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I am submitting herewith a thesis written by Patricia Dreyer Parr entitled "Effects of Orocol TL (A Corrosion Inhibitor) on Germination and Growth of Bush Beans." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology.

John P. Witherspoon, Major Professor

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# EFFECTS OF OROCOL TL (A CORROSION INHIBITOR) ON GERMINATION AND GROWTH OF BUSH BEANS

A Thesis

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Degree

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Dedication

To my grandmother,
Ray E. Ogden,
who had unswerving confidence
in my ability to do anything I
set out to do - and told me so.

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## ABSTRACT

This research is part of a continuing series of studies conducted at Department of Energy (DOE) facilities to determine the environmental effects of hexavalent chromium from cooling tower drift on biological systems. Previous studies provided evidence of the transport of chromium in cooling tower drift to the landscape. Specifically, the purpose of this research was to address the potential for germination and growth effects in bush beans (Phaseolus vulgaris L. var Bush Blue Lake) from hexavelent chromium in Orocol TL (a proprietary chromated, zinc-phosphate compound added to DOE cooling water systems for corrosion inhibition) at low and high concentrations in the soil by adjusting soil pH and the percent of organic matter.

Germination effects were determined in bean plants grown in soils adjusted to differing pH ranges (4-4.5, 5-5.5, 6.5-7), levels of organic matter (1.8%, 3%, 5%) and Orocol TL amendments (control of 0 µg/g, 10 µg/g, 500 µg/g chromium). Growth responses (effects) were determined from bush bean plants cultured in the same soil treatment combinations as described for the germination study. Plants were harvested when the plant died or at the end of eight weeks and partitioned into leaves, stems and roots. Following weight determinations, the leaves, stems and roots were analyzed for total chromium content, and the results compared using analysis of variance and multiple comparison procedures.

Results of the germination experiment showed a significant ($P < 0.05$) effect from organic matter, soil chromium concentration

and the interaction between organic matter and chromium concentration. At the higher concentration of chromium (500 µg/g), there was a lower percent germination. Plants grown in high organic matter (5%) had much higher percent germination even at the high level of chromium addition. The soil pH did not have a significant effect alone or in interaction with organic matter and chromium concentration.

Plants in the growth effects study that received 500 µg/g chromium wilted immediately and did not recover indicating that the hexavalent chromium exceeded the reducing and adsorbing capacity of the soil and roots. Chromium concentrations in the roots of plants of the 500 µg/g chromium treatment with a soil pH range of 4-4.5 were significantly ($P < 0.05$) lower than at the other pH levels. Plants of the 0 and 10 µg/g treatment groups were harvested at the end of eight weeks. There was a significantly ($P < 0.05$) greater amount of chromium in the leaves, stems and roots of the plants receiving the 10 µg/g chromium addition as compared to the controls. Dry weights of roots of plants grown in 3% organic matter were significantly ($P < 0.05$) less than the 5% treatment and the dry weights for roots grown in the soil pH range of 6.5-7 were significantly ($P < 0.05$) lower than at pH 5-5.5. Leaf dry weight was significantly greater ($P < 0.05$) in the order of soil pH 5-5.5 > pH 6.5-7 > pH 4-4.5 and was significantly less ($P < 0.05$) in soils amended to 10 µg/g chromium as compared with controls. There were no significant dry weight differences in the stems.

High levels (500 µg/g) of hexavalent chromium in soil (as Orocol TL) affected germination and growth, while a high level of organic matter significantly reduced chromium toxicity on germination. At lower chromium concentrations there was significant uptake by all plant parts, with a corresponding reduction in biomass of leaves. Consequently, adjustments of soil pH from 4.0 to 7.0 appear to have no significant effect on chromium uptake in plants. Increasing the organic matter level to 5%, while decreasing the toxicity of high chromium levels to germinating seed, did not affect chromium uptake.

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# INTRODUCTION

The use of closed-cycle cooling towers is one method of dissipating waste heat. Closed-cycle cooling towers, which involve the use of evaporative cooling to reject heat, have become increasingly popular as an alternative to once-through cooling for electrical generating stations because of the reduced impact to aquatic systems without returning hot water directly to the source. However, the operation of closed-cycle cooling has the potential for causing adverse effects to terrestrial biota from cooling tower drift. As the demand for electrical power increases, so will the need for cooling towers as a method of dissipating waste heat. Consequently, cooling towers will become an increasingly familiar sight on our landscape.

Depending on the quality of the makeup water and plant-specific operational procedures, constant water treatment may be necessary to decrease the possibility of corrosion within the cooling system. This often involves the addition of corrosion inhibitors and scale retardants. At the Department of Energy (DOE) uranium enrichment facilities in Oak Ridge, Tennessee, Paducah, Kentucky and Portsmouth, Ohio, waste heat is dissipated by mechanical draft cooling towers that use a proprietary chromate, zinc-phosphate corrosion inhibitor (Orocol TL marketed by Betz Laboratories, Inc.) in the recirculating cooling water systems (Fowlkes and Webb, 1972).

A fraction of the circulating water becomes entrained in the exit air flow as small droplets and is carried out beyond the towers as drift. The drift contains concentrations of dissolved minerals and

chemicals similar, if not identical, to concentrations of the recirculating water (Laulainen, 1978), which is maintained at 20 µg/g hexavalent chromium (as $CrO_4^{2-}$) in the recirculating streams of the DOE uranium enrichment facilities. This loss of recirculating water presents a pathway for transfer of potentially toxic elements into the terrestrial environment.

Previous research at the Oak Ridge Gaseous Diffusion Plant (ORGDP) cooling towers has provided quantitative evidence of the transfer of chromium from drift to vegetation (detectable above background to at least 1600 m from the towers) and to soil (with above background concentrations out to 180 m from the towers), and identified some effects of chromium in drift on vegetation (Taylor et al., 1975; Parr et al., 1976; Taylor et al., 1976; Taylor and Parr, 1978; Taylor, 1980). While contamination of vegetation from deposition of drift on aerial parts may represent a short-term effect, accumulation of chromium in the soil poses a possibility of greater and potentially longer term consequences. Since the chromium in drift is in water droplets, it was hypothesized that Orocol TL dissolved in a soil-water solution would be suitable to assess effects of chromated drift without sacrificing chemical composition.

The purpose of this research was to determine the potential for increasing or decreasing germination and growth effects in plants from chromium in Orocol TL at low and high concentrations in the soil by adjusting soil pH and the percentage of soil organic matter.

# CHAPTER I

# LITERATURE REVIEW

## I. Chromium in Soils

Chromium availability. Chromium is present in all soil types with total chromium concentrations ranging from less than 5 µg/g to a few percent. However, availability of the chromium in the soil may be quite low (0.01 to 4 µg/g) according to Towill et al. (1978). Serpentine soils have a level of chromium as high as 1 to 2% (10,000-20,000 µg/g). On serpentine soils, infertility and lack of vegetation has been associated with chromium and nickel toxicity (Soane and Saunder, 1959). Analyses of these soils have shown that the level of available chromium is also very low, although high concentrations occur in indigenous plants.

Most chromium in the soil is in an insoluble form or tightly bound. The trivalent form of chromium (Cr-III) is the most stable oxidation state of the element. Hexavalent chromium (Cr-VI), less stable than the trivalent state, is more mobile and has the potential for causing environmental problems. However, hexavalent chromium is reduced to the stable trivalent state in the presence of organic matter. Shewry and Peterson (1976) found that both trivalent and hexavalent forms of chromium (as $^{51}Cr^{+3}$ and $^{51}CrO_4^{2-}$) were strongly bound in the soil. In a study by Grove and Ellis (1980a), water extractable chromium compounds appeared to convert to insoluble compounds of Cr-III in soils.

Chromium and soil pH. The pH of the soil may have an effect on the reduction and adsorption of chromium. Hexavalent chromium is reduced to Cr-III in acidic soils more rapidly than in alkaline soils. At pH 5.2, Cary et al. (1977b) reported a faster reduction to Cr-III from Cr-VI than at pH 7.2 and 7.5. Adsorption of Cr-VI and Cr-III by kaolinite and montmorillonite clay minerals was found to be highly dependent on pH. Griffin et al. (1977) found also that chromium adsorption decreased as pH increased, with no adsorption above pH 8.5. In the pH range of 1.5-4.0, 30 to 300 times more Cr-III than Cr-VI was adsorbed by clay minerals. Adsorption of hexavalent chromium was markedly reduced as the pH was raised into the alkaline range, and likely would become mobile at a high pH.

Chromium and organic matter. Studies of hexavalent chromium (as dichromate) in soils indicate that it is reduced to the less mobile form by organic matter (Skeffington et al., 1976; Cary et al., 1977b; Gemmell, 1972) even at a pH above 7 (Bartlett and Kimble, 1976b). Artiole and Fuller (1979) determined that both organic matter and Fe-II are effective electron donors for reducing hexavalent chromium to the trivalent state. Bartlett and Kimble (1976a) found reduction did not occur in soils very low in organic matter unless an energy source was added. For plants growing in contaminated soil, Gemmell (1972) reported that peat was superior to sand or soil in reducing chromium toxicity. He suggested that reduction of toxicity may be attributed to chromate reduction and/or pH effect depending on the chemical state of the substrate.

Bartlett and Kimble (1976b) concluded that Cr-VI added to soil will remain mobile only if its concentration exceeds both the adsorbing and reducing capacities of the soil.

Chromium accumulation in soil. Various studies have shown that deposited chromium may accumulate in the top few centimeters of soil. Goodroad and Caldwell (1979) found chromium concentration decreased with depth in a Nicollet clay loam soil. Chromium and nickel increased in the 0-7.5 cm depth of soils following sludge application in a mixed hardwood forest (Sidle and Kardos, 1977). Municipal sludges typically contain chromium, zinc, copper, cobalt, lead and other elements in concentrations higher than those found in typical agricultural soils (Hinesly et al., 1976; Furr et al., 1976). Williams et al. (1980) found that even with increasing sludge rates, values of chromium concentration decreased with depth. At the Oak Ridge Gaseous Diffusion Plant (ORGDP) in Tennessee, increased quantities of chromium in the top 6 cm of soil were evident out to 180 m from the cooling towers, ranging from 10 times background at 15 m to 3 times background at 60 m. Concentrations considered available to plants out to 180 m from the towers varied from 3-27 μg/g chromium. Beyond 180 m, chromium concentrations were similar to background levels (Taylor et al., 1975).

It has been shown that it is possible for trivalent chromium to oxidize to hexavalent chromium under some field soil conditions. Bartlett and James (1979) suggest that the key to oxidation appears to be the presence of oxidized Mn which serves as an electron acceptor.

## II. Chromium in Vegetation

Effects of chromium on germination. Few studies of the effects of chromium on seed germination have been reported. Delayed germination of corn at 80 and 320 µg/g hexavalent chromium (as $Na_2Cr_2O_7$) was reported by Mortvedt and Giordano (1975). In an earlier study, Rediske (1956) reported germination of barley seed at levels of 10, 50 and 100 µg/g Cr-VI in the soil, with no germination at 500 µg/g chromium. Once the barley seed germinated, it grew well, but was always later in development. It was hypothesized that the effect was probably due to inhibition of diastase by chromium, thus preventing the seed from properly mobilizing the reserve starch necessary for initial growth.

