STUDY OF THE EFFECTS OF HEAVY METALS ON SEED GERMINATION AND PLANT GROWTH ON ALFALFA PLANT (*Medicago sativa*) GROWN IN SOLID MEDIA

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ABSTRACT

Preliminary studies have shown that alfalfa plants (*Medicago sativa*) can grow in some heavy metal-contaminated soils. Based on that, we studied the individual effects of several doses of Cd(II), Cr(VI), Cu(II), Ni(II), and Zn(II) on the growth of live alfalfa plants using solid media. The doses used in this study were 0, 5, 10, 20, and 40 ppm. The seed germination and plant growth was significantly affected by Cd(II) and Cr(VI) at 10 ppm, as well as by Cu(II) and Ni(II) at 20 ppm and higher concentrations (P<1%). Zn(II) did not affect seed germination. The roots of the plants exposed to 5 ppm-dose of Cd(II), and 5 and 10 ppm-dose of Cr(VI), Cu(II), Ni(II), and Zn(II), grew more than the roots of the control treatment by more than 30%. Exposures of 5 ppm of Cd(II) reduced the shoot size by 16% as compared to the control. While Cr(VI), Cu(II), Ni(II), and Zn(II) increased the shoot size by 14.0%, 60.0%, 36.0%, and 7.7%, respectively; only Zn(II) promoted the shoot growth at the doses of 20 and 40 ppm.

Key words: *phytoremediation, alfalfa,* Medicago sativa*, heavy metals, solid media*

INTRODUCTION

Heavy metal contamination affects the biosphere in many places worldwide (Cunningham, et al., 1997; Raskin and Ensley, 2000; Meagher, 2000). Metal concentrations in soil range from less than 1 mg/kg (ppm) to high as 100,000 mg/kg, whether due to the geological origin of the soil or as a result of human activity (Blaylock and Huang, 2000). Excess concentrations of some heavy metals in soils such as Cd(II), Cr(VI), CuII), Ni(II), and Zn(II) have caused the disruption of natural aquatic and terrestrial ecosystems (Gardea-Torresdey et al., 1996; Meagher, 2000). Currently, cleanup processes of heavy metal pollution are expensive and environmentally destructive (Nanda et al., 1995; Moffat, 1995; Meagher, 2000). Recently, scientists and engineers have started to generate cost-effective technologies that include the use of microorganisms, biomass, and live plants in the cleaning process of polluted areas (Miller, 1996; Boyajian and Carreira, 1997; Dushenkov et al., 1997; Ebbs and Kochian, 1998; Wasay et al., 1998; Gardea-Torresdey et al., 1996).

Some heavy metals at low doses are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition for most of the plants species (Fernandes and Henriques, 1991; Claire et al., 1991). Researchers have observed that some plants species are endemic to metalliferous soils and can tolerate greater than usual amounts of heavy metals or other toxic compounds

(Banuelos et al., 1997; Blaylock and Huang, 2000; Raskin and Ensley, 2000; Dahmani-Muller et al., 2000). Several studies have been conducted in order to evaluate the effects of different heavy metal concentrations on live plants (Thompson et al., 1997; Reeves and Baker, 2000; Raskin and Ensley, 2000). Most of these studies have been conducted using seedlings or adult plants (Flores et al., 1999; Lee et al., 1999; Chatterjee and Chatterjee; 2000; Gratton et al., 2000; Öncel et al., 2000; Pichtel et al., 2000). In a few studies, the seeds have been exposed to the contaminants (Claire et al., 1991; Vojtechova and Leblova, 1991; Xiong, 1998). The present manuscript reports data regarding the ability of alfalfa seeds to germinate and grow in media containing Cd (II), Cr (VI), Cu (II), Ni (II), and Zn (II) ions.

