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Abiotic Factors Affecting the Toxicity of Lead to Fungi

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The toxicity of lead (Pb) to fungi in pure culture was influenced by several abiotic factors: pH, inorganic anions, clay minerals, and particulate (humic acid) and soluble organic matter. The toxicity of Pb was potentiated under acidic conditions (pH 5 and 6), and phosphate or carbonate anions reduced the toxicity, apparently as a result of the formation of sparingly soluble Pb salts. Clay minerals (montmorillonite > attapulgite > kaolinite) and particulate humic acid protected against the toxicity of Pb, presumably as the result of sorption, by cation exchange, of the Pb to the exchange complexes, which reduced its availability for uptake by the fungi. Soluble organics, such as tryptone, yeast extract, cysteine, succinic acid, and increasing concentrations of neopeptone, also reduced the toxicity of Pb.

Industrialization and domestic activities have resulted in increased mobilization and deposition of heavy-metal pollutants, including lead (Pb), in natural habitats. Elevated concentrations of Pb in soils and vegetation have been attributed to Pb-containing products from the combustion of leaded gasoline (22), liquid and vapor wastes from coal-burning and metal-smelting activities, and Pb-arsenate pesticides and phosphate fertilizers (21). The average concentration of Pb in a variety of soils in Canada ranged from 100 μg/g in soil from rural areas, to 200 to 300 μg/g in soils from urban areas, to 300 to 400 μg/g in soils near highways, and up to 21,000 μg/g in soils near a secondary smelter (24). The average background concentration of Pb in soils from Western Canada was 9.1 μg/g, with a range from 0.6 to 180.4 μg/g, whereas soils near a battery factory had concentrations ranging from 106 to 59,580 μg/g (19). Elevated concentrations of Pb in soils near smelting complexes were also found at Palmerton, Pa. (8), and at Avonmouth, England (25).

Most studies of the toxicology of Pb have focused on human beings (15) and plants (20), and there have been few studies on the effects of Pb on microorganisms. Some bacteria appear to be capable of immobilizing substantial quantities of Pb in their cell walls/cell membranes without injurious effects on cell viability (42), and some strains of *Staphylococcus aureus* contain plasmids that confer resistance to several heavy metals, including Pb, as well as to penicillin (28). Other bacteria (36, 45) methylated Pb (added as trimethyl Pb acetate) to volatile tetramethyl Pb, which was inhibitory to growth and photosynthesis of algae. Inorganic Pb (i.e., Pb^{2+}) also inhibited growth and photosynthesis of marine algae (7, 26) and cyanobacteria (26), nitrogen fixation by cyanobacteria (17), germination of spores and mycelial growth of fungi (10, 37-39), and growth of protozoa (11, 33).

Heavy-metal pollutants, including Pb, also affect many subtle microbial activities and interactions. A concentration of 5 μmol of Pb per g of soil (as Pb acetate) inhibited nitrogen mineralization (23), whereas 121 μmol of Pb acetate per g of soil increased nitrification in a mull soil (43). The addition of 2 mg of Pb per g of soil (as Pb acetate), either coincident with or after amendment with maltose, starch, or sucrose, decreased microbial synthesis of α-glucosidase, amylase, and invertase, respectively (12). The microbiota of soils (L. M. Hartman, Am. J. Bot. **61**[Suppl.]: 23, 1974) and of the leaf surface of plants (14) from sites heavily contaminated with Pb and other heavy metals had a lower species diversity than did soils and plants from noncontaminated sites.

Studies with cadmium (2-4), zinc (5), and mercury (H. Babich and G. Stotzky, Crit. Rev. Microbiol., in press) have shown the influence of abiotic environmental factors on attenuating or potentiating the toxicity of heavy metals to microbes. As there is relatively little information on the responses of fungi to Pb (32) and on the influence of environmental factors on Pb toxicity, the effect of some physicochemical characteristics (i.e., pH, inorganic anionic composition, clay minerals, soluble and insoluble organic matter) on the toxicity of Pb to representative soil fungi in pure culture was studied.

MATERIALS AND METHODS

Source and maintenance of fungi. Filamentous fungi were obtained from the culture collection of the

Laboratory of Microbial Ecology at New York University. *Botrytis cinerea, Cunninghamella echinulata, Aspergillus giganteus, Aspergillus niger, Fusarium solani, Penicillium brefeldianum, Trichoderma viride,* and *Rhizoctonia solani* were grown and maintained on Sabouraud dextrose agar (Difco), pH 5.6, at 25°C.

Description of experiments. Fungi were grown on Sabouraud dextrose agar, and after incubation for several days at 25°C, circular plugs (3 to 5 mm in diameter) made with a sterile metal cork borer were transferred, with the fungal growth up, to the center of petri dishes containing a minimal nutrient agar (1.0% glucose, 0.5% neopeptone, 0.1% NH_4NO_3, and 1.5% agar [Difco], adjusted to pH 5 with HCl) unamended or amended with Pb as $Pb(NO_3)_2$. To study the influence of pH on Pb toxicity, the medium was adjusted to pH 5 (with HCl), 6, 7, 8, or 9 (with NaOH). To determine the influence of phosphate (PO_4^{3-}) and carbonate (CO_3^{2-}) anions on Pb toxicity, the medium was supplemented with 10^{-3} M Na_2HPO_4 or Na_2CO_3. In some studies, the clay minerals kaolinite (Kaolin, Fisher Scientific Co.), montmorillonite (Bentonite, Fisher Scientific Co.), and attapulgite (Attagel, Minerals and Chemicals, Phillipp Corp.) were added to the medium, unamended and amended with Pb. The cation-exchange capacity of the clays, in milliequivalents per 100 g of clay, was 5.8 for kaolinite, 34.0 for attapulgite, and 97.7 for montmorillonite (41). In other studies, soluble organics (peptone, neopeptone, tryptone, yeast extract [all Difco], cysteine, and succinic acid) and insoluble particulate organic matter (humic acid [Aldrich Chemical Co., Inc.]) were added to the medium, with and without Pb.

