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Threshold levels of cadmium for soil respiration and growth of spring wheat (*Triticum aestivum* L.), and difficulties with their determination

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Summary. Inconsistent results were obtained from comparative studies on the impact of increasing Cd contamination in three soils on growth of spring-wheat plantlets and soil respiration. With identical soil Cd loads, plant growth was increasingly inhibited in the following sequence: Neutral sandy hortisol (pH 7.0) < phaeosem (pH 6.9) < acidic cambisol (pH 5.6), suggesting a strong dependence on pH. In contrast, oxidation of a glucose-glutamate mixture by these soils was increasingly inhibited in the sequence: Acidic cambisol < neutral sandy hortisol < phaeosem. Inhibition of plant growth was correlated with the extractability of Cd from soils by 0.1 *M* $CaCl_2$. However, comparison of dose-response curves with dose-extractability and dose-uptake curves suggested the presence of a soil factor that modified plant uptake of available Cd. This factor, possibly the concentration of antagonistic cations, was apparently also active within the plants. The inconsistency in the responses of plant growth and of soil respiration with respect to the soil Cd load was ascribed to microbiological soil properties exceeding the importance of Cd availability. Relatively high in vitro sensitivity of prokaryotes and their biochemical interdependence together with relatively high diversities of streptomycetes and fungi were paralleled by a relatively strong inhibition of soil respiration.

Key words: Cadmium – Threshold levels – Soil respiration – Microbial diversity – *Triticum aestivum* L. – Dose-response curve

Heavy metals, in contrast to potential organic toxicants, must be regarded as permanent stressors, since they are not subject to degradation and disappear very slowly if at all, from a contaminated habitat. Therefore, if heavy-metal contamination exceeds a given biological availability, the indigenous community of organisms may be permanently affected. In particular, this principle applies to the subcommunity of soil microorganisms.

Our knowledge of the impact of heavy metals on soil microorganisms has grown considerably in the last decade. There have been reports of decreases in microbial numbers or biomass (Bisessar 1982; Brookes and McGrath 1984; Stadelmann et al. 1984), in species diversity (Nordgren et al. 1983; Stadelmann et al. 1984; Barkay et al. 1985), and in soil enzyme or respiration activities (Chang and Broadbent 1981; Muskett and Jones 1981; Tyler 1981; Debosz et al. 1985; Doelman and Haanstra 1986; Skujins et al. 1986). As a consequence of these effects, reduction of soil degradative capabilities and accumulation of soil organic matter have been observed (Coughtrey et al. 1979; Tyler 1981; Haanstra and Doelman 1984).

Little information seems to be available, however, on the toxic threshold levels of heavy metals for microorganisms or microbial processes in soil (Stadelmann et al. 1982). Since Cd is the heavy metal causing most public concern, studies were conducted to see whether threshold levels could be determined. Spring wheat was included in these studies not only as a macroscopic measure but also because the microorganisms in arable soils largely depend on energy supplied by the crops.

Materials and methods

Experimental soils and contamination with Cd. Properties of soil samples collected from the Ap horizons of three soils near Braunschweig are shown in Table 1. Solutions of Cd acetate were added to sieved soil (2 mm) in doubling concentrations to give contamination levels of between 0.0156 and 2.0 mmol kg^{-1} dry soil (1.76 and 228.8 mg Cd kg^{-1}). Acetate is an energy source for

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Table 1. Soil properties

	Phaeosem	Neutral sandy hortisol	Acidic cambisol
Organic matter (%)	2.19	2.62	1.67
pH	6.9	7.0	5.6
Water-holding capacity (%)	55.5	67.8	44.5
Sand (%) 2000-63 µm	3.85	88.38	38.70
Silt (%) 63–2 µm	74.90	8.46	54.28
Loam (%) <2 µm	21.25	3.16	7.02

microorganisms and has eight available electrons per molecule (Payne 1970). Therefore, all soil samples treated with less than 2 mmol Cd acetate kg^{-1} (4 mmol acetate) were supplied with glucose (24 available electrons per molecule) to reach the energy status of the most contaminated soil sample. Using the acetate salt of Cd had the advantage that after 1 week's incubation the acetate moiety was oxidized, thus excluding anions that might have increased the biological availability of the heavy metal. After this preincubation, the soils were allowed to dry at room temperature to force the added Cd to react with soil constituents and to prevent the microbial biomass in the contaminated soils from eventually decreasing more rapidly than in the control. This was assumed to be necessary as the soil respiration experiments were expected to take a minimum of 12 weeks. Any decrease in microbial biomass due to drying was considered of minor importance, since this was likely to occur in all samples. Dry soil samples were stored at 2 °C until used.

