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Behavior of Chromium in Soils: IV. Toxicity to Microorganisms[1]

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ABSTRACT

The toxicity of Cr to soil bacterial isolates was studied by measuring turbidity of liquid cultures supplemented with Cr(VI) or Cr(III). Ten to 12 ppm Cr(VI) was found inhibitory to most isolates growing in either a soil-extract medium and or in a semisynthetic medium. Gram negative bacteria were more affected than gram positive bacteria by Cr(VI) both at this level and at 1 ppm. Toxicity due to Cr(III) was not observed at similar levels.

Nellis loam (Typic Eutrochrept) and Hinesburg fine sandy loam (Aquic Hapiorthod) samples were treated with 10 and 100 ppm Cr(VI) and 100 ppm Cr(III). All three treatments significantly decreased CO_2 evolution from that of controls for the 3-week duration of the experiment. Extractable Cr(VI) in these soils decreased rapidly during this time, indicating that reduction of the added Cr(VI) was occurring.

The toxicity of low levels of Cr(VI) to gram negative bacteria in aqueous culture indicates that soil microbial transformations, such as nitrification, may be affected. The fact that both Cr(III) and Cr(VI) inhibited microbial activity in soil shows that more caution may be needed in handling Cr-containing wastes, regardless of the form of Cr.

Additional Index Words: chromium(VI), chromium(III), soil bacteria.

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Increasing use of soil as a repository for human waste products has brought about increased interest in the effects on the soil of high concentrations of potentially toxic substrances. Chromium (Cr) often is found in waste sludges from tanning, metallurgic, and other industries. Chemical aspects of the reactions of Cr with soils have been reported by Bartlett and Kimble (1976a, 1976b) and Bartlett and James (1979).

Trivalent Cr formerly was thought to be the only stable form of Cr in soils because of rapid reduction of added Cr(VI). Work with field-moist soils, however, has shown that some oxidation of added Cr(III) takes place in aerobic, nonacid soils and Cr(VI) can be extracted from soil samples at least 1 year after the Cr(III) addition (Bartlett and James, 1979). Therefore, the toxicities and mobilities of both species must be considered.

It appears that Cr(III) is less toxic than CR(VI). Inorganic Cr(III) is not mobile in soils and is not readily taken up by cells, probably because of its low solubility and tendency to form large hydroxy polymers at neutral pH levels. On the other hand, Cr(VI) is mobile in soils and it penetrates cell membranes readily. Once inside cells, it is reduced; its toxicity probably results from oxidation of cell components (National Research Council, 1974). After reduction to Cr(III) inside the cell, it may interfere with protein function. Mutations have been observed in bacteria grown in the presence of Cr(VI), indicating DNA susceptibility (Petrilli and De Flora, 1977).

Henry and Smith (1946) studied the toxic effects of dichromate-sulfuric acid cleaning mixture on microorganisms and found that 1 ppm Cr(VI) was toxic to *Staphylococcus aureus* in synthetic media, while 10 ppm was needed for complete inhibition in nutrient broth. Rudolfs (1950) reviewed the literature on toxic metals in sewage treatment and recommended a 5-ppm limit for Cr(VI) in sewage. Barth et al. (1967), using a model of an activated sludge plant, found that a 10-ppm continuous dose of Cr(VI) in the influent reduced plant efficiency by 5%, as measured by effluent chemical oxygen demand (COD). Slater and Reisenauer[3] found that both Cr(VI) and Cr(III) reduced CO_2 evolution when added to a moist Yolo soil. The depression caused by Cr(VI) lessened with time, but not that caused by Cr(III).

Because of the lack of reports dealing with Cr toxicity to soil microorganisms, the present study was undertaken to provide an initial indication of the effect of Cr on soil microbial activities. The toxic action of Cr in soils needs to be better understood to provide guidelines for the soil application of Cr-containing wastes.

