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Sensitivity of Various Bacteria, Including Actinomycetes, and Fungi to Cadmium and the Influence of pH on Sensitivity

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A variety of microorganisms, including gram-negative and gram-positive eubacteria, actinomycetes, yeasts, and filamentous fungi, were tested for their sensitivity to cadmium (Cd). In general, the actinomycetes were more tolerant to Cd than were the eubacteria; gram-negative eubacteria were more tolerant to Cd than were gram-positive eubacteria. The period of exponential growth of the eubacteria and actinomycetes was extended in the presence of Cd. Wide extremes in sensitivity to Cd were noted among the fungi; there was no correlation between the class of fungus and tolerance to Cd. Fungal sporulation was more sensitive to Cd than was mycelial growth, as spore formation was inhibited at Cd concentrations that were noninhibitory to mycelial proliferation. The toxicity of Cd to the eubacteria, actinomycetes, and fungi appeared to be pH dependent, as toxicity was generally potentiated at pH 8 or 9.

Current interest in the state of the environment has resulted in increased research to evaluate the global impacts of pollution on the biosphere. Among the numerous pollutants, cadmium (Cd), an element with no known biological function, is of major concern. Industrial utilization of compounds containing Cd has accelerated the mobilization and transport rates of Cd, which far exceed the rates of natural cycling processes. These rates have led to increased deposition of Cd in aquatic and terrestrial environments, with subsequent increased uptake and accumulation of Cd in the biota.

Most of the research on Cd has concentrated on animal studies (e.g., 10, 11), with less investigation into the response of plants and microorganisms. The few studies on the effects of Cd on microorganisms have focused on a limited number of microbial representatives. For example, the effects of Cd on bacteria have been studied primarily with *Staphylococcus aureus* strains which carry a plasmid that confers resistance both to penicillin and to Cd and other heavy metals (e.g., 8, 18, 22) and with *Escherichia coli* (1, 9, 17, 23). There are few data on the response of algae (5; G. Burnison, P. T. S. Wong, Y. K. Chau, and B. Silverberg, Proc. Can. Fed. Biol. Soc. 18:46, 1975, and F. I. MacLean, O. J. Lucis, Z. A. Shaikh, and E. R. Jansz, Fed. Proc. 31:699, 1972) and protozoa (21) to Cd and apparently no information on the sensitivity of actinomycetes to Cd. Most investigations on the tolerance of fungi to Cd have been directed to the use of Cd as a fungicide (e.g., 15, 16).

This research was designed to establish base line concentrations of Cd that are inhibitory or lethal to microbes found in various natural habitats. Too often, studies on the effects of a pollutant use only a few representative microbial species and then attempt to predict, from the responses elicited by the few test organisms, the overall response of the total microbiota to the pollutant. The concentration of Cd in soils from contaminated areas may vary from 20 (12) to over 1,000 μg/g (6). This vast range in concentrations of Cd suggests a corresponding range of microbial sensitivity and adaptability to Cd. To attain a better understanding of the range of microbial tolerance to Cd, a variety of microorganisms, i.e, gram-negative and gram-positive bacteria (including actinomycetes), yeasts, and filamentous fungi, were evaluated for their sensitivity to Cd.

Studies on the effects of anthropogenic toxicants on the microbiota have shown that the toxicity of a pollutant is dependent on the physicochemical characteristics of the environment into which the pollutant is deposited. For example, with some gaseous pollutants (e.g., SO_2, NO_2, NH_3), deposition in a well-buffered environment will elicit different effects on the biota than deposition in a poorly buffered environment (see 2, 3). Consequently, the influence of pH on the toxicity of Cd to microorganisms was also investigated.

MATERIALS AND METHODS

Source and maintenance of microorganisms. Microorganisms were obtained from the culture collec-

tion 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 consisting 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.

Media. (i) Broth. Broth I consisted of 5.0 g of glucose, 1.0 g of peptone, 1.0 g of NH_4NO_3, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of $CaCl_2 \cdot 2H_2O$, 0.1 g of yeast extract, and 1,000 ml of deionized, distilled (4×) water, adjusted to pH 7.0 with NaOH. Broth II consisted of 10.0 g of glucose, 2.5 g of peptone, 1.0 g of NH_4NO_3, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of $CaCl_2 \cdot 2H_2O$, 0.2 g of yeast extract, and 1,000 ml of deionized, distilled (4×) water, which was adjusted to pH 6.0 with HCl. Both broths were amended with various concentrations of Cd (as $CdCl_2$). Inorganic phosphorus was omitted from the broths to avoid precipitation of phosphate salts of Cd. There was apparently sufficient phosphorus, as well as potassium, in the peptone and yeast extract to satisfy the requirements of the microorganisms for these nutrients.

(ii) Agar. The tolerance of fungi to Cd was evaluated with the maintenance agar medium amended with Cd (as $CdCl_2$).

Description of experiments. (i) Effect of Cd on growth of eubacteria. Bacteria were inoculated in Erlenmeyer flasks (125 ml) containing 50 ml of broth I and grown overnight (approximately 18 h) on a shaker at 25°C, and the portions (0.5 ml) were inoculated into test tubes (15 cm by 1 cm) containing 4.5 ml of broth I, which had previously been amended with 0, 0.1, 0.5, 1, 5, 10, 50, 100, or 500 μg of Cd per ml. The tubes were placed in a rotating drum (36 rpm) and housed in a 25°C incubator. After 24 h of growth, turbidity was measured at 420 nm (B & L Spectronic 20). Six replicate tubes were employed for each concentration of Cd, and most experiments were repeated twice. In other studies, turbidity was measured every 3 h during a 24-h period.

(ii) Effect of Cd on growth of actinomycetes and yeasts. Similar to (i) above, except that broth II was employed.

(iii) Effect of Cd on growth of filamentous fungi. Fungi were grown in petri dishes containing approximately 20 ml of the agar medium. After incubation for several days at 25°C, circular fungal plugs (9 mm in diameter), made with a sterilized metal cork borer, were transferred to the center of plates containing the agar medium amended with 0, 0.01, 0.1, 1, 10, 100, or 1,000 μg of Cd per ml. The plates were incubated at 25°C and, after appropriate time intervals, the diameters of mycelial growth were measured in four directions. The duration of incubation was dependent on the specific fungus: mycelial diameters were recorded after 9 to 11 days for fungi with slow growth rates, whereas for more rapidly growing fungi, the experiments were terminated when the controls (i.e., without Cd) approached the periphery of the petri dish. Four replicate plates were employed for each concentration of Cd, and all experiments were performed twice.

(iv) Effect of Cd on sporulation of fungi. Fungi were grown for 1 week at 25°C on slants of the agar medium. The slants were then flooded with 0.85% saline and agitated on a Vortex-Genie. With a transfer loop that had been calibrated to deliver 0.01 ml, portions of the culture suspension, which consisted primarily of spores, were transferred and spread over the surface of agar slants containing the agar medium amended with 0, 1, 5, 10, 50, or 100 μg of Cd per ml. The slants were incubated at 25°C for 4 or 7 days, 2 ml of water was then added to each tube, which was agitated on a Vortex-Genie, and spore counts were performed with a Petroff-Hausser bacterial counter. Five replicate tubes were employed for each concentration of Cd, and all experiments were performed twice.

(v) Effect of pH on toxicity of Cd to bacteria. Bacteria were inoculated into Erlenmeyer flasks (125 ml) containing 50 ml of broth I and grown overnight (approximately 18 h) on a shaker at 25°C. Portions (0.5 ml) of the culture were inoculated into test tubes containing 4.5 ml of broth I, which had been previously adjusted to pH 5, 6, 7, 8, or 9 and amended with 0, 0.1, 1, 10, or 100 μg of Cd per ml. (The systems were not buffered, except for the buffering capacity provided by the peptone, and all pH values refer to initial pH.) The tubes were placed in a rotating drum (36 rpm) and housed in a 25°C incubator. After 24 h of growth, turbidity was measured at 420 nm. Five or six replicate tubes were employed for each concentration of Cd at each pH level, and most experiments were repeated twice for fungi with slow growth rates.