Respiration measurements. A CO_2 ananlyser (type U3K-CO_2) of Wösthoff (Bochum, FRG) was used, equipped with a suction pump to allow continuous aeration of six soil samples at 80 ml min^{-1}. The analyser was able to record a maximum CO_2 concentration in air of 0.5% (v/v), corresponding to a CO_2 production rate of 1.07 mmol h^{-1}. A glucose-glutamic acid solution (neutralized with NH_4OH to give a C:N ratio of 12) was added to 80–100 g air-dried soil in a sufficient concentration and quantity so that (1) the respiration peak of the control samples almost reached the technical maximum and (2) all soil samples had a water potential of 40 kPa (pF 2.6). With this substrate mixture, soil respiration increased faster than with glucose alone, and a diauxic pattern was not observed. In contrast to results with amino acids (Alef and Kleiner 1987; Haanstra and Doelman 1984), this mixture was also expected to leave soil pH, and hence Cd availability, unaltered. The soil samples were incubated in 500-ml Erlenmeyer flasks at 22 °C, the air in the head-space being continuously replaced by humidified CO_2-free air. At the maximum increase in the rate of soil respiration in three control samples (*arrow* on Fig. 1), the relative activity in the remaining three contaminated samples was determined. Figure 1 shows that at the time indicated by the arrow, respiratory activity in the contaminated soil relative to the control soil was at a minimum, indicating optimum sensitivity of the method. When dose-response curves for substrate-induced respiration were derived, each point was the result of four such experiments, hence corresponding to 24 individual soil samples.

Growth experiments with spring wheat (Triticum aestivum L.). The cultivar Kokart was used throughout the experiments. Six pregerminated seeds were planted in shallow pots containing 300 g dry soil humidified to a water potential of 40 kPa. The pots were placed in a greenhouse at the beginning of May (four pots for each contamination level, eight pots for the control). Dry weights of shoots and roots were determined after 4 weeks of growth.

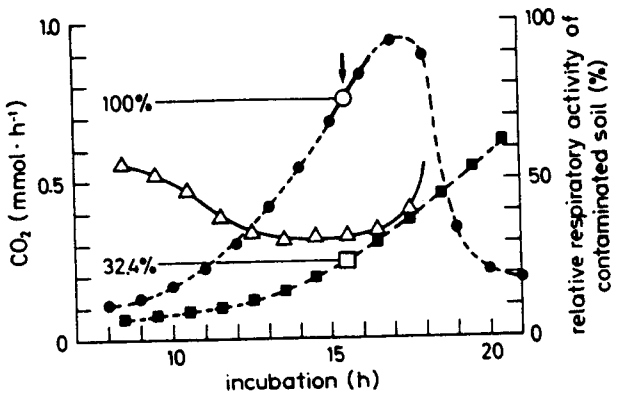

Fig. 1. Method applied to estimate the impact of Cd on substrate-induced soil respiration. ●—●, control soils; ■—■, contaminated soils; △—△, respiratory activity of contaminated soils, relative to control soils at various incubation times. All experiments performed in triplicate. *Arrow* shows maximum increase in rate of soil respiration in three control samples

Quantification of Cd in plants and soils. Wheat shoots were extracted according to Styperek (1986) with a mixture of nitric (65%), perchloric (70%), and sulfuric (96%) acids at a ratio of 8:2:1. To quantify the extractable Cd in soil, 10 g dry soil was shaken with 25 ml 0.1 *M* $CaCl_2$ solution for 2 h at room temperature (Styperek 1986). After centrifugation and appropriate dilution, the Cd concentration was determined in the supernatant by atomic absorption spectrometry (graphite cuvette).

Microbiological analyses. To determine the precentage of Cd-resistant prokaryotes, suspensions of the three soils were plated on agar media (pH 7.0), prepared with and without 35 µM $CdCl_2$ and containing 1 g yeast extract and 0.25 g glucose per liter in addition to a low-phoshate (0.25 g l^{-1}) mineral-salts base (Pochon and Tardieux 1962). The percentage of auxotrophs was determined according to the equation: auxotrophs (%) = $(a-b)/a$, where a is the number of colonies on the complete medium without added Cd and b the number of colonies on a medium containing (per liter) glutamate (0.5 g), aspartate, alanine, and glucose (each 0.25 g).

