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Comparing Cd Toxicity Tests with Plants in Monocultures and Species Mixtures

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Natural ecosystems contain many species interacting in complex, interdependent ways (e.g. predation, competition, symbiosis). Realization of this complexity has led to suggestions that single-species (monoculture) tests of the toxicity of xenobiotic compounds are too unrealistic to be reliable predictors of possible environmental damage (Cairns 1983; Kimball and Levin 1985). Multi-species (mixture) tests have been proposed as a more sensitive, realistic experimental design for toxicity assessment, although some argue that this is not necessarily so (Giesy 1985). More comparative data between the two experimental designs are required.

One possible interaction in mixtures of biologically similar species is interspecific competition, or "interference" (Schoener 1983). Theoretically, there are two ways in which interference may influence the results of multi-species tests, (a) by increasing the total stress experienced by one or more of the test species through competition for a limiting resource (e.g. Menchaca and Hornung 1989), or (b) by reducing toxicity experienced by a test population, either through rapid accumulation of the toxicant by one species (e.g. accumulation of Pb by Navicula algae in mixture with Lemna (Everard and Denny 1985)), or by release of toxicant-chelating substances (e.g. from Cu-tolerant Silene cucubalus (Lolkema et al. 1986)).

The purpose of this study is to assess the effect of interspecific interference on the outcome of a Cd toxicity test with two plant species, the water fern Salvinia minima and the duckweed Spirodela punctata, under different growth regimes (nutrient and Cd levels). Interference indices were calculated to distinguish interference from biomass dilution of Cd, which may reduce toxicity in treatments with higher initial biomasses (see Jarrell and Beverley 1981).

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MATERIALS AND METHODS

The experiment was set up as a replacement series design (see Austin et al. 1988) in which species mixtures containing 1:1 proportions by wt of each species were initiated with monocultures of corresponding biomass. The replacement design was replicated under different nutrient levels (2% and 20% Hoagland and Arnon (1950) medium) and Cd levels (initial Cd concentrations: 0, 10 or 25 ppb). Initial biomass was 200 or 400 mg fresh wt in monocultures and 200 mg f. wt of each species in mixtures (400 mg f. wt total biomass). The experiment continued until most Cd had been removed from solution (30 days). Neither Cd nor nutrient treatments were renewed during the experiment, because interference may most easily be observed when critical substances are in limited supply (Schoener 1983; Harper 1977). Each treatment had three replicates, making a total of 90 replicates.

Plants were grown hydroponically in a growth cabinet at 24°C under incandescent and fluorescent lights on a 16:8 hr light:dark cycle. Treatment solutions (700 ml in plastic containers) were maintained at pH 6.4 ± 0.5 with NaOH and HCl solutions. Because the nutrient and Cd treatments were not renewed, algal growth was minimized by filtering the solutions every five days. Tests showed no loss of Cd due to filtering. At harvest, live plants were washed with deionised water and weighed, then dried at 60°C and reweighed.

Cadmium in solution was measured by graphite furnace AAS on samples acidified to 6% nitric acid. Growth of each species was quantified as relative growth rate (RGR) per wk (Harper 1977) based on initial and harvested fresh weights. RGR data for each species were analysed as a three-way factorial ANOVA for the effects of (i) presence of the second species, (ii) nutrient level, and (iii) Cd level. Tukey tests were used in multiple means comparisons. When higher order interactions were significant, the data were reanalysed by appropriate treatment subgroups. Significance values in the text are at $p<0.05$, unless noted otherwise.

Three interference indices for each species were calculated with harvested dry wt: replacement and additive analyses of relative yield (Austin et al. 1988), and the RIP index derived by Wilson and Keddy (1986), modified by using initial biomass instead of numbers of plants in the denominator. Use of several indices was justified by the uncertainty surrounding analysis of interference by any single method (see Jolliffe et al. 1984; Connolly 1986; Austin et al. 1988). Interference was acknowledged only when two or more indices showed similar outcomes.

RESULTS AND DISCUSSION

Cadmium analysis of solutions in Salvinia monocultures (Fig. 1) showed that plants were exposed to appreciable Cd concentrations for most of the experiment. By the end of the study there was no Cd in the 20% nutrient treatments, but ca. 10% of initial Cd remained in 2% medium. This difference may be explained by lower plant RGR in the latter treatment (see below) resulting in less Cd removed from solution by plant biomass.

