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Reductions in the Toxicity of Cadmium to Microorganisms by Clay Minerals

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The clay minerals montmorillonite and kaolinite protected bacteria, including actinomycetes, and filamentous fungi from the inhibitory effects of cadmium (Cd). Montmorillonite provided greater protection than did equivalent concentrations of kaolinite. The protective ability of the clays was correlated with their cation exchange capacity (CEC). The greater the CEC, the greater the absorbancy of exogenous Cd by the exchange complex and the greater the protection. The greater protection afforded by montmorillonite, as compared to kaolinite, was correlated with its higher CEC. Clays homoionic to Cd did not protect against exogenous Cd, as the exchange complex was already saturated with Cd. Montmorillonite homoionic to Cd was more detrimental to microbial growth than was kaolinite homoionic to Cd, as more Cd was present on and apparently desorbed from the montmorillonite.

Much of the current research on environmental pollution has focused either on the adverse influence of toxicants on the biota or on the physicochemical interactions between pollutants and the environment, and little investigation has been directed toward the triple interaction between pollutants, the environment, and the biota. The physicochemical characteristics of the environment may influence the toxicity of a pollutant by either lessening or magnifying its effect (3, 4). For example, the toxicity of cadmium (Cd) towards bacteria, including actinomycetes, and fungi was potentiated in broth initially adjusted to pH levels of 8 or 9 (5). Conversely, the toxicity of Cd towards bacteria (1, 17) and fungi (12) could be reduced by incorporating zinc or magnesium into the growth media.

Studies with plants have shown that the availability and phytotoxicity of Cd was dependent on several soil factors: pH (2, 11, 16), temperature (6), inorganic mineral composition (6, 13, 14, 18, 28), and cation exchange capacity (CEC) (6, 15). The CEC is a function of the amount and types of organic matter and clay minerals in the soil. The uptake of Cd by wheat was highest in plants grown in soils with a low CEC (reflecting both low organic matter and clay mineral contents) and lowest in plants grown in soils of a higher CEC (reflecting both higher organic matter and clay contents) (19). Apparently, in the soil with a high CEC, more Cd was adsorbed to the exchange complexes, and, hence, less Cd was available for uptake by the wheat plants.

Most microbial activity in soil probably occurs in thin films of water associated with the surfaces of the clay particles or in "necks" between clay particles. The clay fraction in the soil occurs primarily as aggregates or as surface coatings on larger particles (23). The types of clay minerals present in the soil appear to exert an influence on the activity and ecology of microbes in soil microenvironments. Pure culture studies have shown that montmorillonite, but not kaolinite, stimulated bacterial respiration, reduced the lag phase of bacterial growth, protected bacteria against hypertonic osmotic pressures, and served as a buffer by exchanging protons produced during metabolism with basic cations from the clay exchange complex (21, 22, 26). Conversely, fungal respiration in broth media (27) and mycelial growth on agar media (23) were inhibited in the presence of montmorillonite.

The growth rates of bacteria were stimulated and those of fungi were inhibited in soils that either had been amended with montmorillonite or naturally contained a montmorillonite-like clay (23). The spread of the plant pathogens *Fusarium oxysporum* f. *conglutinans* (cabbage wilt) and *Fusarium oxysporum* f. *cubense* (banana wilt) and the establishment of the human pathogen *Histoplasma capsulatum* were restricted in soils that naturally contained a montmorillonite-like clay (23-25). Similarly, the clay composition of soil has been implicated in the geographic distribution of the pathogenic fungi *Cryptococcus neoformans* and *Blastomyces dermatitidis* and the bacteria causing enzootic leptospirosis (23).

The purpose of this study was to determine

the ability of kaolinite and montmorillonite, the hydrous alumino-silicate clay minerals, to affect the toxicity of Cd towards microorganisms.

MATERIALS AND METHODS

Source and maintenance of microorganisms. Microorganisms were obtained from the culture collection of the Laboratory of Microbial Ecology at New York University, the American Type Culture Collection, and the Midwest Culture Service. Bacteria, including actinomycetes, were grown and maintained on nutrient agar (Difco) amended with 1% glucose (pH 6.8); fungi were grown and maintained on an agar medium that consisted of 20.0 g of glucose, 15.0 g of agar, 10.0 g of peptone, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of KH_2PO_4, 0.5 g of NaCl, and 1,000 ml of deionized, distilled (4×) water (pH 5.9). The cultures were stored in a refrigerator at 4°C.

Description of the clay minerals. The montmorillonite (bentonite, Fisher) had a CEC of 97.7 meq/100 g of oven-dried clay and contained approximately the following concentrations (in meq/100 g) of exchangeable cations: H^+, 8.5; K^+, 1.5; Na^+, 49.6; Ca^{2+}, 41.9; and Mg^{2+}, 10.3. The kaolinite (kaolin, Fisher) had a CEC of 5.8 meq/100 g of oven-dried clay and contained approximately the following concentrations (in meq/100 g) of exchangeable cations: H^+, 3.7; K^+, 0.1; Na^+, 0.3; Ca^{2+}, 0.6; and Mg^{2+}, 0.5 (21).

When kaolinite and montmorillonite were made homoionic to Cd, the clay was peptized twice with 0.4 N Na_2CO_3 and centrifuged twice at 10,000 × *g* for 10 min; the pellet was resuspended three times in 0.5 N $CdCl_2$ and then centrifuged three times at 40,000 × *g* for 10 min; the pellet was then washed with deionized, distilled (4×) water by centrifugation at 40,000 × *g* until the supernatant was free of chloride ions and its conductivity remained constant (7, 8).