To determine the streptomycete diversity in the soils, soil suspensions were plated on 5 m*M* 4-hydroxybenzoate agar (pH 7.2). Purified isolates were clustered, with the help of differentiating media, according to four criteria: Melanin formation, colours of aerial and substrate mycelium, and coloration of medium (Reber 1967).

To determine the fungal diversity, microfungi were isolated from each soil by a soil-wash method (Gams and Domsch 1967). Washed soil particles supposed to be free of spores were placed singly into Petri dishes containing 4-hydroxybenzoate agar (5 m*M*, pH 6.0, 300 µg streptomycin ml^{-1}) and incubated in daylight to enhance sporulation. Pure isolates were obtained by streaking spore suspensions on 0.5% malt extract agar (pH 6.0). These were then transferred to agar slants of the same medium. With this method, between one and four different fungi were isolated from each soil particle. The isolates were clustered according to differences in colour and morphology.

The rarefaction method was employed to obtain abundance curves (Odum 1980).

Results

Uptake of Cd by spring wheat and Cd extractability from soil by $CaCl_2$

To obtain an accurate estimate of the soil effects modifying both the biological availability and the toxicity of Cd, we first determined its uptake by spring wheat and its $CaCl_2$ extractability from soil. Between 5 and 100 times more Cd was taken up by the plants than could be extracted chemically (Fig. 2). Further, Cd uptake decreased with increasing additions of Cd to the soil, while $CaCl_2$-extractable Cd showed a generally linear response to the amounts added. At low levels of soil contamination, $CaCl_2$-extractable Cd ranged from 1% (phaeosem) to about 20% (acidic cambisol) of added Cd.

Substrate-induced soil respiration and growth of spring wheat as affected by various Cd levels in soils

Contamination of the three soils with identical quantities of Cd inhibited the substrate-induced soil respiration at different rates. At Cd levels $>50\ \mu g\ g^{-1}$, the greatest inhibition was observed in the phaeosem and the lowest in the acidic cambisol, with an intermediate level in the neutral sandy hortisol (Fig. 3). At lower Cd levels, differences between the phaeosem and the neutral sandy hortisol were negligible.

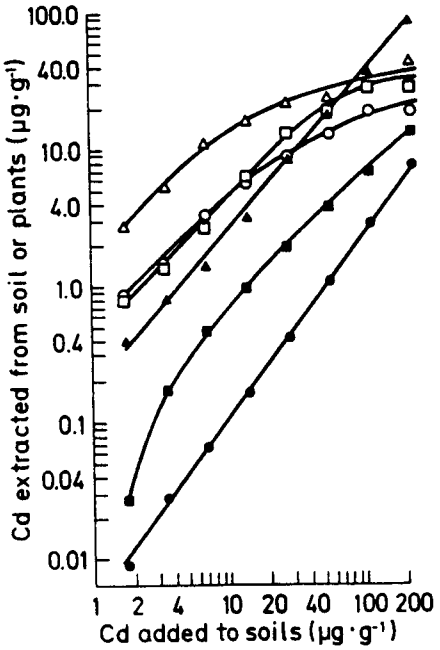

Fig. 2. Relationship between Cd added to soils and Cd either extracted from soils by 0.1 *M* $CaCl_2$ (*closed symbols*) or taken up by shoots of spring-wheat plantlets (*open symbols*). ○—○ and ●—●, Phaeosem; □—□ and ■—■, neutral sandy hortisol; △—△ and ▲—▲, acidic cambisol

In contrast to soil respiration, shoot growth of spring wheat was most affected in the acidic cambisol (Fig. 4). Differences in wheat growth seen between the neutral sandy hortisol and the phaeosem were not con-

