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Comparative Toxicity of Trivalent and Hexavalent Chromium to Fungi

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Chromium (Cr) compounds are used in stainless steel and other alloys, in chrome plating, as pigments and in primer paints, in the chrome tanning of leather products, as mordants in the textile industry, in fungicides, and in wood preservatives. Such extensive industrial use of Cr compounds has resulted in the contamination of aquatic and terrestrial ecosystems with Cr. Although Cr can occur in valence states from -2 to +6, the trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) valencies are the most commonly encountered in the environment. Whereas Cr^{3+} is an essential element for human beings and probably also for plants, Cr^{6+} is highly toxic to the biota and is a suspected carcinogen occurring frequently in the workplace (ANDERSON 1981).

Research on the toxicology of Cr has focused primarily on human beings (LANGARD 1980) and the aquatic macrobiota (EPA 1979), with less research on plants (HUFFMAN & ALLAWAY 1973). However, only limited research has evaluated the toxicology of Cr to the microbiota. For example, from 1.6 to 3.2 ppm Cr^{6+} inhibited growth of the algae, Chlorella variegatus and Chlorococcum humicola (HERVEY 1949); 4.5×10^{-6} and 1.25×10^{-5} M Cr^{3+} reduced by 50% the germination of spores of Alternaria tenuis and Botrytis fabae, respectively (SOMERS 1961); and 2.8 ppm Cr^{3+} reduced conidia production by the fungus, Gnomonia platani (STASKAWICZ & SMITH 1977). Ten to 12 ppm Cr^{6+} inhibited growth of soil bacteria and actinomycetes (ROSS et al. 1981); the numbers of actinomycetes, bacteria, and fungi isolated from soil were decreased by 1, 10, and 100 ppm Cr^{6+}, respectively (DRUCKER et al. 1979); and 0.1 ppm Cr^{3+} reduced the heterotrophic activity of a microbiota from a natural aquatic ecosystem (ALBRIGHT et al. 1972). Strains of Pseudomonas aeruginosa carry a plasmid that confers resistance to Cr^{6+} (SUMMERS & JACOBY 1978), and Gram-negative bacteria resistant to Cr^{6+} have been isolated from a river polluted with Cr (SIMON-PUJOL et al. 1979).

Trivalent and hexavalent Cr exhibit differential

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toxicities to bacteria. For example, Cr^{3+} was less toxic than Cr^{6+} to growth of Klebsiella pneumoniae (BALDRY et al. 1977) and to fermentation by a mixed rumen microbiota (FORSBERG 1978). Furthermore, Cr^{6+}, but not Cr^{3+}, was mutagenic to Salmonella typhimurium (PETRILLI & DE FLORA 1978), Bacillus subtilis (NISHIOKA 1975), and Escherichia coli (VENITT & LEVY 1974). As there has been, apparently, no research on the comparative toxicology of Cr^{3+} and Cr^{6+} to fungi (BABICH & STOTZKY 1980; ROSS et al. 1981), studies were conducted to determine whether the differential toxicities of these two valence states of Cr that were noted for bacteria also occur with fungi.

MATERIALS AND METHODS

1. Preparation of media: Solutions of Cr^{3+} (as $CrCl_3 \cdot 6H_2O$) or Cr^{6+} (as CrO_3), adjusted to pH 5, were sterilized by filtration through a membrane (0.45 µm, Millipore) and added to an autoclaved nutrient medium, consisting of 2% glucose, 1% neopeptone, and 1.5% Bacto-agar and adjusted to pH 5.
2. Mycelial growth rates: Fungi were grown on Sabouraud dextrose agar (SDA), 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 medium unamended or amended with 100, 500, 1000, or 1500 ppm Cr^{3+} or Cr^{6+}. The plates were incubated at 25°C, and mycelial growth rates were measured as described elsewhere (BABICH & STOTZKY 1981). Three replicates were used for each concentration of Cr, and experiments were performed at least twice.
3. Spore germination: Fungi were grown for 1 week on slants of SDA. The slants were flooded with 20 ml of sterile distilled water, agitated on a Vortex-Genie for 10 sec, and then decanted through sterile glass wool. 0.1 ml aliquots of the spore suspension were mixed with molten medium amended with 0, 10, 50, 100, 150, or 200 ppm Cr^{3+} or Cr^{6+}. The plates were incubated for 48 h at 25°C, and germination was determined by the appearance of macroscopic colonies. Two replicates were used for each concentration of Cr, and experiments were performed 3 to 5 times.
4. Sporulation: 0.1 ml of the spore suspension was spread over the surface of slants of the medium amended with 0, 10, 50, 100, 150, or 200 ppm Cr^{3+} or Cr^{6+}. After incubation for 1 week at 25°C to allow for mycelial growth and sporulation, 10 ml of sterile distilled water was added, the tubes were agitated on a Vortex-Genie for 1 min, and spore counts were performed with a Hausser hemocytometer. Two replicates were used for each concentration of Cr, and experiments

TABLE 1

Comparison of the toxicities of trivalent and hexavalent chromium to mycelial growth rates of fungi.

