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Synergistic and Antagonistic Responses of Fern Spore Germination to Combinations of Copper, Cadmium, and Zinc

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The evaluation of toxic responses to combinations of potential pollutants deserves much more attention because a significant percentage of combination toxic substance investigations have revealed higher levels of toxicity than if their combined toxicities were additive. Since most acceptable environmental standards for toxic substances are based on single substance studies, reported synergistic responses are disquieting, as they draw into question the validity of the set standards. Cadmium, copper, and zinc were selected for investigation because there are a number of varying reports on their combined toxic responses, ranging from antagonistic to synergistic.

MATERIALS and METHODS

The same overall procedure presented in FRANCIS & PETERSEN (1983) was employed in this study. Each percent germination value is the average of three replicates. Four combinations of the three metal ions in equal weight ratios (ppm) were tested against Osmunda cinnamomea and Onoclea sensibilis spore germination. The four HM combinations tested were: Cu:Cd, Cu:Zn, and Cu:Cd:Zn. Single toxicity regression line equations for each of the three HM ions generated by FRANCIS & PETERSEN (1983) were used to construct additive model equations of the HM ions' combination toxic response. For the three possible two-way HM ion combinations individual toxic response regression line equations were added together then divided by two to yield the additive model equations. The one possible three-way combination was derived as the sum of the three single HM toxic response equations divided by three.

RESULTS

Percent germination and estimated LC values for Os. cinnamomea and On. sensibilis (Tables 1 and 2) reveal that all combinations with Cu generate within each fern species comparable toxic responses and that the Cd:Zn combination is the least toxic. These data also show that Os. cinnamomea is more susceptible to the HM ions tested than is On. sensibilis. PETERSEN & FRANCIS (1980) found that Os. cinnamomea was more sensitive to Hg toxicity than was On. sensibilis.

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Table 1. Osmunda cinnamomea and Onoclea sensibilis percent spore germination data as a function of combinations of heavy metal ions in equal weight ratios.*

Percent Spore Germination

HM++ (ppm)	Osmunda cinnamomea Cu:Cd	Cu:Zn	Cd:Zn	Cu:Cd:Zn	Onoclea sensibilis Cu:Cd	Cu:Zn	Cu:Zn	Cu:Cd:Zn
0.0	93	92	96	92	95	96	96	96
0.01	88	92	89	92	-	-	-	-
0.05	91	77	85	89	-	-	-	-
0.01	74	75	85	81	-	-	-	-
0.2	39	40	77	58	-	-	-	-
0.3	14	9	61	40	-	-	-	-
0.4	4	0	61	31	-	-	-	-
0.5	0	0	67	4	87	91	92	94
1.0	-	-	56	0	67	85	84	74
2.0	-	-	48	-	16	24	78	7
3.0	-	-	24	-	7	10	38	4
4.0	-	-	5	-	6	11	24	4
5.0	-	-	18	-	2	4	11	2
7.0	-	-	0	-	0	3	2	0
10.0	-	-	-	-	-	0	0	-

* No observations made where there are no numerical values.

Table 2. LC_{50} and LC_{100} values of equal weight combinations of heavy metal ions based on Osmunda cimmamomea and Onoclea sensibilis percent spore germination.*

Osmunda	Cu:Cd	Cu:Zn	Cu:Zn	Cu:Cd:Zn
LC_{50}	0.2	0.2	1.8	0.2
LC_{100}	0.5	0.5	7.0	1.0
Onoclea				
LC_{50}	1.4	1.6	2.7	1.4
LC_{100}	7.0	10.0	10.0	7.0

* HM++ concentrations as ppm.

Regression line equations of the HM combination responses both for the observed data and for the additive toxicity model are presented in Table 3. Equations generated from the observed data yield toxicity rankings based on slop magnitude which is directly proportional to toxicity. HM combination toxic response rankings in decreasing order of toxicity are:

Os. cinnamomea Cu:Cd > Cu:Zn > Cu:Cd:Zn > Cd:Zn

On. sensibilis Cu:Cd:Zn > Cu:Cd = Cu:Zn > Cu:Zn.