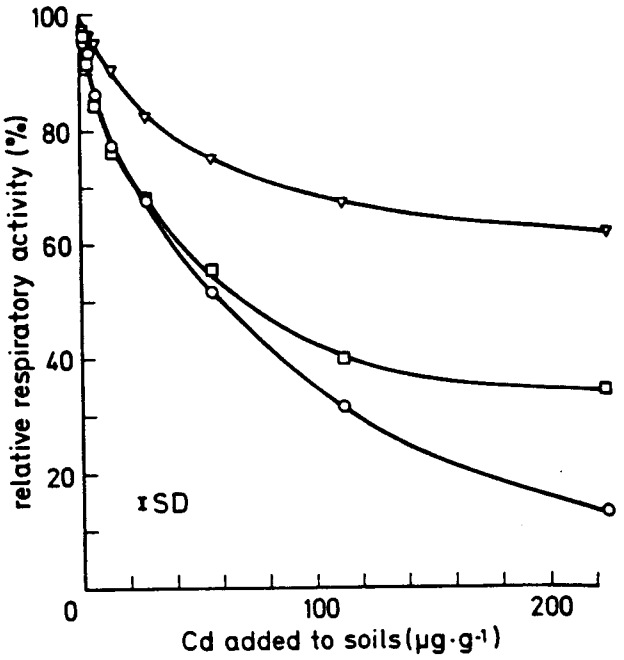

Fig. 3. Relative substrate-induced respiration of a phaeosem (○—○), a neutral sandy hortisol (□—□), and an acidic cambisol (▽—▽), as influenced by added Cd

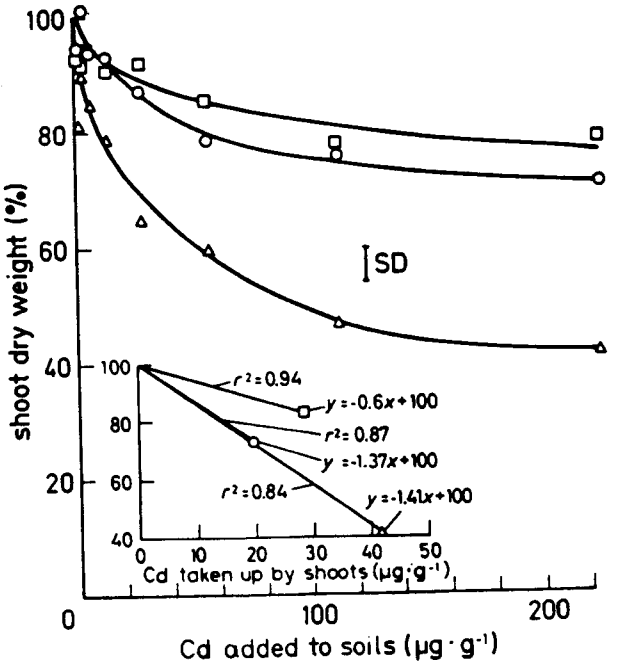

Fig. 4. Relative shoot growth of spring-wheat plantlets in a phaeosem (○—○), a neutral sandy hortisol (□—□), and an acidic cambisol (△—△), as influenced by Cd added to the soils. Inset: Shoot growth as influenced by Cd taken up by plantlets

Table 2. Various threshold levels of Cd, causing decreases in substrate-induced soil respiration in a phaeosem (A), a neutral sandy hortisol (B), and an acidic cambisol (C), as well as in shoot dry weight of spring-wheat plantlets grown in these soils at the 95% confidence level[a]

Cd ($\mu g\ g^{-1}$)	Soil respiration			Shoot dry weight		
	A	B	C	A	B	C
Added to soil	2.7	2.6	7.8	7.0	14.0	1.8
Extracted from soil by 0.1 M $CaCl_2$	0.03	0.15	3.0	0.1	1.5	0.5
Taken up by plants	1.7	1.4	14.0	4.0	8.0	3.8

[a] Values for added Cd pertaining to soil respiration were determined graphically at the intersection of three semilogarithmic dose-response curves with a horizontal line at 100−6 = 94% (4 SD = 6%); those pertaining to wheat growth were the lowest levels of added Cd that reduced growth, as identified by the t-test. Values for $CaCl_2$-extractable and plant-assimilated Cd were read from Fig. 2, and multiplied by 1.56 or 1.23, respectively, 56% and 23% being the mean errors in the quantification of extractable and assimilated Cd

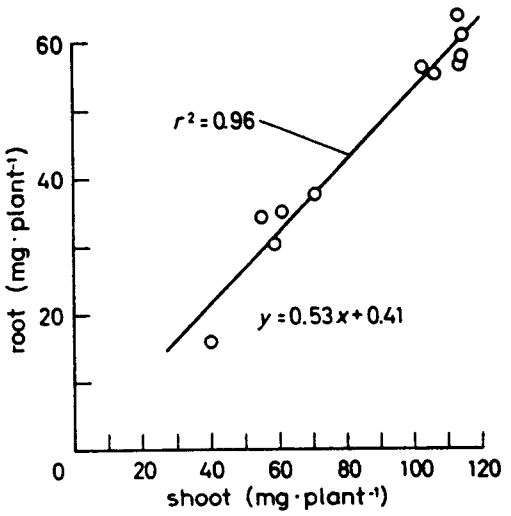

Fig. 5. Relationship between shoot and root dry weight of spring-wheat plantlets growing in an acidic cambisol contaminated by Cd at various levels

