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Effect of Copper, Cadmium, and Zinc on Percent Spore Germination of the Cinnamon Fern (*Osmunda cinnamomea*) and the Sensitive Fern (*Onoclea sensibilis*)

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PETERSEN et al. (1980) reported on a heavy metal (HM) toxicity bioassay based on percent fern spore germination. They determined that the toxicities of the three HM ions tested, Hg, Cd, and Co were directly proportional to their atomic weights. In a subsequent report PETERSEN and FRANCIS (1980) demonstrated species-specific toxicity responses to Hg for three fern and one moss taxa. These two earlier papers also contain background information on previous work using ferns to test for potential pollutants.

WOOD (1974), cited in FORSTNER & WILLIAMS (1979), listed Cu, Cd, and Zn as being very toxic and relatively accessible as opposed to metals that are toxic but very insoluble or very rare or metals that are noncritical. The purpose of this report is to assess the relative toxicities of Cu, Cd, and Zn based on percent spore germination in two fern taxa, Osmunda cinnamomea L. and Onoclea sensibilis L.

MATERIALS AND METHODS

Onoclea sensibilis and Osmunda cinnamomea spores were cultured on a diluted (1/10 strength) and modified (Geigy Fe Sequestrene 330 substituted for $FeSO_4$) liquid Knudson's medium at pH 5.5. Spores of both taxa were cultured separately in a HM concentration series ranging from 0-40 ppm for each of the three divalent ions, Cu, Cd, and Zn. The metal ions were introduced as their salts, $CuSO_4$, $CdCl_2$, and $ZnCl_2$. There were 15,000 spores per petri dish (8 cm dia.) in a 20 mL liquid volume. The petri dishes were sealed in Ziplock plastic bags and cultured in a Sherer growth chamber at 20°C in continuous light (Cool-White Fluorescence, General Electric, at 300 foot candles). Fern spore germination was based on the ability of a spore to produce a rhizoid or to differentiate into 2 or more cells. After seven days, 500 spores were scored per plate. Each percent germination value represents the average of three replicates.

RESULTS AND DISCUSSION

Heavy metal toxicity is based on percent spore germination for the two fern taxa tested (Table 1). The percent spore germination data are expressed as LC_{50} and LC_{100} values (Table 2),

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Table 1. Osmunda cinnamomea and Onoclea sensibilis percent spore germination data as function of heavy metal ion concentration (ppm) for Cu, Cd, and Zn*

	Percent Germination					
	Os. cinnamomea			Os. sensibilis		
HM^{++}, ppm	Cu	Cd	Zn	Cu	Cd	Zn
0	95	96	96	97	98	97
0.01	96	-	-	-	-	-
0.05	95	-	-	-	-	-
0.1	60	-	-	-	-	-
0.2	43	-	-	-	-	-
0.3	8	-	-	-	-	-
0.4	0	-	-	-	-	-
0.5	-	95	93	75	91	88
1.0	-	88	77	59	84	86
2.0	-	59	58	25	67	76
3.0	-	20	38	23	34	68
4.0	-	9	28	20	28	60
5.0	-	6	37	5	3	57
7.0	-	0	18	3	0	50
10.0	-	-	9	3	-	28
20.0	-	-	2	1	-	0
40.0	-	-	0	-	-	-

* (-) tests were not run at these concentrations.

Table 2. Osmunda cinnamomea and Onoclea sensibilis estimated $LC50$ and LC_{100} values based on the percent spore germination data.

	Os. cinnamomea			On. sensibilis		
	Cu	Cd	Zn	Cu	Cd	Zn
LC_{50} (ppm)	0.1	2.2	2.4	1.3	2.5	7.0
LC_{50} (uM)	2	20	37	20	22	107
LC_{100} (ppm)	0.4	7	40	20	7	10
LC_{100} (uM)	6	62	610	310	62	150

as straight line equations (Table 3), derived from regression line analysis of the data, and as graphs of these equations (Figs. 1-6). The equation for a straight line, $y = mx + b$, describes percent spore germination as a function of HM toxicity where,

- y = percent spore germination
- x = HM++ ppm
- m = slope (change in percent spore germination over the change in HM++ ppm)
- b = y-intercept (The percent spore germination value at which the regression line crosses the y-axis).

Table 3. Regression line equations for *Osmunda cinnamomea* and *Onoclea sensibilis* percent spore germination as a function of heavy metal ion concentration for Cu, Cd, and Zn.