MATERIALS AND METHODS

Alfalfa seeds of cultivar Malone were obtained from New Mexico State University, located in Las Cruces, N.M. The seeds were immersed in 3% v/v formaldehyde/deionized water for five minutes to avoid fungal contamination. After that, the seeds were washed with deionized water and placed in Mason jars of one-pint capacity. Each jar contained 250 ml of a medium made with: $Ca(NO_3)_2\,4H_2O$, 3.57×10^{-4} M; H_3BO_3, 2.31×10^{-5} M; $CaCl_2\,2H_2O$, 2.14×10^{-3} M; KH_2PO_4, 9.68×10^{-4} M; KNO_3, 2.55×10^{-4}M; $MgClO_4$, 1.04×10^{-3} M; $FeCl_3$, 6.83×10^{-5} M; and $MnSO_4\,H_2O$, 7.69×10^{-6} M and agar-agar, 1% w/v. The heavy metals: Cd(II) (as $Cd(NO_3)_2\,4H_2O$); Cr(VI), (as $K_2Cr_2O_7$); Cu(II), (as $CuSO_4\,5H_2O$); Ni(II), (as $Ni(NO_3)_2$); and Zn(II), (as $Zn(NO_3)_2\,6H_2O$), were used at the concentrations of 0, 5, 10, 20, and 40 ppm.

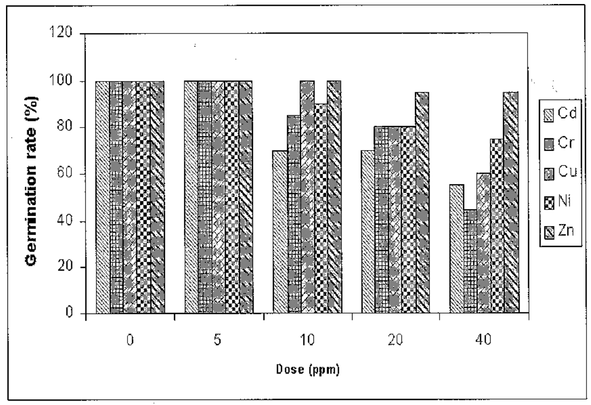

Figure 1. Seed germination of alfalfa plant (cultivar Malone) after two weeks of exposure to heavy metals.

For each treatment, the pH was adjusted to 5.3. Each treatment was replicated three times for statistical purposes. The seeds were set under a photoperiod of 12 hr, and 25/18 °C day/night temperature. The seedlings were harvested after two weeks and the germination rate, and root and shoot length were recorded. The data were analyzed through one-way analysis of variance (ANOVA) to determine the effect of treatments, and least significant difference (LSD) tests were performed to determine the statistical significance of the differences between means of treatments.

RESULTS AND DISCUSSION

Effects of Heavy Metals on Seed Gemination

Figure 1 shows the effects of the concentrations of Cd(II), Cr(VI), Cu(II), Ni(II), and Zn(II) on seed germination of alfalfa (cultivar Malone) grown in solid media (agar). In general, there was a reduction in seed germination as metal concentrations in the growing media increased. The 10 ppm-dose of Cd(II) and

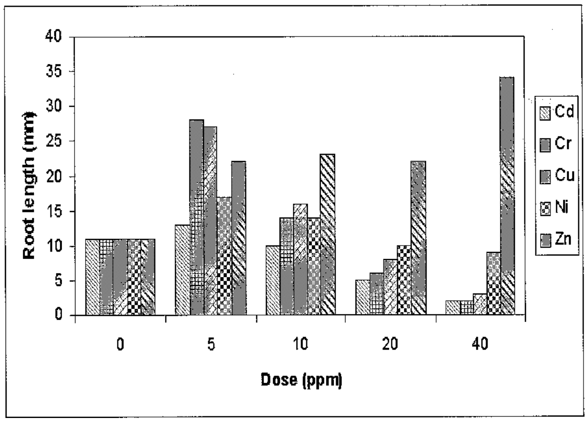

Figure 2. Root length of alfalfa plant (cultivar Malone) after two weeks of culture in heavy metal-enriched media.

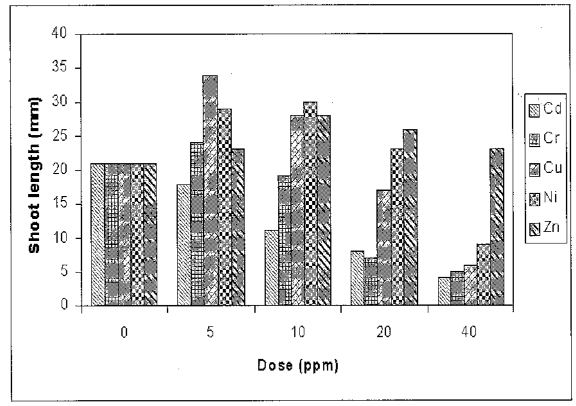

Figure 3. Shoot length of alfalfa plant (cultivar Malone) after two weeks of exposure to heavy metals.