firmed at the 95% confidence level. The inset on Fig. 4 shows the shoot growth of spring wheat as influenced by Cd uptake. It is clear that plantlets grown in the phaeosem and the acidic cambisol exhibited an almost identical reaction towards the amount of Cd taken up, whereas the plants grown in the neutral sandy hortisol appeared to be much less sensitive.

The shape of the dose-response curves in Figs. 3 and 4 suggests that there were no threshold levels of Cd for soil respiration nor for the growth of wheat plantlets. Therefore, we determined the statistically significant Cd levels that marked the beginning of damage to both parameters (Table 2). The data show that in the phaeosem and the neutral sandy hortisol, soil respiration was more sensitive to Cd than the growth of spring wheat plantlets, whereas the reverse was true for the acidic cambisol.

Microorganisms in arable soils are strongly dependent on the root growth of the cultivated plants, especially cereals, since the upper parts of the crops are often removed from the field. We therefore estimated the damage that might occur to root growth following contamination of soils with Cd. For this purpose, the root growth of spring wheat was compared with shoot growth in an experiment with increasing levels of Cd in the acidic cambisol (Fig. 5). Both parts of the plant were equally damaged by Cd, indicating that shoot-growth data can predict root growth under Cd stress.

Differences in microbial communities

The relatively low inhibition of respiration at relatively high levels of extractable Cd in the acidic cambisol together with the reverse findings in the phaeosem suggested that the effects were not only due to the biological availability of Cd but also to differences in microbial communities. To prove this hypothesis, we measured four apparently influential parameters, the relative numbers of Cd-sensitive and auxotrophic prokaryotes and the strain abundances in the populations of fungi and streptomycetes. According to Alexander (1977), auxotrophy is an indication of biochemical interdependence within microbial communities. Table 3 shows that the percentages of Cd-sensitive and auxotrophic prokaryotes in the three soils increased in the sequence: Acidic cambisol < neutral sandy hortisol < phaeosem. The same sequence was obtained with respect to the abundance of strain clusters in the populations of streptomycetes (Fig. 6) and fungi (data not shown), but differences between the fungal popula-

Table 3. Cd resistance and biochemical interdependence within prokaryote communities of a phaeosem (A), a neutral sandy hortisol (B), and an acidic cambisol (C)

	Plate counts (%)		
	A	B	C
35 μM Cd in medium			
I	23.3 ± 4.6	36.0 ± 8.5	46.5 ± 5.9*
II	19.0 ± 5.6	37.0 ± 9.2 +	42.8 ± 9.7
Auxotrophs			
I	44.5 ± 12.9	27.9 ± 8.9	24.2 ± 4.6 +
II	61.6 ± 6.8	45.2 ± 7.2 +	41.3 ± 12.2

Experiments were carried out twice (I, II), see Materials and methods

*$P < 0.01$, +$P < 0.05$, significantly different from corresponding value for soil A

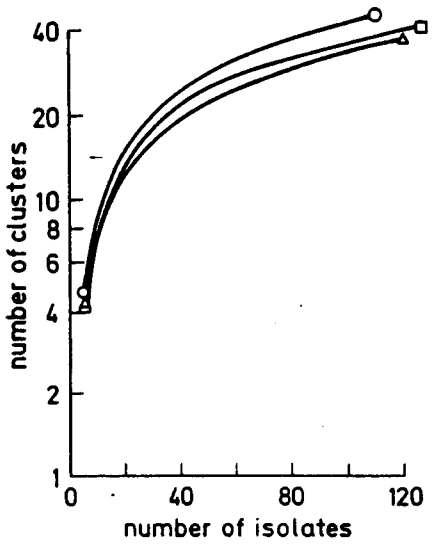

Fig. 6. Abundance of strain clusters in streptomycete populations isolated from a phaeosem (○), a neutral sandy hortisol (□), and an acidic cambisol (△)

tions were insignificant. The results of these microfloristic analyses strongly suggest that inhibition of soil respiration by Cd is correlated not only with the sensitivity of soil microorganisms towards Cd but also with their diversity and biochemical interdependence.

