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Inhibitory Effects of Mercury and Cadmium on Seed Germination of *Enhalus acoroides* (L.f.) Royle

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Toxicity tests, both acute and chronic, are being used to assess the toxicity of chemicals introduced into the environment. Most of the test organisms used are of temperate origins and tests conducted are under such conditions. Data generated from such test has become the basis for the formulation of environmental criteria, but such test organisms and conditions may not be applicable in the tropical region. The U.S. Environmental Protection Agency (EPA) found that biological testing is a scientifically valid approach to control toxic substances in wastewater discharges (Wall and Hanmer 1987). Toxicity tests in the ASEAN (Association of Southeast Asian Nation) region are few and they are only recently gaining ground in the use of establishing environmental criteria in the region. This study presents the use of the seeds of the seagrass *Enhalus acoroides* (L.f.) Royle in a germination test conducted under tropical conditions. This tropical eelgrass is commonly found in the Indian Ocean and in the tropical parts of the Western Pacific. The use of this marine angiosperm as a test organism is of great importance because marine environmental criteria derivation in the ASEAN region, specifically, in the Philippines requires toxicity data from plants as well. Also, this study is the first attempt to fill the gap of toxicity data on marine angiosperm.

MATERIALS AND METHODS

The different test conditions are summarized in Table 1. All the physicochemical parameters measured, fall within the range of characteristics typical of a tropical marine water (CIDA 1993).

The toxicity test was a static renewal chronic exposure for 10 days. The definitive test solutions used (0.0, 5.6, 10.0, 18.0, 32.0, 56.0 and 100.0 mg/L of Hg and Cd in filtered UV-sterilized seawater) were a logarithmic series of concentrations, wherein the lower (5.6 mg/L) and the upper limit (100.0 mg/L) of concentrations were determined from a rangefinding test.

Fruits of *E. acoroides* (L.f.) Royle were collected from the shoreline of Silaki Island in Bolinao, Pangasinan, Philippines. Each fruit contained a range of 8 to 12

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Table 1. Test conditions for seed germination toxicity assay

Test Variables	Conditions
Pre- treatment	1% Hypochlorite soln. for 15 minutes
Test type	renewal
Temperature	28 - 31 ^{0}C
Salinity	31 - 35 ppt
Light quality	ambient light
pH	6 - 8
Test vessel	100 x 85 mm glass culture dish
Control	filtered (0.2μ) UV sterilized natural seawater
Specimen	10 seeds per culture dish
Test replicates	3 / concentrations
Test duration	10 days
Test compounds	mercuric chloride and cadmium chloride

seeds. These were immediately transported to the Marine Science Institute Bolinao Marine Laboratory. Fruits were opened and mature healthy seeds (without defect) were selected and immersed in 1% hypochlorite solution for 15 minutes (Montaño et al. 1994). The seeds were washed with filtered (0.2 μ) UV sterilized seawater. Ten seeds were placed in glass culture dishes containing 20 mL of the different concentrations of test solutions. Seeds were arranged in such a way that each seed did not touch each other nor touch the side of the dish. Three replicates of each test solution were prepared. The length of shoots were measured (in mm.) daily with a caliper. Temperature, salinity and pH were measured every 24 hours and recorded. Solutions were also changed every 24 hours and seeds with molds were treated with 1% hypochlorite solution. Tests were disregarded if more than 10 % of the seeds in control did not germinate.

Actual metal concentration from each test solutions was determined after the 10-day run. These water samples were preserved using concentrated HCl and subjected for analysis. Seagrass seeds were placed in cold storage for metal content analysis. All seeds and metal solutions were analyzed at the Laboratory of the Research and Development Division of the Environmental Management Bureau of the Department of Environment and Natural Resources (EMB-DENR) and the Chemical Oceanography Laboratory of the Marine Science Institute of the University of the Philippines (UP-MSI). These two laboratories regularly participate in local and international intercomparison exercises. The analysis was carried out using the Shimadzu AA680 FAAS with deuterium background correction. Sample analyses were performed using the standard addition technique with the results having a coefficient of variance of < 1.

