responses. Toxic effects on sperm viability are indicated by a reduction in fertilization success.

## Test Procedure

### Spawning of sea urchins

The sea urchins were collected at Koh Kham, Amphoe Sattahip, Chon Buri Province, from between 3 and 10 m depths by snorkel or scuba diving. Eggs and sperm were obtained by injecting 1 mL of 0.5 M potassium chloride (KCl) solution through the peristomeal membrane into the coelomic cavity of the adult sea urchins. If no response was observed after 30 seconds then they were injected with another dose of KCl. Female sea urchins were wet-spawned by placing them aboral side down on top of 100-mL glass beakers filled with seawater. Males were dry-spawned by placing them aboral side down in small petri dishes with about 5 mL of seawater. The sea urchins were allowed to spawn for 10-20 minutes. Sperm were collected with a pasteur pipette, transferred to a small beaker and stored on ice until ready for use. Eggs were pooled from several sea urchins and washed three times with control seawater.

### Test solutions preparation

Test solutions were prepared while the sea urchins were spawning. Cadmium chloride ($CdCl_2.2H_2O$), zinc sulphate ($ZnSO_4.7H_2O$) and copper sulphate ($CuSO_4.5H_2O$) were used to prepare the test solutions. Filtered (0.45 $\mu$m) and sterilized natural seawater was used as the dilution/control water. All concentrations were expressed as the amount of metal ion (e.g. mg Cd/L, mg Zn/L or mg Cu/L), not the metal salt. Five nominal concentrations in geometric series for cadmium ranging from 0.32 - 32 mg Cd/L ( 0.32, 1.0, 3.2, 10, and 32 mg Cd/L), for zinc ranging from 0.032 - 3.2 mg Zn/L ( 0.032, 0.1, 0.32, 1.0 and 3.2 mg Zn/L), and for copper ranging from 0.001 - 0.1 mg Cu/L ( 0.001, 0.0032, 0.01, 0.032 and 0.1 mg Cu/L ) plus a control, were prepared for each toxicity test. The aliquots of 10-mL of each test solution were transfered to the corresponding labelled(16x100-mm) glass test tubes using a 10-mL automatic pipettor. Each treatment consisted of three replicates. The experiments were repeated three times for each metal. Water quality parameters (temperature, pH, dissolved oxygen and salinity) were measured at the beginning of the test in the control, low, medium and high test concentrations for each metal. Subsamples of each test solution were collected at the beginning of each test for heavy metal analysis, extracted immediately with ammonium pyrrolidine dithiocarbamate (APDC) and methyl isobutyl ketone (MIBK), and then back-extracted with 4N nitric acid. The metal concentration was measured using a Hitachi 180-30 atomic absorption spectrophotometer.

### Preparing standard gamete densities

An initial sperm suspension were prepared by diluting "dry" sperm with control seawater. The sperm were examined under a compound microscope; they should be numerous and active. The sperm density was determined in the suspension using a Neubauer haemocytometer. The egg density was determined by counting the number of eggs in 1-mL samples of homogeneous egg suspension using a Sedgewick-Rafter counting chamber under the microscope, and then adjusted to 2,000 eggs/mL.

**Pre-trial fertilization test**

Prior to beginning of the test, a sperm:egg ratio yielding 70-90% fertilization in control seawater was determined. The following recommended sperm:egg ratios were prepared for the pre-trial test: 200:1, 600:1, 1,200:1, 2,000:1 and 6,000:1. The following formula was used to prepare the sperm concentrations, and then corrected for the fact that only 0.1 mL of sperm were added to each test container:

$$C_1 V_1 = C_2 V_2$$

where:
$C_1$ = density of initial sperm suspension
$V_1$ = volume of initial sperm suspension
$C_2$ = desired sperm concentration
$V_2$ = volume of final sperm concentration

For example, preparation of a 200:1 sperm:egg ratio involves addition of a 0.1-mL aliquot containing 400,000 sperm (i.e., sperm density of 4,000,000/mL). If the initial sperm suspension density was 100 million sperm/mL and a 100-mL volume with a density of 4 million sperm/mL were desired ( for a 200:1 ratio), then the volume of initial sperm suspension required would be:

$$V_1 = \frac{(4 \text{ million sperm/mL})\ (100\text{mL})}{(100 \text{ million sperm/mL})} = 4 \text{ mL}$$