Description of experiments. (i) Effect of clay minerals on toxicity of Cd towards fungi. Fungi were grown in petri plates containing approximately 20 ml of the fungal maintenance agar medium. After a few days of incubation at 25°C, circular fungal plugs (9 mm in diameter) were made with a sterilized metal cork borer. In initial experiments, the fungal plugs were transferred to the center of petri plates containing the agar medium amended with 0, 10, 100, or 1,000 μg of Cd per ml (as $CdCl_2$) and either 2% (wt/vol) kaolinite, 2% (wt/vol) montmorillonite, or no clay. The plates were incubated at 25°C, and after the appropriate time intervals the diameters of mycelial growth were measured in four directions. Four replicate plates were employed for each concentration of Cd and clay, and experiments were performed at least twice.

In subsequent experiments, 0, 1, 3, 5, 10, 15, or 20% kaolinite, 0, 1, 3, or 5% montmorillonite (higher concentrations were too viscous and could not be prepared), and 0, 10, 100, or 1,000 μg of Cd per ml were incorporated into the medium. Four to eight replicate plates were employed for each concentration of Cd and clay, and most experiments were repeated twice.

(ii) Effect of clay minerals on toxicity of Cd towards bacteria. Bacteria, including actinomycetes, were inoculated into Erlenmeyer flasks (125 ml) containing 50 ml of nutrient broth (Difco) amended with 1% glucose and grown overnight (approximately 18 h) on a shaker at 25°C. A 1.0-ml portion of each culture was spread over the surface of petri plates containing approximately 20 ml of nutrient agar that had been amended with 1% glucose. After incubation for 2 days at 25°C, circular plugs (4 mm in diameter) made with a sterilized metal cork borer were inverted and placed on the center of petri plates containing nutrient agar, 1% glucose, 0, 1, 10, 100, or 1,000 μg of Cd per ml (as $CdCl_2$), and either 2% kaolinite, 2% montmorillonite, or no clay. The plates were incubated at 25°C for 10 days, after which time colony diameters were recorded. Six to nine replicate plates were employed for each concentration of Cd and clay, and experiments were performed twice.

(iii) Effect of clay minerals, homoionic to Cd, on toxicity of Cd towards fungi. The techniques employed were similar to those used in studies of the influence of clay minerals containing mixed cations on Cd toxicity, except that the clay minerals were homoionic to Cd and the fungal plugs were 4 mm in diameter. The plugs were placed on the agar medium amended with 0 or 10 μg of Cd per ml (as $CdCl_2$) and 0, 1, 3, or 5% clay, either homoionic to Cd or containing mixed cations. Six to eight replicate plates were employed for each concentration of Cd and clay.

RESULTS

Effects of kaolinite or montmorillonite on the toxicity of Cd towards bacteria. In the absence of Cd, montmorillonite and, to a lesser extent, kaolinite stimulated the growth of *Bacillus megaterium* and *Agrobacterium tumefaciens* but had no effect on the growth of *Nocardia corallina*. In addition, montmorillonite and, to a lesser extent, kaolinite decreased the inhibitory effects of Cd towards the bacteria (Table 1). To distinguish between the influence of the clays on Cd toxicity from the direct influence of the clays on bacterial growth, the "percentage of the control" values in Table 1 were calculated using growth on agar containing no Cd and either no clay, 2% kaolinite, or montmorillonite as the controls for the Cd treatments.

In the absence of clays, the growth of *B. megaterium* was reduced to 83% of the control in the presence of 1 μg of Cd per ml, to 17% of the control with 10 μg of Cd per ml, and to 0% (i.e., no growth) with 100 μg of Cd per ml. However, in the presence of 2% kaolinite, growth was 87, 51, and 2%, and with 2% montmorillonite, growth was 93, 63, and 31% of the controls at 1, 10, and 100 μg of Cd per ml, respectively. In the presence of 10 μg of Cd per ml and no clay, the growth of *A. tumefaciens* was reduced to 78% of the control, but in the presence of 2% kaolinite or montmorillonite, growth was 93% of the controls. In the absence

TABLE 1. *Influence of cadmium in the absence and presence of 2% kaolinite or 2% montmorillonite on growth of bacteria on agar*