(vi) Effect of pH on toxicity of Cd to actinomycetes and filamentous fungi. Actinomycetes were inoculated into Erlenmeyer flasks (125 ml) containing 50 ml of broth II and grown for 1 or 2 days on a shaker at 25°C. Fungi were grown for 1 week at 25°C on agar slants. The slants were then flooded with 0.85% saline and agitated on a Vortex-Genie. Portions (0.1 ml) of the actinomycete or fungal suspension were inoculated into test tubes containing 10 ml of broth II, which contained either 0 or 10 μg of Cd per ml, and adjusted to pH 4, 5, 6, 7, 8, or 9. (The media were not buffered, except for the buffering capacity provided by the peptone, and pH refers to the initial pH values.) The tubes were placed in a rotating drum (36 rpm) and housed in a 25°C incubator. After several days of growth (5 days for actinomycetes and 2 or 3 days for fungi), dry weights were determined: the contents of each tube were filtered through tared membrane filters (0.45 μm, Millipore) and rinsed three times with distilled water, the membranes were dried overnight in tared aluminum weighing cups at 100°C and weighed, and the weight of each organism was calculated. Three replicate tubes were employed for each concentration of Cd at each pH level.

In studies of the effects of pH on Cd toxicity, pH levels refer only to the pH at the time of inoculation, as broth I and broth II were essentially unbuffered systems, although some buffering was provided by

the peptone. Cadmium forms many complex inorganic, ionic, and molecular species (4) and has an affinity for organic ligands and chelating agents present in commercial laboratory media (19). Consequently, the composition of the broths was as simple as possible while still permitting abundant growth.

RESULTS

Response of bacteria to Cd. Inhibition of the growth of gram-positive bacteria was first noted at 0.5 μg of Cd per ml with *Brevibacterium linens*, at 1 μg of Cd per ml with *Bacillus megaterium*, at 5 μg of Cd per ml with *Corynebacterium* sp., and at 10 μg of Cd per ml with *Bacillus cereus*. Complete inhibition of growth was noted at 50 μg of Cd per ml with *B. linens* and *B. megaterium*, at 100 μg of Cd per ml with *Corynebacterium* sp., and at 500 μg of Cd per ml with *B. cereus*. It was difficult to ascertain the concentration of Cd inhibitory to *Micrococcus agilis*, as this organism exhibited poor growth in the unamended (i.e., without Cd) broth. However, the growth of *M. agilis* appeared to be initially inhibited at 1 μg of Cd per ml and completely inhibited at 10 μg of Cd per ml (Fig. 1).

There was also extreme variability in sensitivity to Cd among the gram-negative bacteria. The inhibition of growth was first noted at 0.5 μg of Cd per ml with *Agrobacterium tumefaciens* and *Rhizobium meliloti*, at 1 μg of Cd per ml with *Chromobacterium orangum* and *Alcaligenes faecalis*, at 5 μg of Cd per ml with *Proteus vulgaris*, and at 10 μg of Cd per ml with *Enterobacter aerogenes*. Complete inhibition of growth was noted at 50 μg of Cd per ml with *R. meliloti*, *A. tumefaciens*, and *C. orangum* and at 500 μg of Cd per ml with *A. faecalis* and *E. aerogenes*. Total inhibition of growth of *P. vulgaris* was not observed, even at 500 μg of Cd per ml, the highest concentration employed (Fig. 2).

Actinomycetes also showed a wide range in sensitivity to Cd. Inhibition of growth was first evident at 0.5 μg of Cd per ml with *Nocardia*

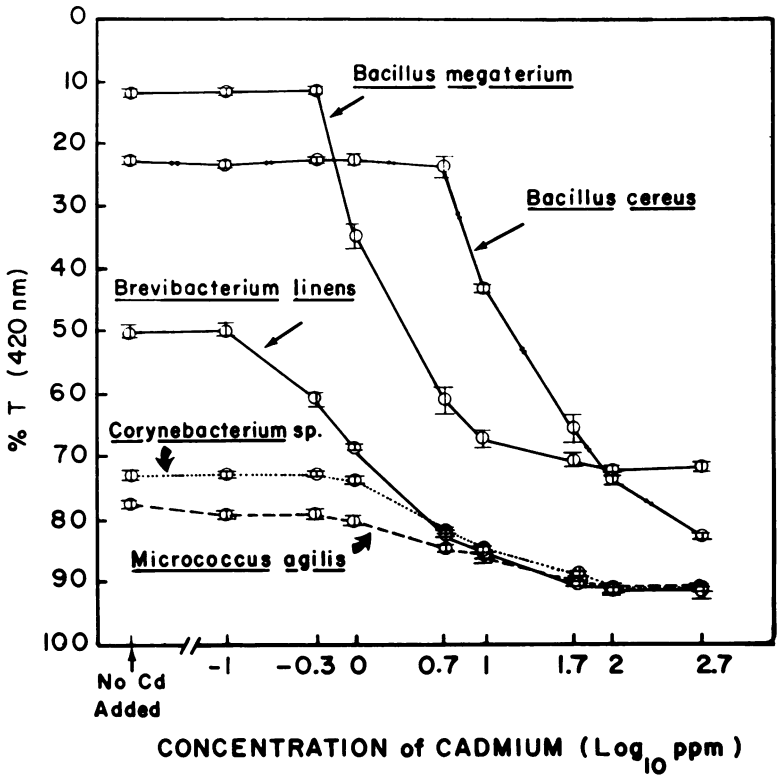

FIG. 1. *Effect of cadmium on growth of gram-positive bacteria. Measurements of the percentage of transmittance were performed at 420 nm after 24 h of growth. Initial measurements of the percentage of transmittance were 82.0 ± 1.00 for B. cereus, 71.8 ± 1.31 for B. megaterium, 88.0 ± 1.00 for M. agilis, 91.5 ± 0.50 for Brevibacterium linens, and 95.0 ± 1.00 for Corynebacterium sp. Mean ± standard error of the mean.*

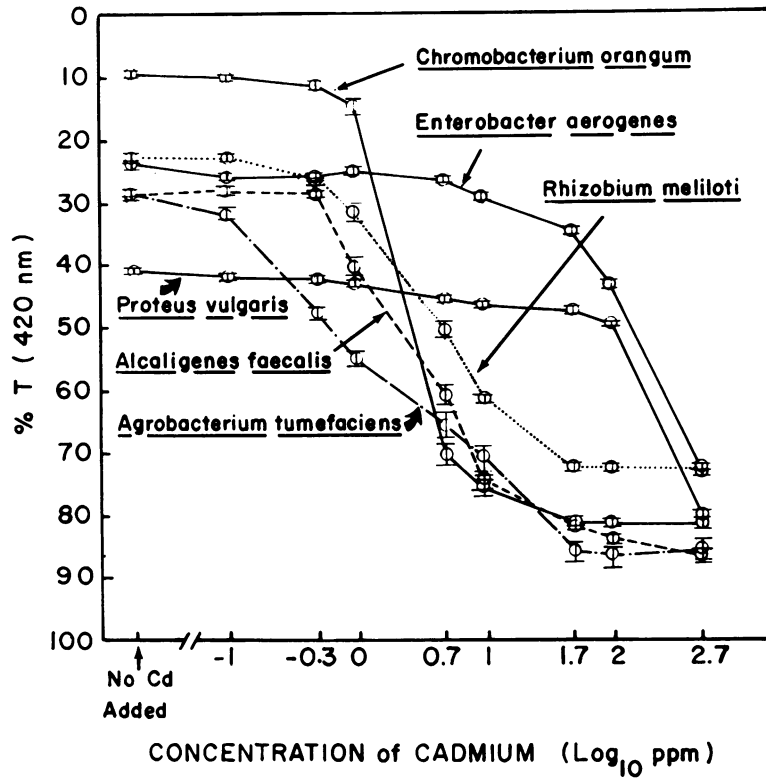

FIG. 2. *Effect of cadmium on growth of gram-negative bacteria. Measurements of the percentage of transmittance were performed at 420 nm after 24 h of growth. Initial measurements of the percentage of transmittance were 72.0 ± 0.58 for R. meliloti, 79.0 ± 1.00 for C. orangum, 87.0 ± 0.00 for A. faecalis, 86.0 ± 0.00 for A. tumefaciens, 87.7 ± 0.56 for P. vulgaris, and 73.0 ± 0.26 for E. aerogenes. Mean ± standard error of the mean.*

corallina, at 1 μg of Cd per ml with *Streptomyces flavovirens*, and at 5 μg of Cd per ml with *Nocardia paraffinae*. Total inhibition of growth occurred at 100 μg of Cd per ml with *N. corallina*, whereas slight growth with *S. flavovirens* and *N. paraffinae* was noted at 500 μg of Cd per ml, the highest concentration employed. It was difficult to determine the toxicity of Cd towards *Micromonospora chalcea*, as this organism exhibited poor growth even in unamended (i.e., without Cd) broth. However, *M. chalcea* appeared to be extremely sensitive to Cd, as initial inhibition occurred at 0.1 μg of Cd per ml and total inhibition at 1 μg of Cd per ml (Fig. 3).

