

## The toxicity of cadmium to barley plants as affected by complex formation with humic acid

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### Abstract

An 'alternating solution' culture method was used to study the effects of chloride ions and humic acid (HA) on the uptake of cadmium by barley plants. The plants were transferred periodically between a nutrient solution and a test solution containing one of four levels of HA (0, 190, 569 or 1710  $\mu\text{g cm}^{-3}$ ) and one of five levels of Cd (0, 0.5, 1.0, 2.5 or 5.0  $\mu\text{g cm}^{-3}$ ) in either a 0.006 M NaNO<sub>3</sub> or 0.006 M NaCl medium. Harvest and analysis of shoots and roots was after nineteen days. The distribution of Cd in the test solutions between Cd<sup>2+</sup>, CdCl<sup>+</sup> and HA-Cd was determined in a separate experiment by dialysis equilibrium.

In the nitrate test solutions Cd uptake was clearly controlled by Cd<sup>2+</sup> concentration and was therefore reduced by HA complex formation. In the absence of HA, chloride suppressed Cd uptake indicating that Cd<sup>2+</sup> was the preferred species. However complex formation with Cl<sup>-</sup> enhanced uptake when HA was present because of an increase in the concentration of inorganic Cd species relative to the nitrate system.

The ratio root-Cd/shoot-Cd remained at about 10 across a wide range of shoot-Cd concentrations, from about 3  $\mu\text{g g}^{-1}$  (sub-toxic) up to 85  $\mu\text{g g}^{-1}$  (80% yield reduction). The ability of the barley plants to accumulate 'non-toxic' Cd in their roots was thus very limited. Humic acid also had no effect on Cd translocation within the plant and the root/shoot weight ratio did not vary with any treatment.

At shoot-Cd concentrations in excess of 50  $\mu\text{g g}^{-1}$ , K, Ca, Cu and Zn uptake was reduced, probably the result of root damage rather than a specific ion antagonism. The highest concentration of HA also lowered Fe and Zn uptake and there was a toxic effect with increasing HA concentration at Cd = 0. However the lowest HA level, comparable with concentrations found in mineral soil solutions, only reduced yield (in the absence of Cd) by < 5% while lowering Cd uptake across the range of Cd concentrations by 66%–25%.

### Introduction

Much concern over heavy-metal contamination has centred on cadmium as an element which, relative to other metals, displays both exceptional toxicity (Friberg *et al.*, 1971) and a high mobility within soils and plants (Frankland and Khan, 1983).

Concentrations of Cd in UK soils normally lie in the range 0.05–1.0  $\mu\text{g Cd g}^{-1}$  (Page, 1981). However, contamination resulting in elevated levels of

Cd may come from various sources. Carboniferous black shales can have up to 200  $\mu\text{g Cd g}^{-1}$  and soils developed on this parent material have been found to contain up to 20  $\mu\text{g Cd g}^{-1}$  (Thornton, 1980). Cadmium enrichment of soil is usually associated with industrial pollution however, especially from the spoil heaps of nineteenth century mining activity in the S.W. of England, north Wales, Derbyshire and the Pennines. An analysis of topsoils contaminated by mine spoil in north east Clwyd, for example, showed a wide range of metal concentra-

tions including Cd levels of up to  $540 \mu\text{g Cd g}^{-1}$  (Davies and Roberts, 1978). Cadmium is a particularly volatile element in Zn ores and so enrichment of soils around Zn smelters in the Swansea valley and near Bristol has occurred (Martin *et al.*, 1980). In this context, concentrations in soils of over  $1700 \mu\text{g Cd g}^{-1}$  have been recorded (Buchauer, 1973). Agricultural activity also contributes to Cd accumulation in soil, through the addition of superphosphate with  $50\text{--}170 \mu\text{g Cd g}^{-1}$  (De Haan and Swerman, 1976) and sewage sludge. An analysis of 42 sewage sludges from England and Wales (Berrow and Webber, 1972) revealed that Cd concentrations were very variable but could extend up to  $1500 \mu\text{g Cd g}^{-1}$ . Furthermore, using the mean concentrations of metals given by Berrow and Webber (1972), the average ratio of Cd concentration in sewage sludge to that in soil is around 2000 compared with only about 10–50 for Ni, Cu, Pb, Sn and Zn.

In soil, the availability of Cd to plants will be affected by both the solid–solution equilibrium of  $\text{Cd}^{2+}$  and the extent of complex formation with organic and inorganic ligands in the soil solution. The implications of complex formation with a ligand added to the soil depend largely on the nature of the ligand and the desorption kinetics of  $\text{Cd}^{2+}$ . For example, provided the ratio of adsorbed Cd to solution Cd is large, the addition of a wholly soluble ligand which cannot be adsorbed by plant roots will only cause a temporary reduction in the availability of Cd. Once sufficient  $\text{Cd}^{2+}$  has desorbed from the solid phase to re-establish equilibrium, the presence of the complexing agent becomes irrelevant to the availability of  $\text{Cd}^{2+}$ . Alternatively, if the complexed form of Cd can be adsorbed by plants, then the net result of ligand addition to the soil is an increase in the availability of Cd with time, to a new final level dictated by  $\text{Cd}^{2+}$  equilibrium with the soil solid phase and with the ligand. The latter situation is one which may arise as a result of the disposal of sewage sludge at sea, in which cadmium has been found to undergo virtually complete dissolution, probably as a result of the formation of chloro-complexes ( $\text{CdCl}^+$ ,  $\text{Cd}(\text{Cl})_2^0$ ), (Laxen, 1982).