Bacterium	Treatment[a]	% of control at cadmium concn (μg/ml) of:				
		0	1	10	100	1,000
B. megaterium	No clay	100 ± 2.6[b]	83 ± 1.0	17 ± 2.8	0	0
		(9.2 ± 0.24)[c]	(7.7 ± 0.09)	(1.6 ± 0.26)	(0)	(0)
	2% K	100 ± 1.5	87 ± 3.1	51 ± 2.4	2 ± 0.4	0
		(20.9 ± 0.30)	(18.2 ± 0.60)	(10.6 ± 0.51)	(0.5 ± 0.09)	(0)
	2% M	100 ± 1.8	93 ± 2.3	63 ± 1.4	31 ± 1.2	0
		(22.8 ± 0.40)	(21.1 ± 0.52)	(14.4± 0.34)	(7.0 ± 0.28)	(0)
A. tumefaciens	No clay	100 ± 0.9	102 ± 1.3	78 ± 1.8	33 ± 1.5	0
		(7.8 ± 0.07)	(8.0 ± 0.10)	(6.1 ± 0.14)	(2.6 ± 0.12)	(0)
	2% K	100 ± 1.2	103 ± 1.6	93 ± 2.7	66 ± 1.9	0
		(8.6 ± 0.11)	(8.8 ± 0.14)	(7.9 ± 0.24)	(5.6 ± 0.16)	(0)
	2% M	100 ± 2.2	100 ± 1.5	93 ± 1.1	92 ± 1.4	0
		(9.3 ± 0.20)	(9.3 ± 0.14)	(8.6 ± 0.10)	(8.6 ± 0.13)	(0)
N. corallina	No clay	100 ± 4.9	95 ± 5.2	43 ± 0.9	0	0
		(9.9 ± 0.49)	(9.5 ± 0.53)	(4.4 ± 0.27)	(0)	(0)
	2% K	100 ± 5.1	100 ± 4.8	51 ± 1.7	0	0
		(9.8 ± 0.50)	(9.8 ± 0.44)	(4.8 ± 0.15)	(0)	(0)
	2% M	100 ± 4.7	98 ± 4.5	54 ± 2.4	24 ± 1.7	0
		(9.9 ± 0.47)	(9.7 ± 0.44)	(5.3 ± 0.23)	(2.4 ± 1.7)	(0)

[a] Abbreviations: K, Kaolinite; M, montmorillonite.

[b] Mean percentage of the control ± standard error of the mean. Based on control plates which contained no Cd and no clay, no Cd and 2% kaolinite, or no Cd and 2% montmorillonite.

[c] Mean diameter of growth (in millimeters) ± standard error of the mean. Calculated after 10 days of incubation.

of clay, 100 μg of Cd per ml reduced growth to 33%, whereas with 2% kaolinite or montmorillonite, growth was 66 and 92%, respectively, of the controls. *N. corallina* grew in 100 μg of Cd per ml in the presence of 2% montmorillonite, but not with 2% kaolinite or in the absence of either clay. The concentrations of clays employed did not protect any of these organisms against 1,000 μg of Cd per ml (Table 1).

Effects of kaolinite or montmorillonite on the toxicity of Cd towards fungi. In the absence of Cd, 2% kaolinite had essentially no effect on the mycelial growth of *Fomes annosus, Pholiota marginata, Botrytis cinerea, Aspergillus niger, Phycomyces blakesleeanus, Trichoderma viride, Chaetomium* sp., *Thielaviopsis paradoxa*, and *Scopulariopsis brevicaulis* but enhanced the growth of *Schizophyllum* sp. In the absence of Cd, 2% montmorillonite suppressed the growth of *Schizophyllum* sp., *F. annosus, P. marginata, T. viride, Chaetomium* sp., and *P. blakesleeanus*, had no significant effect on the growth of *T. paradoxa* and *B. cinerea*, slightly enhanced the growth of *A. niger*, and greatly stimulated the growth of *S. brevicaulis*.

To distinguish between the influence of the clays on Cd toxicity from the direct stimulatory or inhibitory influence of the clays on fungal growth, the data were expressed as a percentage of the control values using the growth on agar containing no Cd and either no clay, 2% montmorillonite, or 2% kaolinite as the controls for the Cd treatments. The data in Table 2 clearly indicate that montmorillonite and, to a lesser extent, kaolinite protected the fungi against inhibitory or lethal concentrations of Cd. For example, 10 μg of Cd per ml reduced the growth of *T. paradoxa* to 11% of the control in the absence of clay, but, in the presence of 2% kaolinite or montmorillonite, growth was 91 and 99%, respectively, of the controls; at 100 μg of Cd per ml, *T. paradoxa* grew only on agar amended with 2% montmorillonite. The inhibitory effect of Cd towards *P. blakesleeanus* was lessened in the presence of the clay minerals, and slight growth with 1,000 μg of Cd per ml occurred only in the presence of 2% montmorillonite (Table 2).

These studies were extended to include greater concentrations of kaolinite or montmorillonite. In the absence of Cd, kaolinite concentrations from 1 to 20% did not appreciably alter mycelial growth of *Chaetomium* sp., *B. cinerea, T. viride*, or *S. brevicaulis*, but stimulated that of *Schizophyllum* sp. In the absence of Cd, 3 and 5% montmorillonite decreased the mycelial extension of *Chaetomium* sp., *B. cinerea, Schizophyllum* sp., and *T. viride* and, at 1% and greater, stimulated the growth of *S. brevicaulis*.

Kaolinite, at concentrations of 5% and

TABLE 2. *Influence of cadmium in the absence and presence of 2% kaolinite or 2% montmorillonite on growth of fungi on agar*