Cr	Mycelial growth rate, in mm/day[a] (% of control)[b]		
	Arthrobotrys conoides	Aspergillus giganteus	Oospora sp.
no Cr added	4.2±0.45 (100±10.5)	2.6±0.14 (100±6.1)	5.8±0.07 (100±1.2)
100 ppm Cr^{3+}	3.2±0.25[c] (76±6.7)	2.9±0.28 (112±10.8)	5.3±0.19 (93±3.2)
500 ppm Cr^{3+}	0.3±0.13[c] (8±3.1)	1.0±1.15[c] (37±5.8)	2.3±0.19[c] (40±3.3)
1000 ppm Cr^{3+}	0[c] (0)	0[c] (0)	0[c] (0)
1500 ppm Cr^{3+}	0[c] (0)	0[c] (0)	0[c] (0)
100 ppm Cr^{6+}	0.3±0.17[c] (7±4.0)	0.4±0.32[c] (15±12.3)	0[c] (0)
500 ppm Cr^{6+}	0[c] (0)	0[c] (0)	0[c] (0)
1000 ppm Cr^{6+}	0[c] (0)	0[c] (0)	0[c] (0)
1500 ppm Cr^{6+}	0[c] (0)	0[c] (0)	0[c] (0)

(continued)

were performed 3 times.
5. Statistics: The data are expressed as the arithmetic mean ± the standard error of the mean, and the Student's t test was performed, with $P < 0.05$ being considered signficant.

RESULTS AND DISCUSSION

Mycelial growth rates were inhibited more by Cr^{6+} than by Cr^{3+}. Incipient inhibition of growth (i.e., the level of Cr at which statistically significant inhibition of growth occurred initially) of Arthrobotrys

TABLE 1

(continued)

Cr	Mycelial growth rate, in mm/day[a] (% of control)[b]		
	Rhizopus stolonifer	Penicillium vermiculatum	Trichoderma viride
no Cr added	39.0±0.00 (100±0.0)	2.0±0.22 (100±11.0)	16.0±1.41 (100±8.8)
100 ppm Cr^{3+}	39.0±0.00 (100±0.0)	2.0±0.17 (100±8.5)	19.3±1.72 (120±10.7)
500 ppm Cr^{3+}	20.1±1.81[c] (51±4.6)	1.9±0.18 (96±9.0)	16.8±1.45 (105±9.1)
1000 ppm Cr^{3+}	0[c] (0)	0[c] (0)	16.1±1.25 (100±7.8)
1500 ppm Cr^{3+}	0[c] (0)	0[c] (0)	13.2±1.73 (82±10.8)
100 ppm Cr^{6+}	1.6±0.63[c] (4±1.6)	2.6±0.08[c] (130±4.0)	11.3±0.39[c] (71±2.4)
500 ppm Cr^{6+}	0[c] (0)	0[c] (0)	2.1±0.96[c] (13±6.0)
1000 ppm Cr^{6+}	0[c] (0)	0[c] (0)	1.8±0.82[c] (11±5.1)
1500 ppm Cr^{6+}	0[c] (0)	0[c] (0)	0[c] (0)

a Mean ± standard error of the mean.
b Control plates contained no added chromium.
c Statistically significant at $P < 0.05$ when compared to control plates not amended with chromium.

conoides was at 100 ppm Cr^{3+}, of Aspergillus giganteus, Rhizopus stolonifer, and Oospora sp. at between 100 and 500 ppm Cr^{3+}, of Penicillium vermiculatum at greater than 500 ppm Cr^{3+}, and of Trichoderma viride at a level greater than 1500 ppm Cr^{3+}. Growth of A. conoides, A. giganteus, Oospora sp., R. stolonifer, and P. vermiculatum was completely inhibited at 1000 ppm Cr^{3+}, but T. viride still grew on media amended with 1500 ppm Cr^{3+}. With Cr^{6+}, incipient inhibition of growth of all

TABLE 2

Comparison of the toxicities of trivalent and hexavalent chromium to spore formation by fungi.

