Bull. Environm. Contam. Toxicol. 27, 474-480 (1981)

Manganese Toxicity to Fungi: Influence of pH

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Studies on the interactions between microorganisms and manganese (Mn) have focused either on the effect of microbial activities on the biogeochemical cycling of Mn or on the requirement for Mn as a micronutrient. Thus, microbes, primarily bacteria (EHRLICH 1971) but also some fungi (TIMONIN et al. 1972), are involved in the oxidation of divalent Mn. Microbes are also involved in the reduction of manganic oxides and manganese dioxide to divalent Mn (EHRLICH 1971). Manganese is required for endospore formation in Bacillus megaterium (WEINBERG 1964), photosynthetic activity of Chlamydomonas reinhardi (TEICHLER-ZALLEN 1969), development of conidia in Penicillium clavigerum (TINNELL et al. 1974), and mycelial growth of Aspergillus niger (KISSER et al. 1980).

Manganese is, however, also a pollutant, as increased anthropogenic activities have accelerated the cycling of Mn through the biosphere. Manganese is used in the production of steel and cast-iron, the manufacture of bronze, and the production of copper, steel, and aluminum alloys. Manganese is emitted into the atmosphere from blast and electric Mn-ferroalloy furnaces, from steel furnaces, and from pig-iron furnaces. Mine effluents, fly ash, and sewage sludge fertilizers are the main sources of contamination of soils by Mn (MENA 1980). The toxicity of Mn to microbes, especially to fungi, has not been extensively evaluated (ROSS 1975), and the influence of abiotic factors on the toxicity of Mn to microbes has received even less attention (BABICH & STOTZKY 1980). The inhibition of growth of Escherichia coli by Mn (SILVER et al. 1972) and the mutagenicity of Mn to Saccharomyces cerevisiae (PUTRAMENT et al. 1975) was inhibited by magnesium. The present research evaluated the effects of Mn on mycelial proliferation of fungi and the effect of pH on Mn toxicity.

0007-4861/81/0027-0474 $01.40

MATERIALS AND METHODS

Fungi were grown on Sabouraud dextrose agar, and after incubation for several days at 25°C, circular plugs cut with a sterile cork borer (4 to 8 mm I.D.) were transferred, with the fungal growth up, to the center of petri dishes containing a medium consisting of 2% glucose, 1% neopeptone, and 1.5% Bacto-agar (in double distilled water), unamended or amended with 10, 50, 100, 500, 1000, 1500, or 2000 ppm Mn (as $MnCl_2 \cdot 4H_2O$) and adjusted to pH 5. The plates were incubated at 25°C, and mycelial growth rates were calculated as described elsewhere (BABICH & STOTZKY 1978). To study the effects of pH on Mn toxicity, the medium was adjusted, with HCl or NaOH, to pH 5.5, 6.5, 7.5, or 8.5; levels of Mn that were inhibitory at pH 5 were used. At the pH levels used in these studies, Mn occurs predominantly as the free divalent ion, as $Mn(OH)_2$ precipitates initially at pH levels of 8.5 to 8.8 (KING 1959). Three replicates were used for each concentration of Mn or each pH, and experiments were performed at least twice.

RESULTS AND DISCUSSION

The fungi exhibited wide differences in their sensitivities to Mn. Incipient inhibition (i.e., the level of Mn at which growth inhibition was noted initially, $P < 0.05$) for *Scopulariopsis brevicaulis* and *Aspergillus giganteus* occurred at 100 ppm Mn; for *Rhizopus stolonifer*, *Arthrobotrys conoides*, *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma viride*, and *Penicillium vermiculatum* at 500 ppm Mn; for *Cephalosporium* sp. at 1000 ppm Mn; and for *Gliocladium* sp. at 1000 to 1500 ppm Mn; growth of *Aspergillus clavatus* was not inhibited even at 2000 ppm Mn. No growth of *S. brevicaulis* occurred at 500 ppm Mn and of *R. stolonifer* at 1500 ppm Mn (Table 1).

The levels of Mn causing incipient and/or total inhibition of mycelial growth of the fungi studied were comparable to the levels reported to inhibit mycelial proliferation of some phylloplane fungi: incipient growth inhibition of *Gnomonia platani*, *Pleurophomella* sp., and *Aureobasidium pullulans* and total growth inhibition of *Chaetomium* sp. occurred at 416.9 ppm Mn; 833.4 ppm Mn was totally inhibitory to mycelial growth of *G. platani* and *A. pullulans* (SMITH et al. 1978).