Toxicity of plant uptake of chromium. Uptake of chromium by vegetation growing on contaminated soils presents a potential pathway for movement into the food chain. Toxicity of chromium has been reported in plants at levels as low as 1 µg/g (Soane and Saunder, 1959), although some investigators have found that low amounts of chromium may actually stimulate growth. Growth of oats and barley was stimulated by 100 µg/g chromium, but 50 µg/g was toxic to wheat (Sullivan, 1969) and 100 µg/g was toxic to bush beans (Wallace et al., 1976). Hexavalent chromium (as potassium dichromate) was toxic to ryegrass at levels of 500 µg/g (Breeze, 1973). Dijkshoorn et al. (1979) reported that chromium became toxic for clover at a concentration ten times lower than nickel, but had a 100-fold lower relative uptake.

Soil pH and chromium uptake. The pH of the soil affects the reduction and adsorption of chromium in the soil, and consequently the availability of chromium to plants. Most investigators have found increased chromium uptake in plants grown in acidic soils, although hexavalent chromium is reported to be reduced to Cr-III in acidic soils more rapidly than in alkaline soils (Cary et al., 1977b; Griffin et al., 1977). In soils with high chromium amendments, Dowdy and Larson (1975a) found greater uptake from acidic soil. Optimal uptake of Cr-VI (as $^{51}CrO_4^{2-}$) for barley seedlings was between a pH of 5.5 and 6.5 (Shewry and Peterson, 1974). In limed soils, Dijkshoorn et al. (1979) determined there were three-fold lower plant: soil concentrations than in acid soil, indicating poor accessibility of the metals to the root surface. Bolton (1975) found that for ryegrass grown in an acid soil limed to pH 4.3-6.8, there was no effect on the chromium concentration. In a study by Mortvedt and Giordano (1975), corn yields were not affected by 5 and 20 µg/g chromium at either pH 5.5 or 7.0. However, yields decreased at both pH levels with 80 µg/g hexavalent chromium (as $Na_2Cr_2O_7$). Sullivan (1969), on the other hand, reported very little chromium available to plants in soils with a pH of less than 4.0. Gemmell (1973) reported indications that severe alkalinity aggravated chromium toxicity in a sand substrate.

Interaction with other metals. The interaction of chromium with other elements in the soil may affect their uptake. Dowdy and Ham (1977) applied metals individually and in combination to soybeans (hexavalent chromium as $Na_2Cr_2O_7$). The high chromium sludge

application did not increase tissue chromium concentration. Chromium uptake was not affected either by the presence of other metals. Likewise, chromium concentrations in corn foliage were not affected by chromium in sludge (Mordvedt and Giordano, 1975), and chromium, nickel, cobalt and cadmium content in barley was not significantly different due to type or rate of sludge applied (Vlamis et al., 1978). Furr et al. (1976) reported higher chromium levels in three crops grown on sludge-treated soil as compared to controls the first year after application. Chromium was noticeably lower the second year.

Cunningham et al. (1975) reported, however, that increasing chromium concentrations, significantly decreased plant tissue concentrations of copper, zinc, nickel, cadmium and manganese. Turner and Rust (1971) found that 0.05 µg/ml chromium in nutrient solution and 10 µg/g in soil culture experiments interferred with uptake of calcium, potassium, magnesium, lead, boron and copper in tops of soybeans. Wallace et al. (1976) reported that in bush beans at 50 and 100 µg/g chromium, iron and zinc concentrations were decreased, and potassium, calcium and magnesium concentrations decreased slightly. 100 µg/g chromium decreased molybdemum concentrations in leaves. Shewry and Peterson (1976) found that nickel and chromium were positively correlated with magnesium in the roots.

Grove and Ellis (1980b) found that the amount of water-soluble iron was lowered on all soils by Cr-III and sludge amendments. Hexavalent chromium initially lowered water soluble iron, but later raised it above control levels. Shewry and Peterson (1974) report that

calcium ions are necessary for maximum uptake of hexavalent chromium (as $^{51}CrO_4^{2-}$), but found no effect on transport.

Hunter and Vergnano (1953) found a relationship between excessive chromium and low levels of nitrogen in plants and an increase of phosphate concentration in the tissue extract. Chromate uptake appears to be inhibited by sulfate ions (Shewry and Peterson, 1974; Skeffington et al., 1976).

In a study by Grove and Ellis (1980b), the amount of water soluble manganese increased on soils treated with water soluble Cr-III. The addition of inorganic chromium compounds to soils induced changes in soil pH, subsequently affecting chemistry of soil manganese.

Effects of chromium on plant growth. An increase in chromium has been reported to cause chlorosis in plants (DeKock, 1956; Parr et al., 1976) accompanied by reduction in iron, manganese uptake and dryweight (Rediske, 1956). Oat plants grown in a nutrient solution of 5 µg/g chromium in a study by Hunter and Vergnano (1953), were basically normal, but exhibited a slight diffuse chlorosis. At 10 µg/g, plants were small and most leaves were slightly chlorotic. At 25 and 50 µg/g, growth was stunted and leaves were narrow and brownish red and exhibited small necrotic areas. Roots were poorly developed with concentrations greater than 10 µg/g chromium. In a later study by Shewry and Peterson (1974), one of the first effects of chromium in barley seedlings was inhibition of root growth; shoot growth was decreased at higher levels. However, DeKock (1956) reported only a small reduction in biomass of mustard roots, although some were stunted.

In a soil culture experiment by Turner and Rust (1971), 10 µg/g hexavalent chromium (as potassium dichromate) significantly reduced dry matter yields. In barley plants with trivalent chromium added (as $KCr(SO_4)_2$), the level at which the yield was reduced 10% (dry matter ) was approximately 10 µg/g chromium (Davis et al., 1978). At 200 µg/g chromium (from chromate smelter waste) in sand, Gemmell (1972) recorded a 50% inhibition of plant growth. Oats contaminated with chromium applied as Cr-VI ($K_2Cr_2O_7$) and Cr-III [$Cr_2O_3$ and $Cr_2(SO_4)_3$] showed a reduction in yield, but chlorosis was not induced (Anderson et al. 1973). Wallace and Romney (1977) found bush bean yields decreased at all levels of Cr-III added as $Cr(SO_4)_3$. However, barley yields increased significatly with increasing rates of sludge applications high in chromium in a study by Dowdy and Larson (1975a).

Hexavalent chromium appears to enter the plant more quickly than trivalent chromium, thus having the potential of causing greater damage. Rediske reported in 1956, that Cr-III was less toxic than Cr-VI when applied to the soil surface, requiring about 10 times greater concentration to produce the same effects. More recent studies have verified his findings. In a study by Mortvedt and Giordano (1975), 320 µg/g hexavalent chromium (as $Na_2Cr_2O_7$) resulted in severe toxicity in corn, indicating greater plant availability of inorganic chromium. Trivalent chromium [as $Cr_2(SO_4)_3$] was less toxic to corn than hexavalent chromium (as $Na_2Cr_2O_7$) at the rate of 80 µg/g chromium. Both were toxic at 320 µg/g. Skeffington

et al. (1976) found, also, that Cr-VI (as $CrO_4^{2-}$) inhibited growth of roots and shoots of barley seelings more than Cr-III.

High concentrations of hexavalent chromium from cooling tower drift was associated with a 75% reduction of leaf growth in potted tobacco plants placed around the towers (Parr et al., 1976).

Translocation of chromium in plants. Many investigators have determined that plants growing in chromium contaminated soil or solution will take up the chromium in the roots, but very little, if any, is translocated elsewhere (Myttenaere and Mousny, 1974; Wallace et al., 1976; Cary et al., 1977a; Wallace and Romney, 1977; Lepp and Eardley, 1978; Parr and Taylor, 1980).

Plants cultured in solutions containing Cr-VI (as $Na_2^{51}CrO_4$) accumulated most of the absorbed radioactivity in the roots as expected (Lyon et al., 1969). Also, the Cr-VI appeared to be reduced to Cr-III during passage from culture solution to plant leaves. The state of this reduction is unknown, but was hypothesized to be due to a small difference in the physiological status of the plant. Shewry and Peterson (1974) determined that most of the $^{51}Cr$ was in the roots of barley seedlings grown in solution at various pH levels and hypothesized that the majority of the $^{51}Cr$ was located in the vacuoles of the root cells. The roots of higher plants may be a barrier to translocation of some metals to shoots of plants and a mechanism for binding chromium in or on roots may not only decrease toxicity to the plant, but retention in roots could decrease entry into the food chain (Wallace and Romney, 1977).

Cary et al. (1977a) found that plants accumulated chromium from nutrient solution, but as expected, retained most of this in the roots. They also determined that plants or tissues that tend to accumulate iron also accumulate chromium. Uptake of Cr-III from the solution tended to level off at about 50 µg/ml solution chromium. In all treatments, over 99% of the $^{51}Cr$ removed from the solution was still in the roots at the conclusion of the experiment.

However, Garcia et al. (1979) found translocation of chromium from soil to corn tissues of plants. The highest concentration was in the roots, but there were increases also in the leaves.

Roots of several crop species supplied with $^{51}Cr$-III in solution contained more chromium than those supplied with $^{51}Cr$-VI, but the reverse was found in the shoots in a study by Lahouti and Peterson (1978). This indicated that although plants supplied with $^{51}Cr$-VI accumulated less in the roots, a larger proportion (although a small amount) was transported to the shoots. The study also indicated that the valence of the chromium was more important in determining distribution of the element than the plant species.

Foliarly applied chromium as Cr-III ($CrCl_3$) and Cr-VI ($Na_2CrO_4$) did not translocate from the leaves of bean and lettuce plants (Levi et al., 1973). The Cr-III penetrated more rapidly than the Cr-VI. They hypothesized that the location in leaf cells of the chromium together with its chemical binding and/or precipitation in an insoluble form, may explain the lack of transport of chromium in plant tissues. Soane and Saunder (1959) hypothesized that chromium is less

readily translocated to the leaves of the plant because of possible interactions between chromium, phosphorus and calcium in the soil-plant system.

In foliar applications of Cr-VI to leaves of soybeans, Parr and Taylor (1980) determined that only a small fraction (1-7%) of the chromium applied was translocated to roots. Similarly, Pickrell and Ellis (1980)found $^{51}Cr$ was absorbed through soybean leaves and translocated, in small amounts (4%). Absorption was rapid, but translocation slow.

Accumulation of chromium in plants. In the Croal Valley of Great Britain which was polluted by an old chromate salt manufacturing company, Breeze (1973) found the chromium entered the shoots of plants grown on soil layers 15 cm deep. In oats grown on soil with 256 µg/g, chromium concentrations in the oat tops was 49 µg/g (Anderson et al., 1973).

Of the food crops studied, the leafy vegetables that tended to accumulate iron (i.e., spinach, turnip leaves) appeared to be the most effective in translocating chromium to leaves (Cary et al., 1977a). Dowdy and Larson (1975b) suggested that since potatotes and carrots are excellent non-accumulators of metals, they may be suitable crops for utilizing sludge amended soils.

