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A Heavy Metal Biossay Based on Percent Spore Germination of the Sensitive Fern, *Onoclea sensibilis*

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This is the first in a series of reports on the effects of heavy metals on the fern gametophyte developmental system. We report herein on a heavy metal bioassay based on percent fern spore germination. We selected two of the top three heavy metal pollutants -- mercury and cadmium -- for investigation and deleted lead because of its low solubility. Cobalt was also included in the investigation to determine if there is a correlation between atomic weight and metallic toxicity.

Fern gametophytes are photosynthetic organisms that are easily cultured in water or on agar containing essential inorganic nutrients. Even at maturity they are relatively small so that several hundred can be cultured on the standard petri dish. Like all small organisms with minimal culturing requirements -- bacteria, many fungal and algal species, etc. -- fern gametophytes are admirably suited to the experimental design demands of manipulation and replication. However, fern gametophytes have a more complex morphogenesis than the aforementioned organisms, though their morphogenesis is not so involved as that of their sporophytic counterpart or that of any other vascular plant. In other words, fern gametophytes have the comparable experimental malleability of bacteria, fungi, and algae, with the added distinction of being a more complex form of life morphologically. Three review articles on the use of fern gametophytes as experimental organisms in the investigation of developmental problems reveal that they are well suited for the purpose (BOPP 1967, MILLER 1968, and BRUNDES 1973).

Specific investigations on the effects of metallic ions and other potential pollutants on fern spores and on fern gametophytes are few. However, there are three bright spots in fern spore research with direct application to pollution monitoring. One of these is the work of KLEKOWSKI (1976a and b) and KLEKOWSKI and BERGER (1976) in which meiotic chromosomal behavior during fern sporogenesis in populations of Osmunda regalis was correlated with the presence of toxic substances in a polluted aquatic environment. The second was the investigation of HOWARD and HAIGH (1970) on the effect of increasing doses of X-ray radiation on the first mitotic division of the spores of Osmunda regalis. The third investigation by EDWARDS and MILLER (1970, 1972a and b) was on the quantitative effects of ethylene on Onoclea sensibilis spore germination and gametophyte growth.

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In addition to being excellent experimental organisms, fern gametophytes seem especially well suited for environmental pollution studies since they exist in an aquatic-terrestrial interface ecosystem which provides application for both water and land pollution studies.

<u>Onoclea sensibilis</u> L., the sensitive fern, was selected as the experimental organism in this investigation because it is a common species of marshes and roadside ditches and has a wide distribution (GLEASON and CRONQUIST 1963) which makes it suitable as an indicator species. It also produces copious quantities of long-lived spores.

MATERIALS AND METHODS

<u>Onoclea sensibilis</u> spores were cultured in full strength liquid Knudson's medium at a pH of 5.5 along a heavy metal ion concentration series ranging from 0 - 60ppm for each of three heavy metal divalent ions -- Hg, Cd, and Co. The heavy metals were introduced as their chloride salts. There were 10,000 spores per petri dish (8cm dia) in a 20ml liquid volume. Plates were sealed in ziplock clear plastic sandwich bags and cultured in a Sherer growth chamber at 20C in continuous light (Cool-White Fluorescence - General Electric, at 300 foot candles). Spore germination was based upon the ability of a spore to produce a rhizoid. After 8 days, 1,000 spores were scored per plate. The control plates (0ppm specified heavy metal ion) served to determine overall spore viability. Three replicates were run for each heavy metal tested. Data was analyzed statistically using regression line, variance, and co-variance analyses.

RESULTS AND DISCUSSION

Percent spore germination as a function of heavy metal ion ppm for Hg, Cd, and Co data are presented in Table 1. Each data set is graphed (Figs. 1, 2, and 3). Each of the three curves can be perceived as two lines of markedly different slopes. Between the range of approximately 1 - 10 ppm of heavy metal ion there is a relatively steep negative slope where the percent spore germination drops from 80% to approximately 10%. After this there is a change to a very gradual negative slope that goes to extinction (0% germination) at 60 ppm of heavy metal ions. For each of the heavy metals tested the curve or line within the heavy metal range of 1 - 10 ppm represents a straight forward dose response curve (i.e. heavy metal concentration is inversely proportional to percent germination). However the abrupt change in response that results in a very gradual slope to extinction above the 10 ppm level is an anomalous situation that should be explained.