For a sperm:egg ratio of 200:1, the sperm density required ($C_2$) is:

$$C_2 = \frac{(2{,}000 \text{ eggs/mL})\ (200 \text{ sperm/egg})}{0.1 \text{ mL}} = 4{,}000{,}000 \text{ sperm/mL}$$

The sperm densities required to achieve the other sperm:egg ratios are as follows:

| | |
|---|---|
| 600:1 ratio | 12,000,000 sperm/mL |
| 1,200:1 ratio | 24,000,000 sperm/mL |
| 2,000:1 ratio | 40,000,000 sperm/mL |
| 6,000:1 ratio | 120,000,000 sperm/mL |

Each sperm concentration was prepared. To start the pretrial test, added 0.1 mL of each sperm concentration to the corresponding test tube. Covered with parafilm, inverted to mix and incubated for 10 mintues. At the end of the sperm exposured, added 1.0 mL of the egg suspension ( density adjusted to 2,000 eggs/mL) to each tube. Covered with parafilm, inverted each test tube to mix the contents and incubated for another 10 mintues. At the end of the sperm and egg exposure, the test was terminated by adding 1 mL of 50% buffered formalin to each test tube to preserve the fertilized eggs. Subsample of 100 eggs were counted at each sperm:egg ratio and the percentage of fertilized and unfertilized eggs were recorded. The sperm:egg ratio yielding 70-90% fertilization (preferably closer to 90%) was used in the definitive test.

### Definitive test

A fresh sperm suspension was prepared at the concentration that yielded 70- 90% fertilization in the pre-trial test. Each test tube was inoculated with a 0.1-mL aliquot of sperm suspension and allowed to incubate for 10 minutes. A 1-mL aliquot of egg suspension was then added to each test tube and, after a 10 minutes fertilization period, the test was terminated by adding 1 mL of 50% buffered formalin to each test tube to preserve the eggs. Fertilized and unfertilized eggs were counted for each test tube under a compound microscope to determine mean percent fertilization success for each treatment by examining subsample of 100 eggs per replicate for presence or absence of a normal fertilization membrane. Eggs with only partial membrane formation were counted as fertilized and damaged eggs were not counted.

### Data analysis

The test endpoint was based on adverse effects on fertilization success. For the test to be valid, mean control fertilization success must be ≥ 50%. Mean percent unfertilized eggs and mean net percent unfertilized eggs were calculated by using the following equations. Abbott 's formula (for correcting responses) was used to calculate mean net unfertilized eggs.

$$\text{Mean Unfertilized Eggs (\%)} = \frac{\text{(Total no. unfertilized eggs for all reps.)}}{\text{(Total no. eggs for all reps.)}} \cdot 100$$

$$\text{Mean Net Unfertilized Eggs (\%)} = \frac{\text{(\% Test Response - \% Control Response)}}{\text{(100 - \% Control Response)}} \cdot 100$$

Significant differences ($P < 0.05$) in percentage of unfertilized eggs for each sample were determined using the TOXSTAT computer program[13]. The data were transformed prior to statistical analysis using an arcsine square root transformation as recommended for binomial data expressed as percentages[14]. Each data set was tested for normality and homogeneity of variance prior to detailed analysis, using a Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variance to determine whether parametric or non-parametric tests should be used. The NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) values were determined using an analysis of variance and Dunnett's *t*-test. All EC50 (median Effective Concentration) values were calculated using the EFFL computer program (IBM/AT Version 1.0), which uses procedures described by Stephan[15].

The NOEC (No Observed Effect Concentration) is the highest tested concentration at which fertilization success did not differ significantly from that in the control. The LOEC (Lowest Observed Effect Concentration) is the lowest tested concentration at which fertilization success differed significantly from that in the control. The EC50 (median Effective Concentration) is the concentration that causes a 50% reduction in fertilization success.