# CHAPTER II

## METHODS AND PROCEDURE

The design of the experiment incorporated three treatments of organic matter, pH and chromium concentration (added as Orocol TL) to determine what effects the treatments, either singly or in interaction, would have on seed germination, chromium uptake and plant growth. Each of the three treatments were tested at three levels.

Soil used for the experiment was the lower A horizon (A2) of a Fullerton cherty silt loam. Fullerton was chosen as the soil because it is fairly common in east Tennessee and is an acidic soil.

Organic matter content in local Fullerton soil is about 1.8% (Peters et al., 1970), which was used as the first level of the organic matter treatment. Peat moss was chosen as the organic matter addition because it is fairly acidic with a pH of about 3.8 to 4.0. In local soils, 5% is similar to the organic matter content of the A1 horizon (Peters et al., 1970).

The pH of the Fullerton soil was measured as 4.0 to 4.5 and was used as the low level of the pH treatment. The pH was determined using a 1:1 soil to water ratio and an Orion pH meter. Soil was limed to raise the pH to 6.5-7.0, the optimum pH range for bean plants (Federation of American Societies for Experimental Botany, 1966), as the high pH level. For the intermediate level, the pH was adjusted to 5.0-5.5.

Chromium was added to the soil in the form of Orocol TL, a proprietary chromate, zinc-phosphate compound which is used as a corrosion inhibitor in many cooling systems.

A pH of 2.75 was measured for the Orocol TL alone. To determine if it might affect pH of soil, the pH of four soil samples was measured, then Orocol TL was added to bring the soil chromium concentration up to 10 µg/g in two samples and to 500 µg/g in the two other samples. There was no difference in the pH values indicating the Orocol TL did not affect the pH at either level of chromium concentration in the soil.

The Orocol TL contains chromium in the hexavalent form (Fowlkes and Webb, 1972). The more mobile and toxic hexavalent chromium can be quickly reduced to the less mobile trivalent form in the soil (Bartlett and Kimble, 1976b; Cary et al., 1977b). Determining the level necessary at which all the hexavalent chromium would not be immediately reduced in the soil leaving enough mobile to get into the plants was the first subproject.

Hexavalent chromium (as $K_2Cr_2O_7$) was added in varying amounts to a beaker with 10 g of dry soil which had been moistened with 3 ml distilled water. Fifty ml of 1 M HCl was then added and the beaker was placed on a shaker for 15 minutes. The soil solution was then filtered immediately through nucleopore filters using a vacuum system.

To measure hexavalent chromium colorimetrically, a reagent was made up of: 200 mg s-diphenyl carbazide in 100 ml 95% ETOH adding,

while stirring, a solution of 40 ml $H_2SO_4$ and 360 ml distilled water (Urone, 1955; Taras et al., 1974). One ml of reagent was added to 0.5 ml of the soil solution and 7.5 ml distilled water and measured colorimetrically at 540 nm using a Bausch and Lomb Spectronic 20.

Additions of 110 and 389 µg/g hexavalent chromium to the soil resulted in recovery of 26.8 and 26.5% Cr-VI respectively. For the 813 µg/g addition there was a recovery of 35.2% of the Cr-VI. The spectrometer was barely off-scale at the 1000 µg/g addition of hexavalent chromium. Based on these results, 500 µg/g chromium was determined to be a concentration high enough for 25-35% of it to remain mobile with the potential to enter the plant, and was chosen for the high level chromium addition. In the experiment, Orocol TL was added to the soil as the hexavalent chromium source. For a low chromium addition, 10 µg/g Cr-VI was used. The third level of chromium was a control with no Orocol TL amended to the soil.

Germination studies were conducted using the three soil treatments: organic matter, pH and chromium concentration. Each treatment was tested at three levels: organic matter at 1.8, 3, and 5%; pH at 4.0-4.5, 5.0-5.5, and 6.5-7.0; chromium at 0 and approximately 10 (9.7) and 500 (496) µg/g added as Orocol TL, for a total of 27 combinations. Three replicates of each required a total of 81 pots. Experimental design for the germination test is shown in Table 1.

A layer of coarse silica was placed in the bottom of plastic pots to provide some drainage, however, the pots did not have drainage holes in order to prevent the leaching out of the chromium through watering.

Table 1. Experimental Design for Germination Test

| Treatments | |
|---|---|
| Chromium (µg/g) Amendment | |
| | 0 |
| | 10 |
| | 500 |
| Organic Matter (percent) | |
| | 1.8 |
| | 3 |
| | 5 |
| Soil pH | |
| | 4.0-4.5 |
| | 5.0-5.5 |
| | 6.5-7.0 |

27 treatment combinations

3 plants/treatment = 81 pots

5 seed/pot

Biological Endpoint: percent germination at the end of a 14 day period.

Bush beans (Phaseolus vulgaris L. var. Bush Blue Lake) were chosen as the test species because of their ease of growth, local availability and use in effects studies by other investigators. Five bean seeds were planted in each pot which contained 300 g of the appropriate soil treatment. Percent germination at the end of a 14 day period was recorded.

Following the germination study, a growth effects study was conducted using the same treatments and levels as the germination study. Three replicates of each of the 27 treatments required a total of 81 plants. Experimental design for the growth effects study is shown in Table 2. Nine-day old bean plants (Phaseolus vulgaris L. var. Bush Blue Lake) were transplanted into 1.9 kg of the appropriate soil mixture in pots with no drainage to reduce loss of chromium through watering soil. Plants were then watered to the same moisture level and placed in the greenhouse.

Measurements of internodal growth were made every two to three days up to day 41 post treatment. Because of the size of the plants at that time, measurements of internodal growth were discontinued and total plant height was recorded. Leaves, stems, and roots were harvested when the plant died or at the end of 8 weeks. Roots were carefully washed to completely remove all traces of soil. Roots of a separate group of bean plants grown in chromium contaminated soil were carefully washed once, twice or three times, then analyzed for chromium content to determine if the one root washing was sufficient. Chromium

Table 2. Experimental Design for Growth Effects Study

| Treatment combination | | | |
|---|---|---|---|
| Cr amendment (µg/g) | Organic matter | Soil pH | Plants/treatment |
| 0 | 1.8 | 4-4.5 | 3 |
| 0 | 1.8 | 5-5.5 | 3 |
| 0 | 1.8 | 6.5-7 | 3 |
| 0 | 3 | 4-4.5 | 3 |
| 0 | 3 | 5-5.5 | 3 |
| 0 | 3 | 6.5-7 | 3 |
| 0 | 5 | 4-4.5 | 3 |
| 0 | 5 | 5-5.5 | 2* |
| 0 | 5 | 6.5-7 | 3 |
| 10 | 1.8 | 4-4.5 | 3 |
| 10 | 1.8 | 5-5.5 | 3 |
| 10 | 1.8 | 6.5-7 | 3 |
| 10 | 3 | 4-4.5 | 3 |
| 10 | 3 | 5-5.5 | 3 |
| 10 | 3 | 6.5-7 | 3 |
| 10 | 5 | 4-4.5 | 3 |
| 10 | 5 | 5-5.5 | 4* |
| 10 | 5 | 6.5-7 | 3 |
| 500 | 1.8 | 4-4.5 | 3 |
| 500 | 1.8 | 5-5.5 | 3 |
| 500 | 1.8 | 6.5-7 | 3 |
| 500 | 3 | 4-4.5 | 3 |
| 500 | 3 | 5-5.5 | 3 |
| 500 | 3 | 6.5-7 | 3 |
| 500 | 5 | 4-4.5 | 3 |
| 500 | 5 | 5-5.5 | 3 |
| 500 | 5 | 6.5-7 | 3 |

*One control plant was mistakenly amended with 10 µg/g Cr.

Biological endpoints: chromium concentrations in leaves, stems and roots; total biomass (dryweight) of leaves, stems and roots; plant growth (height).

concentrations did not differ significantly with the number of washes, indicating that surface contamination was removed in the initial wash.

Plant tissues were oven-dried (24 h) at 100°C, assessed for total biomass, then placed in a furnace for ashing (550°C) overnight. The ashed material was removed and cooled to room temperature. In a fume hood, 2 ml concentrated nitric acid was added to the ash for digestion (about 15 minutes). Using 0.1 N nitric acid, the solution was then washed into a funnel lined with Whatman No. 42 ashless filter paper and diluted in distilled water to 25 ml. Leaves, stems and roots were analyzed for total chromium by atomic spectrophotometry using a Perkin-Elmer Model 403 Spectrophotometer.

Statistical analyses of data were performed at the computer facilities of Oak Ridge National Laboratory using the Statistical Analysis System (SAS) 79.3 computer package (Helwig and Council, 1979) for analysis of variance and multiple range tests.

# CHAPTER III

## RESULTS AND DISCUSSION

### I. Germination Effects

Treatment effects on seed germination were statistically analyzed by analysis of variance. The data were transformed in order to stabilize the variance, using the arcsin transformation for binomial proportions (Snedecor and Cochran, 1972), before the analysis of variance calculations. Results of the analysis indicated significant ($P < 0.05$) main effects due to organic matter content and chromium concentrations along with their interaction. Soil pH did not appear to affect the response. In order to determine whether treatment combinations differed significantly from each other, a multiple comparison procedure (Fisher's Least Significant Difference method) was used (Chew, 1977). Results (Fig. 1) showed that seed germination between treatments containing 1.8% and 3% organic matter were similar, whereas seed germination in 5% organic matter was significantly ($P > 0.05$) higher. Germination of seeds planted in 500 µg/g Cr-VI was significantly ($P < 0.05$) lower than germination in soils containing 0 or 10 µg/g chromium, however, the germination within the 0 and 10 treatments did not differ significantly from each other.