The tolerance of bacteria to Cd was apparently species specific, as variability in sensitivity to Cd was noted for species of the same genus. For example, *B. megaterium* did not grow at 50 μg of Cd per ml, whereas *B. cereus* grew at 100 μg of Cd per ml. Similarly, *N. paraffinae* was more tolerant to Cd than was *N. corallina*.

The Cd concentration at which inhibition of growth was first noted was not directly related to the Cd concentration that caused the total inhibition of growth. For example, the inhibition of growth of *P. vulgaris* and *E. aerogenes* was first evident at 5 and 10 μg of Cd per ml, respectively, whereas 500 μg of Cd per ml permitted some growth of *P. vulgaris* but was totally inhibitory to *E. aerogenes*.

As the differential effects of Cd may have reflected an adverse influence on growth kinetics, the growth of *A. tumefaciens* in broth I, and of *N. corallina* in broth II, was measured at 3-h intervals. Whereas 0.1 μg of Cd per ml only slightly reduced the rate of growth of *A. tumefaciens*, 0.5 to 10 μg of Cd per ml extended the period of exponential growth, with a more pronounced effect occurring in broth amended with 5 or 10 μg of Cd per ml. Growth was completely suppressed at 50 or 100 μg of Cd per ml (Fig. 4). While 0.1 μg of Cd per ml did not adversely affect the growth rate of *N. corallina*, 0.5 and 1 μg of Cd per ml prolonged the time of exponen-

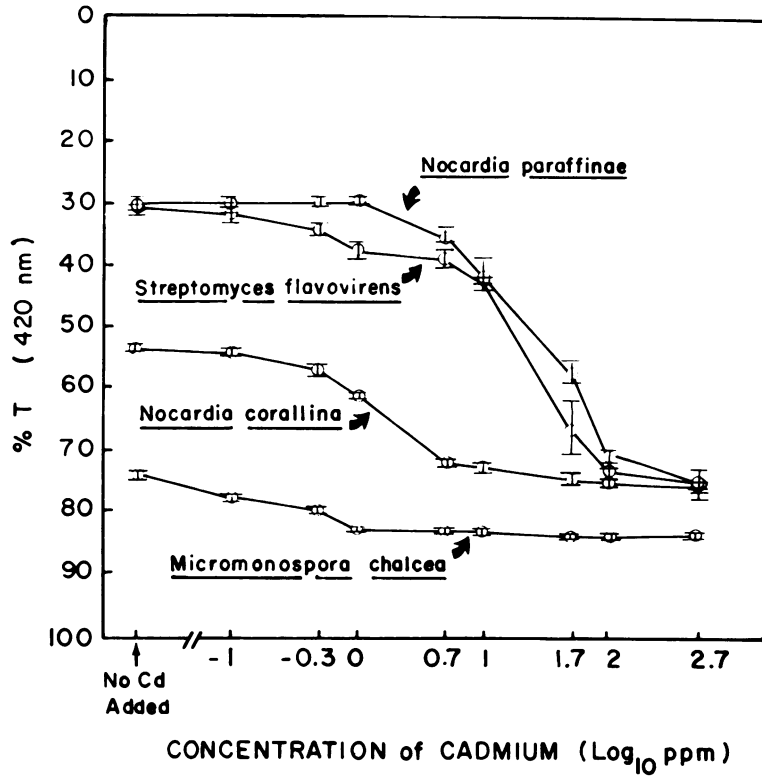

FIG. 3. *Effect of cadmium on growth of actinomycetes. Measurements of the percentage of transmittance were performed at 420 nm after 24 h of growth. Initial measurements of the percentage of transmittance were 79.0 ± 0.63 for N. corallina, 83.8 ± 0.70 for M. chalcea, 84.5 ± 0.56 for S. flavovirens, and 84.0 ± 0.37 for N. paraffinae. Mean ± standard error of the mean.*

tial growth, and 5 and 10 μg of Cd per ml greatly depressed the rates of growth. At 50 or 100 μg of Cd per ml, growth of the actinomycete was totally suppressed (Fig. 5).

Response of fungi to Cd. Inhibition of the growth of yeasts was first noted at 0.1 μg of Cd per ml for *Schizosaccharomyces octosporus* and at 0.5 μg of Cd per ml for *Saccharomyces cerevisiae, Saccharomyces cerevisiae* var. *ellipsoides*, and *Rhodotorula* sp. At 500 μg of Cd per ml, the highest concentration employed, the inhibition of growth was noted with *S. octosporus*, slight growth was noted with *S. cerevisiae* and *S. cerevisiae* var. *ellipsoides*, and moderate growth was evident with *Rhodotorula* sp. (Fig. 6).

The 22 filamentous fungi evaluated, representing phycomycetes, ascomycetes, basidiomycetes, and *Fungi Imperfecti*, were broadly grouped into three categories based on their sensitivity to Cd. (i) The first category consisted of fungi that were capable of growth in the presence of up to 10 μg of Cd per ml but inhibited by 100 μg of Cd per ml: *Botrytis allii, Botrytis cinerea, Penicillium vermiculatum, Aspergillus fischeri, Aspergillus giganteus, Aspergillus janus, Thielaviopsis paradoxa, Fomes annosus*, and *Pyncidiophora dispersa* (Table 1; Fig. 7). (ii) The second category consisted of fungi that were capable of growth in the presence of up to 100 μg of Cd per ml but inhibited by 1,000 μg of Cd per ml: *Aspergillus niger, Aspergillus flavipes, Scopulariopsis brevicaulis, Pholiota marginata, Schizophyllum* sp., *Phycomyces blakesleeanus, Fusarium oxysporum* f. *conglutinans*, and *Chaetomium* sp. (Table 1; Fig. 8). (iii) The third category consisted of fungi that were capable of growth in the presence of 1,000 μg of Cd per ml, the highest Cd concentration employed: *Rhizopus stolonifer, Trichoderma viride, Penicillium asperum, Sphaerostilbe repens*, and *Cunninghamella echinulata* (Table 1; Fig. 9).

As noted with bacteria, differential sensitivities to Cd were evident for species of the same genus. For example, *P. asperum* grew on agar amended with 1,000 μg of Cd per ml, whereas the growth of *P. vermiculatum* was greatly re-

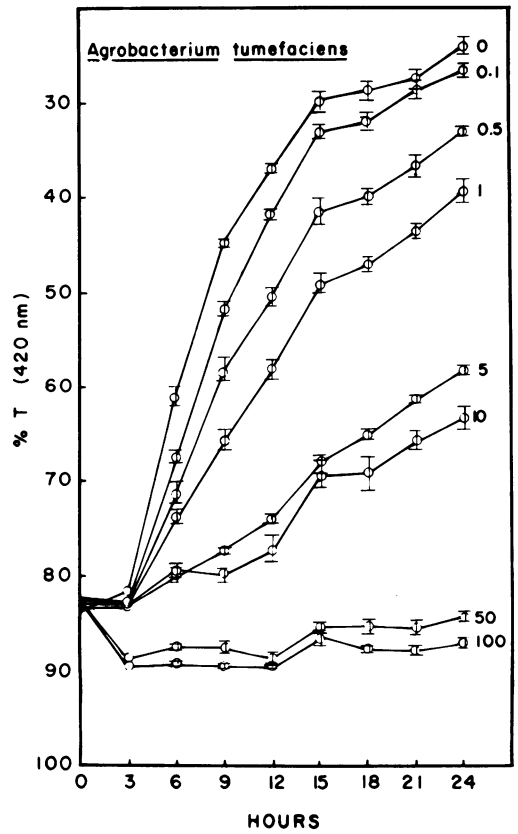

FIG. 4. *Effect of 0, 0.1, 0.5, 1, 5, 10, 50, and 100 μg of cadmium per ml on growth rate of A. tumefaciens. Mean ± standard error of the mean.*

duced by 10 μg of Cd per ml. The order of sensitivity to Cd noted for the various species of *Aspergillus* was: *A. fischeri* > *A. giganteus* > *A. janus* > *A. niger* > *A. flavipes*.