Table 3. Regression line equations for *Osmunda cinnamomea* and *Onoclea sensibilis* percent spore germination as a function of heavy metal ion concentration (ppm) both for the observed data and for the additive heavy metal ion combination toxicity model.*

Osmunda cinnamomea			
Observed		Additive Toxicity Model	
Cu	y = -376x + 110	———	
Cd	y = - 34x + 124	———	
Zn	y = - 23x + 104	———	
Cu:Cd	y = -402x + 115	Cu:Cd(add)	y = -205x + 117
Cu:Zn	y = -304x + 99	Cu:Zn(add)	y = -200x + 107
Cd:Zn	y = - 40x + 93	Cd:Zn(add)	y = - 29x + 114
Cu:Cd:Zn	y = -211x + 102	Cu:Cd:Zn(add)	y = -144x + 113

Onoclea sensibilis			
Observed		Additive Toxicity Model	
Cu	y = - 38x + 96	———	
Cd	y = - 20x + 104	———	
Zn	y = - 9x + 95	———	
Cu:Cd	y = - 48x + 113	Cu:Cd(add)	y = - 30x + 100
Cu:Zn	y = - 47x + 121	Cu:Zn(add)	y = - 24x + 96
Cd:Zn	y = - 23x + 113	Cd:Zn(add)	y = - 15x + 100
Cu:Cd:Zn	y = - 59x + 128	Cu:Cd:Zn(add)	y = - 22x + 98

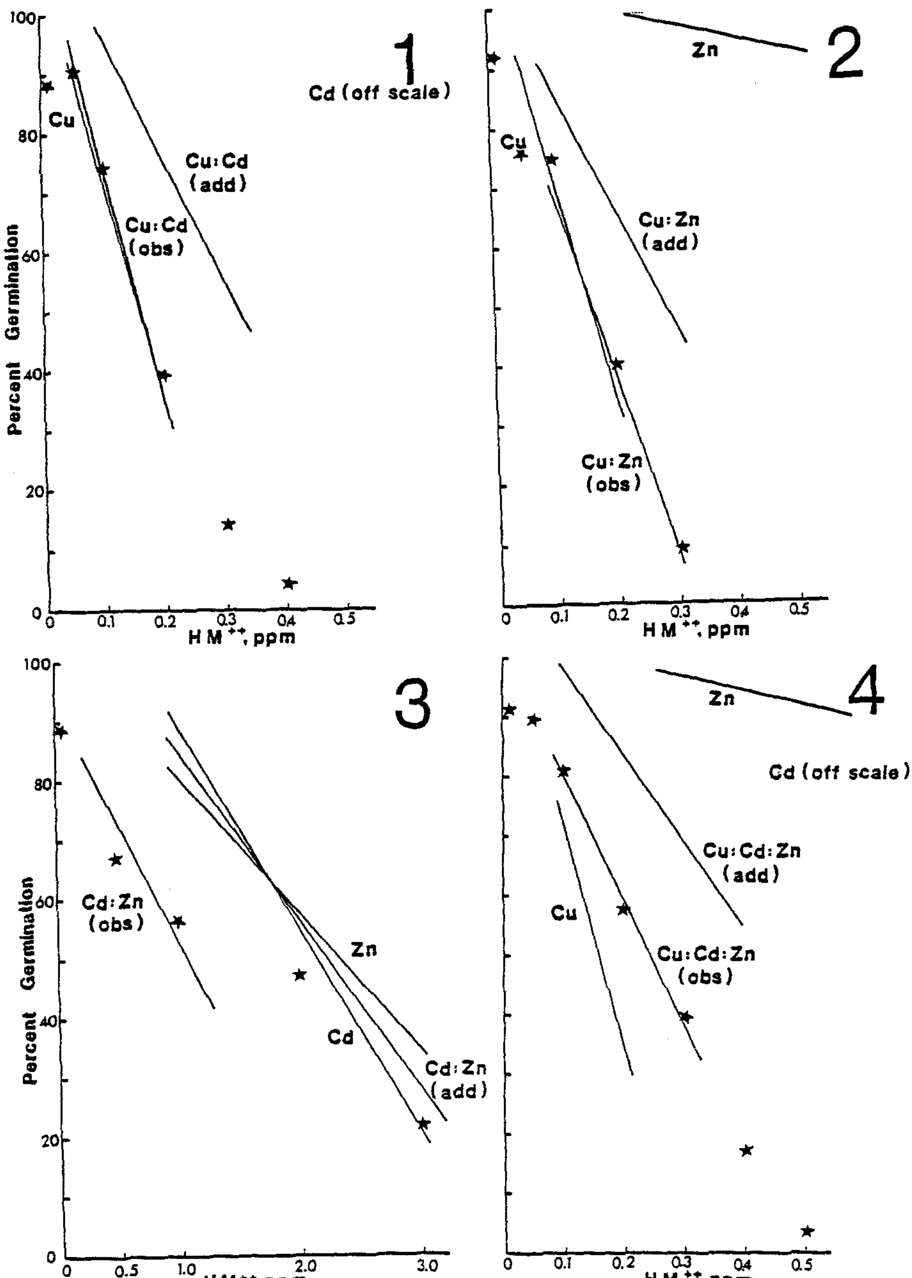
* Single heavy metal ion equations are derived from data presented in FRANCIS & PETERSEN (1983).