Although there was a definite inhibition of germination in all of the 500 µg/g chromium soil combinations, the addition of organic matter to the 5% level significantly increased the probability of the seeds germinating (Table 3). This indicated that the addition of

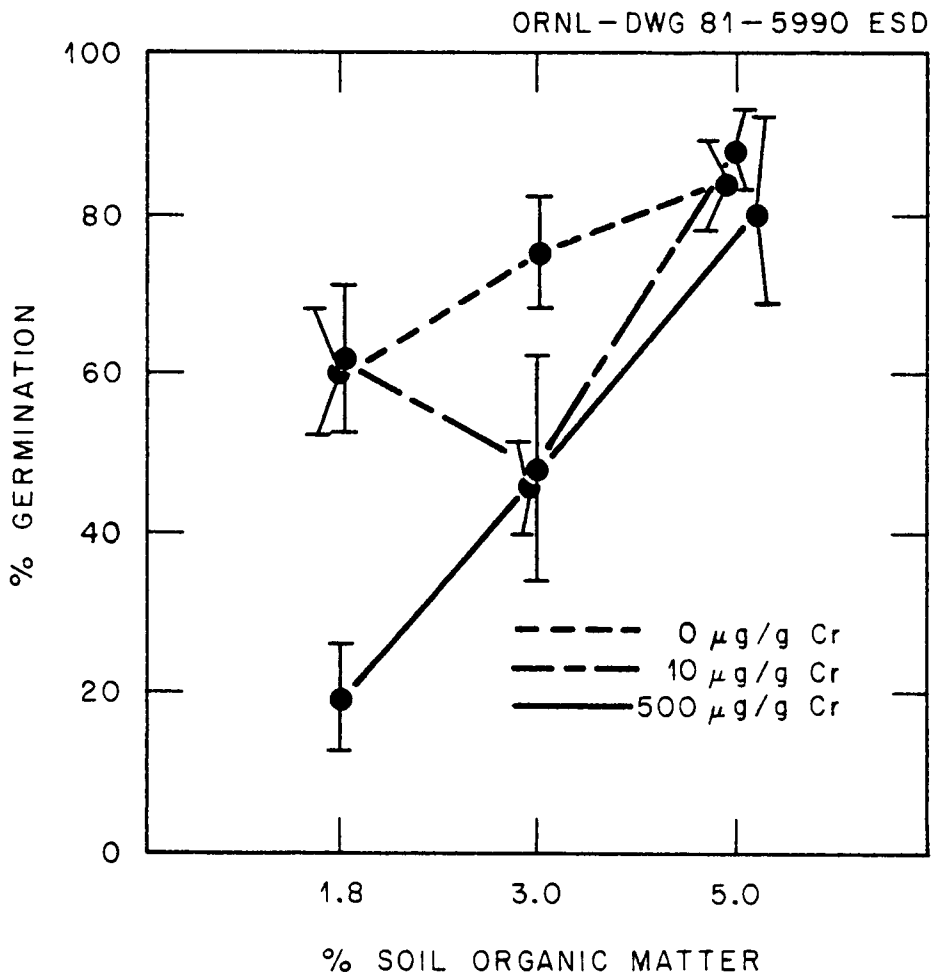



Fig. 1. Percent germination ± SE of bush bean seed in soil amended with Orocol TL. Each point is the mean of nine pots.

Table 3. Percent germination of bush bean seed in soil amended with Orocol TL.*

| | | Cr Amendment = 0 | | |
|---|---|---|---|---|
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 53 | 80 | 47 |
| | 3 | 60 | 80 | 73 |
| | 5 | 93 | 80 | 80 |
| | | Cr Amendment = 10 µg/g | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 67 | 60 | 60 |
| | 3 | 53 | 27 | 67 |
| | 5 | 93 | 93 | 93 |
| | | Cr Amendment = 500 µg/g | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 20 | 20 | 47 |
| | 3 | 40 | 40 | 60 |
| | 5 | 100 | 93 | 47 |

*Each number is based on the mean of three pots.

organic matter might impart some ameloriating effect by reducing the hexavalent chromium to the less toxic, trivalent species.

## II. Growth Effects

The effect of Orocol TL on plant growth was determined by comparing chromium concentrations and biomass of leaves, stems and roots. In order to achieve a normal distribution of the estimated residuals from the analysis of variance model, a log transformation was used on each of the observed chromium concentration values. However, no transformation was found to be necessary for the biomass data. After determining which main effects (chromium concentration in soil, organic matter content and soil pH) differed significantly from each other, Duncan's multiple range test was used to identify significant differences within the main effects.

Effect of 500 µg/g Cr-VI amendment. Beans transplanted into the soil treatment combinations with 500 µg/g Cr-VI wilted immediately and did not recover. Leaves, stems and roots were collected and analyzed for chromium content. Although all plant parts contained extremely high amounts of hexavalent chromium (Table 4), plant roots in the 500 µg/g treatments with a pH of 4.0-4.5 contained significantly ($P < 0.05$) less chromium than the other treatments (Fig. 2). The cause of the lower accumulation in these roots in unclear. Most investigators have reported increased chromium uptake in plants grown in acidic soils (Shewry and Peterson, 1974; Dowdy and Larson, 1975a; Dijkshoorn et al., 1979), and that, normally, chromium tends to

Table 4. Chromium concentrations (µg/g) ± SE in plant tissues of the high (500 µg/g) chromium treatment.

| | | LEAVES | | |
|---|---|---|---|---|
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 6373 ± 364 | 5046 ± 1544 | 7961 ± 2463 |
| | 3 | 4030 ± 229 | 4526 ± 615 | 9523 ± 3642 |
| | 5 | 4853 ± 970 | 6762 ± 3492 | 6586 ± 2291 |
| | | STEMS | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 3799 ± 1449 | 3880 ± 1265 | 4937 ± 1830 |
| | 3 | 2505 ± 788 | 3887 ± 996 | 5998 ± 2541 |
| | 5 | 2184 ± 277 | 5070 ± 1190 | 3683 ± 1390 |
| | | ROOTS | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 1855 ± 147 | 3035 ± 531 | 3490 ± 694 |
| | 3 | 1518 ± 75 | 3152 ± 685 | 3742 ± 1216 |
| | 5 | 1429 ± 142 | 3022 ± 398 | 2392 ± 655 |

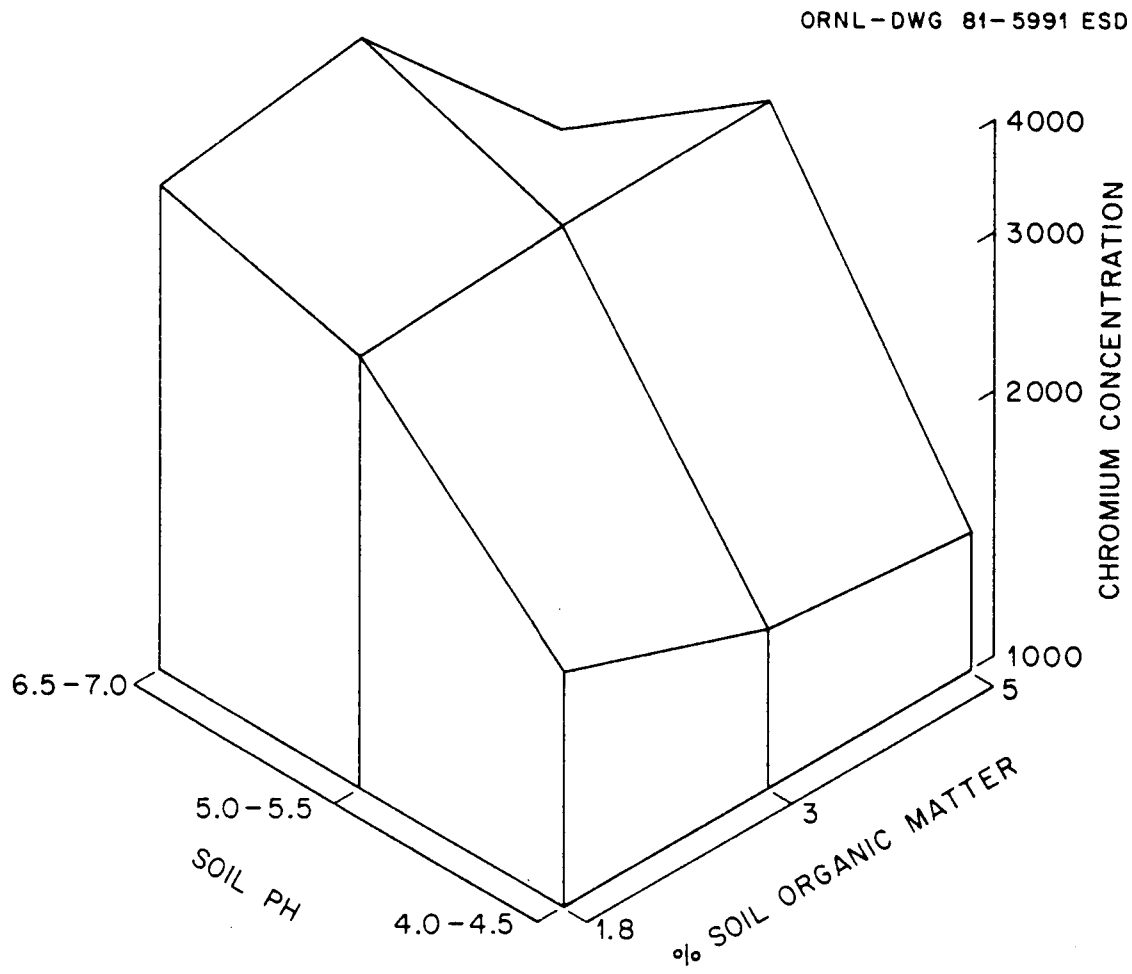



Fig. 2. Chromium concentrations in the roots of the high (500 μg/g) chromium treatment.

accumulate in the roots (Myttenare and Mousney, 1974; Wallace et al., 1976; Cary et al. 1977a; Wallace and Romney, 1977).

Of the 943 mg Cr-VI amended to the soil, 0.0004-0.110% was transported to the leaves and 0.003-0.016% remained in the roots (Table 5). Concentration factors for leaves, stems and roots of each treatment were determined on a dry weight basis from the ratio of the mean chromium concentration (µg/g) in the treated plant part, minus the mean chromium concentration (µg/g) in the controls to the chromium (µg/g) amended to the soil (Table 6). A consistent trend is evident within the 500 µg/g treatment plants with the lowest factors in the roots, increasing in the stems and highest in the leaves. The quantity of hexavalent chromium present in the soil evidently was sufficient to exceed the adsorbing and reducing capacity of the soil and roots (Bartlett and Kimble, 1976b). Enough chromium, therefore, remained in the mobile, hexavalent state and was transported quickly throughout the plant.

Effect of 10 µg/g Cr-VI amendment. Bean plants grown in soil amended to concentrations of 10 µg/g Cr-VI contained significantly ($P < 0.05$) greater concentrations of chromium in all plant tissues when compared to those grown in non-amended soils regardless of the percent of organic matter or soil pH treatment.

Concentrations in leaves (Fig. 3) of plants grown in non-amended soil ranged from 0.02-0.40 µg/g chromium as compared to 0.16-21.77 µg/g Cr in soil amended to 10 µg/g Cr-VI (Table 7).

Table 5. Percent translocation of chromium from soils amended with 500 µg/g hexavalent chromium.

| | | LEAVES* | | |
|---|---|---|---|---|
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | .068 | .054 | .110 |
| | 3 | .034 | .038 | .101 |
| | 5 | .0004 | .036 | .063 |
| | | STEMS* | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | .016 | .016 | .021 |
| | 3 | .008 | .016 | .025 |
| | 5 | .007 | .022 | .016 |
| | | ROOTS* | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | .006 | .013 | .015 |
| | 3 | .003 | .010 | .016 |
| | 5 | .005 | .010 | .010 |

*Computed using the mean of 3 plants for each treatment; untransformed data.