The RGR data indicated that nutrient and Cd levels and the presence of another species were significant factors in plant growth (Table 1a). The RGRs of both species were significantly ($p<0.001$) greater in 20% nutrient medium than in 2% medium at all Cd levels. The effect of Cd on RGR, however, differed between species and with nutrient level. For Salvinia in 2% medium, RGR decreased significantly at 10 ppb Cd and again at 25 ppb. In contrast, in 20% medium, growth at 10 and 25 ppb Cd was similar.

The effect of the presence of Spirodela on Salvinia also varied with Cd and nutrient levels. In Cd-free solution, the RGR of Salvinia was not significantly affected by Spirodela. In Cd treatments with 2% nutrient medium, however, Salvinia grew significantly more rapidly in 200 mg mixture than in 200 mg monoculture, at a rate comparable with its 400 mg monoculture. In 20% medium, RGRs in Cd-dosed mixtures and monocultures were not significantly different.

Spirodela was less sensitive to Cd than was Salvinia in 2% medium, showing a significant decline in RGR only at 25 ppb Cd. The presence of Salvinia at this Cd and nutrient level caused a significant increase in the RGR of Spirodela, but not in other treatments. The increase was not as great as that occurring between the 200 mg and 400 mg monocultures of Spirodela. This indicates that the effect on Spirodela of adding 200 mg of Salvinia was less than that of an equivalent biomass of Spirodela. In contrast, the effect on Salvinia of adding Spirodela was similar to that of equivalent amount of Salvinia. Thus, Spirodela had a greater effect on Salvinia than the reverse. In 20% medium, Spirodela also grew more rapidly in Cd-dosed monocultures with higher initial biomass (c.f. 200 and 400 mg monocultures), whereas Salvinia did not.

For the interference indices (Table 1b), values greater than 1.0 suggest improved growth of a species due to interference from a second species (Wilson and Keddy 1986; Austin et al. 1988). Additive analysis and the RIP index agreed that, in 2% nutrient treatments, Spirodela in 25 ppb Cd and Salvinia in 10 ppb Cd showed

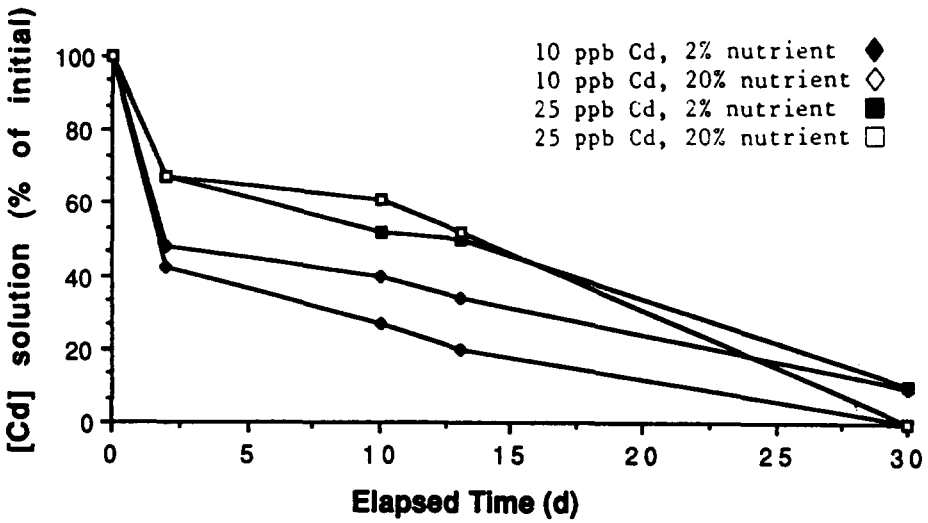

Figure 1. Cadmium concentrations in Salvinia monocultures during the experiment. (Data are means of three replicates)

improved growth due to species interference. In contrast, in 20% medium, species interactions resulted in reduced growth of both species (indices <1.0). The replacement analysis index was mostly between 0.5 and 1.0, an equivocal outcome which does not allow conclusions regarding interference to be drawn (Austin et al. 1988).