Graphs of the observed regression line equations along with the corresponding additive model equations (Figs. 1-8) enable one to interpret toxicity responses of HM combinations. If a regression line is generated that lies below the additive model regression line the HM combination is synergistic, while one above the additive model regression line is antagonistic. All four equal weight combinations of Cu, Cd, and Zn elicited definite synergistic toxic responses in the Os. cinnamomea system (Figs. 1-4). The three combinations with Cu mimic the Cu toxic response even though in the two-way combination one half the amount of Cu is present and in the three-way one third the amount is present (Figs. 1, 2, and 4). The combination toxic responses of the On. sensibilis system were less clear-cut. All four combinations yielded antagonistic responses along the lower concentration range, approximately below the values of 1 -1.5 ppm total HM concentration. At the higher end of the range synergistic responses were observed (Figs. 5-8).

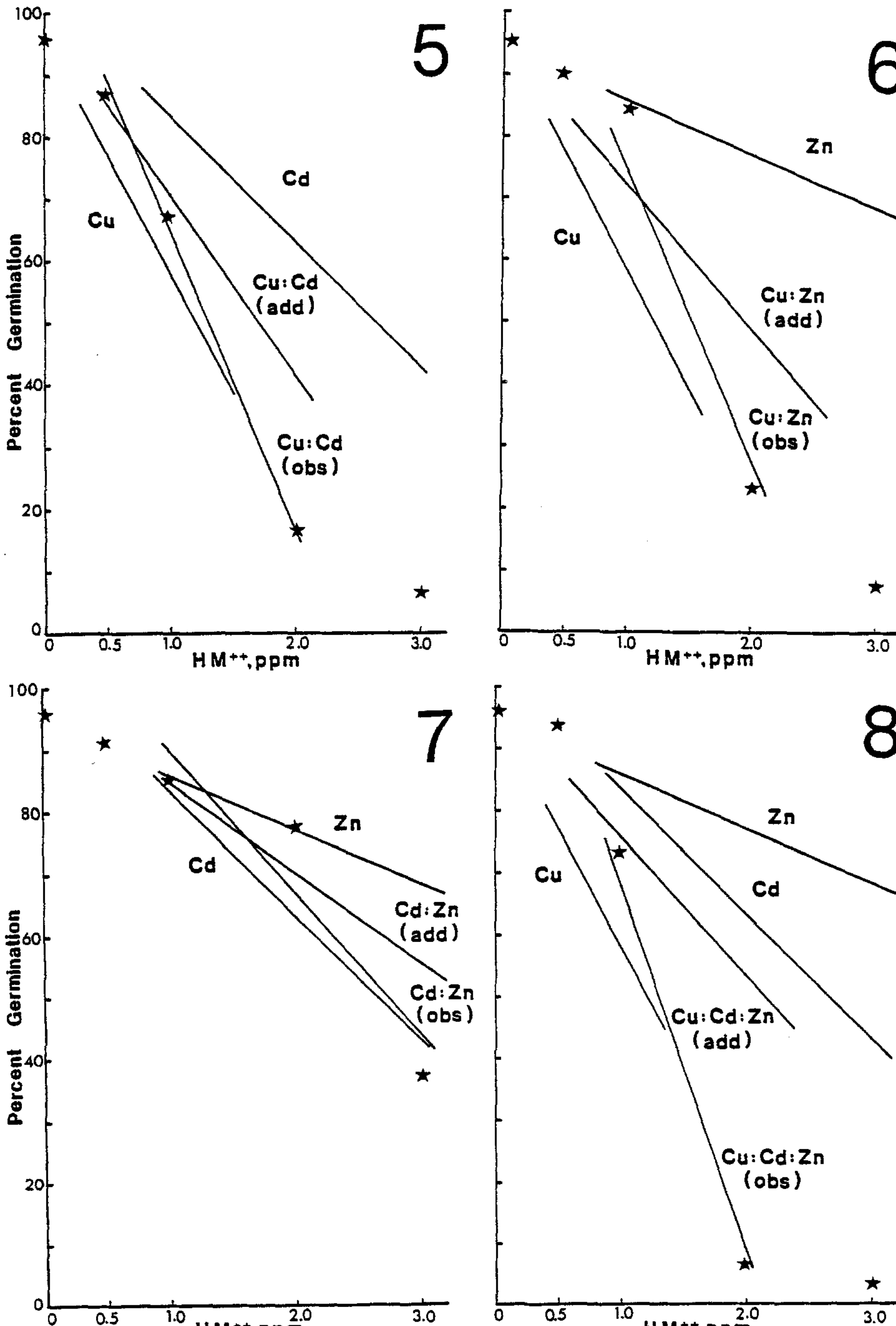
DISCUSSION

All equal weight combinations of Cu, Cd, and Zn exhibited synergistic toxic responses in both the Os. cinnamomea and On. sensibilis systems. However, at the lower concentrations of the HM combinations On. sensibilis exhibited antagonistic toxic responses. A number of investigators have reported on the combined toxic responses of Cu, Cd, and Zn. For a discussion on this for aquatic animals see WESTERNHAGEN et al. (1979).