MATERIALS AND METHODS

Representative soil bacteria were isolated from field-moist samples of a Nellis loam (Typic Eutrochrept), an Eldridge loamy fine sand (Aquic Udorthent), and several other Vermont soils. The isolates were identified to genera according to *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974). A wide range of commonly isolated genera was obtained.

An aqueous soil extract was prepared by autoclaving 1 kg of moist Nellis soil in 1 liter of H_2O for 1 hour. After centrifugation, 0.5 g K_2HPO_4 per liter was added to the supernatant. Before use, the extract was diluted 1.33 × with distilled H_2O. A semisynthetic medium also was used and contained 0.5 g KNO_3, 0.5 g $(NH_4)_2SO_4$, 0.2 g $MgHPO_4 \cdot 3H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 2.0 g casamino acids, and 2.0 g dextrose per liter of H_2O. The pH of each autoclaved medium was adjusted to 6.5 with sterile KOH. This pH, slightly below the optimum for most bacteria, was chosen to increase solubility of Cr(III) above that in neutral solution.

Both nonaerated and aerated cultures were studied. Bacteria in nonaerated cultures were grown in 18- by 150-mm disposable culture tubes which had been selected for optical uniformity at 660 nm using a spectrophotometer. Inoculum was prepared in these same tubes by growing the isolates in the medium to be used for the test. After 48 hours incubation, the optical density (O.D.) was adjusted to 0.05 by dilution with sterile medium. One-tenth milliliter of this inoculum and 0.2 ml of sterile 7.5, 75, or 750 ppm Cr(VI) as $K_2Cr_2O_7$ or 75, 750, or 7,500 ppm Cr(III) as $CrCl_3$ were added to 14.7 ml of media that had been sterilized in the tubes. All treatments were duplicated. After 24, 48, and 72 hours at 25°C, the tubes were vortexed and O.D. determined at 660 nm. This wavelength was used to minimize effects of the yellow color of the medium on the turbidity readings (DeMoss and Bard, 1957).

In the aerated experiments, 250-ml Erlenmeyer flasks containing 45 ml of culture solution and Cr in proportions similar to the nonaerated cultures were shaken continuously at room temperature (23–33°C).

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[3] J. P. Slater and H. M. Reisenauer. 1979. Toxicity of Cr(III) and Cr(VI) added to soils. Agron. Abstr. p. 38.

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Table 1—Toxicity of Cr(VI) in soil extract medium as indicated by optical densities of 48 hour culture suspensions.

Bacteria	Number of isolates	Aerated: Initial Cr(VI) in solution, ppm 0	1	11	Nonaerated: Initial Cr(VI) in solution, ppm 0	0.1	1	10
		optical density†						
Gm⁺ bacteria								
Bacillus sp.	1	0.26a	0.19b	0.07c				
	6				0.06a	0.06b	0.06a	0.03c
Arthrobacter sp.	2	0.25a	0.25a	0.14b				
	2				0.08a	0.08a	0.07a	0.03b
Corynebact. sp.	1	0.23a	0.22a	0.20a				
	2				0.05a	0.05a	0.05a	0.04b
Nocardia sp.	1	0.19a	0.18a	0.0 b				
	1				0.04a	0.04a	0.04a	0.0 b
Streptomyces sp.	1	0.19a	0.17a	0.06b				
	6	0.20a	0.21b	0.10c				
Average of all Gm⁺ bacteria	11				0.06a	0.06b	0.06a	0.03c
Gm⁻ bacteria								
Pseudomonas sp.	2	0.12a	0.12a	0.01b				
	2				0.07a	0.06a	0.04b	0.0 c
Acinetobacter sp.	1	0.09a	0.0 b	0.0 b				
	1				0.08a	0.09a	0.0 b	0.0 b
Moraxella sp.	1				0.07a	0.05b	0.0 c	0.0 c
Flavobact. sp.	1				0.05a	0.04a	0.08a	0.01b
	3	0.11a	0.08b	0.0 c				
Average of all Gm⁻ bacteria	5				0.07a	0.06b	0.02c	0.0 c

† Values followed by the same letter in a row are not significantly different at the 5% level (nonorthogonal contrasts).