## RESULTS

The sperm:egg ratio for the pre-trial test yielding 90% fertilization in control seawater was determined to be 2,000:1 for the three set of tests. The results of fertilization of sea urchin eggs exposed to various concentrations of cadmium, zinc and copper are summarized in Tables 1-3. The statistical endpoints for each test (NOEC, LOEC, EC50) are presented in Table 4.

The mean control fertilization success of sea urchin eggs ranged from 77.3 to 95.0% for the three sets of tests. The percentage of fertilized eggs generally decreased with increasing metals concentration. The NOEC values for cadmium, zinc and copper ranged from 0.95 to 3.28 mg Cd/L, <0.03 to 0.08 mg Zn/L, and <0.001 to 0.01 mg Cu/L, while the LOEC values ranged from 3.23 to 9.00 mg Cd/L, 0.03 to 0.28 mg Zn/L, and 0.001 to 0.032 mg Cu/L, respectively. The 20-min EC50 values for cadmium ranged from 5.21 to 7.75 mg Cd/L ( mean of 6.28 mg Cd/L ), for zinc ranged from 0.16 to 0.49 mg Zn/L ( mean of 0.38 mg Zn/L ), and for copper ranged from 0.009 to 0.032 mg Cu/L ( mean of 0.017 mg Cu/L ). The results indicated that copper appears to be the most toxic metal to fertilization of *D. setosum* followed by zinc and cadmium, respectively.

The water quality parameters measured in the samples prior to testing for the three sets of tests for all three heavy metals were in the ranges: temperature, 25.5 - 28.8°C; salinity, 32-33 ppt; pH, 8.2-8.4; dissolved oxygen, 6.4-7.2 mg/L as detailed in Table 5. Due to the small test solution volumes and the short test duration, water quality parameters were not normally measured at the end of the test.

**Table 1** Effect of various cadmium concentrations on fertilization of sea urchin, *Diadema setosum*. (Sperm:egg ratio = 2,000:1)

| Experiment No. | Nominal Conc. (mg Cd/L) | Actual Conc. (mg Cd/L ) | Mean % Fertilized Eggs | Mean % Unfertilized Eggs[1] | Mean Net % Unfertilized Eggs |
|---|---|---|---|---|---|
| 1 | 32 | 31.8 | 12.3 | 87.7* | 87.0 |
| | 10 | 6.12 | 45.7 | 54.3* | 51.9 |
| | 3.2 | 3.28 | 85.0 | 15.0 | 10.5 |
| | 1.0 | 0.89 | 94.0 | 6.0 | 1.1 |
| | 0.32 | 0.34 | 93.0 | 7.0 | 2.1 |
| | Control | ND | 95.0 | 5.0 | 0.0 |
| 2 | 32 | 31.3 | 2.3 | 97.7* | 97.1 |
| | 10 | 9.0 | 25.0 | 75.0* | 69.0 |
| | 3.2 | 3.23 | 64.3 | 35.7* | 20.3 |
| | 1.0 | 0.95 | 70.0 | 30.0 | 13.2 |
| | 0.32 | 0.34 | 71.3 | 28.7 | 11.6 |
| | Control | ND | 80.7 | 19.3 | 0.0 |
| 3 | 32 | 31.3 | 6.0 | 94.0* | 92.2 |
| | 10 | 9.0 | 18.0 | 82.0* | 76.7 |
| | 3.2 | 3.23 | 60.3 | 39.7 | 22.0 |
| | 1.0 | 0.95 | 64.0 | 36.0 | 17.2 |
| | 0.32 | 0.34 | 65.0 | 35.0 | 16.0 |
| | Control | ND | 77.3 | 22.7 | 0.0 |

[1] Asterisks indicate values where responses were significantly ( $P < 0.05$ ) higher than the control.