Fungus	Time (days)	Treatment[a]	% of control[b] at cadmium concn (μg/ml) of:			
			0	10	100	1,000
F. annosus	7	No clay	100 ± 0.5	52 ± 1.9	0	0
		2% K	100 ± 2.2	44 ± 4.3	0	0
		2% M	100 ± 3.8	102 ± 3.0	0	0
T. paradoxa	2	No clay	100 ± 2.6	11 ± 1.3	0	0
		2% K	100 ± 2.2	91 ± 2.6	0	0
		2% M	100 ± 2.8	99 ± 2.8	18 ± 0.6	0
B. cinerea	3	No clay	100 ± 2.0	8 ± 0.5	0	0
		2% K	100 ± 1.1	66 ± 1.0	0	0
		2% M	100 ± 6.2	102 ± 6.2	28 ± 3.0	0
A. niger	5	No clay	100 ± 1.2	16 ± 0.6	3 ± 0.4	0
		2% K	100 ± 0.5	56 ± 2.4	10 ± 0.2	0
		2% M	100 ± 2.7	95 ± 2.1	47 ± 5.1	0
P. marginata	9	No clay	100 ± 1.8	12 ± 1.1	6 ± 0.1	0
		2% K	100 ± 1.1	60 ± 2.4	11 ± 1.0	0
		2% M	100 ± 1.8	95 ± 2.4	38 ± 2.3	0
Schizophyllum sp.	7	No clay	100 ± 2.0	70 ± 6.6	13 ± 6.0	0
		2% K	100 ± 4.0	83 ± 5.2	18 ± 3.4	0
		2% M	100 ± 3.8	90 ± 3.1	79 ± 4.0	0
P. blakesleeanus	2	No clay	100 ± 0.8	80 ± 1.5	16 ± 0.5	0
		2% K	100 ± 1.4	90 ± 2.6	29 ± 0.6	0
		2% M	100 ± 2.3	100 ± 3.7	90 ± 2.7	3 ± 0.0
S. brevicaulis	9	No clay	100 ± 1.0	11 ± 0.8	4 ± 0.8	0
		2% K	100 ± 3.7	63 ± 2.0	11 ± 0.6	0
		2% M	100 ± 1.1	99 ± 0.8	48 ± 4.0	3 ± 0.2
Chaetomium sp.	5	No clay	100 ± 0.5	78 ± 1.2	27 ± 1.1	0
		2% K	100 ± 2.8	76 ± 0.5	36 ± 0.4	0
		2% M	100 ± 1.7	103 ± 1.6	75 ± 1.1	24 ± 1.5
T. viride	2	No clay	100 ± 1.8	22 ± 0.7	7 ± 0.1	2 ± 0.2
		2% K	100 ± 1.9	93 ± 2.8	24 ± 1.5	1 ± 0.5
		2% M	100 ± 2.3	105 ± 2.3	99 ± 5.6	33 ± 6.2

[a] Abbreviations: K, Kaolinite; M, montmorillonite.

[b] Mean percentage of the control ± standard error of the mean. Based on control plates which contained no Cd and no clay, no Cd and 2% kaolinite, or no Cd and 2% montmorillonite.

greater, and montmorillonite, at concentrations of 1% and greater, significantly decreased the toxicity of 10 and 100 μg of Cd per ml towards *Schizophyllum* sp.; however, with 1,000 μg of Cd per ml, growth occurred only in the presence of 3 and 5% montmorillonite (Fig. 1). In the presence of 10, 100, or 1,000 μg of Cd per ml, kaolinite or montmorillonite, even at 1%, significantly enhanced the growth of *T. viride*. The protective effect of montmorillonite was greater than that of kaolinite with all concentrations of Cd (Fig. 2). Kaolinite, at 3% and above, and montmorillonite, even at 1%, significantly decreased the toxicity of 10 and 100 μg of Cd per ml towards *Chaetomium* sp. With 1,000 μg of Cd per ml, 3 and 5% montmorillonite or 15 and 20% kaolinite afforded some protection against Cd, with montmorillonite having the greater effect (Fig. 2). With 10 or 100 μg of Cd per ml, kaolinite or montmorillonite, at 1% and greater, significantly decreased the toxicity of Cd towards *S. brevicaulis*; however, with 1,000 μg of Cd per ml, only montmorillonite, at 3 and 5%, afforded some protection against Cd (Fig. 3). Kaolinite, at 3% and above, and montmorillonite, even at 1%, significantly decreased the toxicity of 10 μg of Cd per ml towards *B. cinerea*; with 100 μg of Cd per ml, kaolinite, at 5% and greater, and montmorillonite, even at 1%, afforded protection; none of the concentrations of clays employed, however, was sufficient to protect against the inhibitory effects of 1,000 μg of Cd per ml (Fig. 3).

Effects of cation exchange capacity on the toxicity of Cd towards fungi. To determine the mechanisms whereby montmorillonite protected against the toxic effects of Cd to a greater extent than did equivalent concentrations of kaolinite, the concentrations of the clays were converted to reflect their CEC. In all cases (e.g., *B. cinerea* and *Chaetomium* sp. [Fig. 4]), tration of Cd, as the exchange sites on the clay