It has been suggested that strongly complexed trace metals are intrinsically less toxic than weakly complexed forms (Cossa, 1976; Allen *et al.*, 1980; Petersen, 1982). In addition, it seems likely that

humus acid-metal complexes, because of their high molecular weight, are less likely to be able to enter plant roots than monomeric inorganic complexes. In this study, we attempt to quantify the effect of Cd complex formation with humic acid (HA) and with chloride ions on the availability of the metal to barley plants. Dialysis equilibrium was used to derive empirical equations relating the distribution of Cd (between HA-Cd and free  $\text{Cd}^{2+}$ ) to HA concentration, Cd concentration and ionic strength, at constant pH. Barley plants were grown in solution culture, being alternately placed in a standard nutrient solution and in a test solution containing one of four concentrations of HA and one of five concentrations of  $\text{Cd}(\text{NO}_3)_2$ . The background electrolyte of the test solutions was either  $\text{NaNO}_3$  or  $\text{NaCl}$ . The barley plants were harvested when 19 days old and the Cd concentration and yield of both shoots and roots were related to the calculated Cd speciation in the test solutions.

## Materials and methods

### 1. Humic acid

Humic acid was extracted by the method of Schnitzer and Khan (1972) from soil of the Sonning series (Jarris, 1967) (acid brown earth under deciduous woodland). After dialysis against acid and distilled water, the HA had an ash content of  $< 2\%$ .

### 2. Solution culture experiment

Barley seeds (*Hordeum vulgare* L. cv. Ark Royal) were selected within a narrow weight range (50–55 mg) and germinated in moist vermiculite. After six days, seedlings were selected for uniformity and grown by an ‘alternating solution’ method (Hewitt, 1966) in a nutrient solution and in a test solution containing HA and  $\text{Cd}(\text{NO}_3)_2$  in either  $\text{NaNO}_3$  (0.006 M) or  $\text{NaCl}$  (0.006 M). The Long Ashton ‘standard complete nutrient solution’ (Hewitt, 1966) was used, in 250-cm<sup>3</sup> polythene containers covered in Al foil to exclude light. Plants were supported by cotton wool wedges through holes in the container lids, and the solutions were aerated by capillary lines from an air pump. Three plants

were grown in each container. The test solutions, in 50-cm<sup>3</sup> covered polythene pots, contained 0, 190, 569 or 1710  $\mu\text{g HA cm}^{-3}$ ; 0, 0.5, 1.0, 2.5 or 5.0  $\mu\text{g Cd cm}^{-3}$  and either 0.006 M NaCl or 0.006 M NaNO<sub>3</sub> as electrolyte. Humic acid was initially dissolved in NaOH, but the final pH of all test solutions was adjusted to 6.0 with HNO<sub>3</sub> or NaOH. Plants were regularly moved between nutrient and test solutions by transferring the supporting container lid; roots were thoroughly rinsed in distilled water before each transfer. The time (hours) spent in each solution followed the sequence: 48n, 24t, 48n, 36t, 48n, 48t, 48n, 36t, 48n, 24t, 48n; where 'n' and 't' denote time spent in the nutrient and test solutions respectively. During the total growth period of nineteen days, both test and nutrient solutions were replenished three times. The positions of the solution containers were randomly chosen in a growth room set at 60% relative humidity with temperature restricted to 22°–25°C and a sixteen hour light period per twenty four hours.

At the end of the nineteen day growth period, the plants were harvested; roots and shoots were separated, dried at 80°C and weighed. All plant tissue samples were digested in a mixture of concentrated 'Aristar' grade HClO<sub>4</sub>, HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> (Jackson, 1958). Total Cd, Zn, Fe, Ca and K in the plant digests were determined by standard AAS and Cu was measured by flameless AAS.

### 3. Determination of free Cd<sup>2+</sup> in test solutions

An attempt was made to measure free Cd<sup>2+</sup> with a cadmium (CdS) electrode but the presence of HA adversely affected the operation of the electrode. In order to calculate the proportion of free Cd<sup>2+</sup> ( $\alpha_{\text{Cd}}$ ) in the HA test solutions, dialysis equilibrium was used to derive empirical equations relating  $\alpha_{\text{Cd}}$  to the concentration of total Cd ( $\text{Cd}_T$ ), HA concentration and ionic strength (I) for both the nitrate and chloride systems. Humic acid concentrations of 190, 569 and 1710  $\mu\text{g HA cm}^{-3}$  were made up in 0.1, 0.2 and 0.3 M NaNO<sub>3</sub> or NaCl, adjusted to pH 6 (HNO<sub>3</sub>/NaOH) and 20-cm<sup>3</sup> aliquots sealed in air-free dialysis tubing. Each tube was added to a 20-cm<sup>3</sup> aliquot of Cd(NO<sub>3</sub>)<sub>2</sub> solution (0.5, 1.0, 2.5 or 5.0  $\mu\text{g Cd cm}^{-3}$ , with similar background electrolyte, in 100-cm<sup>3</sup> polythene bottles and shaken for 72 hours. The equilibrium Cd concentration in the

solution outside the dialysis bag was determined by AAS and, by difference, the concentration of Cd inside the bag was calculated. The proportion of free Cd, in the presence of HA, was calculated as:

$$\alpha_{\text{Cd}} = \frac{(\text{Cd}^{2+})}{(\text{Cd}^{2+}) + (\text{HA} - \text{Cd})}$$

