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Cellulolytic Activity and Root Biomass Production in Some Metal-Contaminated Soils

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ABSTRACT

A technique involving dye release was used to measure the activity of cellulose decomposing fungi in agar medium as affected by metal toxicity. The technique was developed for use in metal-contaminated soils. Cellulolytic activity was inhibited by toxic metals in the soil environment. In studies involving crop plants, Cd and Pb reduced rhizosphere activity in terms of both root biomass production and activity of the cellulose decomposers. Root biomass was reduced more than cellulolytic activity. Cadmium was much more toxic than lead.

INTRODUCTION

The biogeochemical distributions of heavy metals in different reservoirs in the environment have been studied by a number of investigators in Great Britain, notably Webb (1978), and some data related particularly to soil–plant systems have been collated in a recent report (Khan, 1980). The rhizosphere soil is such a reservoir in which contaminant metals interact with soil constituents (organic and inorganic) and plant roots, thus providing a direct route for heavy metals to enter the food chain.

This paper reports studies on rhizosphere activity as affected by toxic heavy metals, particularly lead, a highly immobile element, and cadmium, a more soluble element readily translocated in biological systems. The rhizosphere activity has been measured using cellulose degradation and root biomass production. Cellulose has been selected as an appropriate substrate for biodegradation studies in the root zone,

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since it constitutes between 40 and 60% of plant residue in the soil. The cellulolytic activity has been determined using a dye release assay developed and modified for soil work.

MATERIALS AND METHODS

Soils

Several soils, collected from uncontaminated and contaminated areas of Great Britain, have been used in this study. Brown earths from Dytchleys Field Station and Weald Country Park, both in Essex, are uncontaminated soils and represent typical agricultural soils in this country. Soils from Shipham, Somerset, have been sampled from a highly contaminated site opposite the village cemetery, much disturbed from past mine workings; and also from a less contaminated site near Rowberrow Lane in the village. Levels of Cd in the soil in certain parts of Shipham were exceedingly high, up to $600\,\mu g\,g^{-1}$. An alluvial soil, collected from the Grogwynion mining area near Aberystwyth, Dyfed, Central Wales, is also a disturbed soil mixed with stone fragments. Soils were air-dried, ground to pass through a 2 mm sieve and homogenised.

A field description of the soils, their physical and chemical characteristics, the levels of heavy metals and the analytical procedures involved in such measurements, have been presented earlier (Khan & Frankland, 1983).

Biodegradation of cellulose by dye release assay

The technique involving dye release (Poincelot & Day, 1972) was used to examine if the cellulolytic activity of fungi can be assayed in metal-contaminated agar medium. Plates were prepared with agar at pH 6·0 (0·5 g $[NH_4]_2SO_4$, 1·0 g KH_2PO_4, 0·5 g KCl, 0·2 g $MgSO_4$, $7H_2O$, 0·1 g $CaCl_2$, 0·5 g yeast extract, 4·0 g starch, 15 g agar, per 1000 ml). Aqueous solutions of metal salts were added to molten agar, Zn and Cu added as sulphate, and Ni and Cd as chloride (metal concentrations shown in Table 1). Cellophane films (2 × 5 cm strips, British Cellophane Ltd, Grade 325P) were dyed with Remazol Blue, overlaid onto agar medium, and then inoculated with pure cultures of fungi. The staining procedure for cellophane films was as described by Moore *et al.* (1979). There were two

TABLE 1
Effects of Zn, Cu, Ni and Cd on the Cellulolytic Activity of Two Fungi

Metal treatment[a] (*μg millilitre*$^{-1}$)	*Metals added*	*Per cent cellulose decomposition (Per cent inhibition relative to control)* Trichoderma viride *7 days*	*30 days*	Chaetomium *sp.* *30 days*
Control		30·4	100·0	100·0
Zn100	$ZnSO_4$	13·4 (55·9)	79·7 (20·3)	65·0 (35·0)
Zn500	$ZnSO_4$	8·7 (71·4)	60·9 (39·1)	28·3 (71·7)
Cu100	$CuSO_4$	11·4 (62·5)	52·2 (47·8)	29·3 (70·7)
Cu500	$CuSO_4$	5·0 (83·5)	38·3 (61·7)	17·8 (82·8)
Ni100	$NiCl_2$	8·0 (73·7)	45·1 (54·9)	18·5 (81·5)
Ni200	$NiCl_2$	5·0 (83·5)	36·0 (64·0)	9·8 (90·2)
Cd100	$CdCl_2$	11·1 (63·5)	—	—
Cd200	$CdCl_2$	8·4 (72·4)	—	—