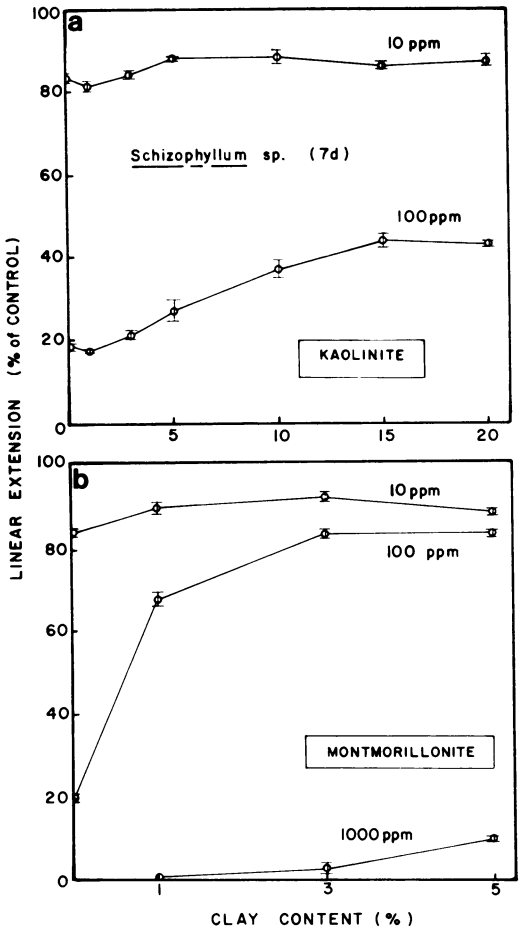

FIG. 1. *Effect of various concentrations of kaolinite (a) or montmorillonite (b) on growth of Schizophyllum sp. in the presence of 0, 10, 100, or 1,000 μg of Cd per ml. Percentages for the cadmium-kaolinite studies were based on control plates which contained no Cd and an equivalent concentration of kaolinite, and percentages for the cadmium-montmorillonite studies were based on control plates which contained no Cd and an equivalent concentration of montmorillonite. Mean ± standard error of the mean.*

increasing the CEC of the media increased the protection against Cd. The increased protection afforded by montmorillonite, as compared to equivalent concentrations of kaolinite, was correlated with the higher CEC of the montmorillonite. Apparently, by exchanging the Cd in the agar for the cations on the clay, the clays reduced the amount of Cd available for microbial uptake. The nature of the curves in Fig. 4 illustrate typical saturation kinetics, i.e., as the CEC was increased, more Cd was apparently adsorbed on the clay exchange complex until a point at which all the exogenous Cd was adsorbed was reached, and increasing the CEC beyond this point did not provide additional protection.

Effects of kaolinite or montmorillonite, homoionic to Cd, on toxicity of Cd towards fungi. *A. niger* and *P. marginata* were grown in the absence and presence of 10 μg of Cd per ml on agar amended with 1, 3, or 5% kaolinite or montmorillonite that either contained mixed cations on the exchange complex or were homoionic to Cd. Kaolinite or montmorillonite (1% and above) with mixed cations on the exchange complex protected *A. niger* against 10 μg of Cd per ml, with montmorillonite providing greater protection compared to equivalent concentrations of kaolinite. However, the additions of either clay when homoionic to Cd, both in the absence and presence of exogenous Cd, were detrimental to *A. niger*, with homoionic montmorillonite being more toxic than equivalent concentrations of homoionic kaolinite. In the absence of exogenous Cd, homoionic kaolinite at 1%, but not at 3 or 5%, permitted some growth of *A. niger*, whereas even 1% montmorillonite homoionic to Cd inhibited growth. In the presence of exogenous Cd, no growth occurred with either kaolinite or montmorillonite homoionic to Cd.

Similar results were obtained with *P. marginata*. In the absence of exogenous Cd, some growth occurred with 1, 3, or 5% kaolinite homoionic to Cd, whereas slight growth occurred only with 1% homoionic montmorillonite. In the presence of exogenous Cd, growth occurred only with homoionic kaolinite.

To clarify the mechanisms whereby montmorillonite homoionic to Cd was more detrimental to fungal growth than equivalent concentrations of homoionic kaolinite, the concentrations of clay were converted to reflect their CEC. Both in the absence and presence of exogenous Cd, increasing the CEC (by increasing the concentration of the homoionic clays) resulted in an increased inhibition of *A. niger* (Fig. 5) and *P. marginata* (Fig. 6), and the greater inhibition of montmorillonite homoionic to Cd was correlated with the higher CEC of the montmorillonite. In the absence of exogenous Cd, the Cd on the homoionic clays was apparently exchanged by either the cations present in the nutrient medium (e.g., H^+, K^+, Na^+, or Mg^{2+}) or by the protons produced during fungal metabolism. Increasing the concentration of clay or employing clay with a higher CEC resulted in a greater desorption of Cd and, hence, in increased toxicities. The exchange of the exogenous Cd did not decrease the ambient concen-