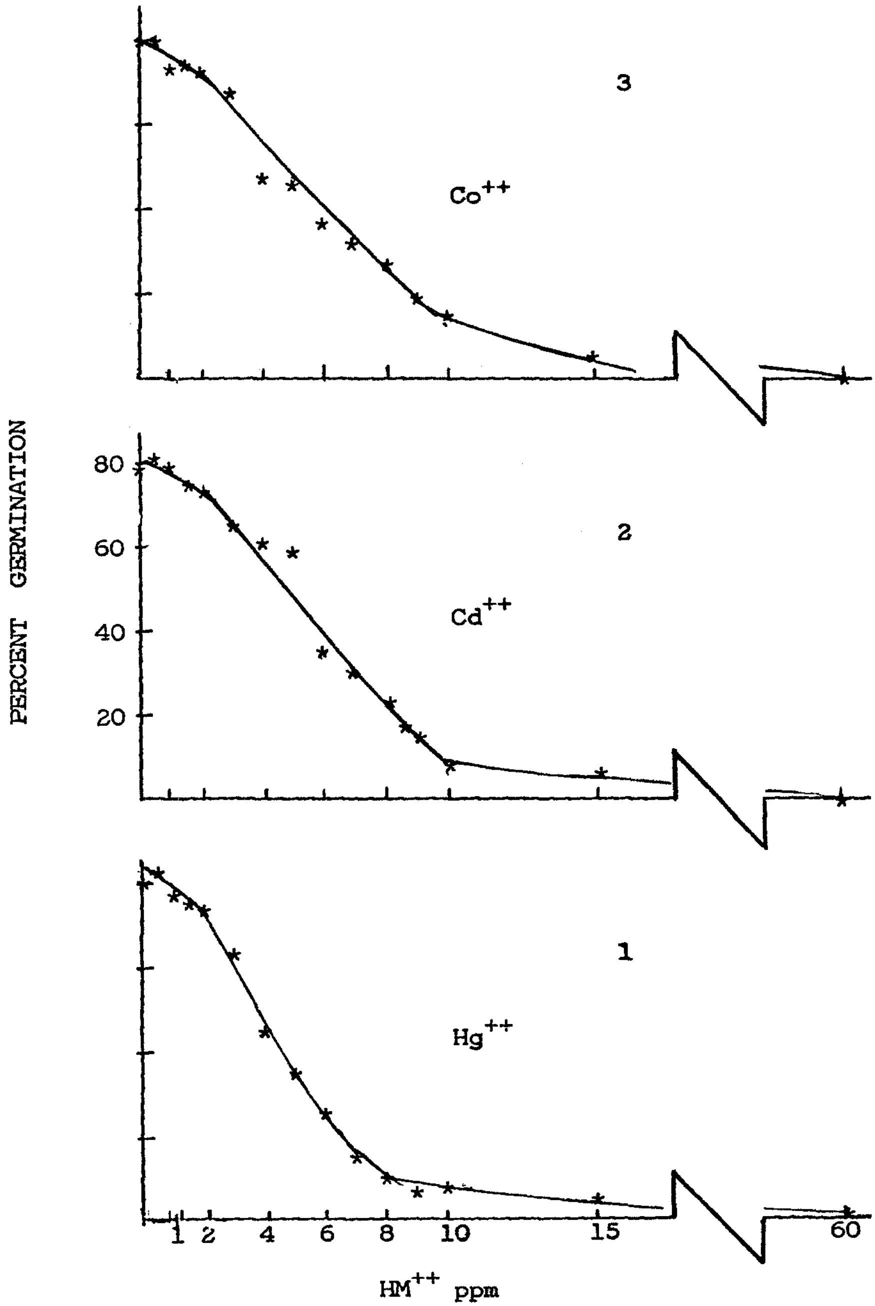
A number of possibilities for this "lag phase" to extinction presented themselves. The two that were investigated were (1) the possibility that there is a natural heavy metal resistance in the O. sensibilis spore population employed and (2) the possibility that a percentage of the spores are in an advanced pre-germinated state and complete germination before heavy metal ions can stop the process.

A pilot study to test for natural resistance turned up negative information. A microscopic examination of the O. sensibilis spore stock revealed that approximately 7% of the spores had cracked spore coats and most of these had a partially protruding green prothallial cell, which seems to indicate an advanced state of pre-germination or even germination without a rhizoid. In addition to this, a control plate and a 10 ppm Cd plate were prepared and the percent germination was monitored on a daily basis for 10 days.