[a] Effects of all metal treatments over control significant at 1% level.

experiments, each laid out with two replicates, and incubated at 25 °C. The first experiment was carried out for 7 days using four metals, Zn, Cu, Ni and Cd, and involved *Trichoderma viride*. The second experiment continued for 30 days using three metals, Zn, Cu and Ni, and involved two fungi, *Trichoderma viride* and *Chaetomium* sp. The cultures of fungi were obtained from the Mycology Laboratory of Queen Mary College.

At the specific time intervals the cellophane films were sampled and first autoclaved in distilled water to remove the dye released during the decomposition process, and then the remaining dye was extracted from the film with 0·2M NaOH at 100 °C and the absorbance measured at 595 nm using a spectrophotometer. The amount of dye originally present in the film, less the amount extracted by the alkali, was taken to represent the quantity of cellulose decomposed by the micro-organisms involved.

Development of dye release technique for soils studies

The dyed cellophane film was encased in nylon mesh before placement in the soil. The rates of cellulose decomposition were measured in an uncontaminated soil (brown earth from Dytchleys Field Station) using petri dishes, to determine the effects of (a) time at 0, 10, 20, 30 and 40 days after film placement in soils (soil moisture level at 50% water-holding

capacity) and (b) soil moisture levels at 0, 10, 25, 50 and 75% water-holding capacity of soil (30 days decomposition time). The experiment was carried out in duplicate in a growth cabinet at 25°C.

Pot studies were carried out using (a) sterilised soils and (b) waterlogged soils (0·5 cm of standing water above the surface) to examine if the cellophane film was decomposable under these conditions. The experiments were carried out in duplicate in a growth cabinet at 25°C. Three hundred grams of soil per pot were used in these and subsequent pot studies.

Soil experiments in the glasshouse

Studies without growing plants

Four soils used in the experiment were a surface soil (0–20 cm, Ap horizon) from Weald Park; surface soils (0–15 cm) from two sites in Shipham, near Rowberrow Lane and opposite the cemetery; and a surface soil (0–15 cm) from the Grogwynion mining area near Aberystwyth. To the Weald Park soil Cd was added as $CdCl_2$ at 0, 10, 50 and 100 $\mu g\ Cd\ g^{-1}$ levels, and Pb added as $PbCl_2$ at 0, 100, 500 and 1000 $\mu g\ Pb\ g^{-1}$ levels. There were two additional metal treatments at 10 and 50 $\mu g\ Cd\ g^{-1}$ combined with 1000 $\mu g\ Pb\ g^{-1}$ levels. Soils from Shipham and Grogwynion mining areas were used without any heavy metals added. A basal dressing of 100 $\mu g\ N\ g^{-1}$, 100 $\mu g\ P\ g^{-1}$ and 100 $\mu g\ K\ g^{-1}$ was given to all pots. Soils were irrigated with distilled water to field capacity with saucers underneath, and left to equilibrate at 25°C for 15 days. The experiment was laid out with four replicates in a randomised design. At the end of the equilibrium period, one dyed cellophane film encased in nylon mesh was buried in each pot. The films were withdrawn after 30 days for dye release assay.

Studies with growing plants

Three crop experiments using oat *Avena sativa* L., cv Maris Tabard, wheat *Triticum aestivum* L., cv Sicco and radish *Raphanus sativum* L., cv Cherry Belle were carried out to measure the cellulose decomposers' activity and the root biomass production in soils contaminated by adding metals.