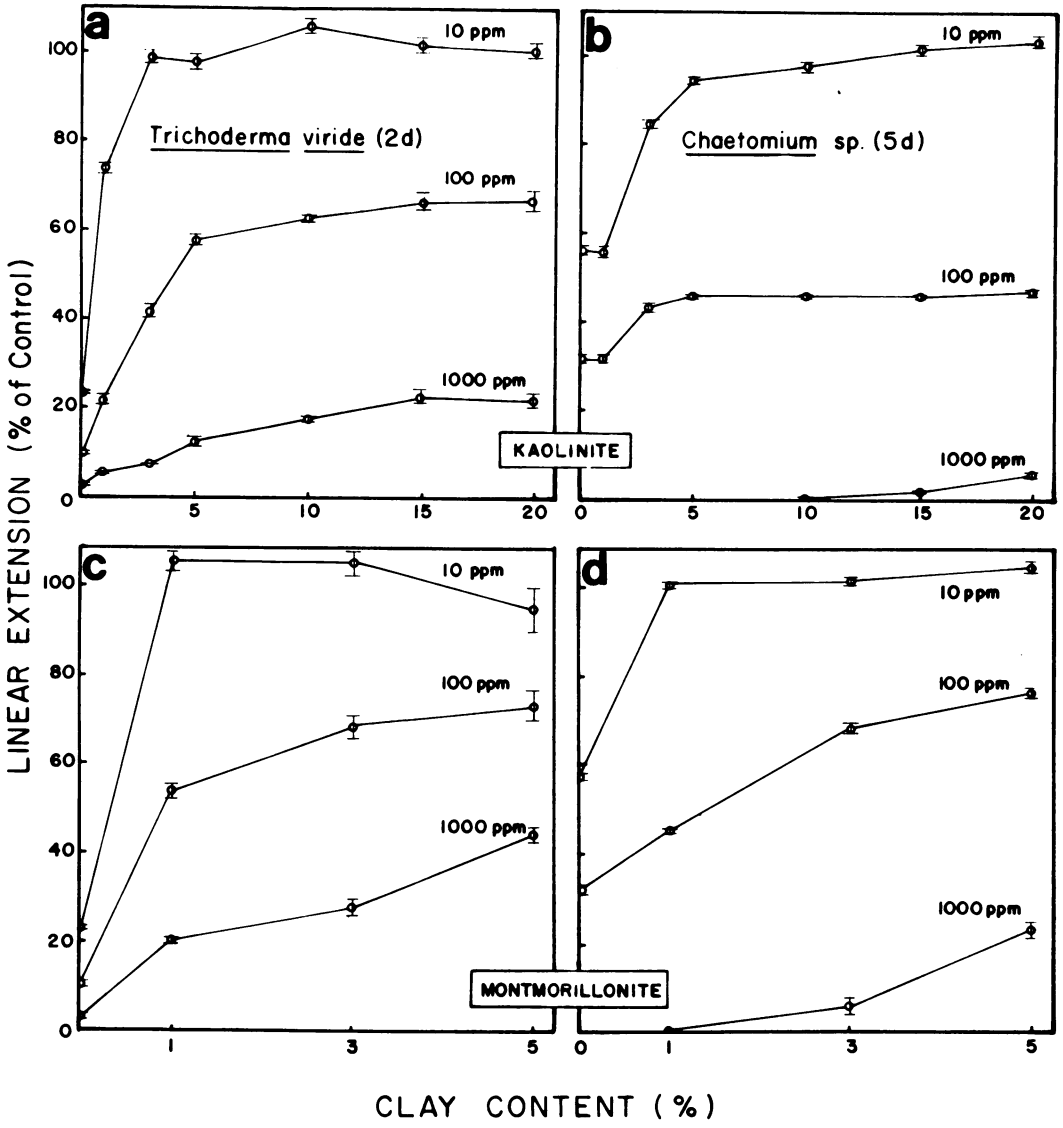

FIG. 2. *Effect of various concentrations of kaolinite or montmorillonite on growth of Trichoderma viride [(a) and (c), respectively] and Chaetomium sp. [(b) and (d), respectively] in the presence of 0, 10, 100, or 1,000 μg of Cd per ml. Percentages for the cadmium-kaolinite studies were based on control plates which contained no Cd and an equivalent concentration of kaolinite, and percentages for the cadmium-montmorillonite studies were based on control plates which contained no Cd and an equivalent concentration of montmorillonite. Mean ± standard error of the mean.*

complex of the homoionic clays were already saturated with Cd, but the exchange of Cd from the clays by other cations in the medium increased the ambient concentration.

DISCUSSION

In the absence of Cd, the growth of *B. megaterium* and *A. tumefaciens*, but not of *N. coralina*, was stimulated by montmorillonite and, to a lesser extent, by kaolinite. Studies employing respirometric techniques have also shown the stimulatory effects of clays, montmorillonite in particular, on bacterial growth. This stimulation was correlated with the buffering capacity, which, in turn, was a function of the CEC of the clays (22, 26). In the absence of Cd, the growth of the majority of fungi tested was