The concentration of Cd causing the initial inhibition of growth was not correlated to the concentration that caused the total inhibition of growth. For example, *F. annosus* (Fig. 7) did not grow at 100 μg of Cd per ml, but at 10 μg of Cd per ml had greater growth (i.e., 52% of the control) than did *A. niger* (i.e., 16% of the control; Fig. 8), which exhibited slight growth at 100 μg of Cd per ml. Similarly, *Chaetomium* sp. did not grow at 1,000 μg of Cd per ml but exhibited growth (i.e., 27% of the control) at 100 μg of Cd per ml (Fig. 8), whereas *T. viride* showed some growth at 1,000 μg of Cd per ml but exhibited less growth (i.e., 7% of the control) at 100 μg of Cd per ml (Fig. 9).

During the studies of the effects of Cd on mycelial growth, retardation of pigmentation development in the colonies was noted at Cd concentrations that were not inhibitory to mycelial proliferation. These alterations in pigmentation were probably a reflection of the inhibition of sporulation processes by Cd. Studies to determine the influence of Cd on spore production by *A. niger*, *T. viride*, and *R. stolonifer* showed that the initial suppression of sporulation occurred at a concentration of Cd lower than that required to inhibit mycelial proliferation. When *A. niger* was grown on agar amended with 1 μg of Cd per ml, spore production was reduced to 35% of the control, whereas this concentration of Cd did not significantly reduce mycelial proliferation. When *T. viride* was grown on agar amended with 5 μg of Cd per ml, spore production was reduced to 20% of the control, whereas an equivalent reduction in mycelial proliferation was not evident until 10 μg of Cd per ml was used. When grown on agar amended with 1 μg of Cd per ml, sporulation of *R. stolonifer* was reduced to 65% of the control, whereas inhibition of mycelial proliferation was not evident until 10 μg of Cd per ml was used (Fig. 10).

Effect of pH on Cd toxicity. When grown for

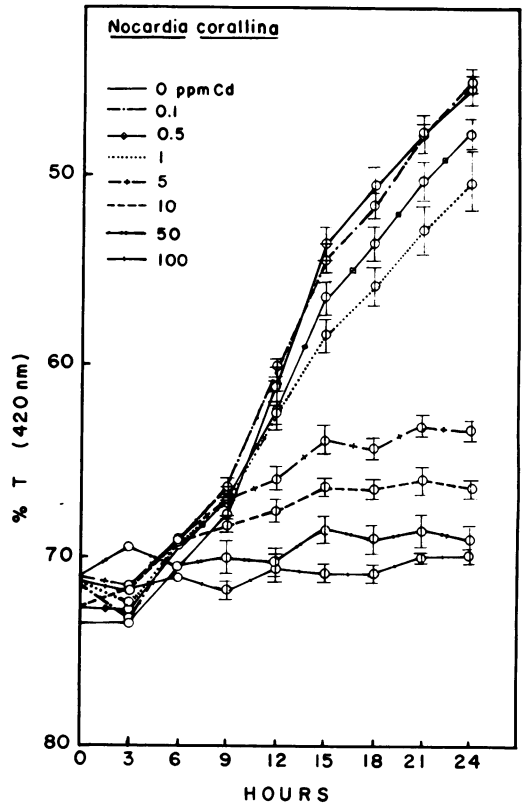

FIG. 5. *Effect of cadmium on growth rate of N. corallina. Mean ± standard error of the mean.*

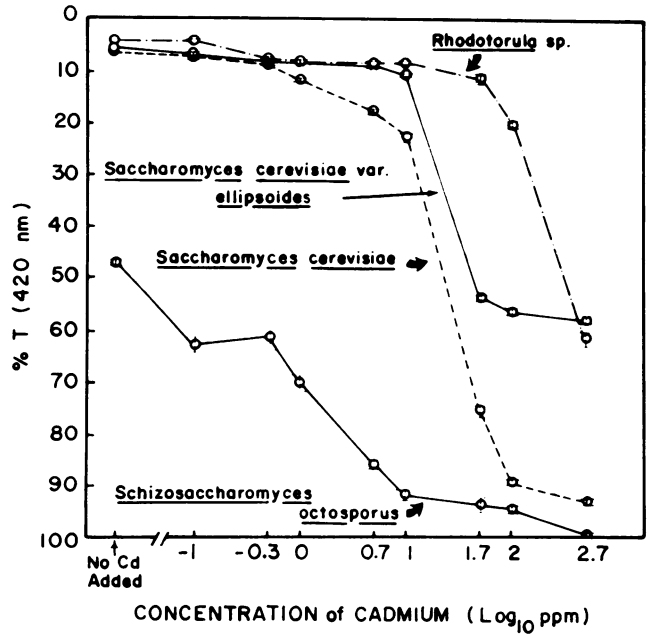

FIG. 6. *Effect of cadmium on growth of yeasts. Measurements of the percentage of transmittance were performed at 420 nm after 24 h of growth. Initial measurements of the percentage of transmittance were 96.5 ± 0.22 for S. cerevisiae, 62.5 ± 0.22 for S. cerevisiae var. ellipsoides, 98.0 ± 0.26 for S. octosporus, and 80.7 ± 0.95 for Rhodotorula sp. Mean ± standard error of the mean.*

24 h in broth amended with 0, 0.1, 1, 10, or 100 μg of Cd per ml, *A. tumefaciens* was not affected by 0.1 μg of Cd per ml from pH 5 to 9. At 1 μg of Cd per ml, growth was inhibited, but the extent of inhibition was equivalent at all pH levels. At 10 μg of Cd per ml, however, inhibition of growth was more pronounced at pH 6 through 9 than at pH 5: growth was completely inhibited with 10 μg of Cd per ml only at pH 9. At 100 μg of Cd per ml, growth was totally suppressed at all pH levels (Fig. 11).

The growth of *A. faecalis*, after 24 h, was reduced in broth unamended with Cd and adjusted to pH 8 or 9. The inhibition of growth of Cd was initially noted with 0.1 μg of Cd per ml at pH 9 and with 1 μg of Cd per ml at pH 5 through 8. Although growth occurred with 10 μg of Cd per ml at pH 5, 6, or 7, growth was totally suppressed at pH 8 or 9. With 100 μg of Cd per ml, no growth occurred at any pH (Fig. 11).

In the absence of Cd, the growth of *B. cereus*, after 24 h, was slightly inhibited at pH 9. Inhibition of growth by Cd was first evident with 0.1 μg of Cd per ml at pH 8, with 1 μg of Cd per ml at pH 7, and with 10 μg of Cd per ml at pH 5. With 10 μg of Cd per ml, total suppression of growth was evident at pH 8 and 9, while growth occurred at pH 5, 6, and 7. There was essentially no growth, at all pH levels, with 100 μg of Cd per ml (Fig. 11).

Streptomyces olivaceus grew only slightly at pH 4, whether in the presence or absence of Cd. There was no apparent interaction between Cd and pH, as, with 10 μg of Cd per ml, growth was reduced to a greater extent at pH 5, 8, and 9 (i.e., approximately 30% of controls) than at pH 6 and 7 (i.e., approximately 45% of controls) (Fig. 12).

N. paraffinae exhibited only slight growth at pH 4 or 5, whether with or without Cd. At pH levels of 6, 7, and 8, 10 μg of Cd per ml caused only a slight reduction in growth (i.e., approximately 85% of controls at pH 6 or 8 and 95% of controls at pH 7). At pH 9, however, 10 μg of Cd per ml was extremely inhibitory, as growth was only 18% of controls (Fig. 12).

At pH 4, 5, and 6, 10 μg of Cd per ml reduced the growth of *R. stolonifer* to 65 to 70% of controls and, at pH 7 and 8, to 75 to 80% of controls. However, at pH 9, 10 μg of Cd per ml decreased growth to 10% of the control (Fig. 13).