Discussion

The present investigation revealed inconsistencies in the reactions of soil microorganisms and spring wheat following contamination of three soils with Cd. At identical soil Cd levels, soil respiration was increasingly inhibited in the sequence: Acidic cambisol < neutral sandy hortisol < phaeosem (Fig. 3); the sequence neutral sandy hortisol < phaeosem < acidic cambisol was obtained for wheat growth (Fig. 4). Differences between the phaeosem and the neutral sandy hortisol were insignificant with respect to wheat growth, but significant with respect to soil respiration at Cd levels $> 50\ \mu g\ g^{-1}$. In the acidic cambisol, however, the impact of Cd was most severe on plant growth and least severe on soil respiration. We therefore sought to determine which factors influenced the toxicity of Cd in soil and how the inconsistency between soil respiration and growth of spring wheat could be explained.

The curves of Figs. 2 and 4, and above all their relative position to each other, indicate that the toxicity of Cd towards plants is much more closely associated with the plant Cd uptake than with the extractability of Cd from soil. This assumption is supported by the linear regressions of shoot growth on Cd uptake (inset Fig. 4) and the lack of a similar linear relationship between shoot growth and extractable Cd (data not shown). The discrepancy between extractability and plant uptake of Cd is possibly due to some soi factor (e.g. the concentration of antagonistic cation: altering the Cd uptake by plants). It is likely that thi: factor was also capable of modifying to toxicity of Cc after uptake by the plants. Otherwise, the regression: of shoot growth on Cd uptake would have been iden tical for each soil (inset, Fig. 4).

Because of the inconsistencies between plan growth and soil respiration, Cd availability and th unidentified soil factor apparently had less effect or the three microbial communities. A likely explanatior was that the microorganisms in the three soils differec with respect to their resistance to Cd. In fact, a low ir vitro resistance of prokaryotes to Cd, as in the phae osem, corresponded with a strong inhibition of soi respiration by Cd and vice versa. However, inhibitior of soil respiration was also paralleled by a high degree of biochemical interdependence within the prokaryot population and also by a relatively high diversity in th streptomycete population. Although we do not yet fully understand the interdependence of the soil microbiological characteristics, the present experimenta results allow us to conclude that diverse and biologically controlled microbial communities are especiall sensitive to soil contamination with Cd. Since so man different factors are able to modify the toxicity of Cc in soil, it appears almost impossible to predict the exact damage that might be inflicted on plant growth o soil respiration by a certain level of Cd in soil, whethe the Cd level is estimated as total or extractable Cd o as the amount taken up by a plant.

No threshold levels of Cd for soil respiration anc growth of spring-wheat plantlets could be determinec from dose-response curves in the present investigation This finding is in contrast to the results of othe workers. A dose-response curve presented by Stadelmann et al. (1982) suggested that soil respiration in a loamy sand (pH 5.6) was not affected at a load o $< 0.3\ \mu g$ $NaNO_3$-extractable Cd g^{-1} soil, whereas Beckett and Davis (1977), Klein et al. (1981), anc Sauerbeck (1982) all found that the yield of mature cereals was not decreased until concentrations > 5 mg Cd g^{-1} plant dry weight were reached. The discrepancy in these results may be due, on one hand, to differences in the sensitivity of soil respirometric methods and, on the other hand, to differences in the tolerance of young and mature cereals to Cd.

Formation of microbial biomass is directly proportional to the available energy (Payne 1970), anc therefore a decrease in plant growth, especially in roo growth, will undoubtedly result in a corresponding reduction of soil microbial biomass. Since both shoo and root growth of spring wheat were equally reduced under Cd stress (Fig. 5), the data shown in Fig. 4 car

be used to estimate the relative decrease in C availability to microorganisms for a given Cd load in the three soils. Assuming a total Cd load of 10 μg g^{-1}, a decrease in microbial biomass of almost 20% could be predicted for the acidic cambisol, but only about 8% for the two other soils, due to reduced primary production. In addition to this indirect effect, Cd toxicity is likely to have a direct effect on the microorganisms. As suggested by the respiration experiments (Fig. 1), the direct effects of Cd are mainly a reduction in the energy flux within a microbial population. Therefore, when there are only limited supplies of C in soils, an increase in the death rate of microorganisms and a decrease in microbial biomass are likely to occur. Studies by Brookes and McGrath (1984) revealed that the combined direct and indirect effects of heavy metals can reduce the soil microbial biomass by as much as 50%.

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