ND = Non-detectable

**Table 2** Effect of various zinc concentrations on fertilization of sea urchin, *Diadema setosum*. (Sperm:egg ratio = 2,000:1)

| Experiment No. | Nominal Conc. (mg Zn/L) | Actual Conc. (mg Zn/L ) | Mean % Fertilized Eggs | Mean % Unfertilized Eggs[1] | Mean Net % Unfertilized Eggs |
|---|---|---|---|---|---|
| 1 | 3.2 | 3.13 | 1.7 | 98.3* | 98.3 |
| | 1.0 | 0.91 | 3.3 | 96.7* | 96.5 |
| | 0.32 | 0.28 | 39.0 | 61.0* | 59.0 |
| | 0.1 | 0.08 | 62.3 | 37.7 | 34.4 |
| | 0.032 | 0.03 | 82.7 | 17.3 | 13.0 |
| | Control | ND | 95.0 | 5.0 | 0.0 |
| 2 | 3.2 | 3.65 | 2.0 | 98.0* | 97.5 |
| | 1.0 | 0.97 | 4.7 | 95.3* | 94.2 |
| | 0.32 | 0.41 | 60.3 | 39.7* | 25.2 |
| | 0.1 | 0.13 | 66.0 | 34.0* | 18.2 |
| | 0.032 | 0.03 | 68.7 | 31.3* | 14.9 |
| | Control | ND | 80.7 | 19.3 | 0.0 |
| 3 | 3.2 | 3.65 | 2.0 | 98.0* | 97.4 |
| | 1.0 | 0.97 | 4.7 | 95.3* | 94.0 |
| | 0.32 | 0.41 | 52.0 | 48.0* | 32.8 |
| | 0.1 | 0.13 | 60.7 | 39.3* | 21.6 |
| | 0.032 | 0.03 | 54.7 | 45.3* | 29.3 |
| | Control | ND | 77.3 | 22.7 | 0.0 |

[1] Asterisks indicate values where responses were significantly ( $P < 0.05$ ) higher than the control.
ND = Non-detectable

**Table 3** Effect of various copper concentrations on fertilization of sea urchin, *Diadema setosum*. (Sperm:egg ratio = 2,000:1)

| Experiment No. | Nominal Conc. (mg Cu/L) | Mean % Fertilized Eggs | Mean % Unfertilized Eggs[1] | Mean Net % Unfertilized Eggs |
|---|---|---|---|---|
| 1 | 0.1 | 7.3 | 92.7* | 92.3 |
| | 0.032 | 64.3 | 35.7* | 32.3 |
| | 0.01 | 79.7 | 20.3 | 16.1 |
| | 0.0032 | 76.3 | 23.7 | 19.7 |
| | 0.001 | 94.3 | 5.7 | 0.7 |
| | Control | 95.0 | 5.0 | 0.0 |
| 2 | 0.1 | 1.7 | 98.3* | 97.9 |
| | 0.032 | 9.3 | 90.7* | 88.4 |
| | 0.01 | 56.3 | 43.7* | 30.2 |
| | 0.0032 | 50.3 | 49.7* | 37.6 |
| | 0.001 | 67.3 | 32.7 | 16.5 |
| | Control | 80.7 | 19.3 | 0.0 |
| 3 | 0.1 | 4.7 | 95.3* | 94.0 |
| | 0.032 | 5.0 | 95.0* | 93.5 |
| | 0.01 | 43.0 | 57.0* | 44.4 |
| | 0.0032 | 55.0 | 45.0* | 28.9 |
| | 0.001 | 60.7 | 39.3* | 21.6 |
| | Control | 77.3 | 22.7 | 0.0 |

[1] Asterisks indicate values where responses were significantly ( $P < 0.05$ ) higher than the control.
ND = Non-detectable

**Table 4** Summary of statistical endpoints measured in sperm cell fertilization of sea urchin, *Diadema setosum*.

| Metals | Experiment No. | NOEC[1] | LOEC[1] | 20-min EC50[2] (95% confidence interval) |
|---|---|---|---|---|
| Cadmium | 1 | 3.28 (3.2) | 6.12 (10) | 7.75 (6.52 - 9.21) |
| (mg Cd/L) | 2 | 0.95 (1.0) | 3.23 (3.2) | 5.88 (4.79 - 7.23) |
| | 3 | 3.23 (3.2) | 9.00 (10) | 5.21 (4.15 - 6.54) |
| | Mean ± SD | 2.49 ± 1.33 | 6.12 ± 2.89 | 6.28 ± 1.3 |
| Zinc | 1 | 0.08 (0.1) | 0.28 (0.32) | 0.16 (0.13 - 0.20) |
| (mg Zn/L) | 2 | < 0.03 (< 0.032) | 0.03 (0.032) | 0.49 (0.40 - 0.60) |
| | 3 | < 0.03 (< 0.032) | 0.03 (0.032) | 0.48 (0.39 - 0.60) |
| | Mean ± SD | < 0.05 ± 0.03 | 0.11 ± 0.14 | 0.38 ± 0.19 |
| Copper | 1 | 0.01 | 0.032 | 0.032 (0.026 - 0.038) |
| (mg Cu/L) | 2 | 0.001 | 0.0032 | 0.009 (0.007 - 0.012) |
| | 3 | < 0.001 | 0.001 | 0.009 (0.007 - 0.012) |
| | Mean ± SD | < 0.004 ± 0.005 | 0.012 ± 0.017 | 0.017 ± 0.013 |