Synergistic toxic responses with Cu, Cd, and Zn have been demonstrated by other investigators using various test organisms. SPRAGUE & RAMSAY (1965) employing trout reported increased mortality with combinations of Zn and Cd. WISSMAR (1972) found that all two and three way combinations of Cu, Cd, and Zn acted synergistically to inhibit carbon fixation in freshwater phytoplankton. WISSMAR (1972) also noted the overriding effect of Cu on the combined toxic responses, which is comparable to the Cu effect reported herein for the Os. cinnamomea system. HUTCHINSON & CZYRSKA (1972) reported a synergistic response for Cu and Zn based on leaf production in two aquatic floating plants, Lemna valdivania (duckweed) and Salvinia natans (fern). Synergistic responses with Zn and Cu based on salmon egg survival were detected by WEDEMEYER (1968) and EISLER & GARDNER (1973) reported synergistic responses of the top-minnow, Fundulus heteroclitus to trimetal combinations of Cu, Cd, and Zn. D'AGOSTINA & FINNEY (1974) measuring parameters of growth and reproduction in the copepod, Tigriopus japonicus reported synergistic responses to combinations of Cu and Cd. SELLERS et al. (1975) reported synergistic responses to mixture of Cu and Cd based on trout ventilatory activity and finally, WALLACE & ROMNEY (1977) found synergistic responses of Phaseolus vulgaris leaf production to combinations of Cu and Zn.