Details of the metal treatments have been described earlier (Khan & Frankland, 1983). The rates and chemical forms of the metals added to soils in three experiments are also shown in Table 3. The basal dressing of

N, P and K and the equilibrium time involved in these crop studies were as described in studies without plants. Potted soils were used in studies with oat and wheat. The oat experiment was carried out with two replicates using the surface soil from Dytchleys. There were a total of eight metal treatments, including a control. The wheat experiment was carried out with three replicates using the surface soil from Weald Park. There were a total of eleven metal treatments, including a control. In both studies involving oat and wheat two seedlings germinated in vermiculite were planted in each pot, and thinned to one seedling 7 days after planting. The radish experiment involved soil columns (10 cm diameter) and was laid out with three replicates. Each column contained the surface soil (Ap horizon) from Weald Park held in the upper 10 cm portion and the sub-soil (B horizon) in the lower 10 cm portion of the plastic pipe. There were a total of five metal treatments including a control. Three germinated radish seeds were sown in each column, and thinned to two seedlings 7 days after sowing. The three crop experiments were laid out separately in a glasshouse at 25 °C, using a randomised design in each case. Plants were harvested 42 days after planting, roots being washed and dried at 60 °C. In all three experiments one dyed cellophane film was buried in each pot or in the surface 5 cm soil of each column, before planting. The films were withdrawn 30 days after placement, the decomposition of cellulose being measured by dye release assay. The cellophane films appeared, in certain cases, to be physically affected, particularly in less contaminated soils. This could be due to the effects of soil animals and plant roots. The cellulolytic activity in the root-zone soil was therefore attributed to the sum total of micro- and macro-organisms.

The soils from pots and columns were air-dried and analysed for the EDTA extractable (0·05M NH_4-EDTA at pH 7·0) and water-soluble fractions of Cd and Pb using atomic absorption spectrophotometry, the lower concentrations in the graphite furnace and the higher concentrations in the acetylene flame (Khan & Frankland, 1983).

RESULTS

Agar culture studies using fungi

At the specific time intervals when the cellophane films were sampled, there were visible differences in the amounts of cellulose decomposed in

the film, which could be attributed to the differences in metal treatments. The dye was released with the breakdown of the cellulose molecules; a portion of the dye released was dispersed in the agar. Results showed that Zn, Cu, Ni and Cd inhibited, within 7 days, cellulolytic activity of *T. viride*, which is a common soil-borne fungus (Table 1). Results on decomposition rates measured after 30 days showed inhibitory effects of the three heavy metals studied, Zn, Cu and Ni, on both *T. viride* and *Chaetomium* sp. This experiment showed the feasibility of the use of the dye release technique in metal-contaminated media.

Studies in soils without growing plants

Preliminary observations showed that the decomposition of cellulose measured after 30 days and at soil moisture level of 50% water-holding capacity (Fig. 1) may be taken as the appropriate level of decomposition at which the cellophane film can be sampled from the soil fairly conveniently for the dye release assay. It was also observed that the decomposition of the cellophane film in the soil was biological in nature, there being no decomposition in sterilised soils. When the soil was waterlogged up to 60 days, the film was not decomposed, indicating that the decomposition of cellulose is initially an aerobic process involving mostly fungi (Tribe, 1961) and arthropods.

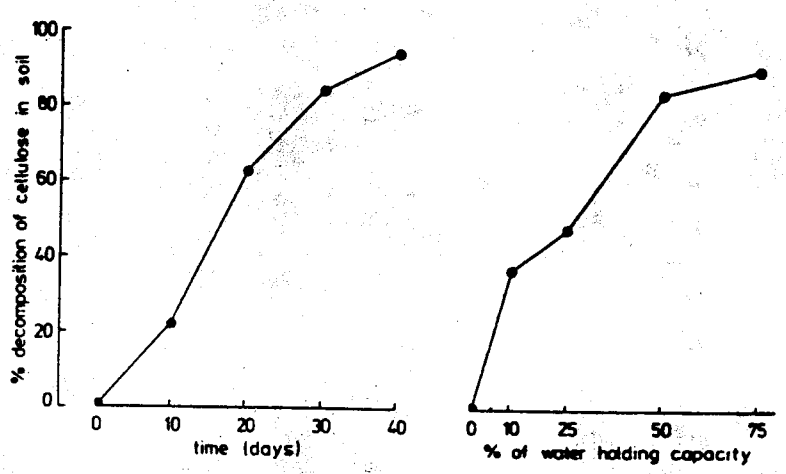

Fig. 1. Effects of time (at 50% of water-holding capacity) and of soil moisture levels (at 30 days time interval) on the decomposition of cellulose in soil.