The test results were expressed as NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) values using the TOXSTAT

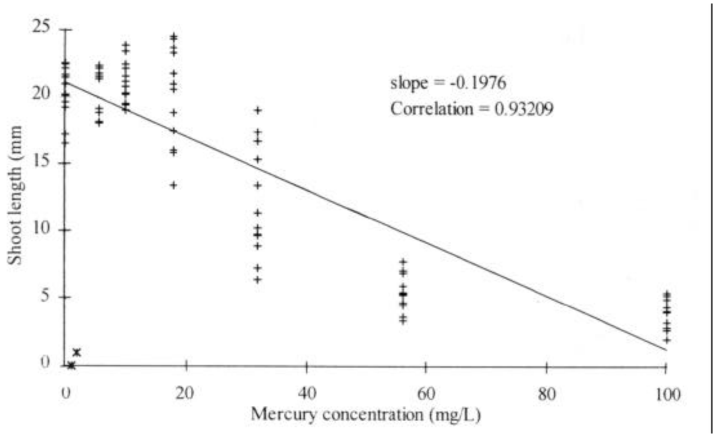

Figure 1a. Correlation between shoot growth and measured Hg concentration

software program (Gulley et al. 1990) and the ICp values using the ICPIN software program (Noerberg-King 1993). The NOEC is the highest concentration tested that does not result in a statistically significant adverse effect relative to the negative control. The LOEC is the lowest concentration tested that did result in a statistically significant adverse effect relative to the negative control. The IC_{25} and IC_{50} values were determined regularly for better interlaboratory comparison of results. These are the concentrations of test material estimated to cause 25% and 50% inhibition on the growth of organism, in this case shoot growth in millimeters. These values were considered better endpoints to measure the degree of adverse effects associated with exposure to the two heavy metals. These measurements are point estimates that allows the computation of a concentration (in mg/L) that solicited a specific response (inhibition) from the test organisms. Linear regression was also performed to clearly show the effect of increasing heavy metal concentration on shoot growth after the 10 day exposure.

RESULTS AND DISCUSSION

The chronic exposure of *E. acoroides* seeds to mercury and cadmium passed the chi-square test (for normality) and the Bartletts test (for homogeneity of variance) provided for in the TOXICITY program. Linear regression analysis showed *r* values that gave an inversely proportional relationship between seed shoot growth and increasing concentration of heavy metals (Figure 1a & b). The high correlation values for mercury (r = 0.93) and cadmium (r = 0.91) indicated a consistent negative trend for seed growth with increasing concentration of heavy metals. The NOEC values were 16.02 ± 2.36 mg/L and 8.73 ± 5.85 mg/L for mercury and cadmium, respectively and the LOEC values were 26.43 ± 5.32 mg/L and 13.54 ± 10.63 mg/L for mercury and cadmium, respectively. The IC_{25} and IC_{50} values for mercury were 17.27 ± 7.97 mg/L and 39.96 ± 18.42 mg/L, respectively and for cadmium 14.22 ± 8.5 mg/L and 38.06 ± 19.36 mg/L, respec-

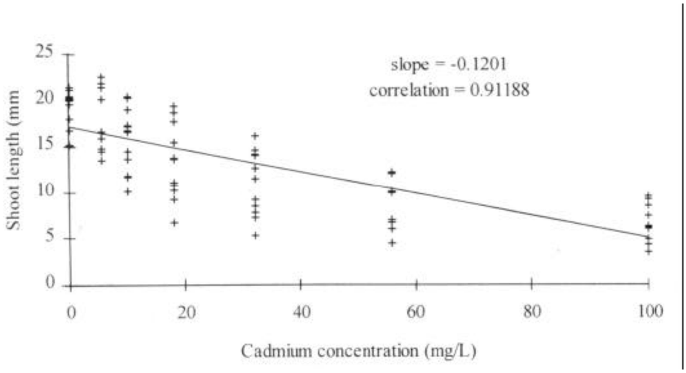

Figure 1b. Correlation between shoot growth and measured Cd concentration

tively. Based on these data, the toxic response of the germinating seeds to cadmium and mercury is not significantly different from each other, although shoot growth under Hg exposure showed a steeper negative slope (Fig. 1a & b) than that of Cd. This occurrence can be attributed to the possibility of a greater threshold of inhibition response exhibited by the seedlings in lower concentration of Hg than that of Cd. The computed ED,, demonstrates the slightly higher values of seeds under Hg (ED_{50} = 31.68 mg/L) than Cd (ED_{50} = 39.97 mg/L), though not significantly different. This proved that the effect of mercury on the growth of seed shoots during germination is slightly more pronounced than in cadmium. However, the onset of inhibition of growth started at lower concentration in Cd than in Hg. This can be quantified by the fact that the average shoot growth of *Enhalus* seeds after 10 days of exposure in 100 mg/L of Hg is only 3.87 mm compared to that of cadmium which is 6.98 mm. Although the effect of Hg on seed growth is more pronounced, it can be safely assumed, based on the inhibition concentration and ED_{50}, data that the seagrass is more sensitive at lower concentration of Cd than Hg due to differences in their threshold of responses. The toxic response of the seagrass is lesser compared with that of a marine microalgae, *Tetraselmis sp.* (Table 3) The summary of results are shown in Table 2.

Generally the assay is less sensitive compared to other tests using different test organisms of different trophic level (Table 3). A comprehensive comparison of sensitivity cannot be made as end points between different tests were not the same, making interlaboratory comparison difficult.