Figs. 1-4. Regression lines of <u>Osmunda cinnamomea</u> percent spore germination as a function of <u>HM ion concentration</u> tested singly and in equal weight combinations. HM combination regression lines are presented from the observed data (obs) and from the additive toxicity models (add). Figs. 1 Cu-Cd, Fig. 2 Cu-Zn, Fig. 3 Cd-Zn, and Fig. 4 Cu-Cd-Zn.



Figs. 5-8. Regression line of Onoclea sensibilis percent spore germination as a function of HM ion concentration tested singly and in equal weight combinations. HM combination regression lines are presently from the observed data (obs) and from the additive toxicity models (add). Fig. 5 Cu-Cd, Fig. 6 Cu-Zn, Fig. 7 Cd-Zn, and Fig. 8 Cu-Cd-Zn.

Additive toxic responses to combinations of Cu, Cd, and Zn have been reported. EATON (1973) working with chronic levels of Cu, Cd, and Zn in the trout found that Zn toxicity was additive when in combination with Cu and Cd. THORP & LAKE (1974) detected additive responses of the freshwater shrimp Paratya tasmaniensis to combinations of Zn and Cd. An investigation on oyster embryo mortality to Cu and Zn (MACINNES & CALABRESE 1978) and one on herring egg and larval mortality in response to Cu and Cd (WESTERNHAGEN et al. 1979) yielded additive toxicities. WALLACE & ROMNEY (1977) detected an additive toxic response to Zn and Cd based on percent leaf yeild in Phaseolus vulgaris.

LLOYD (1961) employing trout reported an antagonistic response to Zn and Cd at low concentrations and a synergistic one at high concentrations. This is comparable to the situation reported for On. sensibilis (Figs. 5-8). EATON (1973) found that trimetal combinations of Cu, Cd, and Zn at chronic concentrations resulted in a lessening of specific Cd toxicity and in an increasing of specific Cu toxicity. At acute concentrations the three metals elicited an additive toxic response. BARTLETT et al. (1974) reported that Cd inhibited the toxicity of Cu in the green alga, Selenastrum capricornutum. Their results are based on growth rate analysis. ROSENTHAL & SPERLING (1974) reported an antagonistic response based on herring egg mortality to Zn and Cd. Finally, DIXON & COMPHER (1977) found that Zn blocked the toxic effects of Cd by allowing limb regenerating in the newt if the wound was first treated with Zn followed by Cd.

It is difficult to reach a consensus on the combined toxic responses of Cu, Cd, and Zn other then to say that more investigators report synergistic responses then either additive or antagonistic ones and that plant systems generate comparable results to animal systems. In many instances plant systems are more amenable to the requirements of experimental design (i.e. replication, manipulation, time to results, and cost) and as such may be more useful in the initial screening of potential pollutants.

A conceptualized multi-model of the combination toxic substance response is presented. The Synergistic-Additive Model does not necessarily require the interaction of reaction between the toxic substances. In this model a particular toxic substance, TS-1 has an array of biochemical target sites (e.g. specific enzymes). Another TS, TS-2, has its own array of targets which may or may not overlap with TS-1's targets. A high degree of overlap elicits an additive toxic response because both substances are affecting the same reactions. On the other hand a low degree of overlap may elicits a synergistic response if certain alternative biochemical pathways not blocked by TS-1 are now being blocked by TS-2. The obstructed alternative pathways greatly acerbate the toxic response - synergism.

Conversely antagonism probably requires the interaction or reaction of toxic substances. TS-1 combines with TS-2 producing a less toxic substance or TS-1 which is less toxic than TS-2 fills a mutual target site thus ameliorating the toxic response - antagonism. The antagonistic response of Hg and Se are probably due to chemical combination. While the antagonism of Zn and Cd in the newt is probably due to mutual site occupation (DIXON & COMPHER 1977).

The formulation of a highly predictive model for HM combination toxic response is probably not near. Such model building would have to be based on detailed biochemical analysis of the toxic response in various organisms, as well as, on detailed physio-chemical analysis of the environment.

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