[1] Actual metal concentrations are reported, with nominal concentrations provided in parentheses except for copper, only nominal concentrations are reported.

[2] The EC50 values were calculated using the trimmed Spearman-Kaber method. Actual metal concentrations are reported for cadmium and zinc and nominal concentrations are reported for copper.

**Table 5** Water quality ranges for sperm cell fertilization tests of sea urchin.

| Parameters | Cd | | | Zn | | | Cu | | |
|---|---|---|---|---|---|---|---|---|---|
| Experiment | No. 1 | No. 2 | No. 3 | No. 1 | No. 2 | No. 3 | No. 1 | No. 2 | No. 3 |
| Temperature (°C) | 25.5 | 28.8 | 28.8 | 25.5 | 28.8 | 28.8 | 25.5 | 28.8 | 28.8 |
| Salinity (ppt) | 33 | 32 | 32 | 33 | 32 | 32 | 33 | 32 | 32 |
| pH | 8.2-8.4 | 8.2-8.3 | 8.2-8.3 | 8.2-8.4 | 8.2-8.3 | 8.2-8.3 | 8.2-8.4 | 8.2-8.3 | 8.2-8.3 |
| Dissolved oxygen (mg/L) | 6.7-7.2 | 6.4-6.7 | 6.4-6.7 | 6.7-7.2 | 6.4-6.7 | 6.4-6.7 | 6.7-7.2 | 6.4-6.7 | 6.4-6.7 |

## DISCUSSION

Heavy metals and other toxicants act on formation of the fertilization membrane, cell division, gastrulation, pluteus formation and many other developmental stages of sea urchin eggs as indicated by Kobayashi[8]. A comparison of the cadmium, zinc and copper toxicity tests on sperm cell fertilization of sea urchin, *D. setosum*, indicated that copper was the most toxic metal, followed by zinc and cadmium, respectively. The potential toxicity of copper to *D. setosum* was found to be approximately 20 and 300 times greater than zinc and cadmium, respectively. Similar findings have been reported by Kobayashi[8]; while the effects of six toxicants on developmental stages of the sea urchin, *D. setosum* differed somewhat from results with Japanese sea urchins, the relative toxicity at the pluteus stage was similar to previous findings,

*viz.*, Cu > Zn > Ni > Cd > ABS > $NH_3$. According to Vernberg and Vernberg[16], the toxicity ranking for these three metals was as follows: Cu > Zn > Cd.

Nacci *et al.*[17] reported toxicity values (EC50s) of copper, zinc and cadmium on sperm cell tests using the sea urchin, *Arbacia punctulata*, were 0.012, 0.121 and 38,000 mg/L, respectively on the basis of a 60 minutes sperm exposure, followed by a 20 minutes egg fertilization (60+20 minutes) (U.S. reference protocal; EPA[18]) while the present study found the EC50 values of copper, zinc and cadmium to *D. setosum* were 0.017, 0.38 and 6.28 mg/L, respectively, on the basis of 10+10 minutes exposure (Canadian reference protocal; Environment Canada[19]). Comparing these two studies was rather difficult because different exposure times may be affected fertilizing capability of the sperm as presented by Dinnel *et al.*[11] However, cadmium was rather more toxic to *D. setosum* than *A. punctulata*.