Therefore  $\alpha_{\text{Cd}} =$

$$\frac{\text{Measured free Cd conc. } (\mu\text{g cm}^{-3})}{\text{Total calculated Cd conc. in dialysis bag } (\mu\text{g cm}^{-3})}$$

Estimation of the degree of complexation in each test solution used in the growth experiment proceeded as follows.

(a) Linear regression equations relating  $\alpha_{\text{Cd}}$  to ( $\text{Cd}_T$ ) were derived from the four  $\text{Cd}_T$  values used at ionic strengths of 0.1, 0.2 and 0.3 for each of the three HA concentrations (190, 569 and 1710  $\mu\text{g cm}^{-3}$ ).

$$\text{i.e. } \alpha_{\text{Cd}} = a(\text{Cd}_T) + b$$

at a given HA concentration and ionic strength, where a and b are regression constants

(b) Values of  $\alpha_{\text{Cd}}$  were then calculated for the prescribed values of  $\text{Cd}_T$  (0.5, 1.0, 2.5 and 5.0  $\mu\text{g cm}^{-3}$ ) for each concentration of HA at ionic strengths of 0.1, 0.2 and 0.3. Regression equations relating  $\alpha_{\text{Cd}}$  to ionic strength were derived.

$$\text{i.e. } \alpha_{\text{Cd}} = c(I) + d$$

at a given HA concentration (190, 569 or 1710  $\mu\text{g cm}^{-3}$ ) and  $\text{Cd}_T$  concentration (0.5, 1.0, 2.5 or 5.0  $\mu\text{g cm}^{-3}$ ), where c and d are regression constants.

From estimated values of I in the growth experiment test solution,  $\alpha_{\text{Cd}}$  values were calculated.

(c) For the nitrate system it was assumed that:

$$(\text{Cd}^{2+}) = \alpha_{\text{Cd}}(\text{Cd}_T) \quad (1)$$

However, in solutions with NaCl as background electrolyte, 'free' Cd must include both Cd<sup>2+</sup> and cadmium chloride complexes. As the Cl<sup>-</sup> concentration in the test solutions was quite low (0.006 M), only distribution of Cd between Cd<sup>2+</sup> and CdCl<sup>+</sup> was expected. The proportion of Cd complexed by Cl<sup>-</sup> was calculated from the stability constant for a 1:1 complex (log K = 2.00) at I = 0 (Sillén and Martell, 1971). The value of log K was adjusted in the normal way with single ion activity

**Table 1a.** Dry matter yield and Cd concentration of the shoots and roots of spring barley grown in test solutions with different HA and Cd concentrations in 0.006 M NaCl. Values are the mean of three replicates

Test solution ( $\mu\text{g cm}^{-3}$ )				Shoots <sup>a</sup>			Roots		Root/ Shoot	Root-Cd/ Shoot-Cd
HA	Cd <sub>T</sub>	(Cd <sup>2+</sup> )	(CdCl <sup>+</sup> )	DM (g)	%Y	Cd ( $\mu\text{g g}^{-1}$ )	DM (g)	Cd ( $\mu\text{g g}^{-1}$ )		
0	0	0	0	0.46	100	0.36	0.25	1.23	0.54	3.4
0	0.5	0.349	0.151	0.39	85	17.4	0.21	138	0.54	8.0
0	1.0	0.697	0.303	0.25	54	34.5	0.12	593	0.48	17.2
0	2.5	1.743	0.757	0.10	22	63.8	0.04	758	0.40	11.9
0	5.0	3.485	1.515	0.08	17	70.1	0.03	778	0.40	11.1
190	0	0	0	0.45	100	0.57	0.25	0.77	0.56	1.4
190	0.5	0.029	0.013	0.44	98	11.7	0.26	102	0.59	8.7
190	1.0	0.075	0.033	0.40	89	20.8	0.23	213	0.58	10.3
190	2.5	0.312	0.135	0.37	82	33.0	0.24	402	0.65	12.2
190	5.0	1.037	0.446	0.25	56	53.5	0.10	735	0.40	13.7
569	0	0	0	0.33	100	0.27	0.19	1.11	0.58	4.1
569	0.5	0.012	0.005	0.43	130	6.76	0.26	53.4	0.60	7.9
569	1.0	0.028	0.012	0.45	136	10.2	0.28	94.5	0.62	9.3
569	2.5	0.098	0.041	0.41	124	16.4	0.26	158	0.63	9.6
569	5.0	0.293	0.124	0.32	97	28.2	0.19	251	0.59	8.9
1710	0	0	0	0.30	100	0.39	0.17	1.34	0.57	3.4
1710	0.5	0.0086	0.003	0.31	103	2.74	0.18	25.1	0.58	9.2
1710	1.0	0.019	0.008	0.33	110	3.39	0.19	29.1	0.58	8.6
1710	2.5	0.064	0.025	0.30	100	5.17	0.17	45.5	0.57	8.8
1710	5.0	0.175	0.070	0.22	73	9.95	0.11	52.9	0.50	5.3