Cr	Number of spores (5×10^4/ml)[a] (% of control)[b]	
	Penicillium vermiculatum	Aspergillus giganteus
no Cr added	115.7±8.16 (100±7.1)	166.4±15.99 (100±9.6)
10 ppm Cr^{3+}	111.0±8.65 (96±7.5)	157.7±19.29 (95±11.6)
50 ppm Cr^{3+}	108.1±9.37 (93±8.1)	156.0±18.45 (94±11.1)
100 ppm Cr^{3+}	104.5±15.9 (90±13.8)	160.4±25.44 (97±15.3)
150 ppm Cr^{3+}	108.7±12.4 (94±10.7)	155.2±33.14 (93±19.9)
200 ppm Cr^{3+}	60.2±6.00 (52±6.0)[c]	156.5±19.77 (94±11.8)
10 ppm Cr^{6+}	81.5±6.51 (70±5.6)[c]	119.0±12.55 (71±7.5)[c]
50 ppm Cr^{6+}	9.6±3.75 (8±3.2)[c]	1.6±1.58 (1±1.0)[c]
100 ppm Cr^{6+}	2.3±1.49 (2±1.3)[c]	0.6±0.58 (0±0.4)[c]
150 ppm Cr^{6+}	1.5±0.98 (1±0.9)[c]	0 (0)[c]
200 ppm Cr^{6+}	0 (0)[c]	0 (0)[c]

a Mean ± standard error of the mean.
b Control tubes did not contain chromium.
c Statistically significant at $P < 0.05$ when compared to control tubes not amended with chromium.

fungi tested, except P. vermiculatum, was evident at 100 ppm and probably would have occurred at lower concentrations. Growth of P. vermiculatum was stimulated

by 100 ppm Cr^{6+}. Growth of A. conoides, A. giganteus, R. stolonifer, and P. vermiculatum was completely inhibited at a level between 100 and 500 ppm Cr^{6+}, and growth of T. viride was completely inhibited between 1000 and 1500 ppm Cr^{6+} (Table 1). The stimulatory effect of Cr^{6+} on mycelial growth of P. vermiculatum was not evident with other fungi. The only other reported stimulatory effect of Cr^{6+} on a fungus occurred also with a species of Penicillium: maximum production of penicillin by Penicillium chrysogenum occurred in a medium amended with 100 ppm Cr^{6+} and good growth, although with a lower level of penicillin production, occurred even at 200 ppm Cr^{6+} (PRATT & DU FRENOY 1947).

Levels of Cr^{3+} from 10 to 150 ppm did not inhibit sporulation of P. vermiculatum, but 200 ppm Cr^{3+} reduced sporulation by approximately 50%. However, incipient inhibition of sporulation of P. vermiculatum occurred at 10 ppm Cr^{6+}, and no spores were produced at 200 ppm Cr^{6+}. Even 200 ppm Cr^{3+} did not inhibit sporulation of A. giganteus, but incipient inhibition of sporulation occurred at 10 ppm Cr^{6+}, and no spores were produced at 150 ppm Cr^{6+} (Table 2).

Incipient inhibition of the germination of spores of P. vermiculatum occurred at 100 ppm Cr^{3+} and at 10 ppm Cr^{6+}, and 50 ppm Cr^{6+} completely inhibited spore germination, whereas 200 ppm Cr^{3+} only reduced germination by about 10%. With A. giganteus, incipient inhibition of spore germination occurred at 50 ppm Cr^{3+}, and some germination occurred at 200 ppm Cr^{3+}; however, with Cr^{6+}, incipient inhibition of spore germination occurred at 10 ppm, and no germination was evident at 50 ppm (Table 3).

Hexavalent Cr was more toxic than equivalent levels of Cr^{3+} to mycelial growth rates, spore formation, and spore germination of the fungi tested. The greater toxicities of Cr^{6+} than of Cr^{3+} to fungi are in agreement with those observed with bacteria (NISHIOKA 1975; BALDRY et al. 1977; FORSBERG 1978; PETRILLI & DE FLORA 1978).

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TABLE 3

Comparison of the toxicities of trivalent and hexavalent chromium to germination of fungal spores on an agar medium.

Cr	Number of spores germinated[a] (% of control)[b]	
	Penicillium vermiculatum	Aspergillus giganteus
no Cr added	93.4±1.98 (100±2.1)	68.3±2.97 (100±4.4)
10 ppm Cr^{3+}	91.8±2.84 (98±3.0)	65.5±2.26 (96±3.3)
50 ppm Cr^{3+}	98.0±4.28 (105±4.6)	52.5±2.90 (77±4.3)[c]
100 ppm Cr^{3+}	87.3±4.14 (94±4.4)[c]	38.6±1.85 (57±2.7)[c]
150 ppm Cr^{3+}	83.5±3.06 (90±3.3)[c]	25.8±3.06 (38±4.5)[c]
200 ppm Cr^{3+}	81.6±4.06 (87±4.4)[c]	13.3±2.59 (20±3.8)[c]
10 ppm Cr^{6+}	78.7±3.93 (84±4.2)[c]	55.9±4.04 (82±5.9)[c]
50 ppm Cr^{6+}	0 (0)[c]	0 (0)[c]
100 ppm Cr^{6+}	0 (0)[c]	0 (0)[c]
150 ppm Cr^{6+}	0 (0)[c]	0 (0)[c]
200 ppm Cr^{6+}	0 (0)[c]	0 (0)[c]

a Mean ± standard error of the mean.
b Control plates were not amended with chromium.
c Statistically significant at $P < 0.05$ when compared to controls not amended with chromium.

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