Only *A. conoides* showed significant ($P < 0.5$) stimulation of mycelial growth by Mn; 10, 50, and 100 ppm Mn increased growth rates over control (0 ppm Mn) values

TABLE 1

Influence of manganese (Mn) on mycelial proliferation of fungi.

Fungus	Radial growth rate, in mm/day (% of control) Mn (ppm)							
	0[1]	10	50	100	500	1000	1500	2000
Scopulariopsis brevicaulis	2.8±0.33 (100±12.9)	2.5±0.33 (91±11.9)	3.0±0.24 (110±8.9)	2.1±0.09 (77±3.3)	0 (0)	0 (0)	0 (0)	0 (0)
Rhizopus stolonifer	35.8±2.13 (100±6.0)	36.1±2.00 (100±5.5)	35.7±2.15 (100±6.0)	36.8±1.67 (103±4.7)	5.3±1.03 (15±2.9)	0.3±0.14 (1±0.4)	0 (0)	0 (0)
Arthrobotrys conoides	5.4±0.07 (100±1.3)	5.8±0.01 (108±2.6)	6.9±0.06 (127±1.0)	6.6±0.08 (122±1.4)	1.7±0.08 (32±1.5)	0.9±0.03 (16±0.5)	0.5±0.12 (8±2.2)	0.2±0.03 (4±0.5)
Aspergillus niger	5.3±0.15 (100±2.8)	5.1±0.11 (96±2.1)	5.0±0.15 (96±2.8)	5.2±0.13 (98±2.5)	3.8±0.16 (72±3.1)	2.5±0.19 (48±3.5)	1.1±0.13 (21±2.4)	0.6±0.09 (10±1.6)
Aspergillus giganteus	2.5±0.16 (100±6.4)	2.5±0.19 (98±7.6)	2.2±0.11 (89±5.6)	2.1±0.14 (82±5.6)	2.0±0.20 (79±7.9)	0.9±0.13 (37±4.9)	0.6±0.07 (22±2.9)	0.3±0.00 (13±0.0)
Trichoderma viride	14.5±0.23 (100±1.6)	15.2±0.53 (105±3.7)	15.5±0.45 (107±3.1)	15.0±0.41 (104±2.9)	11.8±0.59 (82±4.1)	9.5±0.48 (66±3.3)	6.7±0.95 (50±6.6)	3.5±0.58 (24±4.0)

(continued)

TABLE 1

(continued)

Fungus	Radial growth rate, in mm/day (% of control) Mn (ppm)							
	0[1]	10	50	100	500	1000	1500	2000
Penicillium vermiculatum	1.8±0.10 (100±5.6)	1.8±0.11 (102±6.1)	1.8±0.14 (99±7.7)	1.7±0.14 (97±7.7)	1.3±0.19 (72±10.6)	0.9±0.04 (52±2.3)	0.7±0.05 (41±3.1)	0.7±0.05 (39±2.9)
Aspergillus flavus	4.0±0.18 (100±4.5)	4.0±0.13 (99±3.3)	3.8±0.14 (95±3.4)	3.9±0.12 (97±2.9)	3.2±0.10 (80±2.6)	3.1±0.22 (76±5.6)	2.7±0.10 (68±2.6)	2.3±0.16 (56±4.0)
Cephalosporium sp.	1.6±0.03 (100±2.0)	1.7±0.02 (102±0.8)	1.7±0.05 (104±2.9)	1.7±0.05 (103±3.1)	1.7±0.02 (105±1.1)	1.5±0.03 (92±1.6)	1.3±0.02 (80±1.3)	1.2±0.03 (73±1.6)
Gliocladium sp.	2.2±0.04 (100±2.0)	2.1±0.04 (98±1.7)	2.2±0.02 (100±1.0)	2.1±0.07 (96±3.2)	2.0±0.03 (94±1.2)	1.9±0.08 (89±3.8)	1.7±0.02 (79±1.1)	1.6±0.04 (76±1.9)
Aspergillus clavatus	2.0±0.05 (100±2.4)	1.9±0.10 (95±5.0)	2.0±0.08 (98±4.0)	1.9±0.12 (96±6.0)	2.1±0.16 (105±7.9)	2.2±0.22 (108±3.8)	2.0±0.10 (97±5.0)	1.9±0.11 (95±5.6)

1 Control plates did not contain Mn.

TABLE 2

Influence of pH on the toxicity of 350 ppm manganese (Mn) to mycelial proliferation of fungi.