<sup>a</sup> %Y = percentage of the shoot yield obtained for Cd<sub>T</sub> = 0 for single HA concentration

**Table 1b.** Dry matter yield and Cd concentration of the shoots and roots of spring barley grown in test solutions with different HA and Cd concentrations in 0.006 M NaNO<sub>3</sub>. Values are the mean of three replicates

Test solution ( $\mu\text{g cm}^{-3}$ )				Shoots <sup>a</sup>			Roots		Root/ Shoot	Root-Cd/ Shoot-Cd
HA	Cd <sub>T</sub>	(Cd <sup>2+</sup> )	(CdCl <sup>+</sup> )	DM (g)	%Y	Cd ( $\mu\text{g g}^{-1}$ )	DM (g)	Cd ( $\mu\text{g g}^{-1}$ )		
0	0	0	0	0.54	100	0.38	0.27	1.64	0.50	4.3
0	0.5	0.5	0	0.31	57	25.0	0.16	176	0.52	7.1
0	1.0	1.0	0	0.27	50	48.0	0.14	359	0.52	7.5
0	2.5	2.5	0	0.15	28	79.8	0.06	780	0.40	9.8
0	5.0	5.0	0	0.10	19	85.2	0.03	994	0.30	11.7
190	0	0	0	0.52	100	0.44	0.25	1.75	0.48	4.0
190	0.5	0.026	0	0.47	90	8.35	0.26	96.3	0.55	11.5
190	1.0	0.071	0	0.37	71	18.1	0.21	198	0.57	10.9
190	2.5	0.324	0	0.29	56	36.5	0.16	408	0.55	11.2
190	5.0	1.128	0	0.20	38	64.6	0.11	792	0.55	12.3
569	0	0	0	0.34	100	0.35	0.20	1.34	0.59	3.8
569	0.5	0.010	0	0.42	124	4.63	0.22	53.6	0.52	11.6
569	1.0	0.024	0	0.36	106	7.04	0.20	75.6	0.56	10.7
569	2.5	0.092	0	0.28	82	12.2	0.15	103	0.54	8.4
569	5.0	0.284	0	0.25	74	22.8	0.13	223	0.52	9.7
1710	0	0	0	0.27	100	0.35	0.13	1.26	0.48	3.6
1710	0.5	0.008	0	0.28	104	2.26	0.15	12.9	0.54	5.7
1710	1.0	0.018	0	0.27	100	2.70	0.14	18.8	0.52	6.9
1710	2.5	0.052	0	0.23	85	3.90	0.13	33.2	0.57	8.5
1710	5.0	0.130	0	0.23	85	6.43	0.12	65.1	0.52	10.1

<sup>a</sup> %Y = percentage of the shoot yield obtained for Cd<sub>T</sub> = 0 for a single HA concentration.

coefficients calculated from the Debye-Hückel equation appropriate to the estimated ionic strength of each test solution. Thus, the concentration of  $\text{Cd}^{2+}$  in a test solution containing  $\text{Cl}^-$  ions was estimated as:

$$(\text{Cd}^{2+}) = \frac{\alpha_{\text{Cd}}(\text{Cd}_T)}{1 + K(\text{Cl}^-)} \quad (2)$$

## Results and discussions

### *The effect of Cd on yield and Cd uptake*

In the absence of HA, an increase in  $\text{Cd}_T$  resulted in a decrease in yield and increase in the Cd content of both shoots and roots (Tables 1a and 1b). In particular, it was noted that at  $\text{Cd}_T = 2.5 \mu\text{g cm}^{-3}$  roots were extremely stunted and root elongation and the development of laterals were severely inhibited.

The critical concentration of Cd in the shoots of young barley plants at which toxic effects (10% reduction in yield) are shown is quoted as 10–20  $\mu\text{g g}^{-1}$  (Macnicol and Beckett, 1985). The relationship between Cd content and yield is not consistent in this study however as each HA concentration gives rise to a different pattern. High levels of HA lower shoot and root yield over the range of Cd concentrations (Table 1) so that the influence of shoot-Cd content on yield cannot be examined as a single effect. However, the change in % yield within each HA concentration (100% being  $\text{Cd}_T = 0$ ) appears to indicate that a 10% reduction in shoot growth resulting from 10–20  $\mu\text{g Cd g}^{-1}$  is realistic.