Table 2—Toxicity of Cr(VI) and Cr(VI) in solution at [illegible] of incubation in a semisynthetic medium.

Bacteria	Initial Cr(VI) in solution, 24 hours 0	1	12	24 [illegible] 1 [illegible]
	optical density			
Bacillus sp. (Gm⁺)	2.70a†	1.35b	0.24c	[illegible]
Arthrobacter sp. (Gm⁺)	1.60a	1.06b	0.11c	0.15 [illegible]
Streptomyces sp. (Gm⁺)	1.45a	1.55a	0.30b	0.0 [illegible]
Acinetobacter sp. (Gm⁻)	1.10a	0.75a	0.03b	0.33 [illegible]
Pseudomonas sp. (Gm⁻)	1.10a	0.31b	0.01c	1.0 [illegible]

† Values followed by the same letter in each row are not significantly different at the 5% level (nonorthogonal contrasts).

Table 3—Toxicity of Cr(III) in soil extract medium, as indicated by optical densities of 48 hours culture suspensions.

Bacteria	Initial Cr(III) in solution, ppm 0	1	10	100
	optical density			
Aerated				
Bacillus sp. (Gm⁺)	0.14a†	0.15a	0.16a	[illegible]
Arthrobacter sp. (Gm⁺)	0.23a	0.24a	0.25a	[illegible]
Acinetobacter sp. (Gm⁻)	0.08ab	0.07a	0.11b	[illegible]
Pseudomonas sp. (Gm⁻)	0.11a	0.16b	0.15b	[illegible]
Nonaerated				
Bacillus sp. (Gm⁺)	0.06a	0.06a	0.08b	[illegible]
Arthrobacter sp. (Gm⁺)	0.08a	0.10b	0.08ab	[illegible]
Moraxella sp. (Gm⁻)	0.06a	0.06a	0.06a	[illegible]
Pseudomonas sp. (Gm⁻)	0.09a	0.08a	0.06b	[illegible]

† Values followed by the same letter in each row are not significantly different at the 5% level (nonorthogonal contrasts).

Aliquots were transferred to tubes and O.D. determined at 24 and 48 hours. If the O.D. was >0.40, the sample was diluted 10×. At 48 hours, samples were centrifuged and Cr in solution also was determined: oxidized Cr was measured colorimetrically by the s-diphenyl carbazide method (Bartlett and James, 1979) and total Cr by atomic absorption.

Microbial activity in the soil samples was evaluated by measuring CO_2 evolution using NaOH traps. One-kilogram samples (dry wt basis) of field-moist Nellis loam and Hinesburg fine sandy loam (Entic Haplorthod) were placed in 2-liter jars. The pH levels of these two soils were 6.4 and 5.9, respectively. A bottle containing 1.0*N* NaOH was placed on top of the soil in each jar. Treatments in triplicate included $K_2Cr_2O_7$ at 10 and 100 ppm Cr, $CrCl_3$ at 100 ppm Cr, based on the weight of the soil, and controls without Cr. The final H_2O content of the treated soils and the controls was adjusted to exactly 25% for the Nellis and 20% for the Hinesburg (approximately 1/3 atm moisture tension). The jars were incubated in the dark at 25°C and the air inside was freshened daily by removing the caps for a few seconds. At 3- to 5-day intervals, the NaOH in each jar was titrated with HCl after the addition of $BaCl_2$ and phenolphthalein. Oxidized Cr in the treated soil was determined colorimetrically as described above, after extracting moist 2.5 cm³ samples with 0.01*M* KH_2PO_4-K_2HPO_4.