At pH 4, 5, and 6, 10 μg of Cd per ml did not significantly affect the growth of *T. viride*; however, at pH 7 and 8, 10 μg of Cd per ml reduced the growth of *T. viride* to 61 and 67% of

TABLE 1. *Influence of cadmium on growth of fungi on agar*

Category	Fungus	Time (days)	Linear diam extension (mm)[a] at Cd concn (μg/ml) of:						
			0	0.01	0.1	1	10	100	1,000
Group A (sensitive)	*B. allii*	5	53.2 ± 0.40	52.9 ± 0.53	53.3 ± 0.46	53.3 ± 0.53	1.1 ± 0.11	0	0
			(100 ± 1.2)[b]	(99 ± 1.9)	(100 ± 1.7)	(100 ± 1.8)	(2 ± 0.2)	(0)	(0)
	P. vermiculatum	11	51.4 ± 0.62	51.8 ± 0.67	51.4 ± 0.61	46.9 ± 1.78	1.9 ± 0.09	0	0
			(100 ± 1.2)	(101 ± 1.3)	(100 ± 1.2)	(92 ± 3.4)	(4 ± 0.2)	(0)	(0)
	B. cinerea	3	42.3 ± 0.47	42.4 ± 0.51	39.8 ± 0.41	38.7 ± 0.41	3.5 ± 0.18	0	0
			(100 ± 2.0)	(100 ± 2.3)	(94 ± 1.2)	(92 ± 1.4)	(8 ± 0.5)	(0)	(0)
	A. fischeri	9	53.8 ± 0.74	53.3 ± 0.58	53.5 ± 0.75	53.5 ± 0.80	4.7 ± 0.80	0	0
			(100 ± 1.4)	(99 ± 1.1)	(100 ± 1.4)	(100 ± 1.5)	(9 ± 1.5)	(0)	(0)
	T. paradoxa	2	40.5 ± 1.07	39.6 ± 0.99	40.6 ± 1.12	39.8 ± 0.57	4.3 ± 0.51	0	0
			(100 ± 2.6)	(98 ± 2.4)	(100 ± 2.7)	(98 ± 1.4)	(11 ± 1.3)	(0)	(0)
	A. giganteus	7	47.6 ± 0.93	46.1 ± 0.59	47.5 ± 1.64	46.8 ± 1.15	5.5 ± 0.60	0	0
			(100 ± 2.0)	(97 ± 1.2)	(100 ± 3.5)	(98 ± 2.4)	(12 ± 1.3)	(0)	(0)
	A. janus	11	25.3 ± 0.45	25.3 ± 0.49	24.8 ± 0.42	24.8 ± 0.36	4.6 ± 0.39	0	0
			(100 ± 1.9)	(100 ± 2.0)	(99 ± 1.5)	(98 ± 1.4)	(19 ± 1.6)	(0)	(0)
	P. dispersa	11	34.5 ± 0.77	34.6 ± 0.61	35.4 ± 0.78	34.8 ± 0.73	11.6 ± 0.45	0	0
			(100 ± 2.1)	(101 ± 1.8)	(103 ± 2.3)	(100 ± 2.1)	(33 ± 1.3)	(0)	(0)
	F. annosus	7	72.6 ± 0.24	73.4 ± 0.19	72.8 ± 0.22	72.8 ± 0.17	38.0 ± 0.66	0	0
			(100 ± 0.5)	(101 ± 0.5)	(100 ± 0.5)	(100 ± 0.3)	(52 ± 1.9)	(0)	(0)
Group B (moderately resistant)	*A. niger*	5	52.9 ± 0.36	53.6 ± 0.31	52.6 ± 0.34	51.4 ± 0.30	9.1 ± 0.21	1.8 ± 0.13	0
			(100 ± 1.2)	(101 ± 1.0)	(99 ± 1.2)	(97 ± 0.9)	(16 ± 0.6)	(3 ± 0.4)	(0)
	S. brevicaulis	9	48.2 ±0.25	48.8 ± 0.16	48.5 ± 0.22	45.9 ± 0.36	5.1 ± 0.23	2.1 ± 0.20	0
			(100 ±1.0)	(102 ± 0.6)	(101 ± 0.9)	(95 ± 1.4)	(11 ± 0.8)	(4 ± 0.8)	(0)
	A. flavipes	11	31.0 ± 0.82	31.1 ± 0.79	31.1 ± 0.78	31.1 ± 0.80	7.5 ± 0.82	1.6 ± 0.18	0
			(100 ± 2.4)	(100 ± 2.5)	(100 ± 2.5)	(100 ± 2.6)	(24 ± 2.6)	(5 ± 0.6)	(0)
	P. marginata	9	33.8 ± 0.32	34.3 ± 0.28	32.3 ± 0.31	31.1 ± 0.24	4.0 ± 0.22	1.8 ± 0.07	0
			(100 ± 1.8)	(102 ± 1.5)	(96 ± 1.1)	(92 ± 0.1)	(12 ± 1.1)	(6 ± 0.1)	(0)
	Schizophyllum sp.	7	44.3 ± 0.43	44.7 ± 0.45	44.8 ± 0.51	42.5 ± 0.55	31.0 ± 1.40	5.6 ± 0.19	0
			(100 ± 2.0)	(101 ± 2.0)	(101 ± 2.3)	(96 ± 2.5)	(70 ± 6.6)	(13 ± 6.0)	(0)
	P. blakesleeanus	2	59.5 ± 0.33	60.9 ± 0.25	60.3 ± 0.33	60.0 ± 0.32	47.7 ± 0.48	9.1 ± 0.42	0
			(100 ± 0.8)	(102 ± 0.4)	(101± 1.0)	(100 ± 0.7)	(80 ± 1.5)	(16 ± 0.5)	(0)
	F. oxysporum f. *conglutinans*	6	66.1 ± 0.80	66.1 ± 0.97	66.2 ± 0.84	65.9 ± 0.86	26.5 ± 1.54	14.8 ± 0.57	0
			(100 ± 1.2)	(100 ± 1.5)	(100 ± 1.3)	(100 ± 1.3)	(40 ± 3.3)	(22 ± 0.9)	(0)
	Chaetomium sp.	5	47.7 ± 0.15	47.1 ± 0.17	46.9 ±0.18	46.9 ± 0.17	36.6 ± 0.30	12.1 ± 0.28	0
			(100 ± 0.5)	(101 ± 0.4)	(100 ± 0.7)	(101 ± 0.7)	(78 ± 1.2)	(27 ± 1.1)	(0)

Group C (resistant)										
	R. stolonifer	1	46.7 ± 0.35	47.3 ± 0.42	46.3 ± 0.37	47.0 ± 0.33	32.5 ± 0.48	9.5 ± 0.46	2.8 ± 0.08
			(100 ± 1.3)	(101 ± 1.7)	(99 ± 1.4)	(101 ± 1.3)	(48 ± 2.1)	(20 ± 2.0)	(1 ± 0.1)
	T. viride	2	49.9 ± 0.57	49.2 ± 0.51	48.8 ± 0.34	50.3 ± 0.52	10.8 ± 0.28	3.4 ± 0.13	1.2 ± 0.07
			(100 ± 1.8)	(99 ± 1.8)	(98 ± 1.8)	(100 ± 1.9)	(22 ± 0.7)	(7 ± 0.1)	(2 ± 0.2)
	P. asperum	11	60.2 ± 0.69	59.6 ± 0.89	60.4 ± 0.99	59.6 ± 0.56	12.0 ± 0.99	7.6 ± 1.01	4.8 ± 0.60
			(100 ± 1.0)	(99 ± 1.5)	(100 ± 1.6)	(99 ± 0.9)	(20 ± 1.5)	(13 ± 1.6)	(8 ± 1.0)
	S. repens	11	44.3 ± 0.46	44.8 ± 0.76	44.7 ± 0.71	44.0 ± 0.47	38.4 ± 0.90	12.5 ± 0.49	6.3 ± 0.31
			(100 ± 1.0)	(101 ± 1.7)	(101 ± 1.6)	(99 ± 1.1)	(87 ± 2.0)	(27 ± 1.0)	(14 ± 0.7)
	C. echinulata	3	51.9 ± 0.28	53.3 ± 0.26	52.4 ± 0.28	52.0 ± 0.28	41.8 ± 0.25	30.8 ± 0.41	15.5 ± 0.30
			(100 ± 1.0)	(103 ± 0.8)	(101 ± 1.0)	(100 ± 1.0)	(80 ± 0.8)	(59 ± 1.6)	(30 ± 1.0)

[a] Mean diameter of growth ± standard error.
[b] Mean percentage of control ± standard error; based on control plates containing no Cd.

controls, respectively. At pH 9, 10 μg of Cd per ml reduced growth to 6% of the control (Fig. 13).