A higher concentration of Cd was found in roots than in shoots in all cases (Table 1). Surprisingly, for test solutions in which  $\text{Cd}_T > 0$ , there was no apparent change in the ratio 'root-Cd/shoot-Cd' with increasing Cd concentration. Omitting the data for test solutions in which  $\text{Cd}_T = 0$ , the average ratio root-Cd/shoot-Cd was found to be 10.0 ( $\sigma_{n-1} = 2.7$ ) for the chloride system and 9.6 ( $\sigma_{n-1} = 2.0$ ) where sodium nitrate was the electrolyte. Only when  $\text{Cd}_T = 0$ , were the average ratios significantly lower, at 3.1 and 3.9 respectively. It has been suggested that roots are capable of preventing translocation of Cd to shoots by storage of the metal in a relatively innocuous form, such as Cd-oxalate, in intercellular spaces (Hardiman *et al.*,

1984). It is apparent here that even at Cd concentrations which give rise to only slight toxicity, the capacity of the roots to accumulate Cd and prevent translocation to shoots is very limited. Only in the nitrate system with  $\text{HA} = 1710 \mu\text{g cm}^{-3}$  when root-Cd was less than 20  $\mu\text{g g}^{-1}$  and shoot-Cd < 3  $\mu\text{g g}^{-1}$  was there an indication of root-Cd/shoot-Cd increasing with Cd concentration. It has also been suggested that root growth is generally more inhibited than shoot growth as a result of metal toxicity (Frankland and Khan, 1983). The constancy of the root/shoot yield ratios (Table 1) strongly suggests there is no differential toxicity effect across a range of root-Cd concentrations of virtually 1–1000  $\mu\text{g g}^{-1}$ . The average weight ratios of root/shoot were 0.55 ( $\sigma_{n-1} = 0.075$ ) and 0.52 ( $\sigma_{n-1} = 0.065$ ) for the chloride and nitrate systems respectively.

Cadmium appeared able to affect (reduce) the uptake of Zn, Cu, Ca and K only at high shoot concentrations of > 50  $\mu\text{g Cd g}^{-1}$  (Table 2); this is well over the Cd concentration required to produce a toxic effect. Uptake of Fe was unaffected by Cd concentration but was significantly reduced at the highest level of HA. It is often quoted that antagonism occurs between Cd and Zn (Hardiman *et al.*, 1984) although there may not be a simple relationship between the two ions (Gerritse *et al.*, 1983). The alternating solution method used here dictates that the two ions are not present in the same solution and this may affect the nature of any competitive reaction. The reduction in uptake of K, Ca and Cu along with Zn and only at exceptionally high root Cd concentrations (> 700  $\mu\text{g g}^{-1}$ ) suggests an effect of general root damage rather than a specific ion antagonism. The picture is also complicated by the fact that the highest concentration of HA appears to reduce Fe and Zn uptake even though both HA and Cd were not actually present in the nutrient solution.

### *The effect of HA on yield and Cd uptake*

Humic acid lowered Cd uptake at all concentrations of  $\text{Cd}_T$ , providing strong evidence that complex formation effectively de-toxifies the metal, (e.g. Fig. 1). However, there was no apparent relationship between HA and the distribution of Cd between root and shoot (Tables 1a and 1b). This

Table 2. Dry matter yield and concentration of Fe, Zn, Cu and K of the shoots of spring barley grown in solution media with different humic acid and cadmium concentrations ( $\text{Cl}^-$  and  $\text{NO}_3^-$  results combined)

Humic acid concentration ( $\mu\text{g cm}^{-3}$ )	$\text{Cd}_T$ ( $\mu\text{g cm}^{-3}$ )	Dry matter yield (g)	Fe ( $\mu\text{g g}^{-1}$ )	Zn ( $\mu\text{g g}^{-1}$ )	Cu ( $\mu\text{g g}^{-1}$ )	Ca (%)	K (%)	Cd ( $\mu\text{g g}^{-1}$ )
0	0	0.50	96.01	63.43	18.12	0.69	6.90	0.37
	0.5	0.35	98.72	77.54	17.17	0.65	6.91	21.2
	1.0	0.26	119.49	74.38	16.46	0.64	6.31	41.2
	2.5	0.13	93.13	50.39	14.22	0.38	4.50	71.8
	5.0	0.09	92.53	38.58	6.92	0.24	2.62	77.7
190	0	0.49	93.41	64.97	21.17	0.71	7.09	0.51
	0.5	0.46	88.54	73.79	16.58	0.64	6.96	10.0
	1.0	0.39	94.28	80.85	16.72	0.60	6.77	19.5
	2.5	0.33	95.98	87.87	15.12	0.58	6.16	34.8
	5.0	0.24	79.62	50.62	13.40	0.48	5.44	59.0
569	0	0.34	104.49	61.63	18.68	0.69	6.88	0.31
	0.5	0.43	80.99	57.76	16.77	0.63	6.19	5.7
	1.0	0.41	97.46	67.02	15.66	0.60	6.34	8.6
	2.5	0.35	89.99	73.04	22.62	0.66	6.84	14.3
	5.0	0.29	90.56	61.76	22.57	0.62	6.28	25.5
1710	0	0.29	71.17	43.85	18.32	0.70	5.30	0.37
	0.5	0.30	77.26	52.47	17.32	0.66	5.45	2.5
	1.0	0.30	73.25	51.49	20.32	0.68	5.45	3.0
	2.5	0.27	75.78	59.35	14.41	0.67	5.33	4.5
	5.0	0.23	75.58	50.27	16.39	0.63	4.66	8.2

contrasts with the findings of Tyler and McBride (1982) who found that HA lowered the ratio root-Cd/shoot-Cd of corn plants grown in hydroponic solutions containing Cd concentrations of 0.1 or  $2.0 \mu\text{g cm}^{-3}$ . The effect of HA on shoot and root yield is more complex (Fig. 2) and depends on the

total cadmium ( $\text{Cd}_T$ ) concentration. At lower levels of HA, yield increases with HA when Cd is present—presumably due to complex formation of the metal. At higher concentration of HA however, yields of shoots and roots are significantly depressed in the case of both the chloride and nitrate test