After various periods of incubation at 25°C, the diameters of mycelial growth (in four directions) and the radial growth rates (in millimeters per day) were determined (5). Mycelial growth was usually measured on days 2 and 3 after inoculation for *B. cinerea, C. echinulata,* and *T. viride,* on days 3 and 4 for *R. solani* and *A. niger,* and on days 3 and 5 for *F. solani, A. giganteus,* and *P. brefeldianum*; however, measurements of growth were made after longer time intervals on agars containing inhibitory concentrations of Pb. Three petri dishes were used for each concentration of Pb and all other permutations, and each experiment was repeated at least once. The data are expressed as the mean ± standard error of the mean.

RESULTS AND DISCUSSION

R. solani was the most sensitive fungus to Pb, as mycelial growth rates were initially reduced at 10 μg of Pb per ml and progressively decreased as the concentration of Pb was increased until, at 500 μg of Pb per ml, no growth occurred (Table 1). Mycelial growth rates of *A. giganteus* were initially reduced at 50 μg of Pb per ml, and no growth was evident at 500 μg of Pb per ml. *F. solani, T. viride,* and *C. echinulata* exhibited significant decreases in mycelial growth at 100 μg of Pb per ml, and limited growth of *T. viride* and *C. echinulata,* but not of *F. solani,* was evident at 1,000 μg of Pb per ml. *B. cinerea* and

TABLE 1. *Influence of lead on mycelial growth of fungi*

Fungus	Mycelial growth rate (mm/day)[a] at Pb concn (μg/ml) of:					
	0	10	50	100	500	1,000
Rhizoctonia solani	8.4 ± 0.46 (100 ± 5.5)[b]	6.6 ± 0.36 (79 ± 4.3)	3.0 ± 0.52 (36 ± 6.2)	0.6 ± 0.14 (7 ± 1.7)	0 (0)	0 (0)
Aspergillus giganteus	4.8 ± 0.10 (100 ± 2.1)	4.7 ± 0.27 (98 ± 5.6)	2.2 ± 0.15 (46 ± 3.2)	1.0 ± 0.09 (21 ± 1.8)	0 (0)	0 (0)
Fusarium solani	4.9 ± 0.20 (100 ± 4.0)	5.2 ± 0.08 (104 ± 1.6)	4.8 ± 0.09 (98 ± 1.9)	3.4 ± 0.33 (69 ± 6.9)	0.8 ± 0.08 (16 ± 1.6)	0 (0)
Trichoderma viride	13.1 ± 0.16 (100 ± 1.1)	13.1 ± 0.33 (100 ± 2.4)	12.3 ± 0.22 (94 ± 1.7)	10.9 ± 0.13 (83 ± 1.0)	2.0 ± 0.15 (15 ± 1.8)	1.7 ± 0.15 (13 ± 1.1)
Cunninghamella echinulata	9.0 ± 0.54 (100 ± 6.0)	8.5 ± 0.24 (95 ± 2.7)	8.2 ± 0.08 (91 ± 0.8)	6.0 ± 0.21 (67 ± 2.3)	1.1 ± 0.18 (12 ± 2.0)	0.6 ± 0.29 (7 ± 3.2)
Botrytis cinerea	9.3 ± 0.38 (100 ± 3.7)	10.1 ± 0.17 (109 ± 1.8)	9.8 ± 0.37 (106 ± 4.0)	1.3 ± 0.33 (96 ± 3.6)	1.3 ± 0.29 (14 ± 3.2)	0.4 ± 0.08 (4 ± 0.9)
Penicillium brefeldianum	2.6 ± 0.05 (100 ± 1.8)	2.6 ± 0.08 (100 ± 3.2)	2.7 ± 0.05 (102 ± 2.0)	2.6 ± 0.08 (100 ± 3.2)	1.1 ± 0.13 (44 ± 5.0)	0.3 ± 0.11 (10 ± 4.3)
Aspergillus niger	6.0 ± 0.16 (100 ± 2.6)	6.1 ± 0.13 (103 ± 2.2)	6.0 ± 0.14 (100 ± 2.4)	5.7 ± 0.21 (95 ± 3.5)	5.8 ± 0.15 (98 ± 2.5)	6.2 ± 0.16 (105 ± 2.7)

[a] Mean radial growth ± standard error of the mean.
[b] Number in parentheses is mean percentage of control ± standard error of the mean.

P. brefeldianum exhibited reduced growth initially at 500 μg of Pb per ml, and *A. niger* was not inhibited by 1,000 μg of Pb per ml. The relative sensitivity to Pb of the fungi was *R. solani* > *A. giganteus* > *F. solani* > *T. viride*, *C. echinulata*, *B. cinerea* > *P. brefeldianum* > *A. niger* (Table 1). The mycelial growth of some phylloplane fungi, e.g., *Pleurophomella* sp., *Epicoccum* sp., *Pestalotiopsis* sp., and *Aureobasidium pullulans*, was also inhibited by Pb at comparable concentrations (37, 38).