Table 6. Concentration factors for the 500 µg/g chromium soil amendment group.*

| Percent organic matter | Soil pH | Leaves | Stems | Roots |
|---|---|---|---|---|
| 1.8 | 4-4.5 | 12.75 | 7.60 | 3.71 |
| | 5-5.5 | 10.09 | 7.76 | 6.06 |
| | 6.5-7 | 15.92 | 9.87 | 6.98 |
| 3 | 4-4.5 | 8.06 | 5.01 | 3.03 |
| | 5-5.5 | 9.05 | 7.77 | 6.30 |
| | 6.5-7 | 19.05 | 12.00 | 7.48 |
| 5 | 4-4.5 | 9.71 | 4.37 | 2.85 |
| | 5-5.5 | 13.52 | 10.14 | 6.04 |
| | 6.5-7 | 13.17 | 7.36 | 4.78 |

*Concentration factor = $\frac{\text{Cr}\mu\text{g/g } (\bar{x} \text{ treated}) - \text{Cr}\mu\text{g/g } (\bar{x} \text{ controls})}{\text{amount of Cr added to soil}}$

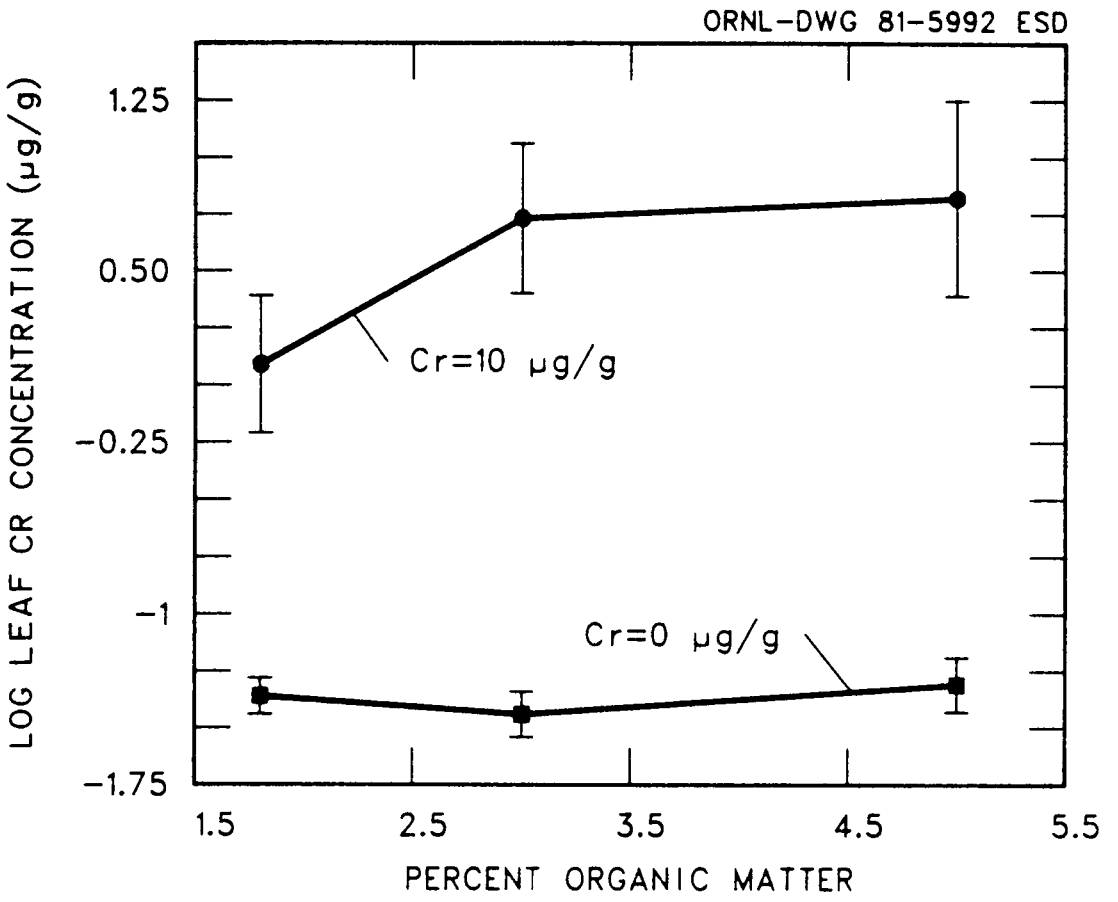



Fig. 3. Chromium concentrations ± SE in leaves of 0 and 10 µg/g treatment groups at the different organic matter levels.

Table 7. Chromium concentrations (µg/g) ± SE in leaves of the 0 and 10 µg/g chromium soil amendment groups.

| | | Chromium Amendment = 0 | | |
|---|---|---|---|---|
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 0.12 ± 0.03 | 0.11 ± 0.02 | 0.11 ± 0.05 |
| | 3 | 0.11 ± 0.04 | 0.09 ± 0.03 | 0.12 ± 0.01 |
| | 5 | 0.06 ± 0.02 | 0.15 ± 0.02 | 0.26 ± 0.08 |
| | | Chromium Amendment = 10 µg/g | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 2.70 ± 1.80 | 1.24 ± 0.79 | 0.58 ± 0.15 |
| | 3 | 1.27 ± 0.41 | 3.41 ± 1.60 | 1.67 ± 0.89 |
| | 5 | 6.99 ± 1.82 | 0.98 ± 0.31 | 7.64 ± 7.06 |

*Values are the mean of three plants; untransformed data; blank values were subtracted prior to calculations.

In the stems (Fig. 4), chromium concentrations in controls were low, not detectable to 1.70 µg/g, and in some cases equaled the procedural blank analyzed along with the samples. Stem concentrations of plants in the 10 µg/g Cr-VI soil ranged from not detectable to 24.64 µg/g chromium (Table 8).

Chromium concentrations of roots (Fig. 5) of plants in the 10 µg/g Cr-VI treatment ranged from 6.56-33.83 µg/g as compared to 0.28-7.35 µg/g for plants in non-amended soil (Table 9). Concentrations of chromium in the roots were significantly ($P < 0.05$) higher than concentrations in the leaves and stems. Previous studies have shown that plants grown in chromium contaminated soil or solution will take up chromium in the roots, but little, if any is translocated elsewhere (Myttenaere and Mousney, 1974; Wallace et al., 1976; Cary et al., 1977a; Lepp and Eardley, 1978).

Only a small fraction of the chromium added to the soil was transported to the leaves and stems with 0.003-0.02% of the 18.4 mg Cr-VI added to the soil reaching the leaves (Table 10) and even less, 0.0003-0.0107% in the stems. Most (0.05-0.10%) of the chromium available to the plants remained in the root mass. Concentration factors were calculated for the 10 µg/g treatment (Table 11). Factors for stems and leaves were all less than one and similar to each other. Factors in roots were higher than in leaves and stems, but not much greater than one, indicating little magnification of chromium from the soil. It has been hypothesized that the roots of higher plants provide not only a barrier to translocation of some metals

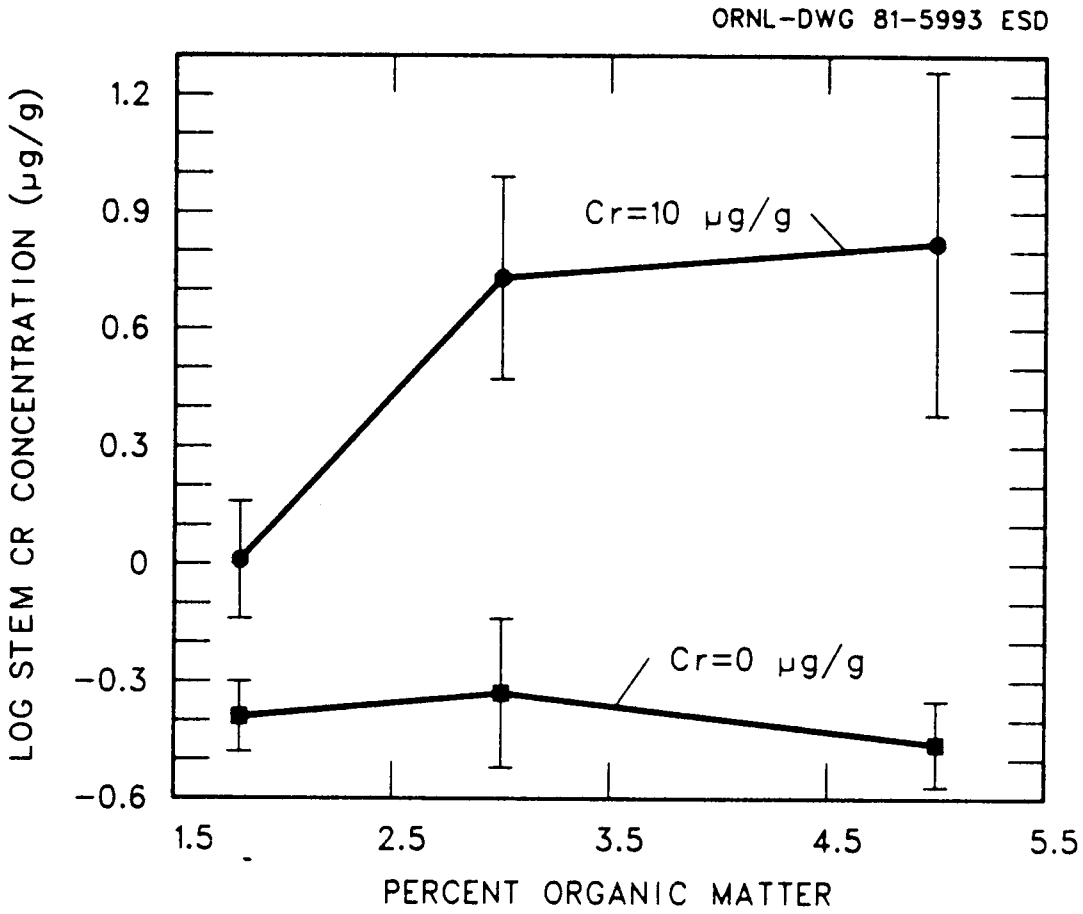



Fig. 4. Chromium concentrations (µg/g) ± SE in stems of the 0 and 10 µg/g treatment groups at the different organic matter levels.

Table 8. Chromium concentrations (µg/g) ± SE in stems of the 0 and 10 µg/g chromium soil amendment groups.*

| | | Chromium Amendment = 0** | | |
|---|---|---|---|---|
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 0.05 ± 0.06 | 0 ± 0.03 | 0.08 ± 0.05 |
| | 3 | 0 ± 0.05 | 0.52 ± 0.59 | 0 ± 0.02 |
| | 5 | 0.04 ± 0.07 | 0.02 ± 0.01 | 0.09 ± 0.03 |
| | | Chromium Amendment = 10 µg/g | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 0.26 ± 0.42 | 0.44 ± 0.15 | 0.47 ± 0.26 |
| | 3 | 0.74 ± 0.17 | 4.05 ± 2.00 | 1.49 ± 0.91 |
| | 5 | 6.78 ± 1.40 | 0.44 ± 0.25 | 8.24 ± 8.20 |

*Values are the mean of three plants; untransformed data.

**Blank values were subtracted prior to calculations; some of the control stems were almost equal to blanks and are reported as 0.