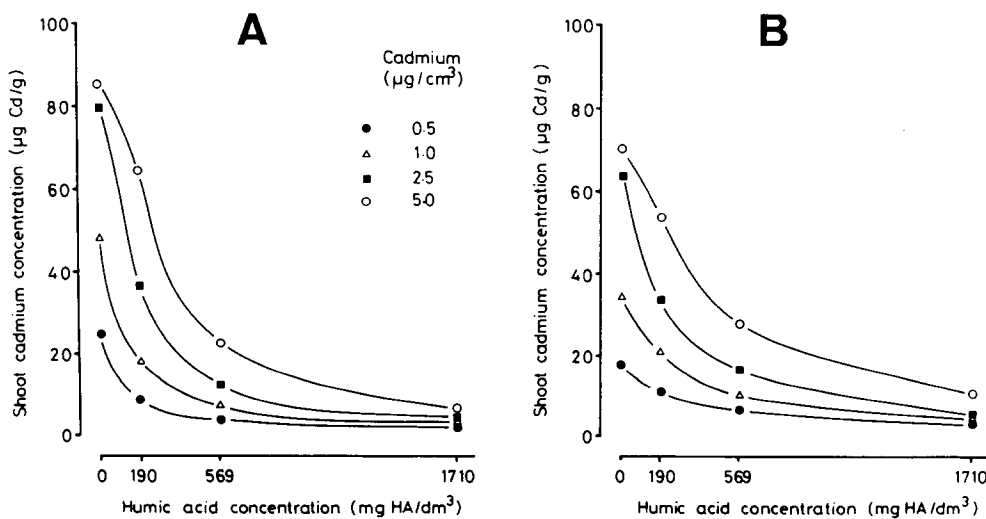


Fig. 1. Concentration of cadmium in spring barley shoots as a function of the concentration of humic acid at four values of  $\text{Cd}_T$ . Background electrolyte is  $0.006 \text{ M NaNO}_3$  (A) or  $0.006 \text{ M NaCl}$  (B).

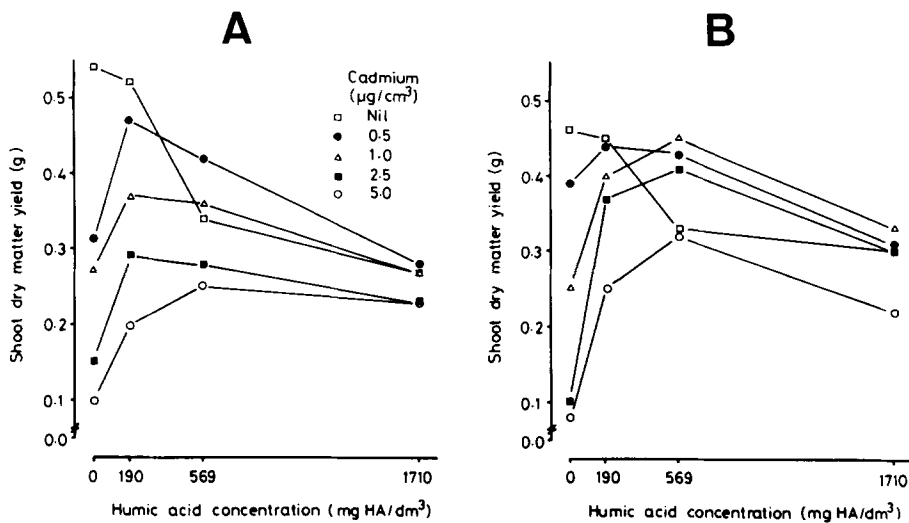


Fig. 2. Dry matter yield of spring barley shoots as a function of the concentration of humic acid at five values of Cd<sub>T</sub>. Background electrolyte is 0.006 M NaNO<sub>3</sub> (A) or 0.006 M NaCl (B).