The results of this study can be compared to other toxicity tests conducted with tropical marine invertebrates. Ong and Din[20] reported that the 96-h LC50 values of copper to the clam, *Donax faba*, and spats of the blood cockle, *Anadara granosa*, were 0.12 and 0.22 mg/L, respectively, and the 96-h LC50 values of cadmium to these two invertebrates were 1.01 and 0.95 mg/L, respectively. Dechaprompun[21] reported that the 48-h EC50 values of copper and cadmium on embryonic development of the oyster, *Crassostrea commercialis*, at 28°C were 0.0094 and 0.5542 mg/L, respectively, and 96-h LC50 values of copper and cadmium for adult oysters at ambient temperature were 2.44 and 2.21 mg/L, respectively. Jaritkhuan and Sawangwong[22] reported the 96-h LC50 values of cadmium, copper and zinc on juvenile tiger prawn, *Penaeus monodon* were 18.82, > 1.60 and > 7.63 mg/ L, respectively. Based on the comparisons, sperm cell tests using sea urchin from the present study were more sensitive to copper than the acute tests using the clam, cockle, oyster and tiger prawn, while the acute tests conducted with the clam, cockle and adult oyster were more sensitive to cadmium than the sperm cell tests. No information was available for zinc toxicity comparisons among these tropical marine invertebrates, except, the sperm cell tests using sea urchin were more sensitive to zinc toxicity than the acute tests using tiger prawn.

The sperm cell test is quick, efficient and requires no extraordinary equipment or procedures, and the test's sensitivity is greater than that of acute toxicity tests, at least for some chemicals (Nacci *et al.*[17]). It was also agreed with Kobayashi[23] that sperm activity is the most sensitive to some chemicals than other developmental stages of sea urchin. Therefore, use of the sperm cell fertilization test is useful in measuring toxicities that can provide a quick and easy long term monitoring tool and help to provide valuable comparative data for toxicant effects in natural marine waters.

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## บทคัดย่อ

พิษของโลหะแคดเมี่ยม สังกะสี และทองแดง ได้ทำการศึกษาโดยใช้วิธีการทดสอบการเข้าไปปฏิสนธิของตัวอสุจิของเม่นทะเล, *Diadema setosum* โดยทำการทดลองที่ระดับความเข้มข้น 5 ระดับ ได้แก่ แคดเมี่ยมในช่วงระหว่าง 0.32 - 32 มิลลิกรัมต่อลิตร สังกะสีในช่วงระหว่าง 0.032 - 3.2 มิลลิกรัมต่อลิตร และทองแดงในช่วงระหว่าง 0.001 - 0.1 มิลลิกรัมต่อลิตร โดยมีกลุ่มควบคุมเพื่อใช้ในการเปรียบเทียบด้วย ศึกษาผลของโลหะเหล่านี้ที่มีต่อความสามารถของตัวอสุจิที่จะเข้าไปปฏิสนธิกับไข่ของเม่นทะเล การทดลองใช้ระยะเวลาช่วงสั้น คือ ปล่อยให้ตัวอสุจิอยู่ในสารละลายทดลองนาน 10 นาที แล้วตามด้วยการเข้าทำปฏิสนธิกับไข่อีก 10 นาที รวมเวลาทั้งหมด 20 นาที ทำการทดลองซ้ำรวม 3 ครั้งสำหรับโลหะหนักแต่ละชนิด ผลการทดลอง พบว่าโลหะทองแดงมีพิษต่อเม่นทะเล, *D. setosum* มากที่สุด รองลงมา ได้แก่ สังกะสี และ แคดเมี่ยม ตามลำดับ โดยมีค่าระดับความเข้มข้นที่เป็นพิษ (EC50) ดังนี้ ทองแดง $0.017 \pm 0.013$ มิลลิกรัมต่อลิตร สังกะสี $0.38 \pm 0.19$ มิลลิกรัมต่อลิตร และแคดเมี่ยม $6.28 \pm 1.32$ มิลลิกรัมต่อลิตร ซึ่งค่าเหล่านี้สำหรับโลหะแคดเมี่ยมและสังกะสี คำนวณโดยใช้ระดับความเข้มข้นที่มีอยู่จริงในน้ำทดลอง ส่วนของทองแดงคำนาณโดยใช้ระดับความเข้มข้นของโลหะที่เติมลงไป