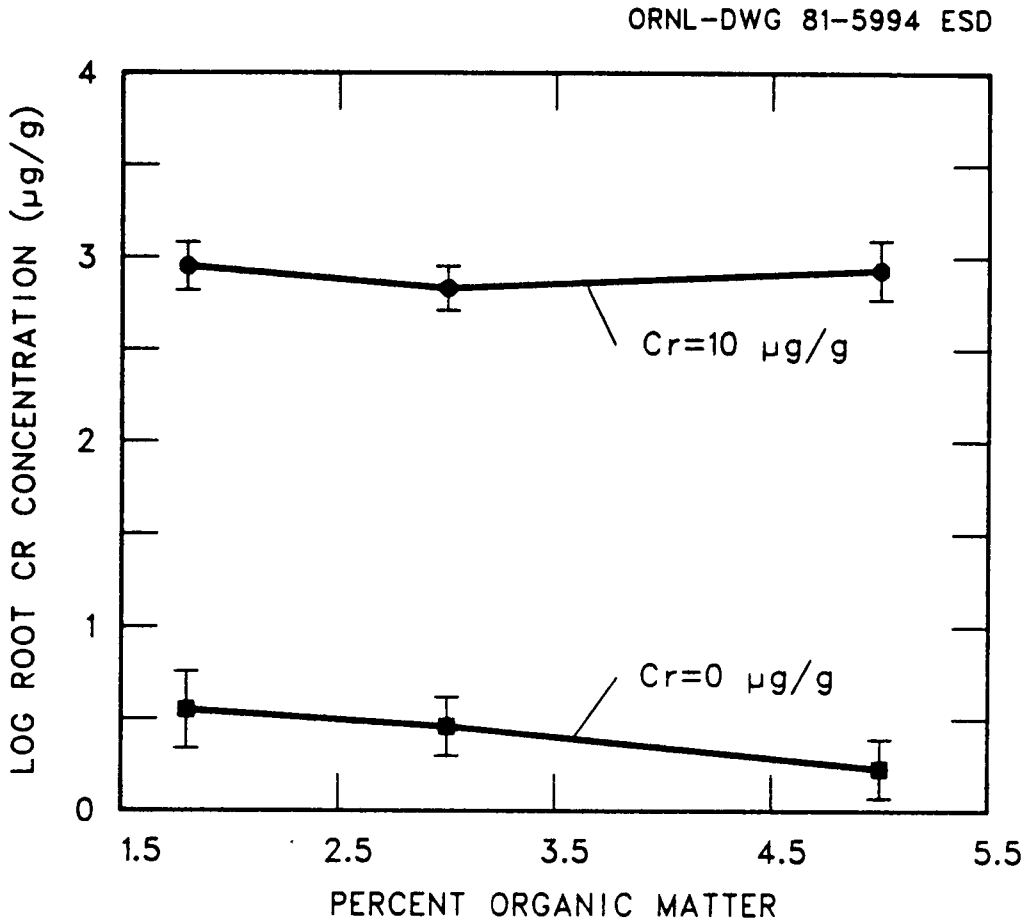



Fig. 5. Chromium concentrations ± SE in roots of the 0 and 10 µg/g treatment groups at the different organic matter levels.

Table 9. Chromium concentrations (µg/g) ± SE in roots of the 0 and 10 µg/g chromium amendment groups.

| | | Chromium Amendment = 0 | | |
|---|---|---|---|---|
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 1.73 ± 0.16 | 3.22 ± 2.07 | 0.85 ± 0.17 |
| | 3 | 2.07 ± 0.93 | 1.17 ± 0.28 | 1.19 ± 0.35 |
| | 5 | 1.27 ± 0.18 | 1.35 ± 0.62 | 0.98 ± 0.52 |
| | | Chromium Amendment = 10 µg/g | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 21.02 ± 4.68 | 15.82 ± 2.50 | 24.91 ± 6.84 |
| | 3 | 16.86 ± 3.78 | 18.83 ± 3.98 | 17.05 ± 4.84 |
| | 5 | 20.13 ± 3.10 | 16.38 ± 5.13 | 26.49 ± 6.07 |

*Values are the mean of three plants; untransformed data; blank values were subtracted prior to calculations.

Table 10. Percent translocation of chromium from soils amended with 0 and 10 µg/g hexavalent chromium.

| | | LEAVES* | | |
|---|---|---|---|---|
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | .011 | .011 | .003 |
| | 3 | .007 | .027 | .009 |
| | 5 | .040 | .006 | .051 |
| | | STEMS* | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | .0003 | .0009 | .0006 |
| | 3 | .0012 | .0068 | .0018 |
| | 5 | .0107 | .0006 | .0117 |
| | | ROOTS* | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | .06 | .09 | .07 |
| | 3 | .06 | .09 | .05 |
| | 5 | .08 | .07 | .10 |

*Computed using the mean of 3 plants for each treatment; untransformed data.

Table 11. Concentration factors for the 0 and 10 µg/g soil amendment groups.*

| Percent organic matter | Soil pH | Leaves | Stems | Roots |
|---|---|---|---|---|
| 1.8 | 4-4.5 | .26 | .02 | 1.93 |
| | 5-5.5 | .11 | .04 | 1.26 |
| | 6.5-7 | .05 | .04 | 2.41 |
| 3 | 4-4.5 | .12 | .07 | 1.48 |
| | 5-5.5 | .33 | .35 | 1.77 |
| | 6.5-7 | .16 | .15 | 1.59 |
| 5 | 4-4.5 | .69 | .67 | 1.89 |
| | 5-5.5 | .08 | .04 | 1.50 |
| | 6.5-7 | .74 | .82 | 2.55 |

*Concentration factor = $\frac{\text{Cr}\mu\text{g/g } (\bar{x} \text{ treated}) - \text{Cr}\mu\text{g/g } (\bar{x} \text{ controls})}{\text{amount of Cr added to soil}}$

within plants, but function as a mechanism for binding the chromium by absorption or adsorption (Wallace and Romney, 1977).

Biomass of plant tissues. Biomass effects due to soil treatments were significant in the roots and leaves, but not apparent in the stems (Table 12). In the roots, average dry weights ranged from 0.30-1.56 g/plant in the non-amended soils, and from 0.29-1.50 g/plant in soils amended with 10 µg/g chromium (Table 13). The organic matter content and the pH of the soil affected biomass significantly ($P < 0.05$) (Figs. 6 and 7). Bean roots grown in an organic matter of 5% had a significantly ($P < 0.05$) greater biomass than at 3%. There was no difference between 1.8% and 3% or between 1.8% and 5% soil organic matter treatments.

A soil pH of 5.0-5.5 produced significantly ($P < 0.05$) greater biomass than pH of 6.5-7.0, but pH 4.0-4.5 was not significantly different from either of the other two pH ranges. The significantly higher chromium accumulation in the roots of the 10 µg/g treatment versus the control (0 µg/g) did not affect the root biomass significantly. Previous studies had shown that one of the first effects of chromium was reduction of root growth (Shewry and Peterson, 1974), although this effect was small in some cases (DeKock, 1956). Other investigatiors (Hunter and Vergnano, 1953) reported poorly developed roots in chromium concentrations greater than 10 µg/g.

Chromium concentration in the leaves ranged from 0.02-0.40 µg/g in plants of non-amended soils and from 0.16-21.77 µg/g in plants from soils amended with 10 µg/g Cr-VI and was negatively correlated

Table 12. Stem biomass (grams) ± SE.

| | | Chromium Amendment = 0 | | |
|---|---|---|---|---|
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 0.30 ± 0.05 | 0.28 ± 0.07 | 0.26 ± 0.01 |
| | 3 | 0.28 ± 0.04 | 0.24 ± 0.03 | 0.25 ± 0.06 |
| | 5 | 0.27 ± 0.06 | 0.41 ± 0.16 | 0.31 ± 0.04 |
| | | Chromium Amendment = 10 µg/g | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 0.21 ± 0.03 | 0.39 ± 0.07 | 0.22 ± 0.03 |
| | 3 | 0.31 ± 0.05 | 0.31 ± 0.03 | 0.22 ± 0.01 |
| | 5 | 0.29 ± 0.06 | 0.26 ± 0.02 | 0.26 ± 0.07 |

Table 13. Root biomass (grams) ± SE.

| | | Chromium Amendment = 0 | | |
|---|---|---|---|---|
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 0.76 ± 0.03 | 0.76 ± 0.08 | 0.65 ± 0.02 |
| | 3 | 0.77 ± 0.13 | 0.54 ± 0.15 | 1.07 ± 0.06 |
| | 5 | 1.19 ± 0.37 | 0.85 ± 0.16 | 0.51 ± 0.09 |
| | | Chromium Amendment = 10 µg/g | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 0.51 ± 0.09 | 1.05 ± 0.26 | 0.53 ± 0.00 |
| | 3 | 0.64 ± 0.18 | 0.84 ± 0.08 | 0.51 ± 0.03 |
| | 5 | 0.77 ± 0.13 | 0.78 ± 0.14 | 0.66 ± 0.17 |

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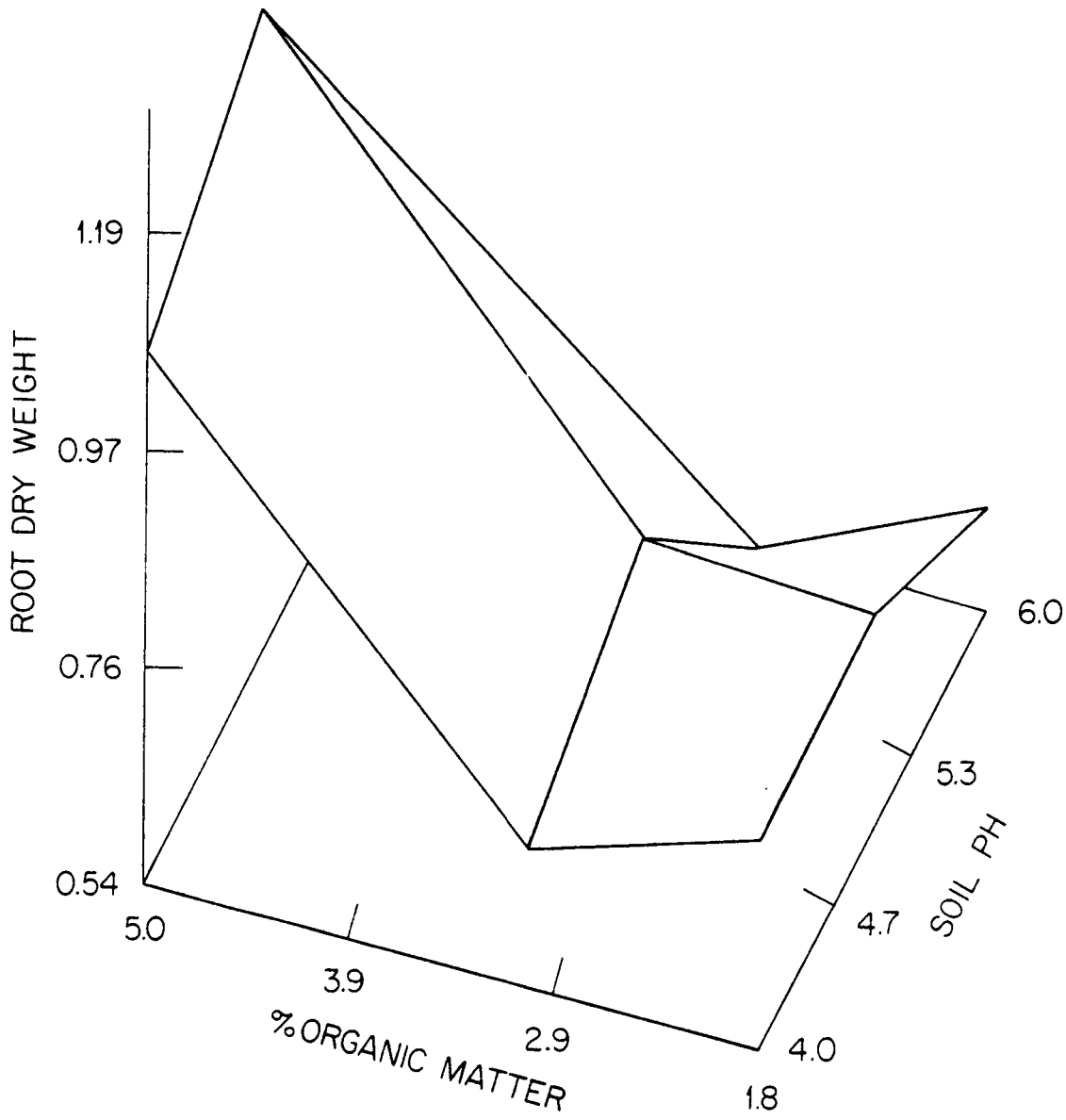



Fig. 6. Effect of soil pH and percent organic matter on biomass of control group (non-amended).