	Os. cinnamomea		*On. sensibilis*
(Cu)	$y = -376x + 110$	(Cu)	$y = -38x + 96$
(Cd)	$y = -34x + 124$	(Cd)	$y = -20x + 104$
(Zn)	$y = -23x + 104$	(Zn)	$y = -9x + 95$

In other words, these equations are an expression of HM toxicity. For example, the regression line equation derived from the *Os. cinnamomea* data for Cu toxicity (Table 3 and Fig. 1) is

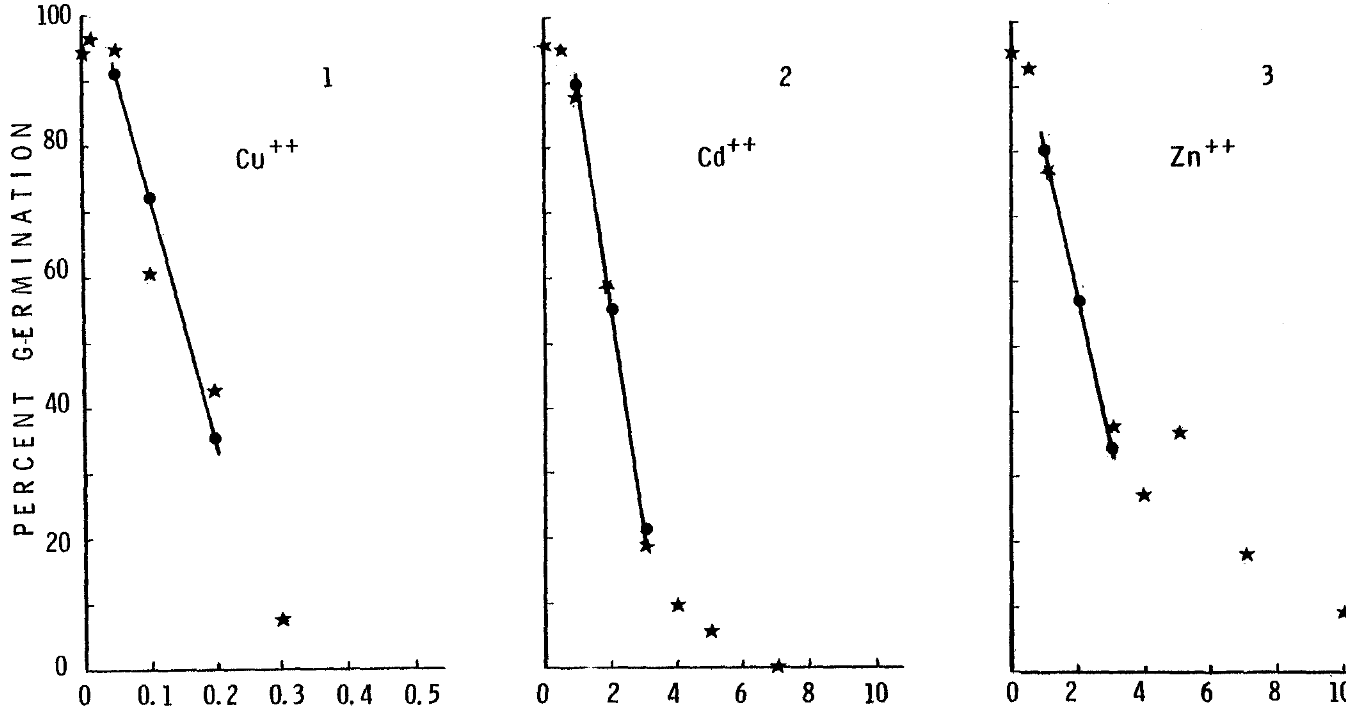
$$\text{(Cu)} \quad y = -376x + 110.$$

The slope, $m = -376$, is a relatively steep negative slope, indicating that Cu is quite toxic. The y-intercept, $b = 110$, in conjunction with the slope indicates the species has a relatively short tolerance range for Cu. This is in part contrasted with the regression line equation derived from the *On. sensibilis* data for Zn toxicity (Table 3 and Fig. 6)