The pH of the medium influenced the response of the fungi to Pb: the toxicity of 1,000 μg of Pb per ml to *T. viride* progressively decreased as the pH was increased from 5 to 9, and the toxicity of 2,000 μg of Pb per ml to *A. niger* was greatest at pH 5 and 6 and was reduced at pH 7, 8, or 9 (Table 2). The inorganic form of Pb is pH dependent: at pH 5 and below, Pb exists as the divalent cation, Pb^{2+}; at pH 6, approximately 50% exists as Pb^{2+} and 50% as the monohydroxylated species, $PbOH^+$; at pH 7, approximately 70% occurs as $PbOH^+$ and 30% as Pb^{2+}; and at pH 8 to 9, almost all the Pb exists as $PbOH^+$ (16). Polynuclear coordination complexes, e.g., Pb_2OH^{3+}, $Pb_3(OH)_4^{2+}$, $Pb_4(OH)_4^{4+}$, and $Pb_6(OH)_8^{4+}$, also form at alkaline pH levels (29, 30). The reduction in the toxicity of Pb to *T. viride* and *A. niger* as the pH of the medium was increased may have reflected the differential toxicities of Pb^{2+} and $PbOH^+$ and the polynuclear complexes, with the latter species being less toxic. However, the decreases in toxicity with increases in pH may also have been a result of the more efficient competition of Pb (whether as Pb^{2+}, $PbOH^+$, or polynuclear complexes) with protons (H^+) for the organic matter in the medium (which became more negatively charged as the pH was increased), and the complexed forms of Pb were less toxic than the free forms (Babich and Stotzky, in press).

TABLE 2. *Influence of pH on the toxicity of lead to mycelial growth of fungi*

Fungus	Treatment	Growth rate (mm/day)[a]	% of control[b]
Trichoderma viride (Pb, 1,000 μg/ml)	pH 5; no Pb	17.9 ± 0.37	100 ± 2.1
	pH 5; Pb	1.3 ± 0.14	7 ± 0.8
	pH 6; no Pb	16.6 ± 0.14	100 ± 0.8
	pH 6; Pb	2.4 ± 0.59	15 ± 3.6
	pH 7; no Pb	15.8 ± 0.38	100 ± 2.4
	pH 7; Pb	3.3 ± 0.15	20 ± 0.9
	pH 8; no Pb	14.2 ± 0.41	100 ± 2.9
	pH 8; Pb	4.3 ± 0.40	30 ± 2.8
	pH 9; no Pb	12.8 ± 0.66	100 ± 5.2
	pH 9; Pb	7.8 ± 0.27	61 ± 2.1
Aspergillus niger (Pb, 2,000 μg/ml)	pH 5; no Pb	6.4 ± 0.13	100 ± 1.9
	pH 5; Pb	1.6 ± 0.71	25 ± 11.2
	pH 6; no Pb	7.1 ± 0.29	100 ± 4.0
	pH 6; Pb	2.2 ± 0.75	31 ± 10.5
	pH 7; no Pb	6.9 ± 0.12	100 ± 1.8
	pH 7; Pb	4.6 ± 0.09	67 ± 1.3
	pH 8; no Pb	6.7 ± 0.10	100 ± 1.5
	pH 8; Pb	4.3 ± 0.28	66 ± 5.6
	pH 9; no Pb	6.8 ± 0.18	100 ± 2.6
	pH 9; Pb	4.4 ± 0.09	64 ± 1.3

[a] Mean radial growth ± standard error of the mean.
[b] Mean percentage of control ± standard error of the mean (control = no Pb at an equivalent pH).

The addition of 10^{-3} M PO_4^{3-} or CO_3^{2-} to the medium reduced the toxicity of 100 μg of Pb per ml to *F. solani* and *A. giganteus*, with the greater reductions occurring with PO_4^{3+} (Table 3), presumably as the result of the formation of sparingly soluble $Pb_3(PO_4)_2$ and $PbCO_3$. A white precipitate was evident upon addition of PO_4^{3-} or CO_3^{2-} to the Pb-amended medium. The toxicity of Pb to growth of *Chlamydomonas reinhardtii* was also reduced by the incorporation of PO_4^{3-} into the medium (35), and the toxicity of Pb to *Tetrahymena pyriformis* was greater in soft (20 mg of $CaCO_3$ per liter) than in hard (400 mg of $CaCO_3$ per liter) water (11), possibly due to the formation of greater quantities of $PbCO_3$ in the hard water. Similarly, the sparingly soluble Pb salts, PbO, PbS, and $PbCO_3 \cdot Pb(OH)_2$, were not toxic to *A. niger*, whereas equivalent concentrations of free Pb^{2+} were highly toxic (46). Apparently, Pb in the form of sparingly soluble inorganic salts is less available for uptake by microbes than is free Pb.

The toxicity of 1,500 μg of Pb per ml towards the mycelial growth of *T. viride* and *C. echinulata* was reduced by the incorporation of 1% (wt/vol) attapulgite or montmorillonite, but not of kaolinite, into the medium. Increasing the concentrations of the clays from 1 to 3% progressively reduced the toxicity of 250 μg of Pb per ml to *A. giganteus*, *R. solani*, *F. solani*, and *C. echinulata*, and at equivalent concentrations of clay, the sequence of protection was montmorillonite > attapulgite > kaolinite (Table 4). Kaolinite, attapulgite, illite, and montmorillonite have been shown to adsorb Pb and, thereby, remove the metal from solution (13, 27). By exchanging the Pb in the medium for the cations (e.g., Ca^{2+}, Mg^{2+}, K^+, Na^+, H^+) on the exchange complex of the clays, the amount of free Pb available for uptake by the fungi was reduced. Montmorillonite and, to a lesser extent, kaolinite also provided protection to bacteria, including

TABLE 3. *Influence of carbonate and phosphate on the toxicity of lead (100 μg/ml) to mycelial growth of fungi*

Treatment	Mycelial growth rate[a] (% of control[b])	
	Fusarium solani	*Aspergillus giganteus*
No Pb	4.9 ± 0.09 (100 ± 1.8)	5.4 ± 0.21 (100 ± 3.9)
Pb	3.6 ± 0.07 (73 ± 1.8)	1.2 ± 0.10 (22 ± 1.9)
10^{-3} M Na_2CO_3 + no Pb	4.3 ± 0.21 (100 ± 4.9)	3.3 ± 0.14 (100 ± 4.2)
10^{-3} M Na_2CO_3 + Pb	3.9 ± 0.06 (92 ± 1.4)	1.9 ± 0.10 (57 ± 3.1)
10^{-3} M Na_2HPO_4 + no Pb	4.4 ± 0.26 (100 ± 5.8)	1.9 ± 0.21 (100 ± 11.2)
10^{-3} M Na_2HPO_4 + Pb	4.2 ± 0.37 (96 ± 8.4)	2.1 ± 0.06 (111 ± 3.2)

[a] Mean radial growth, in millimeters per day, ± standard error of the mean.
[b] Mean percentage of control ± standard error of the mean (control = no Pb and an equivalent concentration of phosphate or carbonate).