TABLE 2
Decomposition of Cellulose in Uncropped Soils

Soils and metal treatment ($\mu g\ g^{-1}$)		*Per cent cellulose decomposition*	*(Per cent inhibition in relation to control)*
Weald Country Park			
Control		43·6	
Cd10	as $CdCl_2$	37·7	(13·5)
Cd50	as $CdCl_2$	36·0*	(17·4)
Cd100	as $CdCl_2$	28·4**	(34·9)
Pb100	as $PbCl_2$	40·0	(8·3)
Pb500	as $PbCl_2$	37·1	(14·9)
Pb1000	as $PbCl_2$	33·8*	(22·5)
Cd10 + Pb1000	as $CdCl_2 + PbCl_2$	31·7**	(27·3)
Cd50 + Pb1000	as $CdCl_2 + PbCl_2$	29·1**	(33·3)
Shipham, Rowberrow Lane, Somerset		36·1*	(7·2)
Shipham, opposite cemetery, Somerset		30·3**	(30·5)
Grogwynion mining area, Dyfed		15·3**	(64·9)

* Significant effect over control 5% level.
** Significant effect over control 1% level.

Studies of the metal-contaminated soils showed that Pb and Cd inhibited the activity of cellulose decomposers, Cd being more toxic than Pb (Table 2). There was no statistical evidence for any specific interaction between Cd and Pb other than an additive one. In soils from the Grogwynion mining area and those from a very contaminated field opposite Shipham cemetery the cellulolytic activity was considerably inhibited in relation to the control soil but the effects were produced apparently due to several heavy metals that were present in the soils, such as Cd, Zn and Pb in Shipham soil, and Pb and Zn in the alluvial soil from the Grogwynion area. The low decomposition rate, as measured in the latter soil, would be partly due to the small amount of plant residues present in the soil which is constituted of coarse stony material. The Shipham soil near Rowberrow Lane, sampled from a normal meadow land in the area and found to be less contaminated, showed little inhibition of the cellulolytic activity. Interestingly, this soil has much higher levels of Cd and Zn than in the alluvial soil from the Grogwynion area (Khan & Frankland, 1983).

TABLE 3
Decomposition of Cellulose and Root Biomass Production in Soils

Metal treatment ($\mu g\ g^{-1}$)	*Metals added*	*Per cent cellulose decomposition*	(*Per cent inhibition in relation to control*)	*Root biomass* (*mg per plant*)	(*Per cent inhibition in relation to control*)
Oat experiment					
Control	—	72·9		408	
Cd10	$CdCl_2$	47·9*	(34·3)	308**	(24·5)
Cd20	$CdCl_2$	47·3*	(35·1)	185**	(30·2)
Cd50	$CdCl_2$	52·1*	(28·5)	145**	(64·5)
Cd100	$CdCl_2$	45·2**	(38·0)	95**	(76·7)
Pb100	$PbCl_2$	76·7	(0·0)	343	(15·9)
Pb500	$PbCl_2$	65·2	(10·6)	258**	(36·8)
Pb1000	$PbCl_2$	51·9*	(28·6)	233**	(42·9)
Wheat experiment					
Control	—	49·8		297	
Cd50	$CdCl_2$	28·4**	(43·0)	115**	(61·3)
Cd100	$CdCl_2$	23·9**	(52·0)	96**	(67·7)
Cd100	$CdSO_4$	19·6**	(60·6)	96**	(67·7)
Cd100	$CdCO_3$	42·8	(14·1)	256*	(13·8)
Cd100	CdO	42·5	(14·7)	156**	(47·5)
Pb500	$PbCl_2$	43·2	(13·3)	253**	(14·8)
Pb1000	$PbCl_2$	38·9*	(21·9)	197**	(33·7)
Pb1000	$PbSO_4$	44·2	(11·2)	275	(7·4)
Pb1000	$PbCO_3$	48·4	(2·8)	259*	(12·8)
Pb1000	PbO	50·5	(0·0)	277	(6·7)
Radish experiment					
Control	—	41·4		1865	
Cd50	$CdCl_2 + CdO(1:1)$	30·5	(26·3)	1270**	(31·9)
Cd100	$CdCl_2 + CdO(1:1)$	25·3*	(38·9)	1070**	(42·6)
Pb500	$PbCl_2 + PbO(1:1)$	39·6	(4·4)	1780	(4·8)
Pb1000	$PbCl_2 + PbO(1:1)$	37·2	(10·1)	1495**	(19·8)