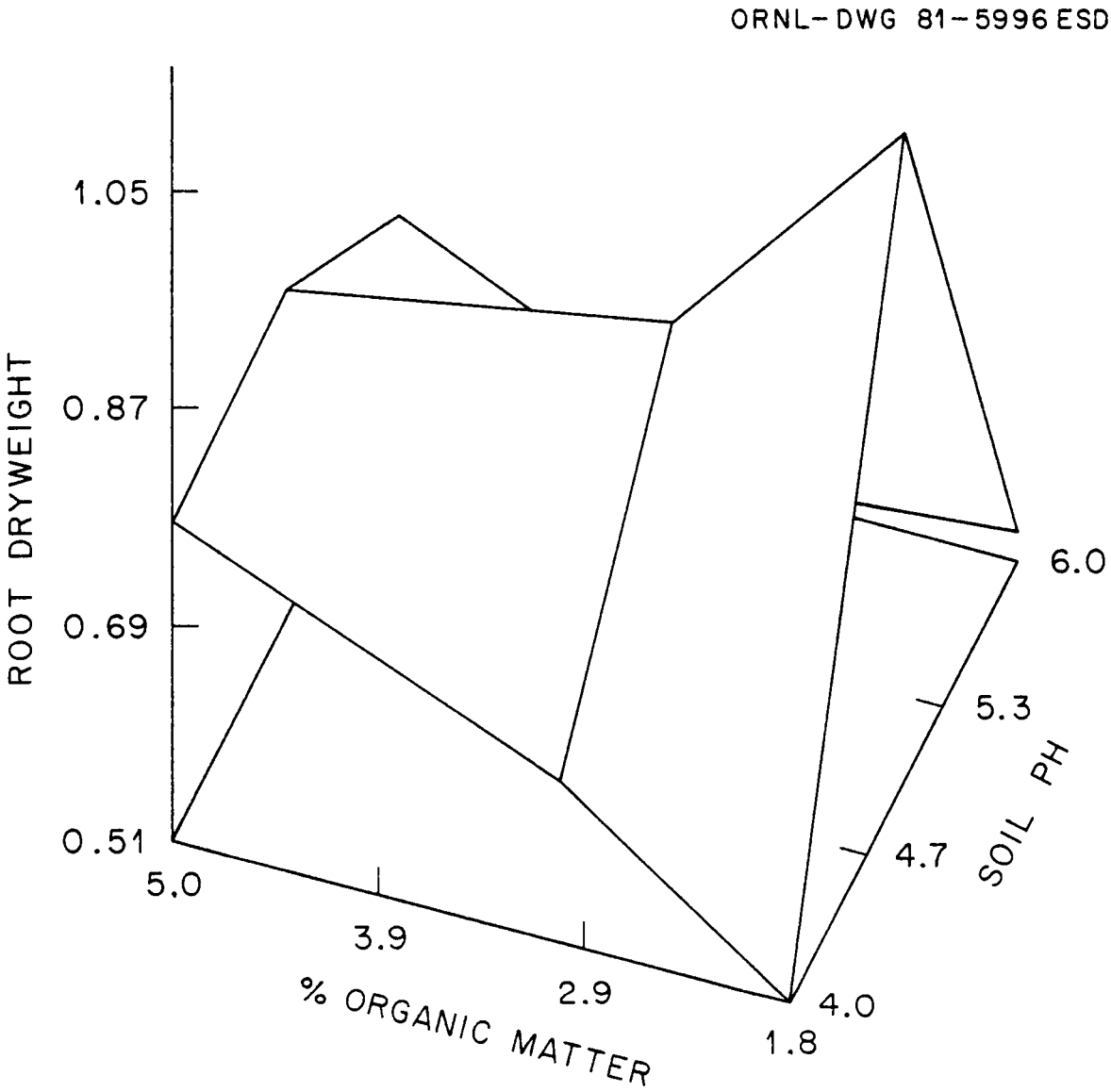



Fig. 7. Effect of soil pH and percent organic matter on biomass of 10 µg/g chromium amendment group.

with biomass (Table 14). The level of organic matter in the soil did not have a significant effect on leaf biomass, however, the pH in the soil had significant ($P < 0.05$) effects at all three levels (Fig. 8). A pH of 5.0-5.5 appeared to be optimum for growth because leaf biomass was significantly ($P < 0.05$) greater than at the other two levels, although a pH of 6.0-7.5 should be the optimum for Phaseolus vulgaris (Federation of American Societies for Experimental Botany, 1966). Leaf biomass of plants grown in a soil of pH 6.5-7.0, although significantly less than at pH 5.0-5.5, was significantly ($P < 0.05$) greater than at pH 4.0-4.5.

The significantly higher accumulation of chromium in the leaves of the 10 µg/g treatment corresponded with a significant ($P < 0.05$) decrease in leaf biomass when compared to controls in non-amended soil. Other investigators have reported a reduction in yield for plants grown in 10 µg/g chromium in soil culture or nutrient solutions (Turner and Rust, 1971; Shewry and Peterson, 1974; Davis et al., 1978).

It is apparent that many of the biomass effects were due not only to the Orocol TL amendment to the soil, but to the condition of the soil. As was anticipated, plants grew best in soil conditions of high organic matter content and a soil pH of 5.0-7.0. Although chromium concentrations were significantly higher in all plant parts (leaves, stems and roots) for the 10 µg/g treated group, only in leaf biomass was there a significant decrease in yield when compared to controls.

Table 14. Leaf biomass (grams) ± SE.

| | | Chromium Amendment = 0 | | |
|---|---|---|---|---|
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 1.00 ± 0.12 | 1.38 ± 0.13 | 1.25 ± 0.02 |
| | 3 | 1.18 ± 0.16 | 1.24 ± 0.16 | 1.18 ± 0.21 |
| | 5 | 1.25 ± 0.13 | 1.61 ± 0.26 | 1.58 ± 0.03 |
| | | Chromium Amendment = 10 µg/g | | |
| | | Soil pH | | |
| | | 4-4.5 | 5-5.5 | 6.5-7 |
| Organic Matter Content (percent) | 1.8 | 0.72 ± 0.10 | 1.58 ± 0.17 | 1.04 ± 0.10 |
| | 3 | 0.95 ± 0.19 | 1.45 ± 0.09 | 0.99 ± 0.05 |
| | 5 | 1.04 ± 0.19 | 1.22 ± 0.10 | 1.22 ± 0.24 |

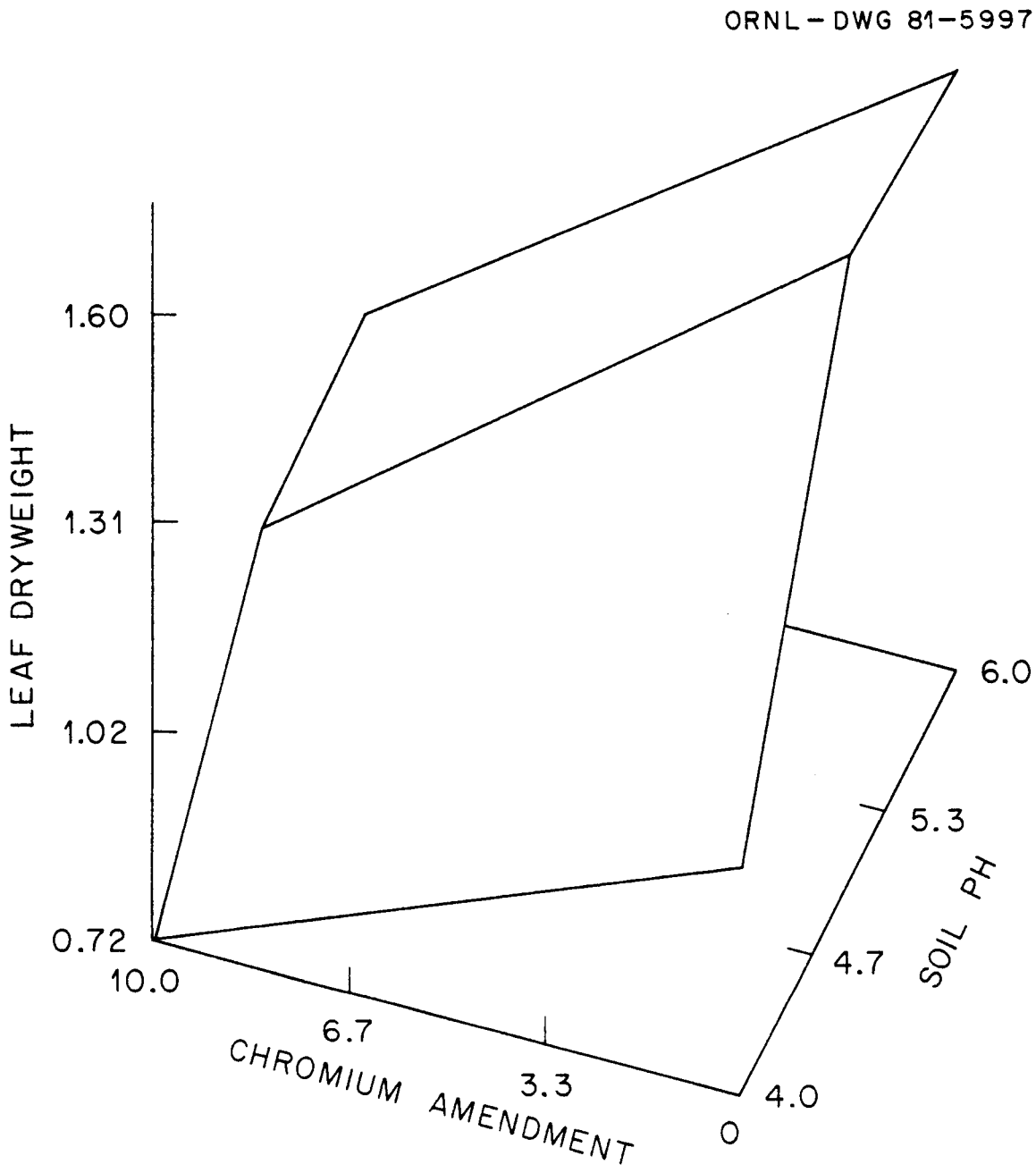



Fig. 8. Effect of soil pH and chromium soil amendment on biomass of leaves.