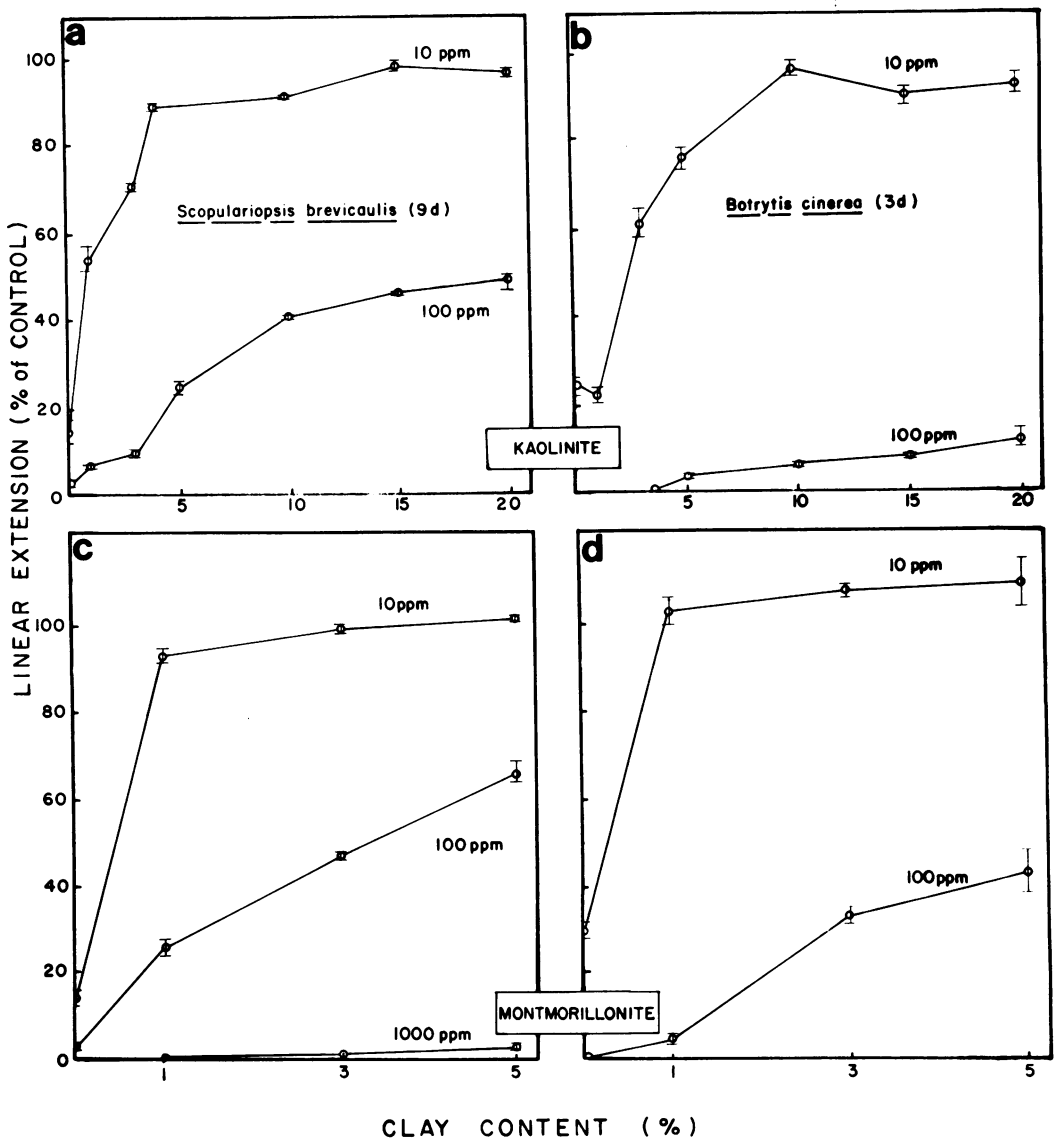

FIG. 3. *Effect of various concentrations of kaolinite or montmorillonite on growth of S. brevicaulis [(a) and (c), respectively] and B. cinerea [(b) and (d), respectively] in the presence of 0, 10, 100, or 1,000 μg of Cd per ml. Percentages for the cadmium-kaolinite studies were based on control plates which contained no Cd and an equivalent concentration of kaolinite, and percentages for the cadmium-montmorillonite studies were based on control plates which contained no Cd and an equivalent concentration of montmorillonite. Mean ± standard error of the mean.*

unaffected by kaolinite and inhibited by montmorillonite. However, exceptions were noted: e.g., the growth of *S. brevicaulis* was stimulated by montmorillonite, and the growth of *Schizophyllum* sp. was enhanced by kaolinite. Similarly, other studies have shown that fungal respiration is generally unaffected by kaolinite and inhibited by montmorillonite, although some exceptions were also noted (27). The mechanisms whereby fungal growth and respiration were inhibited by montmorillonite have not been completely elucidated (23), although increased viscosity and reduced access to oxygen may be important factors (27).

In the presence of Cd, montmorillonite and, to a lesser extent, kaolinite decreased the in-

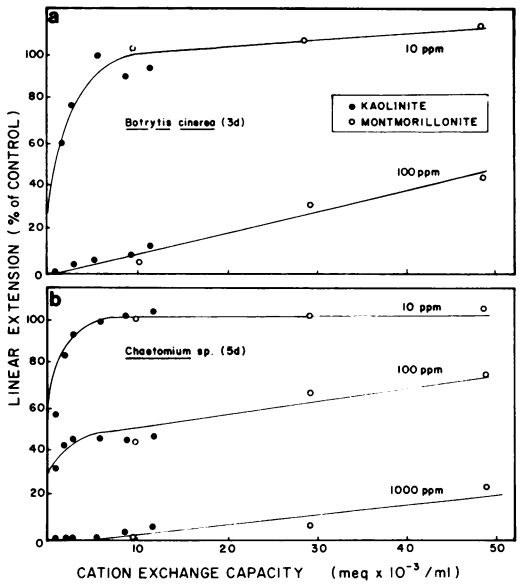

FIG. 4. *Effect of cation exchange capacity on growth of B. cinerea (a) and Chaetomium sp. (b) in the presence of cadmium. Cadmium, in concentrations of 0, 10, 100, or 1,000 μg/ml, was used in combination with 0, 1, 3, 5, 10, 15, or 20% kaolinite or 1, 3, or 5% montmorillonite. Percentages for the cadmium-kaolinite studies were based on control plates which contained an equivalent concentration of kaolinite and no cadmium; percentages for the cadmium-montmorillonite studies were based on control plates which contained an equivalent concentration of montmorillonite and no cadmium.*

hibitory effects of Cd to bacteria, including actinomycetes, and fungi. The greater protection afforded by montmorillonite, as compared to an equivalent concentration of kaolinite, appeared to be correlated with the higher CEC of montmorillonite. The CEC of a clay reflects its capacity to exchange cations on the exchange complex of the clay for cations in the ambient environment. By this exchange mechanism, Cd in the agar media was apparently exchanged for the cations on the clays. Montmorillonite, which has a higher CEC, would remove more Cd ions than would equivalent concentrations of kaolinite. Other studies (F. H. Sweeton and T. Tamura, Agron. Abstr., p. 34, 1975) have shown that montmorillonite adsorbed greater quantities of Cd than did kaolinite.