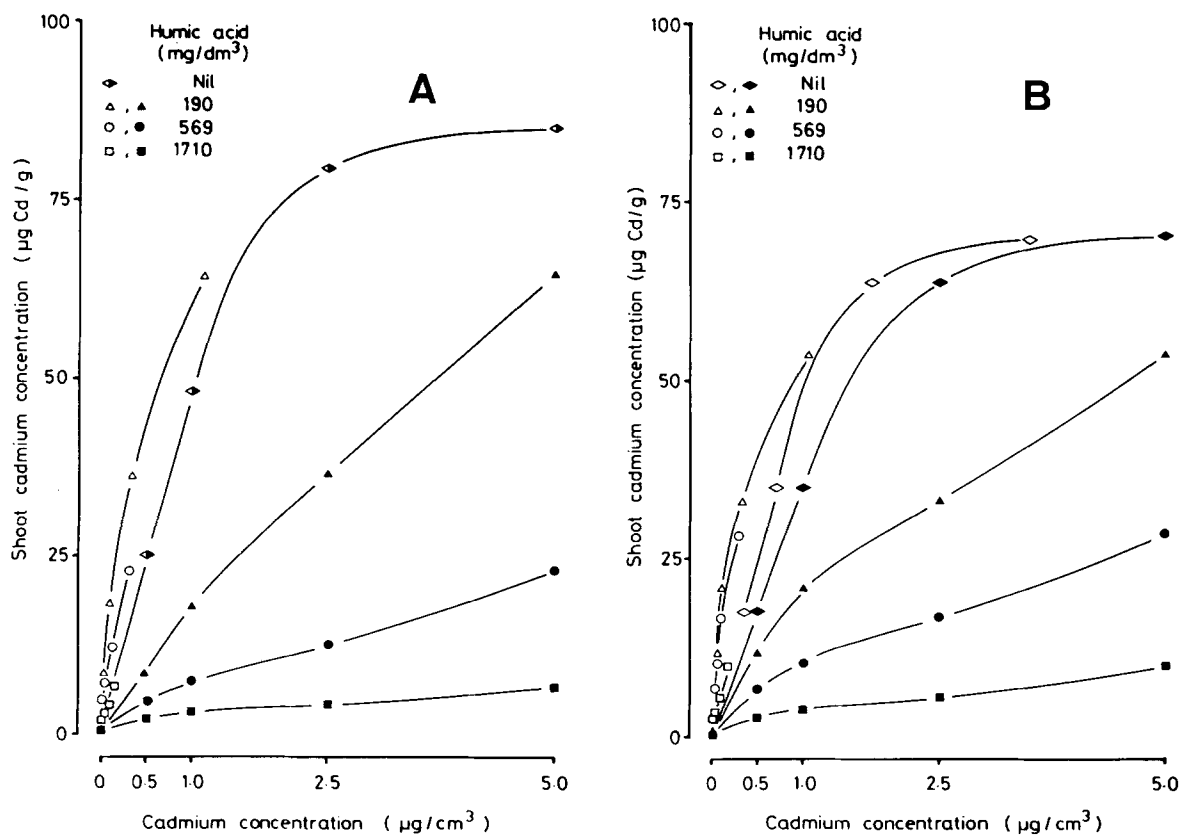


Fig. 3. Concentration of cadmium in spring barley shoots as a function of the concentration of free cadmium, Cd<sup>2+</sup> (open symbols) and Cd<sub>T</sub> (solid symbols) for four levels of humic acid. Background electrolytes are A: 0.006 M NaNO<sub>3</sub>, and B: 0.006 M NaCl.

solutions suggesting that HA has a deleterious effect. Where  $Cd_T = 0$ , depression in yield is continuous with increasing HA. It is apparent therefore that for a given concentration of HA and  $Cd_T$ , the plant yield will be the product of both a beneficial and adverse effect involving HA. Several workers have noted that, while low concentrations of humic and fulvic acids may enhance plant growth, high concentrations tend to lower yield (Fernandez, 1968; Poapst *et al.*, 1970; Vaughan, 1974). We observed that increasing HA (at  $Cd_T = 0$ ) caused a reduction in root length and in root hair development. In addition, high concentrations caused the root systems to be coated with HA which resisted washing in distilled water. This meant that humic acid on the root surface was transferred to the nutrient solutions and may thus have acted as a barrier to the uptake of micronutrient metals. This is born out by the marked reduction in Fe and Zn uptake at the highest HA concentration (Table 2). A rather odd result (Fig. 2 and Tables 1a and 1b) is that yield of shoots and roots at  $Cd_T = 0$  fell below the yield achieved for the lower concentrations of Cd at 569 and 1710  $\mu\text{g HA cm}^{-3}$ . This was found for both the chloride and nitrate systems and suggests that low concentrations of Cd actually had a beneficial effect in the presence of high HA concentrations. One rather tenuous explanation for this might be that Cd effectively blocks sites on the HA-coated roots which would otherwise adsorb micronutrient metals following transfer of plants to the nutrient solution. A similar explanation involving Fe adsorption sites on the root surface has been suggested to explain the growth response to Al ions sometimes found for plants grown in solution culture (Grime and Hodgson, 1968). However, the lack of variation in Fe, Zn and Cu shoot concentration with increasing Cd at high HA levels does not support this hypothesis (Table 2).

#### Cadmium uptake as a function of speciation

Figures 3a and 3b show shoot-Cd concentration plotted against  $Cd_T$  and against calculated free ( $Cd^{2+}$ ) (Tables 1a and 1b). It is clear that the effect of HA virtually disappears when the X-axis is taken as ( $Cd^{2+}$ ) indicating that free ionic  $Cd^{2+}$  is the form in which the metal is adsorbed by plants. The fact

that the line for HA = 0 falls slightly below those with HA may be attributed to the effects of depletion of Cd in the test solutions. Cadmium uptake is greater for a given calculated ( $Cd^{2+}$ ) concentration when HA is present because the HA-Cd complex species will act as a reservoir, desorbing  $Cd^{2+}$  in response to Cd uptake by plant roots. Depletion of Cd in the test solutions was up to 36% in the case of the treatment receiving zero HA and  $Cd_T = 0.5$ .