* Significant effect over control 5% level.
** Significant effect over control 1% level.

Studies in soils with growing plants

The results on cellulose-decomposing activity in root-zone soils involving the growth of three crop plants showed that Cd and Pb inhibited both the root biomass production and the cellulolytic activity (Table 3). This was observed irrespective of the differences in crop plants and soils used, and the differences in the experimental set up involved.

In the oat experiment the effects of Cd were significant but with little changes in effect over the concentration range 10 to 100 $\mu g\,g^{-1}$. Pb reduced cellulose decomposition relative to control only at the 1000 $\mu g\,g^{-1}$ level, although the effect on root biomass was observed at both concentrations of Pb applied. The studies with wheat involved different chemical forms of heavy metals and showed that Cd added in insoluble forms, e.g. carbonate and oxide, did not affect the cellulolytic activity significantly, but the root biomass was reduced by Cd, irrespective of the chemical forms used. The Pb significantly inhibited the cellulolytic activity when the metal was added in soluble chloride form at the 1000 $\mu g\,g^{-1}$ level. The root biomass was, however, reduced by both chloride and carbonate forms of Pb added to the soil. The soil column experiment with radish showed that Cd inhibited the cellulolytic activity only at the 100 $\mu g\,g^{-1}$ level in which 50% of the metal added was in the soluble form, the Pb showing no effect on the decomposition process (Table 3). The root biomass was progressively reduced by Cd, while Pb inhibited the root biomass production only at the 1000 $\mu g\,g^{-1}$ level.

TABLE 4
Correlations of Soil Levels of Cd and Pb with Cellulolytic Activity and Root Biomass

	Correlation coefficients	
	Inhibition of root biomass	*Inhibition of cellulolytic activity*
H_2O soluble ($\mu g\,g^{-1}$)		
Cd	0·7210**	0·6563*
Pb	0·5202	0·6612*
EDTA soluble ($\mu g\,g^{-1}$)		
Cd	0·6640*	0·6667*
Pb	0·3047	0·3377

* Significant at 5% level.
** Significant at 1% level.

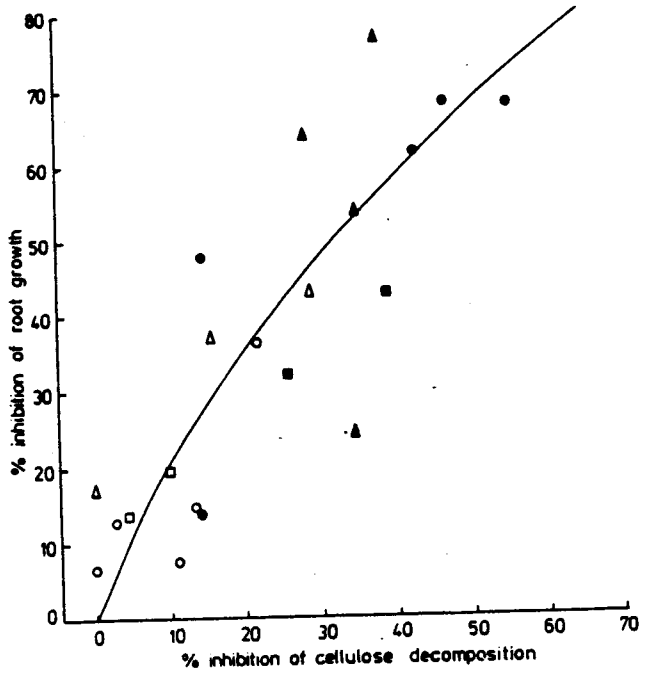

Fig. 2. Relationship between cellulolytic activity and root biomass production in contaminated soils cropped with oat (Cd ▲, Pb △), wheat (Cd ●, Pb ○) and radish (Cd ■, Pb □).