At pH 5, 10 μg of Cd per ml reduced the growth of *A. niger* to 63% of control, and, at pH 4 and 6, growth was reduced to 49 and 46% of controls, respectively. At pH 7, 8, and 9, 10 μg of Cd per ml reduced growth to 34, 23, and 10% of controls, respectively (Fig. 13).

DISCUSSION

The sequence of sensitivity to Cd of the bacteria studied was *M. chalcea* > *M. agilis* > *B. linens* > *R. meliloti* > *A. tumefaciens* > *C. orangum* > *B. megaterium* > *N. corallina* > *Corynebacterium* sp. > *A. faecalis* > *B. cereus* > *E. aerogenes* > *S. flavovirens* > *N. paraffinae* > *P. vulgaris*. In general, actinomycetes were more resistant to Cd than eubacteria, and gram-negative eubacteria were more tolerant to Cd than were gram-positive eubacteria. Other studies (7) have also indicated that gram-positive eubacteria are more sensitive to Cd than are gram-negative eubacteria.

The relative sensitivity to Cd of the filamentous fungi and yeasts studied was: *B. allii* > *P. vermiculatum* > *B. cinerea* > *A. fischeri* > *T. paradoxa* > *A. giganteus* > *A. janus* > *P. dispersa* > *F. annosus* > *A. niger* > *S. brevicaulis* > *A. flavipes* > *P. marginata* > *Schizophyllum* sp. > *P. blakesleeanus* > *F. oxysporum* f. *conglutinans* > *Chaetomium* sp. > *S. octosporus* > *S. cerevisiae*, *S. cerevisiae* var. *ellipsoides* > *Rhodotorula* sp. > *R. stolonifer* > *T. viride* > *P. asperum* > *S. repens* > *C. echinulata*. There was no correlation between the class of fungus and sensitivity to Cd.

The growth of *A. niger* was reduced on agar supplemented with 10 μg of Cd per ml, and with 100 μg of Cd per ml growth was slight, i.e., only 3% of the control. Other studies (9) have shown that growth of *A. niger* was initially reduced in broth amended with 10 μg of Cd per ml and was almost totally inhibited with 80 μg of Cd per ml. *B. cereus* grew in broth amended with 100 μg of Cd per ml, and other studies (9) showed that *B. cereus* was tolerant to 80 μg of Cd per ml, the highest concentration tested. *Rhodotorula* sp., the yeast found in these studies to be most tolerant to Cd, grew in the presence of 500 μg of Cd per ml, and other studies showed that several strains of *Rhodotorula* were more resistant to Cd than to other heavy metals, i.e., Ag, Co, Hg, Ni (1).

The toxicity of Cd to *B. cereus*, *A. faecalis*, *A. tumefaciens*, *N. paraffinae*, *A. niger*, *T. viride*, and *R. stolonifer* was enhanced at alkaline pH levels, whereas with *S. olivaceus*, the toxicity

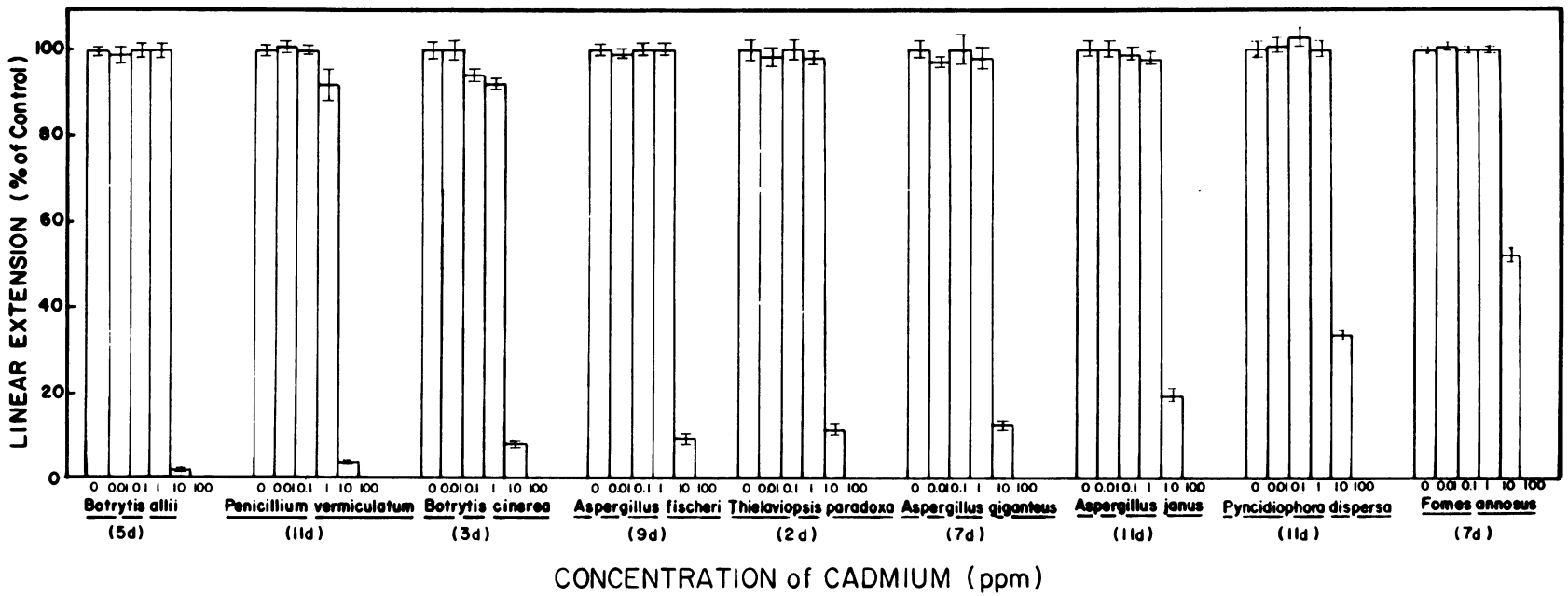

FIG. 7. *Fungi capable of growth on media amended with up to 10 μg of Cd per ml. No growth was observed on media amended with 100 μg of Cd per ml. Percentages were based on control plates which did not contain cadmium. Mean ± standard error of the mean.*