When fungi were grown on agar supplemented with montmorillonite or kaolinite homoionic to Cd, the clays were extremely toxic, even in the absence of exogenous Cd, as the Cd on the clay surface was apparently exchanged with both the cations present in the agar and the protons produced during metabolism. In the presence of exogenous Cd, the toxicity of the clays homoionic to Cd was enhanced, as the Cd ions in the agar were not removed by the clays whose exchange sites were already saturated with Cd. Regardless of the presence or absence of exogenous Cd, increasing the concentration of clays homoionic to Cd was detrimental to fungal growth, as more Cd ions were exchanged from the clay by cations present in the media. Montmorillonite homoionic to Cd was more detrimental than was homoionic kaolinite, as the montmorillonite had a higher CEC and, therefore, exchanged more Cd to the ambient environment.

The availability and, hence, toxicity of Cd to the biota may be dependent on the overall CEC of the environment, which is dependent on the contents of organic matter and clay minerals.

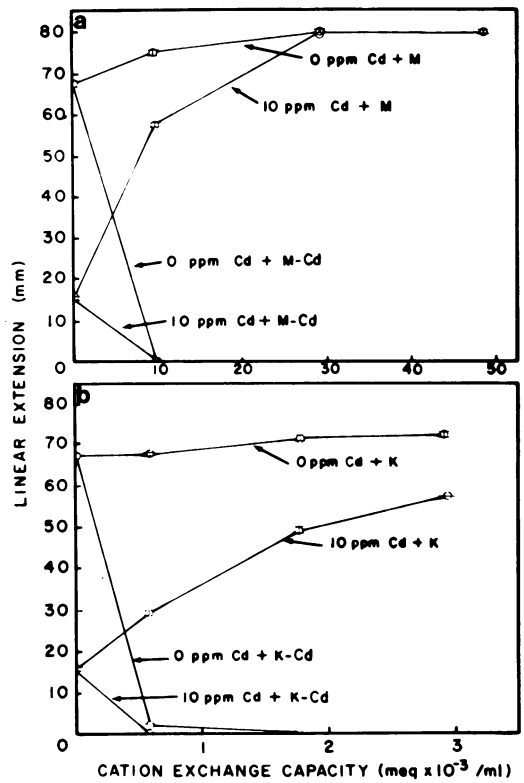

FIG. 5. *Effect of 1, 3, and 5% montmorillonite (a) or kaolinite (b) homoionic to cadmium on growth of A. niger in the absence and presence of exogenous cadmium. Clay concentrations are expressed as cation exchange capacity. Growth, in millimeters, was measured after 5 days of incubation. Abbreviations: M, Montmorillonite containing mixed cations; M-Cd, montmorillonite homoionic to cadmium; K, kaolinite containing mixed cations; K-Cd, kaolinite homoionic to cadmium. Mean ± standard error of the mean.*

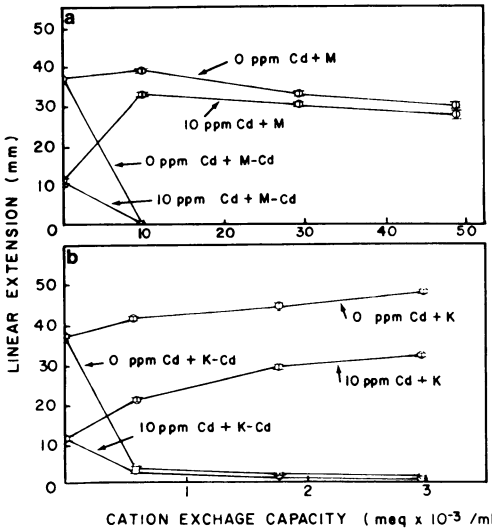

FIG. 6. *Effect of 1, 3, and 5% montmorillonite (a) or kaolinite (b) homoionic to cadmium on growth of P. marginata in the absence and presence of exogenous cadmium. Clay concentrations are expressed as cation exchange capacity. Growth, in millimeters, was measured after 11 days of incubation. Abbreviations: M, Montmorillonite containing mixed cations; M-Cd, montmorillonite homoionic to cadmium; K, kaolinite containing mixed cations; K-Cd, kaolinite homoionic to cadmium. Mean ± standard error of the mean.*

Cadmium is strongly bound to organic matter (2, 10, 15, 20), which may have a CEC as high as 200 meq/100 g (23). Peat and illitic clay soils were particularly strong adsorbents of Cd, whereas hydrous iron oxides and kaolinite were comparatively poor adsorbents (2). The degree of binding of Cd ions in different types of soils followed the sequence: organic soil > heavy clay soil > sandy and silt loam soil > sandy soil (10).

The toxicity of Cd, or any pollutant, is controlled by the physicochemical characteristics of the environment into which the pollutant is deposited. In assessing the toxicity of Cd to the microbiota, it is important to distinguish between the total concentration of Cd in the polluted environment and the amount of Cd available for microbial uptake and accumulation. The amount of available Cd is, in part, dependent on the CEC of the environment, which, in turn, is a function of the types and concentrations of organic matter and clay minerals. Consequently, the deposition of Cd into a soil or sediment containing montmorillonite may result in lower toxicities to both microbes and plants than deposition into an environment lacking this clay mineral.

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