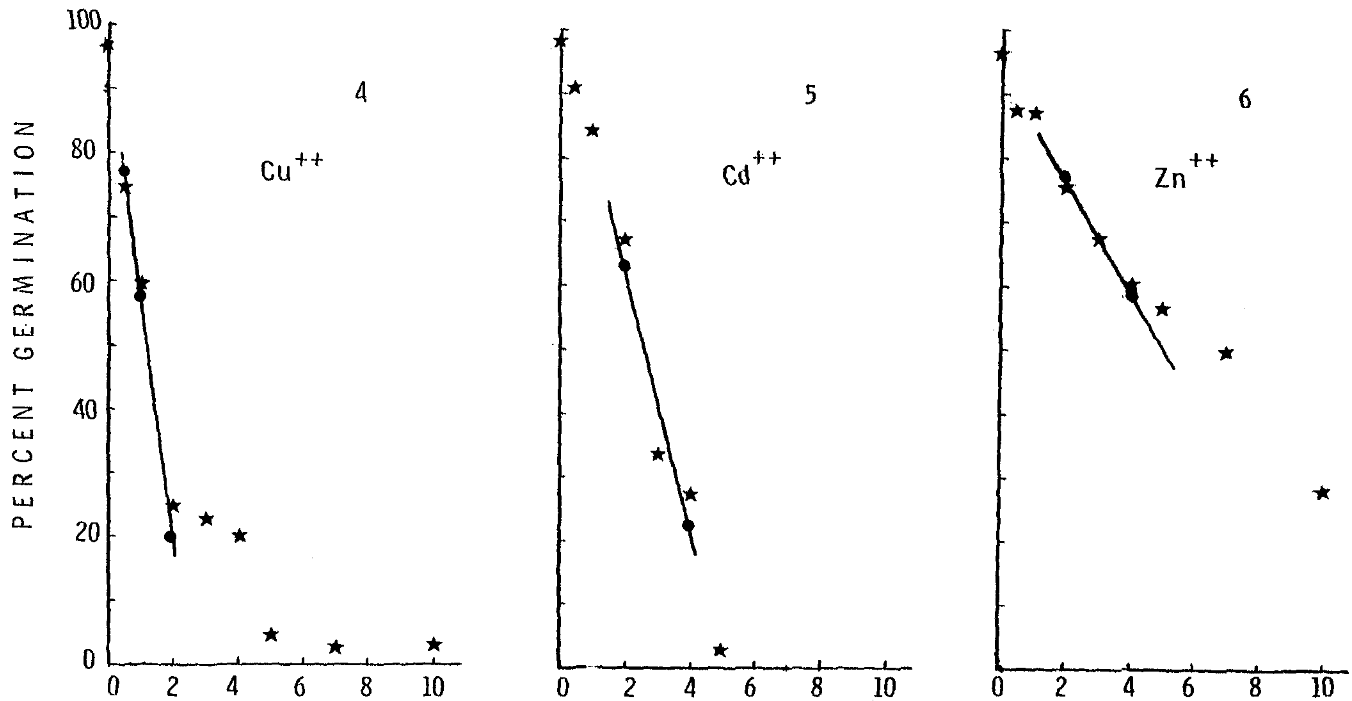
$$\text{(Zn)} \quad y = -9x + 95.$$

The slight negative slope (-9) indicates a low toxicity.

The LC values and regression line euqation for *Os. cinnamomea* (Tables 2 and 3; Figs. 1-3) yield the following heavy metal ion toxicity ranking in decreasing order, Cu > Cd > Zn. Based on LC_{50} values in uM, Cu is 20x more toxic than Zn and 10x more toxic than Cd. Cd is 2 more toxic than Zn. Based on LC_{100}



Figs. 1, 2, and 3. Osmunda cinnamomea percent spore germination as a function of heavy metal ion concentration (mg^{-l}). Fig. 1 (Cu++), Fig. 2 (Cd++), and Fig. 3 (Zn++). Actual data points are (★). Regression line determined points are (●).



Figs. 4, 5, and 6. Onoclea sensibilis percent spore germination as a function of heavy metal ion concentration (mg^{-1}). Fig. 4 (Cu++), Fig. 5 (Cd++), and Fig. 6 (Zn++). Actual data points are (★). Regression line determined points are (●).

values in uM Cu is 100x more toxic than Zn and 10x more toxic than Cd. Cd is 10x more toxic than Zn.