Cr(VI), and the 20 ppm-dose of Cu(II) and Ni(II), significantly reduced the seed germination ($P<1\%$). At a concentration of 40 ppm, Cd(II), Cr(VI), Cu(II), and Ni(II) inhibited significantly seed germination by 45.0%, 55.0%, 40.0%, and 25.0%, respectively. Claire and coworkers (1991) obtained similar results in a study using nickel and other heavy metals on cabbage, lettuce, millet, radish, turnip, and wheat. However, in this study Zn (II) was the only metal that did not significantly reduce the seed germination, even at a concentration of 40 ppm ($P<1\%$).

Effect of Heavy Metals on Root Growth

The data corresponding to the root growth of the alfalfa plant vs. the dose of the heavy metal reported in this paper is shown in Figure 2. The dose of 5-ppm of Cd(II), Cr(VI), Cu(II), Ni(II), and Zn(II) promoted the root growth by 22.0%, 166.0%, 156.0%, 63.0%, and 105.0%, respectively, as compared to the root growth of the control plants. The heavy metals Cr(VI), Cu(II), and Ni(II), and Zn(II) at 10 ppm concentration still increased the root growth over the control root size by approximately 37.0%, 54.0%, 37.0%, and 100.0%, respectively. However, at the same dose, Cd(II) reduced the root size by 6.0% as compared to the control root elongation. Cr(VI), Cu(II), and Ni(II) demonstrated a concentration-dependent inhibition of root growth at the dose of 20 and 40 ppm. Öncel and collaborators (2000) found similar effects using cadmium in wheat seedlings. All Zn(II) concentrations increased the root length by more than 100.0% of the control.

Effect of Heavy Metals on Shoot Growth

The effects of the heavy metals over the shoot growth were different as compared to the effects on root growth (Figure 3). At 5 ppm-dose, Cd(II) reduced the shoot size by about 16.0% as compared with shoot size of the control group. On the other hand, a dose of 5 ppm of Cr(VI), Cu(II), Ni(II), and Zn(II) increased the shoot length in 14.0%, 60.0%, 36.0%, and 7.7%, respectively, related to the growth of the control treatment. However, Cd(II) and Cr(VI), at a 10 ppm-dose, significantly reduced the shoot growth as shown in the control plants (P<1%). When the concentration of these two heavy metals was increased to 20 ppm, the shoot size diminished by 63.0% and 66.0%, respectively. However, these metals at 40 ppm concentration showed lethal effects over the alfalfa plants. These data corresponded with those of Öncel et al. (2000), who found that Cd(II) reduces the chlorophyll *a* and *b* in wheat, whereas Chatterje and Chatterjee (2000) found that Cu(II) and Cr(VI) significantly decreased the water potential and Fe(II) concentration in cauliflower. Cu(II) and Ni(II) exert detrimental effects at the dose of 40 ppm, causing a shoot elongation reduction of 70.0% and 58.0%, respectively. However, Zn(II), at 40 ppm produced a positive effect in shoot growth (10% over control group). These results indicate that low concentrations of Cr(VI), Cu(II), and Ni(II) have micronutrient-like effects on the alfalfa plant. In the case of Zn(II), the data found in this study indicate that this heavy metal has positive effects on the growth of the alfalfa plants, even at moderately high concentrations.

CONCLUSIONS

Based on the results, we concluded that the seed germination of the alfalfa plant (cultivar Malone) is seriously affected by a concentration of 20 ppm of Cd(II), Cr(VI), and by 40 ppm of Cu(II) and Ni(II). The root and shoot growth of the alfalfa plant is stimulated by a concentration of 5 ppm of Cr(VI), Cu(II), Ni(II), and Zn(II). Alfalfa plants did not show any capabilities to germinate and grow in a medium containing 20 ppm of Cd(II) and Cr(VI), and 40 ppm of Cu(II) and Ni(II). However, alfalfa was able to germinate and grow efficiently at any Zn(II) concentration evaluated in this study. These data indicate that the alfalfa plant may be grown directly in soils individually contaminated with moderate amounts of Cd(II), Cr(VI), Cu(II), and Ni(II). Further studies need to be performed in order to establish the maximum amount of Zn(II) that the plants may tolerate, and the ability of the alfalfa plants to germinate and grow in media containing mixtures of these heavy metals.

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