Effect of Cr-VI on rate of growth. The effect of the 10 µg/g amendment of chromium to the soil on the rate of plant growth was calculated to determine if there were any significant differences due to treatment.

Growth of plants in all treatments appeared to have leveled off around day 41 post treatment (Figs. 9 and 10). Rate of growth was calculated for each plant of each treatment by subtracting the first height measurement of the plant from the height measured on day 41 divided by the number of days of growth (41). An analysis of variance comparison showed no significant ($P < 0.05$) differences in the rates of growth due to treatment.

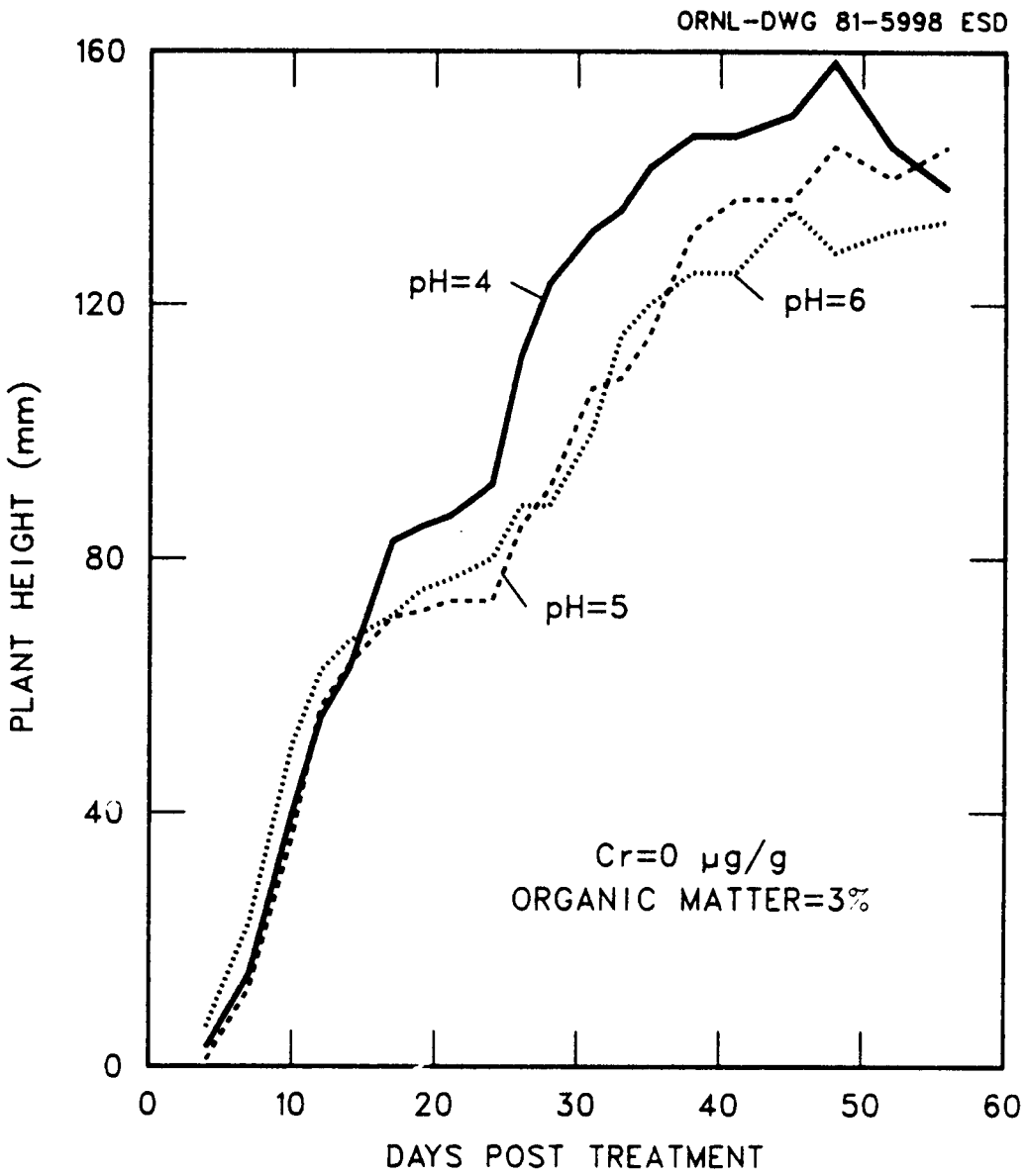



Fig. 9. Effect of pH on rate of plant growth at 3% organic matter in non-amended soil.

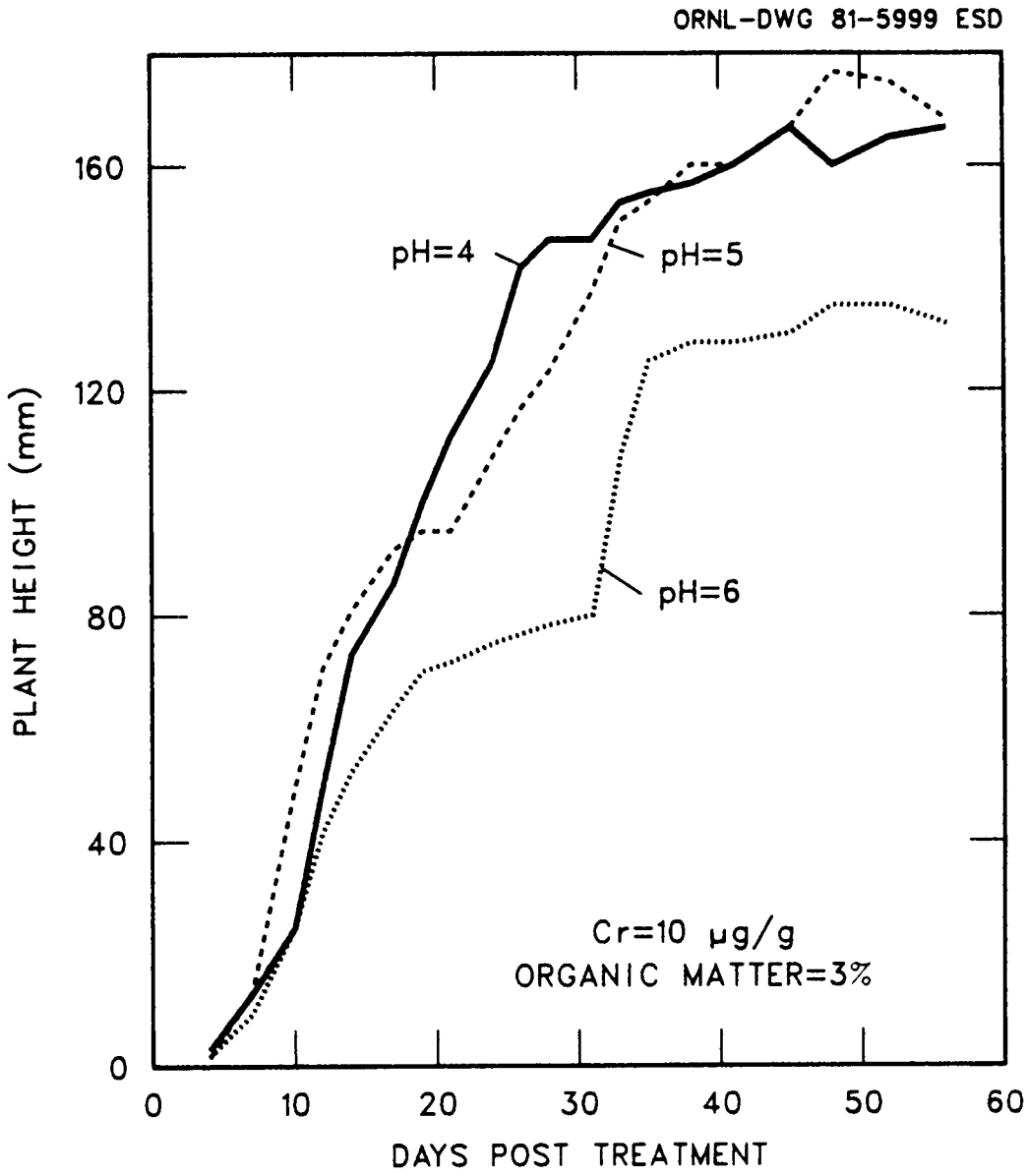



Fig. 10. Effect of soil pH on rate of plant growth at 3% organic matter in soil amended to 10 µg/g chromium.

# CHAPTER IV

# CONCLUSION

Numerous investigations have been conducted at the DOE uranium enrichment facilities in Oak Ridge, Tennessee, and Paducah, Kentucky, to determine environmental effects of cooling tower drift, and in particular hexavalent chromium from Orocol TL, on biological systems. This study was designed to answer specific questions concerning the effects of chromium in Orocol TL on the germination and growth of bush beans in soils of various pH levels and organic matter contents.

Statements of major findings are summarized below:

(1) The chromium (Orocol TL) in the soil significantly inhibited germination of bush beans. That the effect was reduced with an increase in organic matter suggests that the chromium may have reverted to forms that were insoluble and unavailable for plant uptake.

(2) Excessive amounts of Orocol TL (500 µg/g Cr) were immediately toxic to bush beans growing in all soil combinations which may have been related to osmotic or salt effects, but probably indicate that the Cr-VI exceeded the reducing and adsorbing capacity of the soil.

(3) There was significantly greater chromium concentration in leaves, stems and roots of the 10 µg/g chromium soil amendments. However, concentration factors indicated the actual amount translocated was small and that most of the chromium remained in the roots confirming previous studies. Although chromium concentrations were significantly higher in all plant parts, only in the leaves was there a corresponding reduction in biomass.

Based on the results of this study it appears that organic matter (1.8-5%) and soil pH (4-7) in ranges found locally neither increase, nor decrease significantly the uptake of chromium from the soil. However, chromium was taken up by the roots and translocated to stem and leaves, and leaf biomass was significantly reduced. This would suggest that in the siting of mechanical draft towers, the potential problems associated with deposition of chromated drift on the surrounding soil and vegetation should be carefully evaluated, particularly in agricultural areas.

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Patricia Dreyer Parr was born in Coral Gables, Florida on January 4, 1952. Following graduation from Oak Ridge High School (Tennessee) in 1970, she attended Tennessee Wesleyan College in Athens, Tennessee where she received her B.S. in 1974 with a major in Biology and teaching certification for Biology and Psychology.

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Sensitivity of tobacco to chromium from cooling tower drift. 1976. Atmos. Environ. 10:421-423 (with F. G. Taylor and J. J. Beauchamp).

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