Treatment	Radial growth rate, mm/day (% of control) Rhizopus stolonifer	Penicillium vermiculatum	Scopulariopsis brevicaulis
pH 5.5; no Mn	31.9±2.00[1] (100±6.1)	1.9±0.07 (100±3.8)	3.0±0.16 (100±5.6)
pH 5.5; Mn	24.4±1.89 (76±5.9)	1.3±0.05 (69±2.4)	1.6±0.12 (53±4.1)
pH 6.5; no Mn	31.1±2.26 (100±7.3)	1.8±0.08 (100±4.2)	3.4±0.07 (100±2.2)
pH 6.5; Mn	20.8±1.51 (67±4.9)	1.4±0.06 (75±3.2)	2.1±0.03 (61±1.7)
pH 7.5; no Mn	29.7±1.33 (100±4.5)	1.9±0.06 (100±3.2)	3.9±0.03 (100±0.7)
pH 7.5; Mn	16.6±0.80 (56±2.7)	1.3±0.06 (68±3.4)	2.6±0.07 (67±1.7)
pH 8.5; no Mn	27.7±1.60 (100±5.7)	1.9±0.08 (100±4.0)	4.1±0.05 (100±1.2)
pH 8.5; Mn	15.9±1.20 (57±4.3)	1.4±0.05 (74±2.7)	3.0±0.08 (74±2.0)

1 Control plates were adjusted to an equivalent pH but contained no Mn.

(Table 1). A level of 8.3 ppm Mn stimulated mycelial growth of A. pullulans (SMITH et al. 1978).

There was no consistent trend in the effect of pH on Mn toxicity to the fungi. However, each fungus showed a definitive response to Mn at the different pH levels. Thus, increasing the pH from 5.5 to 8.5 did not significantly affect the toxicity of Mn to Gliocladium sp., P. vermiculatum, or A. niger. The toxicity of Mn to R. stolonifer and T. viride was not different at pH 5.5 and 6.5, but increasing the pH to 7.5 or 8.5 significantly enhanced the toxicity (Tables 2 and 3). Enhanced toxicity for cadmium at alkaline pH levels was also noted for R. stolonifer and T. viride (BABICH & STOTZKY 1977). In contrast, the

TABLE 3

Influence of pH on the toxicity of 1500 ppm manganese (Mn) to mycelial proliferation of fungi.

Fungus	Radial growth rate, mm/day (% of control)		
	Aspergillus niger	Gliocladium sp.	Trichoderma viride
pH 5.5; no Mn	6.7±0.09[1] (100±1.4)	1.9±0.03 (100±1.3)	15.0±0.14 (100±1.0)
pH 5.5; Mn	2.7±0.15 (43±4.1)	1.4±0.02 (73±1.3)	7.5±0.68 (50±4.5)
pH 6.5; no Mn	6.2±0.10 (100±1.7)	1.9±0.03 (100±1.6)	14.5±0.13 (100±0.9)
pH 6.5; Mn	2.6±0.15 (42±2.4)	1.5±0.09 (78±4.6)	6.2±0.64 (43±4.4)
pH 7.5; no Mn	6.3±0.04 (100±0.6)	1.9±0.04 (100±2.2)	13.3±0.21 (100±1.6)
pH 7.5; Mn	2.6±0.13 (42±2.1)	1.4±0.08 (71±6.2)	3.5±0.54 (24±4.6)
pH 8.5; no Mn	6.8±0.11 (100±1.6)	1.8±0.02 (100±1.0)	12.6±0.27 (100±2.0)
pH 8.5; Mn	2.9±0.20 (42±3.1)	1.4±0.02 (80±6.4)	4.0±0.55 (31±4.3)

1 Control plates were adjusted to an equivalent pH but contained no Mn.

toxicity of Mn to S. brevicaulis, a basophilic fungus whose growth rates and sporulation increased as the pH was increased, was significantly reduced as the pH was increased (Table 3). This increased tolerance to Mn at alkaline pH levels may have resulted from the enhanced physiological state of this fungus at alkaline pH levels.

Regulatory agencies, when setting standards for environmental contaminants, are now considering the influence of abiotic factors on pollutant toxicity, i.e., different ecosystems may tolerate differing levels of the same pollutant. The lack of a consistent biotic response to Mn toxicity as a function of pH

will probably not enable the establishment of distinct criteria for Mn in environments having different pH values.

ACKNOWLEDGMENTS

This research was supported, in part, by grant R808329 from the United States Environmental Protection Agency.

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Accepted July 28, 1981