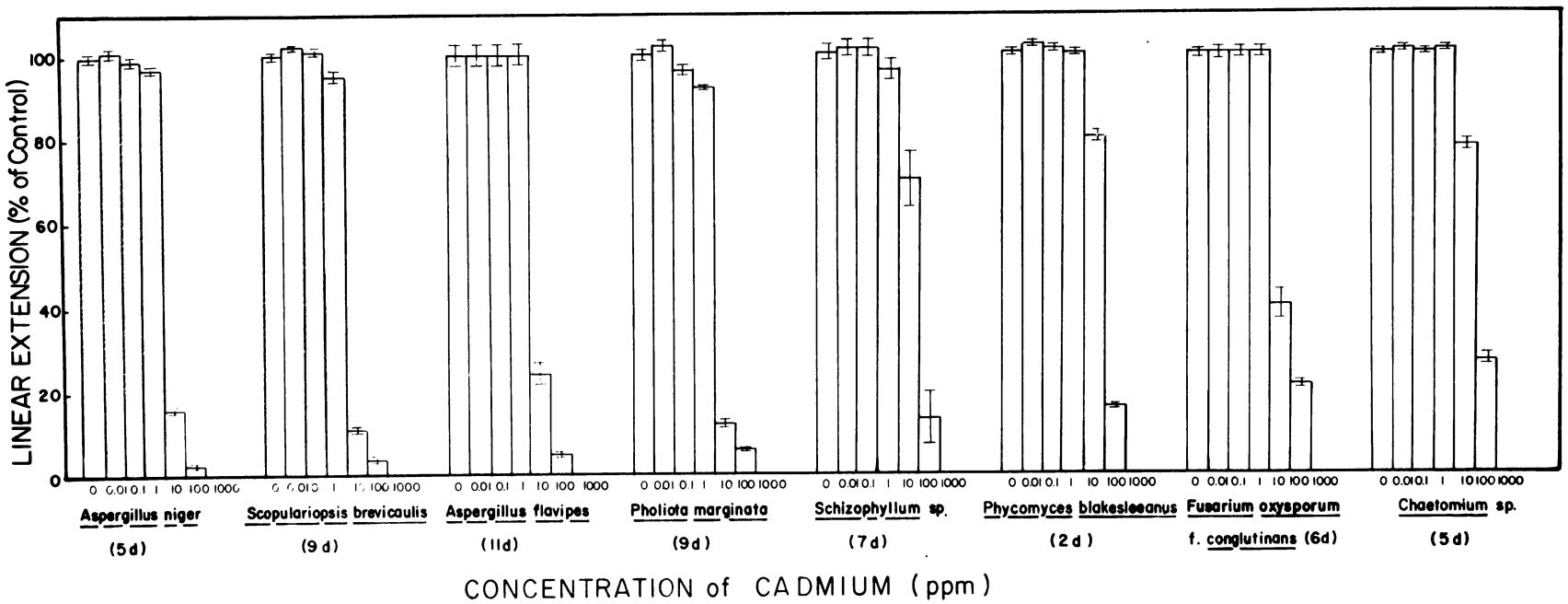

FIG. 8. *Fungi capable of growth on media amended with up to 100 μg of Cd per ml. No growth observed on media amended with 1,000 μg of Cd per ml. Percentages were based on control plates which did not contain cadmium. Mean ± standard error of the mean.*

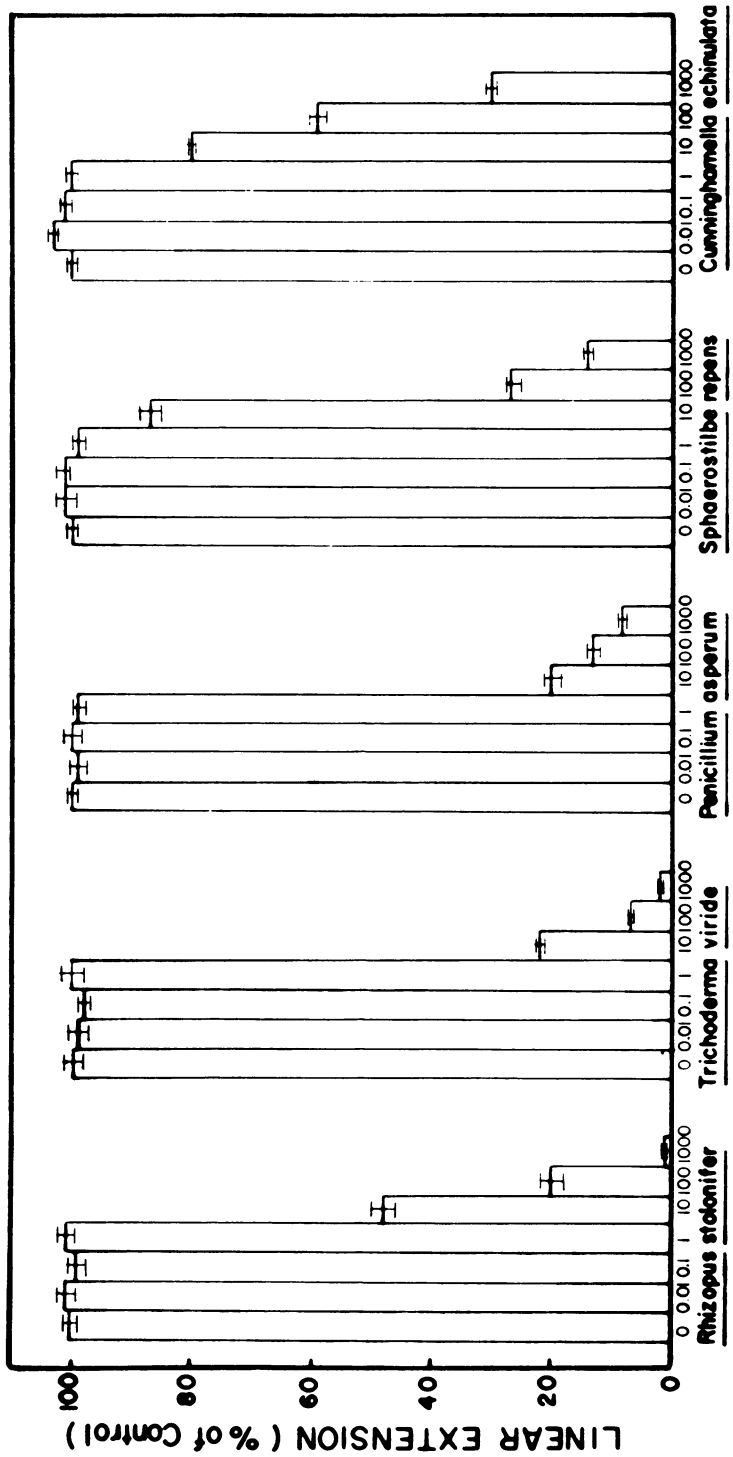

FIG. 9. *Fungi capable of growth on media amended with 1,000 μg of Cd per ml. Percentages were based on control plates which did not contain cadmium. Mean ± standard error of the mean.*

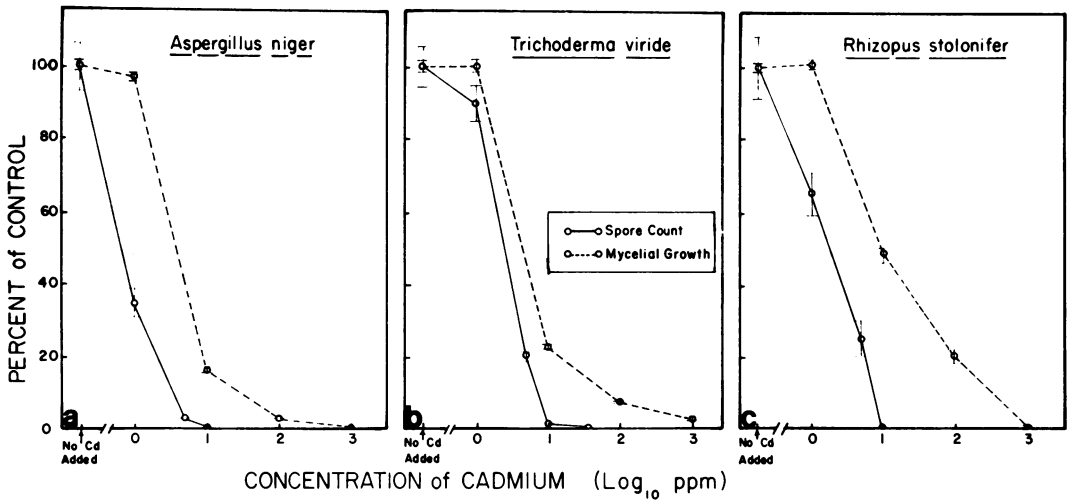

FIG. 10. *Comparative toxicity of cadmium to sporulation and mycelial growth of fungi. Percentages were based on controls which did not contain cadmium. Mean ± standard error of the mean.*