RESULTS AND DISCUSSION

Toxicity in Culture

Optical densities for 48-hour bacterial cultures in soil extract media containing Cr(VI) are given in Table 1. All gram negative (Gm⁻) bacteria tested were almost completely inhibited by the high level of Cr(VI). One ppm Cr(VI) had little or no effect on most gram positive (Gm⁺) bacteria, whereas inhibition was complete for some Gm⁻ bacteria at this Cr level. Growth in nonaerated cultures for four actinomycetes was in the form of macroscopic colonies, making turbidity readings unreliable. Two of these isolates were slightly inhibited by 1 ppm Cr(VI) and completely inhibited by 10 ppm Cr(VI). The other two were somewhat inhibited [illegible] 10 ppm level.

The concentration of Cr(VI) in solution after [illegible] hours (Gm⁺, 0.78 and 9.2 ppm; Gm⁻, 1.15 and 10.9 [illegible] for the 1 and 11 ppm Cr(VI) levels, respectively, [illegible] aerated cultures) indicated that Cr(VI) tolerance [illegible] ently was not the result of its reduction to the less [illegible] Cr(III) in the aerated cultures, although less Cr(VI) [illegible] found in cultures with a higher final cell concentration.

To determine whether or not the soil extract [illegible] was involved in the interaction between the bacteria [illegible] Cr(VI), the aerated experiment was repeated using [illegible] semisynthetic medium. Growth rates were much [illegible] than in the soil extract, and the experiment was [illegible] after 1 day (Table 2). The toxicity of Cr(VI) was [illegible] more pronounced in the synthetic medium than in the [illegible] soil extract medium, and again Gm⁻ bacteria were [illegible] sensitive than Gm⁺ bacteria.

Toxicity of Cr(III) was tested under both aerated [illegible] nonaerated conditions. Mean concentrations of soluble Cr at the end of the experiment were 40.7, 4.6, and 0.[illegible] ppm for the 100, 10, and 1 ppm levels, respectively (Table 3). Large decreases in Cr concentration occurred because of the low solubility of Cr(III) at [illegible] 6.5. Significantly lower growth rates were found for [illegible] of four isolates tested in the nonaerated study at [illegible] 100-ppm level. Higher growth rates at the 10-ppm [illegible] compared with the controls, were found in the aerated study; perhaps Cr(III) increased growth by complexing [illegible] inhibitory compounds present in the soil extract. Optical densities for a *Streptomyces* sp. were unreliable [illegible]

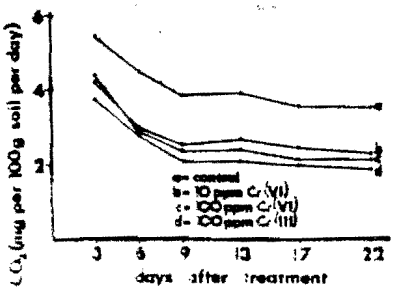

Fig. 1—Evolution of CO_2 in Cr-treated Hinesburg fine sandy loam with time.

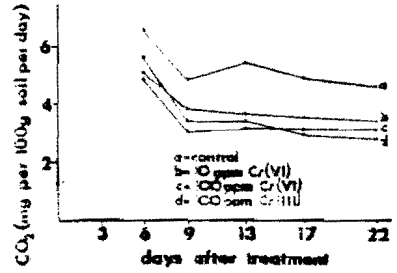

Fig. 2—Evolution of CO_2 in Cr-treated Nellis loam with time.

but no differences in aerated and nonaerated cultures were apparent.

Bacteria included in this study apparently were much more sensitive to Cr(VI) than Cr(III) and Gm^- bacteria were somewhat more inhibited by Cr(VI) than were Gm^+ bacteria.

CO_2 Evolution

Microbial activity of the control soils was higher than that in all Cr-treated Nellis and Hinesburg soils (Fig. 1 and 2). All Cr-treated soils showed decreased respiration as evidenced by decreased CO_2 evolved per day, and differences between Cr-treated soils were small.

Figure 3 shows the decline in extractable Cr(VI), apparently by reduction to Cr(III), in the soils in which it was measurable. The presence of Cr(VI) in the 100 ppm Cr(III)-treated soils after 3 days showed that some oxidation of Cr(III) had occurred in the Nellis soil, but extractable Cr(VI) had disappeared by 13 days. About three-fourths of the Cr(VI) added to the soils treated with 100 ppm Cr(VI) was not extractable after 3 days.