TABLE 4. *Influence of increasing concentrations of kaolinite (K), attapulgite (A), or montmorillonite (M) on the toxicity of lead (250 μg/ml) to mycelial growth of fungi*

Treatment	Mycelial growth rate[a] (% of control[b])			
	Aspergillus giganteus	*Rhizoctonia solani*	*Fusarium solani*	*Cunninghamella echinulata*
No Pb	4.9 ± 0.15 (100 ± 3.7)	7.1 ± 0.33 (100 ± 4.7)	4.9 ± 0.11 (100 ± 2.5)	8.0 ± 0.36 (100 ± 4.5)
Pb	0.4 ± 0.11 (9 ± 2.8)	0 (0)	2.0 ± 0.20 (40 ± 4.0)	1.8 ± 0.09 (23 ± 1.1)
1% K + no Pb	5.1 ± 0.26 (100 ± 5.0)	7.3 ± 0.38 (100 ± 5.3)	5.1 ± 0.11 (100 ± 2.2)	7.1 ± 0.11 (100 ± 1.6)
1% K + Pb	1.8 ± 0.15 (32 ± 2.4)	0.6 ± 0.18 (9 ± 2.5)	2.3 ± 0.13 (45 ± 2.5)	2.1 ± 0.08 (29 ± 1.1)
2% K + no Pb	5.5 ± 0.19 (100 ± 3.5)	7.7 ± 0.62 (100 ± 8.0)	4.8 ± 0.09 (100 ± 1.9)	7.3 ± 0.19 (100 ± 2.6)
2% K + Pb	2.1 ± 0.12 (38 ± 2.3)	1.4 ± 0.28 (17 ± 3.6)	2.6 ± 0.12 (53 ± 2.5)	2.8 ± 0.16 (38 ± 2.2)
3% K + no Pb	5.5 ± 0.19 (100 ± 3.4)	7.5 ± 0.54 (100 ± 7.3)	5.0 ± 0.12 (100 ± 2.3)	7.8 ± 0.20 (100 ± 2.5)
3% K + Pb	2.3 ± 0.08 (41 ± 1.4)	1.7 ± 0.28 (22 ± 3.7)	2.8 ± 0.15 (55 ± 3.0)	3.4 ± 0.17 (44 ± 2.1)
1% A + no Pb	6.0 ± 0.10 (100 ± 1.7)	7.0 ± 0.32 (100 ± 4.6)	5.2 ± 0.05 (100 ± 1.0)	8.8 ± 0.25 (100 ± 2.9)
1% A + Pb	4.2 ± 0.24 (70 ± 4.0)	2.0 ± 0.29 (29 ± 4.1)	3.4 ± 0.12 (66 ± 2.3)	5.1 ± 0.39 (58 ± 4.4)
2% A + no Pb	5.2 ± 0.37 (100 ± 7.1)	8.6 ± 0.19 (100 ± 2.2)	5.1 ± 0.05 (100 ± 0.9)	7.5 ± 0.18 (100 ± 2.4)
2% A + Pb	5.1 ± 0.28 (99 ± 5.3)	3.0 ± 0.26 (35 ± 3.1)	5.0 ± 0.03 (97 ± 0.6)	6.4 ± 0.36 (85 ± 4.8)
3% A + no Pb	4.7 ± 0.12 (100 ± 2.6)	8.0 ± 0.24 (100 ± 2.9)	5.1 ± 0.14 (100 ± 2.8)	7.5 ± 0.14 (100 ± 1.9)
3% A + Pb	4.9 ± 0.06 (104 ± 1.3)	3.4 ± 0.15 (43 ± 2.0)	5.4 ± 0.08 (105 ± 1.6)	7.3 ± 0.28 (97 ± 3.7)
1% M + no Pb	6.0 ± 0.12 (100 ± 2.1)	7.2 ± 0.49 (100 ± 6.7)	5.2 ± 0.09 (100 ± 1.7)	8.2 ± 0.42 (100 ± 5.2)
1% M + Pb	4.9 ± 0.12 (81 ± 1.9)	2.8 ± 0.22 (39 ± 3.0)	5.0 ± 0.03 (97 ± 0.6)	7.7 ± 0.32 (94 ± 3.9)
2% M + no Pb	5.4 ± 0.26 (100 ± 4.8)	7.1 ± 0.53 (100 ± 7.5)	5.2 ± 0.07 (100 ± 1.3)	6.5 ± 0.15 (100 ± 2.4)
2% M + Pb	5.2 ± 0.08 (96 ± 1.5)	4.5 ± 0.35 (63 ± 4.9)	5.2 ± 0.03 (100 ± 0.6)	6.9 ± 0.19 (106 ± 3.0)
3% M + no Pb	4.7 ± 0.05 (100 ± 1.1)	5.9 ± 0.68 (100 ± 11.4)	5.2 ± 0.06 (100 ± 1.1)	6.3 ± 0.33 (100 ± 5.2)
3% M + Pb	4.8 ± 0.11 (103 ± 2.3)	4.6 ± 0.11 (78 ± 1.9)	5.2 ± 0.07 (100 ± 1.3)	6.9 ± 0.20 (110 ± 3.1)

[a] Mean radial growth, in millimeters per day, ± standard error of the mean.
[b] Mean percentage of control ± standard error of the mean (control = no Pb + an equivalent concentration of clay).

actinomycetes, and fungi against inhibitory levels of cadmium (3).