This experiment indicated that although an effect of Cd on plant growth was evident at the same Cd concentrations (10 ppb for Salvinia, 25 ppb for Spirodela) in 2% nutrient monocultures and mixtures, the magnitude of observed toxicity was somewhat different. For both species, plants in Cd-contaminated mixtures showed more rapid growth, because of species interference, than in corresponding monocultures. Nutrient concentration was a significant factor in the results from this experiment. Variations in growth rate due to Cd, or to the effect of species interference, were most obvious in the low nutrient treatment; the high nutrient level tended to reduce differences between Cd levels and between monocultures and mixtures.

These findings regarding interference are comparable to those reported for Salvinia natans and Lemna minor mixtures exposed to Cd (Hutchinson and Czyrska 1975). Although the design of the latter experiment did not allow interference indices to be calculated, it showed that S. natans also grew better and accumulated less Cd in the presence of L. minor than in monoculture. In

Table 1. Relative growth rates (mean ±s.e.) (a) and interference indices (b) for *Salvinia* and *Spirodela*. (SL- dry wt sample lost, preventing index calulation Different letters across rows and within columns indicate $p<0.05$.)

(a) Relative Growth Rate

	Cd Treatment (ppb)					
	0	10	25	0	10	25
	2% Nutrient Medium			20% Nutrient Medium		
Spirodela						
	a	a	b	a	b	b
200 mg monoculture	0.09 ±0.01	0.07 ±0.01	-0.03 ±0.01	0.39 ±0.02	0.23 ±0.01	0.22 ±0.01
	a	a	c	b	b	b
200 mg mixture	0.07 ±0.02	0.05 ±0.05	0.00 ±0.01	0.23 ±0.01	0.19 ±0.03	0.24 ±0.01
	a	a	d	a	a	a
400 mg monoculture	0.13 ±0.01	0.11 ±0.01	0.02 ±0.01	0.39 ±0.03	0.39 ±0.03	0.44 ±0.01
Salvinia						
	a	b	d	a	b	b
200 mg monoculture	0.40 ±0.01	0.12 ±0.02	0.01 ±0.00	0.56 ±0.01	0.42 ±0.02	0.47 ±0.02
	a	c	de	a	b	b
200 mg mixture	0.37 ±0.02	0.24 ±0.03	0.03 ±0.01	0.50 ±0.02	0.44 ±0.03	0.45 ±0.02
	a	c	e	a	b	b
400 mg monoculture	0.41 ±0.01	0.27 ±0.03	0.04 ±0.01	0.53 ±0.01	0.45 ±0.03	0.43 ±0.02

Table 1 cont'd.

			(b) Interference Indices			
Spirodela						
Additive	0.72	1.15	8.25	0.69	0.74	0.93
Replacement	0.48	0.52	0.55	0.46	0.30	0.42
RIP	0.64	0.40	1.12	1.52	-1.22	-0.72
Salvinia						
Additive	0.82	1.87	1.16	0.71	0.86	0.99
Replacement	0.76	0.59	0.49	0.71	SL	1.01
RIP	0.30	1.46	0.89	0.58	SL	0.02

contrast, L. minor grew slower in mixture and accumulated more Cd than in monoculture. This difference between the present and latter studies may be due to the use of different species, or because the present study used higher initial biomasses, which may have been advantageous to the duckweed (see earlier discussion of Spirodela RGR in monocultures).

The findings of Hutchinson and Czyrska (1975), Everard and Denny (1985) and Lolkema et al. (1986) indicate that differential uptake of metals may account for differences in toxicity test outcomes from monocultures and species mixtures. Plants which grew more rapidly under conditions of species interference contained lower metal levels than in monoculture, while other species in the mixture contained more. Although the mechanism of interference was not examined here, this study adds to the existing literature showing that toxicity tests with species mixtures provide results which are at least qualitatively different from those with corresponding monocultures. An outcome common to all these studies is that metal toxicity in one or more test populations was lower in multi-species tests than in single-species tests. The question as which experimental design provides a more "realistic" outcome remains to be addressed.

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