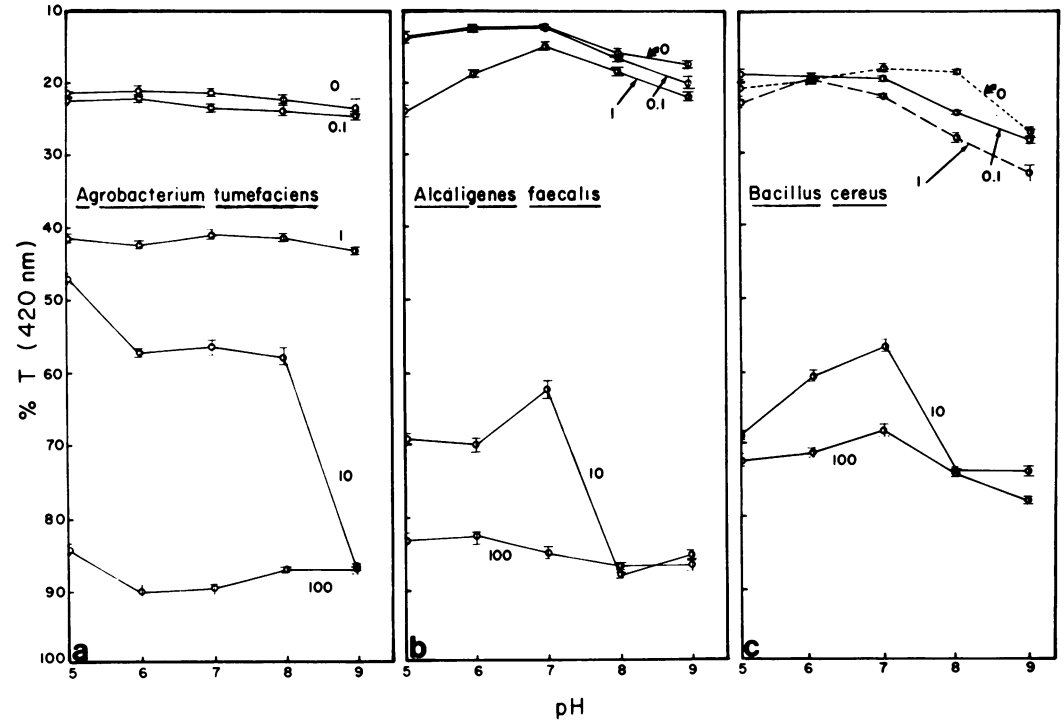

FIG. 11. *Effect of pH on the growth of bacteria in the presence of cadmium. Measurements of the percentage of transmittance were performed at 420 nm after 24 h of growth. Mean ± standard error of the mean.*

of Cd was independent of pH. Although the toxicity of Cd to the majority of microbes tested was potentiated at pH 8 or 9, it is unclear whether this increased toxicity was a reflection of the formation of complex ionic and molecular species of hydroxylated Cd. At pH 8 and below, in a pure aqueous system, Cd exists predominately as the free, divalent ion, Cd^{2+}. The formation of $CdOH^+$ begins at pH 7.5 and that of $Cd(OH)_2$ at pH 9. Thus, at pH 8, most of the Cd

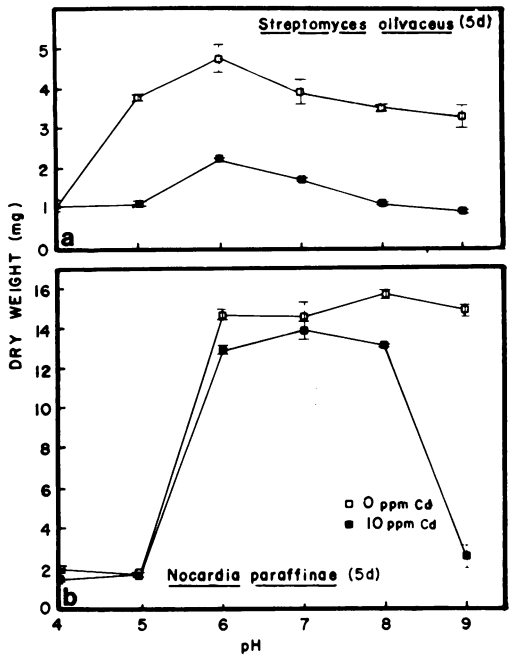

FIG. 12. *Effect of pH on growth of actinomycetes in the absence (□) or presence (■) of 10 μg of cadmium per ml. Dry weight determinations were performed after 5 days of incubation. Mean ± standard error of the mean.*

would be in the form of Cd^{2+}, with little $CdOH^+$ and no $Cd(OH)_2$, whereas at pH 9 the Cd^{2+} and $CdOH^+$ ionic species would predominate, with little $Cd(OH)_2$ being present (13). In addition, hydroxyl ions, in comparison to other anionic ligands, are poor competitors for the divalent Cd^{2+} cation (4), and the Cd^{2+} cation has a strong affinity for organic materials, such as yeast extract (19). Thus, in the complex system of a microbial growth medium, it is unclear whether substantial quantities of $CdOH^+$ or $Cd(OH)_2$ would be formed and, thus, possibly explain the increased Cd toxicity demonstrated at alkaline pH levels.

The wide range in Cd concentration used in these pure culture studies was comparable to the range occurring in polluted environments; e.g., soils near a smelting complex in South Wales contained 26 μg of Cd per g (12), whereas soil near a zinc smelter in Pennsylvania contained from 900 to 1,500 μg of Cd per g (6). The differential tolerances of microorganisms to Cd, in broth or on agar, may be indicative of their differential sensitivities in soil or other environments. For example, *R. meliloti* was relatively sensitive to Cd, as some inhibition was noted at 0.5 μg of Cd per ml, and total inhibition occurred at 50 μg of Cd per ml. Inhibition of species of *Rhizobium* in soils contaminated with Cd could hinder nodulation of leguminous plants, and, in fact, nodule formation by leguminous plants has been inhibited by application of Cd (14).

The adverse effect of Cd on microbial reproductive potential (e.g., reduction in growth rates and inhibition of fungal sporulation) could also influence the establishment, population dynamics and interactions, and general ecology of microbes in natural habitats. Exponential rates of growth of *A. tumefaciens* and *N. corallina* were extended by as little as 0.5 μg of Cd per ml. Other studies have shown that Cd increased the generation time of *E. coli* (17), *Ankistrodesmus falcatus* (G. Burnison, P. T. S. Wong, Y. K. Chau, and B. Silverberg, Proc. Can. Fed. Biol. Soc. 18:46, 1975), *Selanastrum capricornutum* (5), *Colpidium campylum*, *Vorticella microstoma*, and *Opercularia* sp. (22).

Sporulation was apparently more sensitive to Cd than mycelial proliferation, as spore forma-

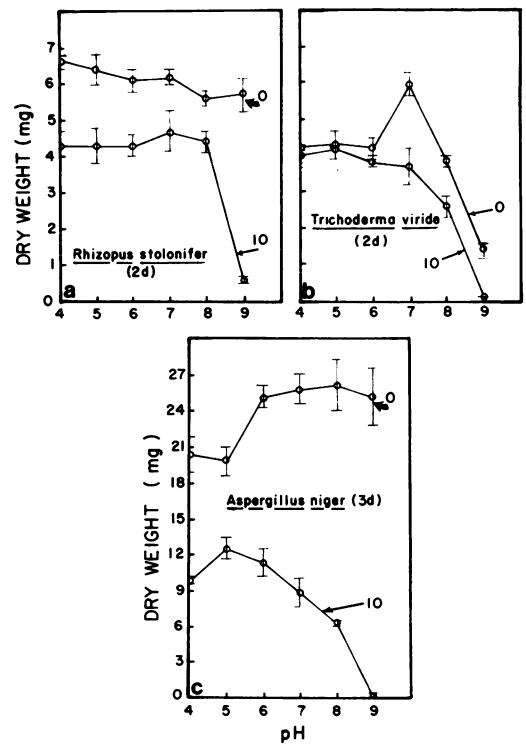

FIG. 13. *Effect of pH on the growth of fungi in the absence and presence of 10 μg of cadmium per ml. Dry weight determinations for R. stolonifer (a) and T. viride (b) were performed after 2 days of incubation and after 3 days of incubation for A. niger (c). Mean ± standard error of the mean.*

tion by *T. viride*, *A. niger*, and *R. stolonifer* was inhibited at concentrations of Cd that were not inhibitory to mycelial growth. Other studies have shown that Cd inhibits sporangial development of the marine fungus, *Thraustochytrium striatum* (20), inhibits mycelial growth of several ectomycorrhizal fungi (e.g., *Amanita muscaria*; J. D. McCreight and D. B. Schroeder, Phytopathology 64:583, 1974) and inhibits spore germination (e.g., of *A. niger*) (16). In assessing the toxicity of Cd to fungi, a distinction must be made between concentrations that are inhibitory to spore germination, to spore formation, and to mycelial proliferation.

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