The low sensitivity of the assay can be explained by the capability of the seagrass for metal uptake. Initial analysis of the metal content of the seeds after 10-day exposure revealed that the seeds are capable of uptake of both metals (Table 4). Metal uptake or bioaccumulation is a natural phenomenon exhibited by marine

Table 2. Summary of results (mg/L)

	Mercury	Cadmium
NOEC	16.0 ± 2.4 mg/L	8.7 ± 5.8 mg/L
LOEC	26.4 ± 5.3 mg/L	13.5 ± 10.6 mg/L
IC_{25}	17.3 ± 8.0 mg/L	14.2 ± 8.5 mg/L
IC_{50}	40.0 ± 18.4 mg/L	38.1 ± 19.4 mg/L
ED_{50}	31.7 mg/L	40.0 mg/L

Table 3. Comparison of sensitivity of different test organisms to mercury and cadmium

TEST ORGANISMS	NOEC	LOEC	IC25	IC50	LC50	SOURCE
Cadmium						
Milkfish					27.3 ppm	Diaz 1994
Clams					1.01 ppm	Ong and Din 1994
Cockles					0.95 ppm	Ong and Din 1994
Seabass					17.38 ppm	Ong and Din 1994
Tetraselmis	1.4-1.6 ppm	3.1-3.2 ppm	1.8-2.9 ppm	3.9 - >10.0 ppm		Gonzales 1994
Coho salmon					3.7 ppb	Chapman and Steveens 1978
Steel head					5.2 ppb	Chapman and Stevens1978
Mercury						
Milkfish					0.38 ppm	Diaz 1994
Tetraselmis	0.05 ppm	0.11ppm	0.05 ppm	0.09 ppm		Gonzales 1994
White shrimp (postlarval)					17 ppb	Green et al. 1976
Amphipod					0.08 ppm	Ahsanullah 1982
Prosobranch Adult Larva					 330 ppb 60 ppb	Thain 1984

organisms. Guthrie et al. (1979) showed that barnacle, clam and polychaetes bioaccumulate Cd and barnacle, clam and polychaete take up Hg. The cadmium uptake of the seeds are higher compared to that of mercury. The bioconcentration factor (Table 4) of the seeds for both metals was also computed by dividing the metal concentration in the organism by the metal concentration in the medium. Results showed that the bioconcentration factor of Cd (40.31) was less than one order of magnitude higher than that of Hg (5.5). According to Buikema Jr. et al.

Table 4. Metal uptake of the seeds in a 10-day exposure

	Mercury	Cadmium
Nominal metal concentration in seawater (mg/L)	10	10
Actual metal concentration in seawater after 10-day exposure (mg/L)	1.4 ± 0.3	9.0 ± 0.2
Metal concentration in seeds after 10-day exposure (mg/Kg)	55.0 ± 14.0	403.1 ± 75.1
Bioconcentration factor (BCF)	5.5	40.31

(1982) the bioconcentration of a particular chemical was related to its persistence and lipophilicity. The persistence of the cadmium in solution correlated well with its tendency to bioconcentrate better than mercury. There was higher losses of mercury in the solution than cadmium after the duration of the assay. This phenomenon might be rarely encountered in temperate countries. This might be due to higher ambient temperature in tropical than in temperate countries that can make the mercury more volatile than that of cadmium. Furthermore, there is also a possibility of adsorption of the metal ions on the surfaces of the test containers.

The bioaccumulation and bioconcentration capability of the seeds quantify the use of seagrass in transplantation to rehabilitate or restore previously impacted coastal areas. The bioremoval property of *E. acoroides* was assessed by Fortes (1983). He concluded that there was a significant gross reduction in the total chemical effluent load of a polluted river as it passes through the seagrass beds. Only the mature leaves and epiphytes were analyzed for the metal load; analysis of the seagrass seed for bioabsorption was not done. Seagrass transplantation was part of an attempt to rehabilitate Calancan Bay which was the site of dumping of copper mine tailings since the 1970's. This was also applied in Manila Bay as continuous deterioration of the bay was observed. Such data from this test protocol could further strengthen the use of seagrass transplantation for environmental mitigation and the claim that the seagrass meadows can act as a "pollutant sink" and therefore contribute to a better quality of water in the coastal zone. This seagrass can also be used as an indicator species for the presence of heavy metals in the marine environment due to its bioaccumulating property.

The rather low sensitivity of this marine seed germination assay is in agreement with the observation of Kapustka and Reporter (1993). According to their study, there are two factors that might be involve in this observation : first, many chemicals may not be taken into the seed; and second, the plant embryo derives its nutrient internally from the seed storage materials making it in a sense isolated from the environment.

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