TABLE 1

Percent spore germination of *Onoclea sensibilis* as a function of heavy metal ions (ppm) for the chloride salts of mercury, cadmium, and cobalt. Values are the averages of three replicates given with the standard deviation.

	PERCENT GERMINATION		
Heavy metal ions ppm	Hg^{++}	Cd^{++}	Co^{++}
0.0	80 $\pm$0.7	78 $\pm$0.6	80 $\pm$0.3
0.5	81 $\pm$0.6	80 $\pm$0.2	80 $\pm$0.5
1.0	76 $\pm$1.0	78 $\pm$0.5	74 $\pm$2.0
1.5	75 $\pm$0.8	74 $\pm$1.5	75 $\pm$3.0
2.0	73 $\pm$0.4	72 $\pm$1.2	72 $\pm$0.7
3.0	62 $\pm$3.0	64 $\pm$4.1	67 $\pm$1.8
4.0	44 $\pm$2.7	60 $\pm$2.0	47 $\pm$4.0
5.0	34 $\pm$1.0	58 $\pm$5.0	45 $\pm$2.4
6.0	25 $\pm$0.8	34 $\pm$3.0	36 $\pm$4.0
7.0	13 $\pm$3.4	29 $\pm$1.7	31 $\pm$0.8
8.0	10 $\pm$4.0	22 $\pm$3.0	27 $\pm$5.0
9.0	6 $\pm$0.2	14 $\pm$2.0	19 $\pm$3.0
10.0	7 $\pm$1.0	6 $\pm$0.5	15 $\pm$2.0
15.0	4 $\pm$0.3	5 $\pm$0.2	5 $\pm$1.0
20.0	1 $\pm$0.0	3 $\pm$0.3	2 $\pm$0.0
40.0	1 $\pm$0.0	1 $\pm$0.0	1 $\pm$0.0
60.0	0 $\pm$0.0	0 $\pm$0.0	0 $\pm$0.0



Figs. 1, 2, and 3. Onoclea sensibilis spore percent germination as a function of heavy metal ion concentration (ppm). Fig. 1 (Hg^{++}), Fig. 2 (Cd^{++}), and Fig. 3 (Co^{++}).

After 4 days 8% of the spores in the Cd plate had germinated (i.e. produced decernible rhizoids) and this 8% figure remained more or less constant thereafter. So it appears that the "lag phase" to extinction is the result of approximately 10% of the spores being in an advanced state of pre-germination and that these spores germinate under what would be normally lethal concentrations of heavy metal ions. Thus the gradual negative slope to 0% germination generated by all three heavy metals is assumed to be an artifact of the experiment due to the advanced pre-germinated state of a percentage of the spores. Because of this, the percent germination data above the 10 ppm concentration is not considered in the statistical analysis and discussion which follow.

Regression line and variance analyses of each data set reveal that for all three heavy metals straight lines are generated having +95% confidence limits between the heavy metal concentration range of 1 - 10 ppm. Analysis of covariance for the three data sets reveals by a +95% confidence limit that they are generating the same line. Table 2 lists the statistically determined percent spore germinations with their corresponding heavy metal ion concentrations expressed both as ppm and μM. When these statistically determined percent germinations are graphed as a

TABLE 2

Statistically determined percent Onoclea sensibilis spore germination data as a function of heavy metal ion concentration expressed as ppm and μM for Hg, Cd, and Co.

Heavy metal ions ppm	μM Hg	μM Cd	μM Co	%G
1	5	9	17	78
2	10	20	34	70
3	15	27	51	62
4	20	36	68	54
5	25	44	85	41
6	30	53	102	38
7	35	62	119	30
8	40	71	136	22
9	45	80	153	14
10	50	89	170	6

function of heavy metal concentrations in terms of μM, an interesting phenomenon is visualized (Fig. 4). Hg (at. wt. 200.6) with a LC-50 of 25μM is twice as toxic as Cd (at.