To determine the mechanism whereby the clays differentially protected the fungi against the inhibitory effects of Pb, the concentrations of the clays were converted to reflect their cation-exchange capacity. The sequence of protection provided by the clays was correlated with the sequence of the cation-exchange capacity of the clays (Fig. 1). The curve of *F. solani* (which was essentially the same as those for *C. echinulata* and *A. giganteus*) showed typical saturation kinetics: i.e., as the cation-exchange capacity was increased, more Pb was adsorbed on the exchange complex of the clays until a point was reached at which the concentration of free Pb inhibitory to fungal growth was reduced and further increases in cation-exchange capacity did

not provide additional protection. With *R. solani*, the fungus most sensitive to Pb, the cation-exchange capacity of the medium was not adequate to sequester completely the inhibitory quantities of free Pb.

Incorporation of particulate humic acid into

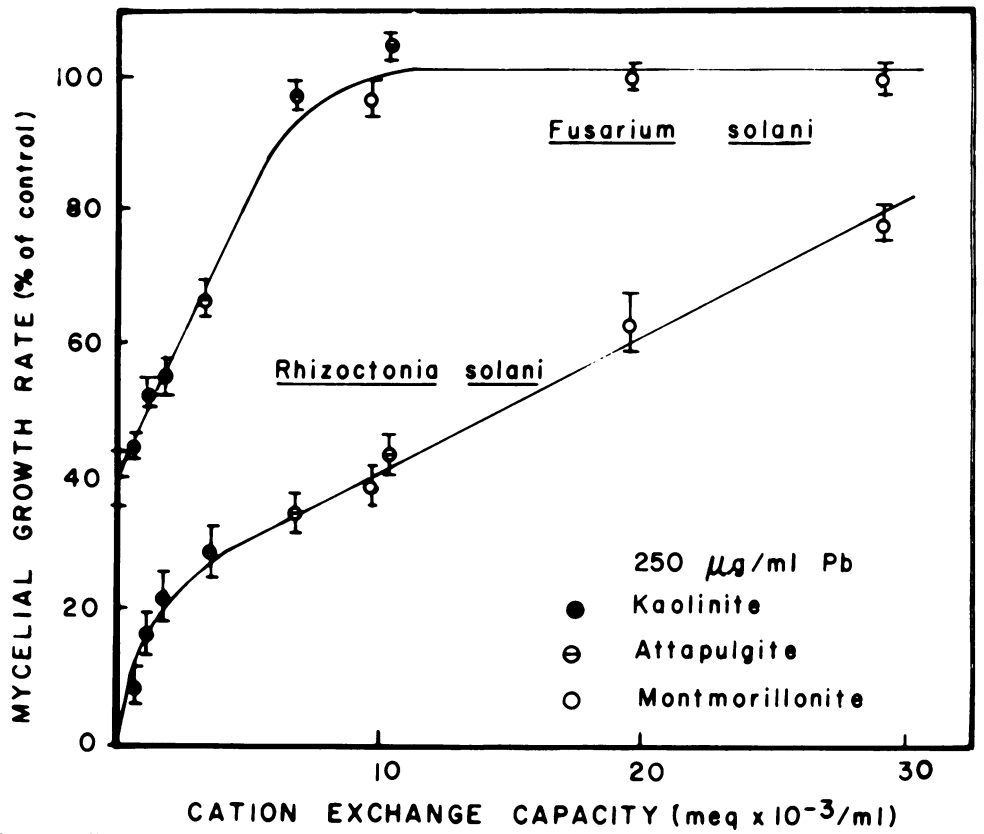

FIG. 1. *Effect of cation-exchange capacity on the growth of Fusarium solani and Rhizoctonia solani in the presence of 250 µg of Pb per ml, as $Pb(NO_3)_2$. Lead was added in combination with 0, 1, 2, or 3% kaolinite, attapulgite, or montmorillonite. Percentages for the Pb-clay studies were based on control plates which contained an equivalent concentration of clay but no Pb. Data are presented as the mean percentage of the control ± standard error of the mean.*

TABLE 5. *Influence of increasing concentrations of humic acids (HA) on the toxicity of lead (750 µg/ml) to mycelial growth of fungi*

Treatment	Mycelial growth rate[a] (% of control[b])							
	Rhizoctonia solani		*Aspergillus giganteus*		*Fusarium solani*		*Cunninghamella echinulata*	
No Pb	8.5 ± 0.42	(100 ± 4.8)	4.3 ± 0.34	(100 ± 8.0)	4.4 ± 0.06	(100 ± 1.4)	8.3 ± 0.17	(100 ± 2.1)
Pb	0	(0)	0	(0)	0	(0)	0.4 ± 0.07	(5 ± 0.8)
0.1% HA + no Pb	10.1 ± 0.46	(100 ± 4.5)	5.7 ± 0.29	(100 ± 5.2)	5.5 ± 0.16	(100 ± 2.9)	7.8 ± 0.28	(100 ± 3.6)
0.1% HA + Pb	0	(0)	0.2 ± 0.09	(4 ± 1.6)	1.0 ± 0.11	(18 ± 2.0)	1.4 ± 0.07	(18 ± 0.9)
0.3% HA + no Pb	10.4 ± 0.52	(100 ± 5.3)	6.5 ± 0.13	(100 ± 2.1)	5.4 ± 0.05	(100 ± 0.8)	8.7 ± 0.06	(100 ± 0.7)
0.3% HA + Pb	1.1 ± 0.16	(11 ± 1.5)	3.0 ± 0.22	(47 ± 0.2)	5.0 ± 0.08	(93 ± 1.5)	7.9 ± 0.24	(91 ± 2.8)
0.5% HA + no Pb	9.6 ± 0.58	(100 ± 6.1)	6.6 ± 0.26	(100 ± 3.8)	5.3 ± 0.05	(100 ± 1.1)	8.8 ± 0.14	(100 ± 3.1)
0.5% HA + Pb	5.7 ± 0.30	(59 ± 3.1)	4.9 ± 0.15	(74 ± 2.3)	5.7 ± 0.04	(105 ± 0.8)	8.3 ± 0.14	(93 ± 1.3)
0.7% HA + no Pb	8.9 ± 0.56	(100 ± 6.2)	6.7 ± 0.16	(100 ± 2.4)	5.4 ± 0.04	(100 ± 0.7)	8.2 ± 0.17	(100 ± 1.5)
0.7% HA + Pb	7.3 ± 0.28	(82 ± 3.1)	5.4 ± 0.20	(81 ± 2.9)	5.6 ± 0.05	(103 ± 0.9)	8.2 ± 0.27	(100 ± 3.2)