Only one concentration of  $Cl^-$  was used so it is not possible to quantify the relative uptake rates of  $Cd^{2+}$  and  $CdCl^+$ . A comparison of the nitrate and chloride test solutions for all levels of HA did not give a single consistent relationship between Cd uptake and solution concentration whether the latter parameter was taken as ( $Cd^{2+}$ ) or ( $Cd^{2+} + CdCl^+$ ), (Tables 1a and 1b). It was noticeable however that at HA = 0, shoot Cd for the chloride solutions ( $Cd^{2+} = 70\% Cd_T$ ) was on average 76% of shoot Cd for the nitrate solutions ( $Cd^{2+} = 100\% Cd_T$ ), (Tables 1a and 1b). This seems to suggest that Cd was adsorbed only as  $Cd^{2+}$ . However, it seems reasonable to expect that Cd can also be adsorbed as  $CdCl^+$  but that the adsorption rate will be considerably less than for

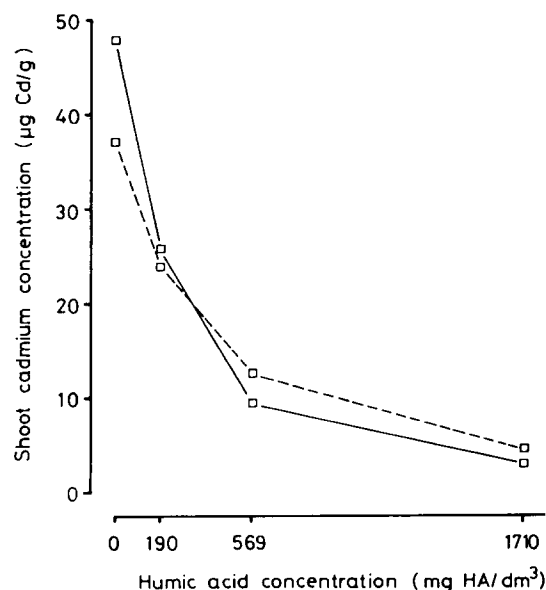


Fig. 4. Concentration of cadmium in spring barley shoots as a function of the concentration of humic acid. Each point represents the average of four  $Cd_T$  levels (12 barley plants) and the background electrolyte is either 0.006 M  $NaNO_3$  (solid line) or 0.006 M  $NaCl$  (broken line).



$\text{Cd}^{2+}$ . There is evidence for this in Fig. 4 in which shoot cadmium concentrations have been averaged over the four  $\text{Cd}_T$  values for both the chloride and nitrate systems. At  $\text{HA} = 0$ , more Cd is adsorbed when the electrolyte is  $\text{NaNO}_3$  indicating a preference for  $\text{Cd}^{2+}$  over  $\text{CdCl}^+$ . By contrast, at high HA, Cd uptake is greater when  $\text{Cl}^-$  is present. Chloride acts as a competing ligand (with HA) so that the total inorganic and hence 'available' Cd ( $\text{Cd}^{2+} + \text{CdCl}^+$ ) will be greater in this case (Tables 1a and 1b).

### Conclusions

The evidence presented suggests that  $\text{Cd}^{2+}$  is strongly preferred over  $\text{CdCl}^+$  for uptake and that HA-Cd complexes are not adsorbed by plants. The concentration of humus acids in the soil solution typically falls in the range  $25\text{--}250 \mu\text{g cm}^{-3}$  (Young and Bache, 1985). Even at this relatively low level Cd speciation and availability will be significantly affected. In addition, it is likely that soluble fulvic acids in the soil solution will exhibit a greater adsorption capacity for Cd than the humic acid used in this study. Although  $\text{CdCl}^+$  ions display a lower rate of uptake than  $\text{Cd}^{2+}$ , the effect of chloride in the presence of organically complexed Cd (and presumably 'solid phase' Cd) is to increase the bio-availability of the metal by raising the total concentration of soluble inorganic Cd.

The barley plants used displayed very little ability to store Cd in roots and prevent translocation to shoots. From 'sub-toxic' concentrations of Cd ( $\approx 3 \mu\text{g g}^{-1}$  in shoots) up to  $85 \mu\text{g g}^{-1}$ , giving an 80% yield reduction, there was no deviation from a root-Cd/shoot-Cd ratio of around 10. Humic acid did not seem to affect the distribution of Cd between roots and shoots either. It seems therefore that the concentration of Cd in barley shoots depends directly on the quantity in the plant roots. Furthermore, the relative toxicity to shoots and roots seems to be unaffected by the amounts adsorbed, or the presence of HA, as shown by the consistent weight ratio (root/shoot) of 0.50–0.55.

Humic acid lowered micronutrient metal uptake at high concentrations (of HA) but displayed a toxic effect even when Fe and Zn uptake did not seem to be restricted. There may have been a specific physiological reaction involved, and it was noted

that HA suppressed the production of root hairs. However, at the lowest concentration of HA used, which was comparable with levels in mineral soil solutions, the reduction in shoot yield (in the absence of Cd) was almost negligible (<5%). By contrast, the reduction in shoot-Cd uptake caused by  $190 \mu\text{g HA cm}^{-3}$  ranged from 66% to 25% depending on  $\text{Cd}_T$ . In polluted fresh water environments, the potential benefits of humus acids are thus likely to outweigh their intrinsic toxicity.

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