Figure 2 shows a positive relationship between the inhibition of cellulolytic activity and the inhibition of root biomass production. Figure 2 was drawn on the assumption of a linear relationship between the probits of the two parameters being compared.

Data are given in Table 4 on the correlation coefficients for the relationship of the decomposition of cellulose and root growth with the extractable metal contents of soils. The extractable metals comprised water-soluble and EDTA-soluble fractions, the latter calculated by deducting the water-soluble amount from that extracted by EDTA.

Analysis of variance was used in assessing the statistical significance of the effects of metal treatments relative to the control, the data being reported in Tables 1 to 3. Treatment means were compared with the control mean by partitioning the sum of squares followed by the use of the standard variance ratio test.

DISCUSSION

It was observed that Cd and Pb inhibited celiulolytic activity in the soil. The degree of inhibition varied considerably within an individual crop experiment, depending on a number of factors including the type and concentrations of the contaminant metals, their chemical forms at the point of entry and subsequent changes that may occur within the rooting zone soils. Compared on the basis of molarity, Cd appears to be much more toxic than Pb with respect to both the root biomass production and the activity of cellulose decomposers. Past evidence for the inhibition of cellulolytic activity in metal-contaminated environment is meagre. More recently, Martin *et al.* (1982) suggested reduced decomposition of litter in forest soils. Tyler (1975) found that Cu and Zn reduced the decomposition of cellulose in a forest soil near a brass mill in Sweden, the process being measured by CO_2 evolution. Using a different method based on the decomposition of cotton cellulose powder buried in woodland soils exposed to atmospheric pollution (acid rain, gases, particulates) from a coking plant in North England, Killham & Wainwright (1981) observed that cellulose decomposition was marginally inhibited in the polluted soil.

Results from crop experiments suggest that the contaminant metals used in this study inhibited root biomass production more than cellulose decomposing activity, treatments causing 50% inhibition of the former, inhibiting the latter by only 32% (Fig. 2). Inhibition of root biomass production will obviously reduce the soil reserve of organic matter, which is essentially formed by the accumulation of plant residues, especially cellulose. However, results showing a significant correlation between the inhibition of root biomass production and the inhibition of cellulose decomposers' activity do not imply a causal relationship. The contaminant metals may have produced two effects, the reduction in the growth of roots and the inhibition of the activity of the cellulose decomposers, the two effects probably being independent of each other.

The implications of the changes in the chemical forms of Cd and Pb in soil, as reflected by the measured amounts of water- and EDTA-soluble material, have been discussed in an earlier paper (Khan & Frankland, 1983). Since the effect of added metal reflects the effect of that proportion of the metal which is available to plant or microbial tissue, correlation coefficients for the relationship of rhizosphere activity with extractable metals in the soil were examined (Table 4). Cd in soils, both water-soluble

and EDTA-soluble, showed significant correlations with the inhibition of rhizosphere activity (root biomass and cellulolytic activity). The EDTA-soluble Pb did not show any correlation, whereas the water-soluble Pb was significantly correlated only with the inhibition of cellulolytic activity in rooting zone soils. The water extraction of soils releases metals held in ionic and molecular forms which are readily available to plant roots, micro-organisms and soil animals in the rhizosphere. The EDTA-soluble metal would comprise not only the chelated fraction but also metal ions in an adsorbed state and those present as insoluble inorganic compounds (Khan & Frankland, 1982). It has yet to be determined which fraction of the EDTA-soluble Cd is having most effect in reducing root biomass production and cellulolytic activity in the rhizosphere soil.

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