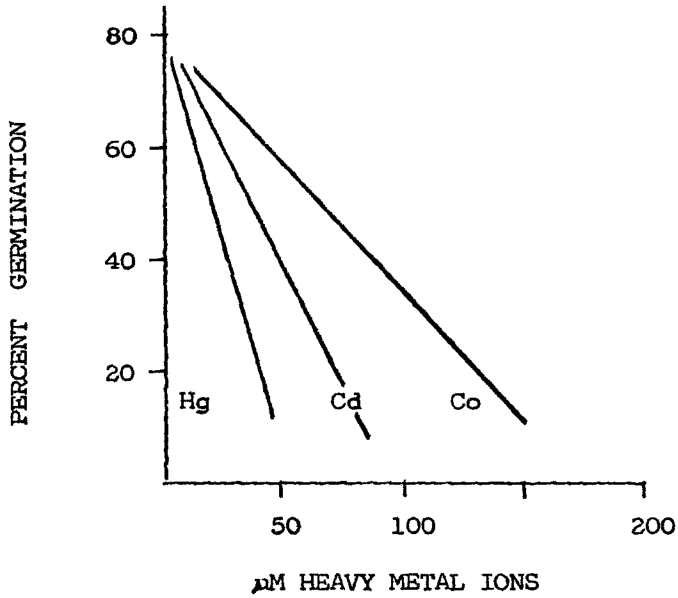


Fig. 4. Onoclea sensibilis percent spore germination as a function of heavy metal ion concentration expressed as μM.

wt. 112.4) with a LC-50 of 44μM. Hg is nearly four times as toxic as Co (at. wt. 58.9) with a LC-50 of 85μM. Thus for these three heavy metal ions, their toxicities are directly proportional to their atomic weights.

However an overall explanation of metallic ion toxicity based solely on atomic weight is undoubtedly incorrect. In fact, we have found that Cu^{++} (at. wt. 63.5) is far more toxic than Hg^{++} based on the percent fern spore germination test (unpublished data). MATHEWS (1904) first proposed that the toxicity of an ion is relative to its electric potential. DANIELLI and DAVIS (1951) considered it more likely that metallic toxicity was a function of the strength of the covalent bond formed between the metal ion and ionic and thiol groups of the cell. Electronegativity, which is a direct function of the sub-orbital positions of an atom's outer electrons, is a measurement of an element's covalent bonding ability. An element's atomic weight is only one factor in the determination of its electronegativity. SOMMERS (1959), employing the fungus Alternaria tenus, and BIESINGER AND CHRISTENSEN (1972), employing Daphnia magna, supported this hypothesis of metallic toxicity as a function of an element's electronegativity.

The fern spore germination test for the detection and evaluation of heavy metal toxicity revealed that the divalent ions of Hg, Cd, and Co generate the same toxicity curves when concentrations are expressed as ppm (mg^{-1}). This means that there is a positive correlation between the atomic weights of these three heavy metal ions and their toxicities. Hg^{++} is twice as toxic as Cd^{++}, and approximately four times as toxic as Co^{++}.

The fern spore germination test is a good bioassay for the detection and evaluation of heavy metal toxicity. It is relatively quick (8 days) and an inexpensive bioassay which is both easily and rapidly set up and scored. It has a high degree of quantitative accuracy within the concentration range of 1 - 10 ppm for the metals tested and under the conditions employed.

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REFERENCES

BIESINGER, K. E. and G. M. CHRISTENSEN. J. Fish. Res. Board Canada 29, 1961 (1972).
BOPP, M. Ann. Rev. Pl. Physiol. 18, 361 (1967).
BRUNDES, H. Ann. Rev. Pl. Physiol. 24, 115 (1973).
DANIELLI, J.F. and J.T. DAVIES. Advan. Enzymol. 11, 35 (1951).
EDWARDS, M.E. and J.H. MILLER. Plant. Physiol. 46 (Suppl.), 32 (1970).
EDWARDS, M.E. and J.H. MILLER. Am. J. Bot. 59, 450 (1972a).
EDWARDS, M.E. AND J.H. MILLER. Am. J. Bot. 59, 458 (1972b).
GLEASON, H.A. AND A. CRONQUIST. Manual of vascular plants of northeastern United States and adjacent Canada. D. Van Nostrand Co., Inc. Princeton, New Jersey (1963).
HOWARD, A. AND M.V. HAIGH. Radia. Biol. 18, 147 (1970).
KLEKOWSKI, E.J. Am. J. Bot. 63, 1024 (1976a).
KLEKOWSKI, E.J. Am. Fern J. 66, 75 (1976b).
KLEKOWSKI, E.J. and B.B. BERGER. Am J. Bot. 63, 239 (1976).
MATHEWS, A.P. Am. J. Physiol. 10, 290 (1904).
MILLER, J.H. Bot. Rev. 34, 361 (1968).
SOMMERS, E. Nature 184, 475 (1959).