Since the bacterial culture studies did not show reduced Cr to have much toxicity, the suppression of microbial activity by added Cr(III) was not anticipated (Fig. 1 and 2). Slater and Reisenauer[3] also found decreased CO_2 evolution in moist Yolo soil with added Cr(III). The fact that CO_2 evolution did not increase as the Cr(VI) disappeared also was unexpected. Four explanations are possible:

1) Cr(VI), even though present for only a short time, may have permanently damaged one portion of the soil population.
2) "Tanning" of available substrate by Cr(III) may have occurred; that is, Cr(III) may have formed cross-linkages between organic compounds in the soil, rendering them unavailable to microbial attack.

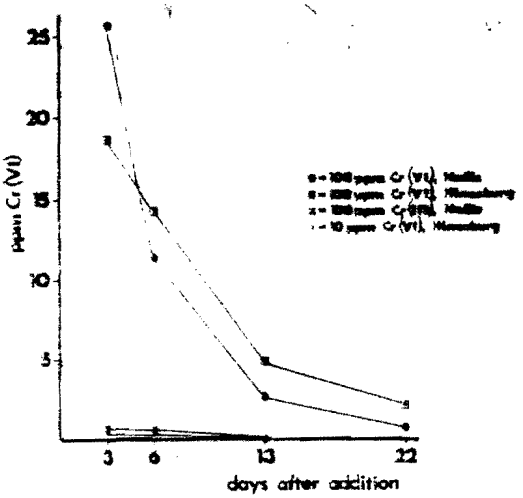

Fig. 3—Concentrations of extractable Cr(VI) in the two soils studied for CO_2 evolution with time after Cr application.

3) An unstudied portion of the soil microbial population, e.g., fungi, may have been highly sensitive to Cr(III).
4) Conditions of growth in soil may be so different from those in pure culture that toxicity studies in one medium cannot be applied to the other.

There is no direct evidence favoring any one of the above conjectures. It is possible that, in some locales, Gm^- bacterial activity could have been completely suppressed. High sensitivity of some Gm^- bacteria to Cr(VI) was shown earlier in this study. It is hard to imagine that 100 ppm Cr(III) "tanned" very much of the available substrate, but this effect cannot be ruled out.

The inhibition of Gm^- bacteria in aqueous culture by low levels of Cr(VI) indicates that soil microbial processes carried out by Gm^- bacteria would likely be affected in soils treated with wastes containing small amounts of Cr, if the Cr becomes oxidized. Rudolfs (1950) observed that nitrification in an activated sludge process was inhibited by Cr at levels as low as 2 ppm. Initial inhibition may, however, be overcome if the microbial population is able to adjust to the stress.

Toxicity of Cr(VI) to most forms of life is documented, whereas Cr(III) is not generally considered a problem. Even though the reasons are not clear, it is evident that both Cr(VI) and Cr(III) additions may be toxic to soil microorganisms. It should not be assumed, therefore, that Cr(III) is harmless when added to soil. More study is needed to determine the nature and extent of the toxicity of both species of Cr in field soils.

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The Response of a Southern Appalachian Forest to Waste Water Irrigation[1]

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ABSTRACT

The operational land treatment system at Unicoi State Park near Helen, Georgia was used for a period of 3 years to study the role of a mature Appalachian forest in renovating municipal waste water. Uptake of some of the nutrients in the waste water was occurring in the vegetation, but no systematic nutrient uptake by any one component of the stand was observed. Overstory trees failed to show any growth response to the irrigation in height or diameter at breast height (dbh), but did show increased stem diameter growth within the canopy. The irrigation was causing significant increases in diameter at 0.3 m and height of understory trees. A detailed stem analysis of 5 treated and 5 control white pine trees (*Pinus strobus* L.) from the mature overstory showed that upper stem diameter growth was increased significantly as a result of the irrigation. This stem volume response to waste water irrigation would not be detected had breast height diameter and total height been the only stem measurements taken. The irrigation is decreasing the biomass, the diversity, and the number of individuals of the ground vegetation.