[a] Mean radial growth, in millimeters per day, ± standard error of the mean.
[b] Mean percentage of control ± standard error of the mean (control = no Pb + an equivalent concentration of humic acid).

the culture medium reduced the toxicity of Pb: 0.5% (wt/vol) humic acid reduced the inhibitory or lethal effects of 250 μg of Pb per ml to *R. solani*, *A. giganteus*, *F. solani*, and *P. brefeldianum*, and 1.0% humic acid reduced the inhibitory effects of 1,500 μg of Pb per ml to *T. viride* and *C. echinulata*. Increasing the concentration of humic acid in the medium from 0.1 to 0.7%

TABLE 6. *Influence of cysteine and succinic acid on the toxicity of lead to mycelial growth of fungi*

Treatment	Mycelial growth rate[a] (% of control[b])			
	Fusarium solani	*Aspergillus giganteus*	*Rhizoctonia solani*	*Cunninghamella echinulata*
No cysteine				
No Pb	4.9 ± 0.9 (100 ± 5.7)	5.4 ± 0.21 (100 ± 3.9)		
Pb, 100 μg/ml	3.6 ± 0.7 (73 ± 1.5)	1.2 ± 0.10 (22 ± 1.9)		
Cysteine, 10^{-3} M				
No Pb	4.5 ± 1.0 (100 ± 2.2)	5.3 ± 0.26 (100 ± 5.0)		
Pb, 100 μg/ml	4.1 ± 0.09 (91 ± 2.0)	1.7 ± 0.12 (33 ± 2.3)		
No succinic acid				
No Pb	4.8 ± 0.09 (100 ± 1.9)	4.1 ± 0.20 (100 ± 4.8)	6.0 ± 0.34 (100 ± 5.7)	8.6 ± 0.29 (100 ± 3.4)
Pb, 200 μg/ml	1.9 ± 0.34 (40 ± 7.1)	0.9 ± 0.12 (23 ± 3.0)	0 (0)	3.6 ± 0.20 (42 ± 1.6)
Succinic acid, 10^{-2} M				
No Pb	4.7 ± 0.17 (100 ± 3.6)	4.6 ± 0.16 (100 ± 3.5)	4.5 ± 0.30 (100 ± 5.7)	9.4 ± 0.12 (100 ± 1.3)
Pb, 200 μg/ml	4.8 ± 0.12 (102 ± 2.6)	2.5 ± 0.23 (54 ± 4.9)	1.9 ± 0.14 (42 ± 3.1)	6.9 ± 0.09 (76 ± 2.4)

[a] Mean radial growth, in millimeters per day, ± standard error of the mean.
[b] Mean percentage of control ± standard error of the mean (control = no Pb and with or without cysteine or succinic acid).

TABLE 7. *Influence of increasing concentrations of neopeptone on the toxicity of lead to mycelial growth of fungi*

Fungus	Treatment	Mycelial growth rate (mm/day)[a]	% of control[b]
Aspergillus giganteus (Pb, 50 μg/ml)	0.5% neopeptone + no Pb	6.6 ± 0.40	100 ± 6.0
	0.5% neopeptone + Pb	4.0 ± 0.20	60 ± 3.0
	1.0% neopeptone + no Pb	7.0 ± 0.12	100 ± 1.7
	1.0% neopeptone + Pb	6.1 ± 0.07	87 ± 1.0
	1.5% neopeptone + no Pb	7.9 ± 0.13	100 ± 1.7
	1.5% neopeptone + Pb	7.4 ± 0.31	93 ± 3.9
Cunninghamella echinulata (Pb, 100 μg/ml)	0.5% neopeptone + no Pb	7.5 ± 0.20	100 ± 2.6
	0.5% neopeptone + Pb	4.9 ± 0.12	66 ± 1.6
	1.0% neopeptone + no Pb	9.4 ± 0.32	100 ± 3.4
	1.0% neopeptone + Pb	7.5 ± 0.36	80 ± 3.9
	1.5% neopeptone + no Pb	9.3 ± 0.26	100 ± 2.8
	1.5% neopeptone + Pb	9.4 ± 0.15	101 ± 1.7

[a] Mean radial growth ± standard error of the mean.
[b] Mean percentage of control ± standard error of the mean (control = no Pb + an equivalent concentration of neopeptone).

TABLE 8. *Influence of different soluble, complex organic nutrients on the toxicity of lead (100 μg/ml) to mycelial growth of Cunninghamella echinulata*

Organic amendment	Mycelial growth rate[a] (% of control[b])	
	No Pb	Pb
Neopeptone	7.5 ± 0.20 (100 ± 2.6)	4.9 ± 0.12 (66 ± 1.6)
Peptone	8.8 ± 0.33 (100 ± 3.7)	5.3 ± 0.12 (61 ± 1.4)
Tryptone	6.8 ± 0.20 (100 ± 3.0)	6.3 ± 0.48 (92 ± 8.6)
Yeast extract	10.4 ± 0.28 (100 ± 2.7)	9.3 ± 0.27 (90 ± 2.6)

[a] Mean radial growth, in millimeters per day, ± standard error of the mean.