The LC values and regression line equations for On. sensibilis (Tables 2 and 3; Figs. 4-6) yield various HM toxicity rankings. The ranking based on LC_{50} (ppm) and on the slopes of the regression line equation is Cu > Cd > Zn, the same as in Os. cinnamomea. However, converting ppm to µM changes the ranking to Cu = Cd > Zn where Cu and Cd are each 5x more toxic than Zn. The On. sensibilis LC_{100} values yield a toxicity ranking of Cd > Zn > Cu. Because of the extremely low germination values along the upper end of the HM concentration series (Table 1) and because a small percentage of the spore employed have been determined to be in an advanced pre-germinated state (PETERSEN et al. 1980) the validity of these LC_{100} values is tenuous and as such more significant should be placed on the LC_{50} values and regression line equations. A sampling of the Cd, Cu, and Zn relative toxicities is presented in Table 4. Of the reports listed in Table 4, Zn is in each case the least toxic and Cu, with two exceptions (BIESINGER & CHRISTENSEN 1972 and some findings of this report, Table 2) is the most toxic by a factor of 10 or more.

PETERSEN et al. (1980) using full strength Knudson's liquid medium indicated an LC50 value for On. sensibilis spores of 5.2 ppm Cd. In this paper with modified and diluted Knudson's liquid medium, the LC_{50} value for On. sensibilis is 2.5 ppm Cd. The ionic concentration of the nutrient medium appears to affect the toxicity of the HM such that a solution with a lower ionic concentration needs a less amount of HM to produce the same level of toxicity as a solution with a higher ionic concentration. (See, FORSTNER & WITTMANN (1979) for a discussion of physiochemical factors effecting heavy metal toxicity).

A comparison of the LC_{50} (µM) values of the two taxa (Table 2) shows that Os. cinnamomea is more sensitive to Cu (X10), Cd (2X), and Zn (3X) than is On. sensibilis. PETERSEN & FRANCIS (1980) found that Os. cinnamomea and Os. claytoniana were nearly equally susceptible to Hg and that they were 5X more sensitive to Hg than was On. sensibilis. However, the moss, Polytrichum commune was 10X more sensitive to Hg than was On. sensibilis. FRANCIS & PETERSEN (unpublished data) found P. commune to be more sensitive to Cu, Cd, and Zn than were On. sensibilis and Os. cinnamomea.

The hypothesis that metal toxicity is a function of an element's electronegativity has been mentioned by other authors (see, PETERSEN et al. 1980). The higher the electron negativity value, the more toxic an element might be. The toxicity ranking of Cu, Cd, and Zn follows this hypothesis with electronegativity values of 1.8, 1.7, and 1.6, respectivity.

Table 4. A sampling of the literature on Cd, Cu, and Zn relative toxicity ratios.[1]

Author(s) and date	Organism	Relative toxicity ratio (times more toxic)
Somers (1961)	*Alternaria tenuis* (fungus)	Cu (10,000): Zn (1)
	Botrytis fabae (fungus)	Cu (100): Zn (1)
Biesinger & Christensen (1972)	*Daphinia magna* (crustacean)	Cu (30): Cu (3): Zn (1)
Coombes & Lepp (1974)	*Funaria hygrometrica* (moss)	Cu (10): Zn (1)
	Marchantia polymorpha (liverwort)	Cu (100): Zn (1)
Rosko & Rachlin (1975)	*Nitzschia closterium* (marine alga)	Cu (10): Zn (1)
Braek et al. (1976)	*Amphidinium carteri* (marine alga)	Cu (10): Zn (1)
Francis & Petersen (Table 2 of this report)	*Onoclea sensibilis* (fern)	Cu (5): Cd (5): Zn (1)
Francis & Petersen (Unpublished)	*Polytrichum commune* (moss)	Cu (100): Cd (3): Zn (1)

1. Relative toxicity ratios are based on LC_{50} values in terms of uM of heavy metal ions.

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REFERENCES

BIESINGER, K. E. and G. M. CHRISTENSEN: J. Fish. Res. Board Canada 29, 1691 (1972).
BRAEK, G. S., A. JENSEN, A. MOHUS: J. Exp. Mar. Biol. Ecol. 25, 37 (1976).
COOMBES, A. J. and N. W. LEPP: Bryologist 77, 447 (1974).
FORSTNER, U. and G. T. W. WITTMANN: Metal pollution in the aquatic environment. Berlin-Heidelberg, Springer-Verlag (1979).
PETERSEN, R. L., D. ARNOLD, D. G. LYNTH, S. A. PRICE: Bull. Environm. Contam. Toxicol. 24, 489 (1980).
PETERSEN, R. L. and P. C. FRANCIS: Am. Fern J. 70, 115 (1980).
ROSKO, J. J. and J. W. RACHLIN: Bull. Torrey Bot. Club. 102, 100 (1975).
SOMERS, E: Ann. Appl. Biol. 49, 246 (1961).
WOOD, J. M.: Science 183, 1049 (1974).

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