Additional Index Words: land treatment, biomass, stem analysis, *Pinus strobus* L.

Brister, G. H., and R. C. Schultz. 1981. The response of a southern Appalachian forest to waste water irrigation. J. Environ. Qual. 10: 148–153.

In 1973 the Unicoi State Park was selected by the Environmental Protection Division of the State of Georgia and the School of Forest Resources of the University of Georgia as the site for a land application demonstration in the southern Appalachian hardwood-pine forests. The site was to become the operational waste water treatment system for the recreational facility and its associated conference center.

Because of the operational nature of the demonstration, only one site and one application rate were used. This demonstration provided an opportunity to investigate the response of a mature forest to waste water and the role played by the forest in nutrient renovation. Renovation is the removal of nutrients, organic matter, and pathogens from applied waste water by the biotic and abiotic components of the site. Although destructive sampling of the stand could not be permitted, inferences on net vegetation nutrient storage are possible under the assumption that the biomass of an individual plant is related to measures of its height and stem diameter (Taras and Clark, 1975). Since there is no replication of irrigated plots, the observed responses do not provide a measure of site variability, but do provid guidance in the southern Appalachian region where in formation on site response to waste water irrigation is scarce or nonexistent. The purpose of this study is, therefore, to determine whether the addition of waste water to this Appalachian forest would increase the growth and nutrient uptake of the vegetation and caus a change in species distribution.

MATERIALS AND METHODS

The demonstration facility is located on a steep forested slope w a north aspect in the Unicoi State Park in the Blue Ridge Mountains near Helen, Georgia. Its elevation ranges from 490 to 520 m with slopes from 9 to 20°. The major soil on the site is classified by the S Conservation Service (USDA) as a nutrient deficient, well drain Hayesville sandy loam, a member of the clayey, oxidic, mesic fam of Typic Hapludults. The soil pH averages 5.7 and cation exchan capacities range from 5 to 14 meq/100 g.

The climate is characterized by warm summers, cool winters, and average rainfall of 1,800 mm with a maximum in spring and a mum in fall. Only a few days of above 32°C or below 0°C tempe tures are recorded each year, and the mean annual temperature 15°C.

Vegetation on the study area consists of a pine-hardwood fo with predominant overstory species of oaks (*Quercus alba* L., *Q. cinea* Muenchh., *Q. falcata* Michx., *Q. montana* Willd., *Q. rubra* *Q. stellata* Wangenh.), white pine (*Pinus strobus* L.) and Virginia p (*Pinus virginiana* Mill.). The lower slopes contain a well-defined, dense understory consisting almost entirely of mountain la (*Kalmia latifolia* L.), and the predominant understory species on upper slopes are blueberry (*Vaccinium vacillans*), huckleb (*Gaylussacia* spp.), dogwood (*Cornus florida* L.), and red ma (*Acer rubrum* L.). There is a distinct vertical stratification within t woody vegetation. The lower stratum rarely reaches 6 m in height and the upper story is consistently taller than this. The stand has an average age of 45 years.

The waste water from Unicoi State Park receives the equivalent of secondary treatment in an oxidation lagoon. The waste water is chlori nated as it is pumped from the lagoon to the irrigation site. There are no heavy metals present in this waste water.

The 0.8-ha study site is divided into two areas approximately 70 long and 55 m wide. The treatment area is irrigated 1 day each wee throughout the year at the rate of 8 mm/hour for a total of 76 mm/day using impact sprinklers on 1.2-m risers. The spray head pressure was < 3.2 kg/cm². The mean monthly quantities of nutrients added to the site are presented in Table 1. Initially, weekly sampling was done, but this was changed in 1976 to a monthly basis. The table

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