[b] Mean percentage of control ± standard error of the mean (control = no Pb + the equivalent organic amendment [0.5%, wt/vol]).

progressively decreased the toxicity of 750 μg of Pb per ml towards the mycelial growth of *R. solani*, *A. giganteus*, *F. solani*, and *C. echinulata* (Table 5). Humic acids have a strong affinity for Pb and can bind substantial quantities, apparently primarily by cation exchange (9, 40). The mechanism of protection provided by humic acid against the toxicity of Pb may have been similar to that of the clay minerals, i.e., adsorption to the exchange complex with subsequent removal, at least temporarily, of Pb from solution and, therefore, limiting of the availability of Pb for uptake by the fungi.

The addition of 10^{-3} M cysteine to the medium reduced the toxicity of 100 μg of Pb per ml to *F. solani* and *A. giganteus* (Table 6). As Pb has a strong affinity for sulfhydryl groups (44), the Pb probably reacted with the cysteine, as evidenced by the darkening of the medium, and Pb complexed to cysteine was less toxic than was free Pb.

The addition of 10^{-2} M succinic acid to the medium also reduced the inhibitory or lethal effect of 200 μg of Pb per ml to *R. solani*, *F. solani*, *A. giganteus*, and *C. echinulata* (Table 6), probably as a result of the chelation of Pb by the dicarboxylic acid. Chelated forms of Pb were also less toxic to growth of *A. niger* (46) and *C. reinhardtii* (35) and were less readily taken up by *Phaeodactylum tricornutum* (34) than was free Pb.

When the concentration of neopeptone in the medium was increased from 0.5 to 1.5%, the toxicity of 50 and 100 μg of Pb per ml to *A. giganteus* and *C. echinulata*, respectively, was decreased (Table 7). When 0.5% peptone was substituted for an equivalent concentration of neopeptone, the toxicity of 100 μg of Pb per ml to *C. echinulata* was slightly increased, but the toxicity was greatly reduced when 0.5% tryptone or yeast extract was substituted for neopeptone (Table 8). Other studies have also shown that soluble organics react with heavy metals and that different organics have differential affinities for the same heavy metal (31).

The present studies, as well as those of others, were conducted in laboratory media and not in a natural microbial environment; however, the results indicate that abiotic environmental factors (e.g., pH, inorganic anions, clay minerals, particulate and soluble organic matter) have the potential to influence the toxicity of Pb, and probably of other heavy metals, to microbes in natural habitats. Thus, in assessing and predicting the toxicity of pollutants to the microbiota, and to the biota in general, attention must be focused on the specific physicochemical abiotic characteristics of the recipient environment, which may attenuate or potentiate the toxicity of the contaminants (1, 6; Babich and Stotzky, in press).

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LITERATURE CITED

1. **Babich, H., and G. Stotzky.** 1974. Air pollution and microbial ecology. Crit. Rev. Environ. Control **4**:353-421.
2. **Babich, H., and G. Stotzky.** 1977. Sensitivity of various bacteria, including actinomycetes and fungi, to cadmium and the influence of pH on sensitivity. Appl. Environ. Microbiol. **33**:681-695.
3. **Babich, H., and G. Stotzky.** 1977. Reductions in the toxicity of cadmium to microorganisms by clay minerals. Appl. Environ. Microbiol. **33**:696-705.
4. **Babich, H., and G. Stotzky.** 1977. Effect of cadmium on fungi and on interactions between fungi and bacteria in soil: influence of clay minerals and pH. Appl. Environ. Microbiol. **33**:1059-1066.
5. **Babich, H., and G. Stotzky.** 1978. Toxicity of zinc to fungi, bacteria, and coliphages: influence of chloride ions. Appl. Environ. Microbiol. **36**:906-914.
6. **Babich, H., and G. Stotzky.** 1978. Effects of cadmium on the biota: influence of environmental factors. Adv. Appl. Microbiol. **23**:55-117.
7. **Berland, B. R., D. J. Bonin, V. I. Kapkov, S. Y. Maestrini, and D. P. Arlhac.** 1976. Action toxique de quatre metaux lourds sur la croissance d'algues unicellularies marines. C.R. Acad. Sci. Ser. D **282**:633-636.
8. **Buchauer, M. J.** 1973. Contamination of soil and vegetation near a zinc smelter by zinc, cadmium, copper, and lead. Environ. Sci. Technol. **7**:131-135.
9. **Bunzl, K.** 1974. Kinetics of ion exchange in soil organic matter. II. Ion exchange during continuous addition of Pb^{2+}-ions to humic acid and peat. J. Soil Sci. **25**:343-356.
10. **Carter, G. A., and R. L. Wain.** 1964. Investigations on fungicides. IX. The fungitoxicity, phytotoxicity, and systemic fungicidal activity of some inorganic salts. Ann. Appl. Biol. **53**:291-309.

11. **Carter, J. W., and I. L. Cameron.** 1973. Toxicity bioassay of heavy metals in water using *Tetrahymena pyriformis*. Water Res. **7**:951-961.
12. **Cole, M. A.** 1977. Lead inhibition of enzyme synthesis in soil. Appl. Environ. Microbiol. **33**:262-268.
13. **Farrah, H., and W. F. Pickering.** 1977. The sorption of lead and cadmium species by clay minerals. Aust. J. Chem. **30**:1417-1422.
14. **Gingell, S. M., R. Campbell, and M. H. Martin.** 1976. The effect of zinc, lead, and cadmium pollution on the leaf surface microflora. Environ. Pollut. **11**:25-37.
15. **Grandjean, P.** 1978. Widening perspectives of lead toxicity. Environ. Res. **17**:303-321.
16. **Hahne, H. C. H., and W. Kroontje.** 1973. Significance of pH and chloride concentration on behavior of heavy metal pollutants: mercury (II), cadmium (II), zinc (II), and lead (II). J. Environ. Qual. **2**:444-450.
17. **Henriksson, L. E., and E. J. DaSilva.** 1978. Effects of some inorganic elements on nitrogen-fixation in blue-green algae and some ecological aspects of pollution. Z. Allg. Mikrobiol. **18**:487-494.
18. **Hutchinson, T. C.** 1973. Comparative studies of the toxicity of heavy metals to phytoplankton and their synergistic interactions. Water Pollut. Res. Can. **8**:68-90.
19. **John, M. K.** 1971. Lead contamination of some agricultural soils in Western Canada. Environ. Sci. Technol. **5**:1199-1203.
20. **Koeppe, D. E.** 1977. The uptake, distribution, and effect of cadmium and lead in plants. Sci. Total Environ. **7**: 197-206.
21. **Lagerwerff, J. V.** 1967. Heavy-metal contamination of soils, p. 343-364. *In* N. C. Brady (ed.), Agriculture and the quality of our environment. American Association for the Advancement of Science, Washington, D.C.
22. **Lagerwerff, J. V., and A. W. Specht.** 1970. Contamination of roadside soil and vegetation with cadmium, nickel, lead, and zinc. Environ. Sci. Technol. **4**:583-586.
23. **Liang, C. N., and M. A. Tabatabai.** 1977. Effects of trace elements on nitrogen mineralization in soils. Environ. Pollut. **12**:141-147.
24. **Linzon, S. N., B. L. Chai, P. J. Temple, R. G. Pearson, and M. L. Smith.** 1976. Lead contamination of urban soils and vegetation by emissions from secondary lead industries. J. Air Pollut. Control Assoc. **26**:650-654.
25. **Little, P., and M. H. Martin.** 1972. A survey of zinc, lead, and cadmium in soil and natural vegetation around a smelting complex. Environ. Pollut. **3**:241-254.
26. **Malanchuk, J. L., and G. K. Gruendling.** 1973. Toxicity of lead nitrate to algae. Water Air Soil Pollut. **2**:181-190.
27. **Morton, S. D., and E. W. Sawyer.** 1976. Clay minerals remove organics, viruses, and heavy metals from water. Water & Sewage Works ref. no. R116-120.
28. **Novick, R. P., and C. Roth.** 1968. Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. J. Bacteriol. **95**:1335-1342.
29. **Olin, A.** 1960. Studies on the hydrolysis of metal ions. 25. The hydrolysis of lead (II) in perchlorate medium. Acta Chem. Scand. **14**:126-149.
30. **Olin, A.** 1960. Studies on the hydrolysis of metal ions. 28. Application of the self-medium to the hydrolysis of lead (II) in perchlorate solution. Acta Chem. Scand. **14**:814-822.
31. **Ramamoorthy, S., and D. J. Kushner.** 1975. Binding of mercuric and other heavy metal ions by microbial growth media. Microb. Ecol. **2**:162-176.
32. **Ross, I. S.** 1975. Some effects of heavy metals on fungal cells. Trans. Br. Mycol. Soc. **64**:175-193.
33. **Ruthven, J. A., and J. Cairns, Jr.** 1973. Response of fresh-water protozoan artificial communities to metals. J. Protozool. **20**:127-135.
34. **Schulz-Baldes, M., and R. A. Lewin.** 1976. Lead uptake in two marine phytoplankton organisms. Biol. Bull. **150**:118-127.
35. **Schulze, H., and J. J. Brand.** 1978. Lead toxicity and phosphate deficiency in *Chlamydomonas*. Plant Physiol. **62**:727-730.
36. **Silverberg, B. A., P. T. S. Wong, and Y. K. Chau.** 1977. Effect of tetramethyl lead on freshwater green algae. Arch. Environ. Contam. Toxicol. **5**:305-313.
37. **Smith, W. H.** 1977. Influence of heavy metal leaf contamination on the *in vitro* growth of urban-tree phylloplane fungi. Microb. Ecol. **3**:231-239.
38. **Smith, W. H., B. J. Staskawicz, and R. S. Harkov.** 1978. Trace metal pollutants and urban-tree leaf pathogens. Trans. Br. Mycol. Soc. **70**:29-33.
39. **Somers, E.** 1961. The fungitoxicity of metal ions. Ann. Appl. Biol. **49**:246-253.
40. **Stevenson, F. J.** 1977. Nature of divalent transition metal complexes of humic acids as revealed by a modified potentiometric titration method. Soil Sci. **123**:10-17.
41. **Stotzky, G.** 1966. Influence of clay minerals on microorganisms. II. Effect of various clay species, homoionic clays, and other particles on bacteria. Can. J. Microbiol. **12**:831-848.
42. **Tornabene, T. G., and H. W. Edwards.** 1972. Microbial uptake of lead. Science **176**:1334-1335.
43. **Tyler, G., B. Mornsjo, and B. Nilsson.** 1974. Effects of cadmium, lead, and sodium salts on nitrification in a mull soil. Plant Soil **40**;237-242.
44. **Vallee, B. L., and D. D. Ulmer.** 1972. Biochemical effects of mercury, cadmium, and lead. Annu. Rev. Biochem. **41**:91-128.
45. **Wong, P. T. S., Y. K. Chau, and P. L. Luxon.** 1975. Methylation of lead in the environment. Nature (London) **253**:263-264.
46. **Zlochevskaya, I. V., and E. G. Rukhadze.** 1968. A study of the toxic action of some lead compounds. Microbiology (USSR) **37**:951-955.