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THE EFFECTS OF HEAVY METALS ON SEED GERMINATION AND PLANT GROWTH ON ALFALFA PLANT (*MEDICAGO SATIVA*)

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Abstract

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The effects of Cd^{+2}, Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2} on Alfalfa Plant (*Medicago sativa*) were studied in this research. The doses of 0, 5, 10, 20, and 40 ppm were used. The seed germination and plant growth were significantly affected by Cd^{+2} and Cr^{+6} at 10 ppm, as well as by Cu^{+2} and Ni^{+2} at 20 ppm and higher concentrations. Zn^{+2} did not affect seed germination. The plant roots were exposed to 5 ppm-dose of Cd^{+2}, 5 and 10 ppm-dose of Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2}. Meanwhile, the dose of 5 ppm of Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2} increased the shoot size by 13.0%, 59.0%, 35.0%, and 6.6%, respectively. Zn^{+2} were only promoted the shoot growth at the doses of 20 and 40 ppm.

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

Introduction

Metal contamination of agricultural soils by atmospheric deposition or by disposal of sewage sludge constitutes a risk of either leaching of metals into the groundwater or excessive accumulation in the top soil (Adriano, 1992; Kabata-Pendias and Pendias, 1992). Metal concentrations in soil range from less than 1 mg/kg 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^{+2}, Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2} have caused the disruption of natural aquatic and terrestrial ecosystems (Gardea-Torresdey et al., 1996; Meagher, 2000). Although some metals are immobile and persistent, other metals are mobile, and, therefore, the potential of transfer either through the soil pro?le down to the groundwater aquifer or via plant-root uptake (bioavailability) is likely. Cadmium and lead, which have no known beneficial effects, may become toxic to plants and animals if their concentrations exceed certain values (Adriano, 1986; Gough et al., 1979). Ni, Cu, and Zn are three essential micronutrients for plant nutrition. Nickel is an essential component of the enzyme urease, but when Ni concentrations in vegetative tissues of plants exceed 50 mg/kg dry weight, plants may suffer from excess Ni and exhibit toxicity symptoms. Once absorbed, Cu apparently accumulates in roots, even in cases where roots have been damaged by toxicity (Adriano, 1986). Zinc phytotoxicity is reported relatively often, especially for acid and heavily sludged

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soils. The physiology and biochemistry of the toxic effects of Zn in plants are likely to be similar to those reported for other heavy metals; however, Zn is not considered to be highly phytotoxic (Kabata-Pendias and Pendias, 1992). Nowadays, cleanup processes of heavy metal pollution are expensive and environmentally destructive (Nanda et al., 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 (Wasay et al., 1998). Many 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).

In a few studies, the seeds have been exposed to the contaminants (Claire et al., 1991; Xiong, 1998). The ability of alfalfa seeds (*Medicago sativa*) to germinate and grow in containing Cd^{+2}, Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2} ions were studied in this research.

Materials and Methods

Alfalfa seeds (cultivar Malone) 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: $Ca(NO_3)_2\ 4H_2O$, $3.57x10^{-4}$ M; H_3BO_3, $2.31x10^{-5}$ M; $CaCl_2\ 2H_2O$, $2.14x10^{-3}$ M; KH_2PO_4, $9.68x10^{-4}$ M; KNO_3, $2.55x10^{-4}$ M; $MgClO_4$, $1.04x10^{-3}$ M; $FeCl_3$, $6.83x10^{-5}$ M; and $MnSO_4\ H_2O$, $7.69x10^{-6}$ M and agar-agar, 1% w/v. The heavy metals of Cd^{+2}, (as $Cd\ (NO_3)_2\ 4H_2O$); Cr^{+6}, (as $K_2Cr_2O_7$); Cu^{+2}, (as $CuSO_4\ 5H_2O$); Ni^{+2}, (as $Ni\ (NO_3)_2$); and Zn^{+2}, (as $Zn\ (NO_3)_2\ 6H_2O$) were used at the concentrations of 0, 5, 10, 20, and 40 ppm in this research. The concentrations of Cd^{+2}, Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2} were determined by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer).

Each treatment was replicated three times for statistical purposes. The pH was adjusted to 5.3 for each treatment. 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 lengths 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 Germination

The effects of the concentrations of Cd^{+2}, Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2} on seed germination of alfalfa (cultivar Malone) grown in solid media (agar) were presented in Figure 1. There was a reduction in seed germination as metal concentrations in the growing media increased in general. The 10 ppm of Cd^{+2} and Cr^{+6}, and the 20 ppm of Cu^{+2} and Ni^{+2}, significantly reduced the seed germination ($P<1\%$). The concentration of 40 ppm, Cd^{+2}, Cr^{+6}, Cu^{+2}, and Ni^{+2} inhibited significantly seed germination by 44.0%, 54.0%, 39.0%, and 24.0%, respectively. Claire et al., (1991) obtained similar results in a study using nickel and other heavy metals on cabbage, lettuce, millet, radish, turnip, and wheat. Meanwhile, Zn^{+2} was the only metal that did not significantly reduce the seed germination, even at a concentration of 40 ppm ($P<1\%$) in this study.

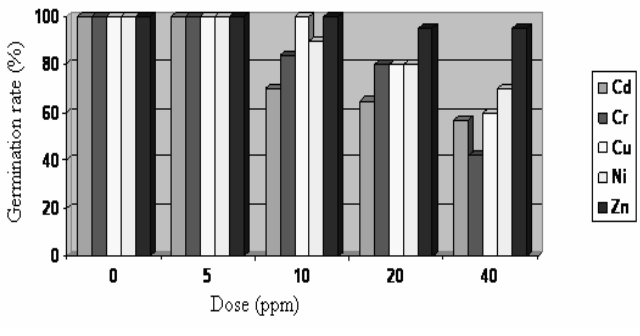

Fig. 1. Seed germination after two weeks of exposure to heavy metals

Effect of Heavy Metals on Root Growth

The root growth was presented in Figure 2. The 5 ppm of Cd^{+2}, Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2} promoted the root growth by 21.0%, 165.0%, 155.0%, 62.0%, and 104.0%, respectively, as compared to the root growth of the control plants. The respectively, Cr^{+6}, Cu^{+2}, and Ni^{+2}, and Zn^{+2} at 10 ppm concentration still increased the root growth over the control root size by approximately 36.0%, 53.0%, 36.0%, and 100.0% in this study. Meanwhile, at the same dose of Cd^{+2} reduced the root size by 5.0% as compared to the control root elongation. Cr^{+6}, Cu^{+2}, and Ni^{+2} showed a concentration dependent inhibition of root growth at 20 and 40 ppm doses. Oncel et al. (2000) obtained similar effects using cadmium in wheat seedlings. All Zn^{+2} concentrations were increased the root length by more than 100.0% of the control.

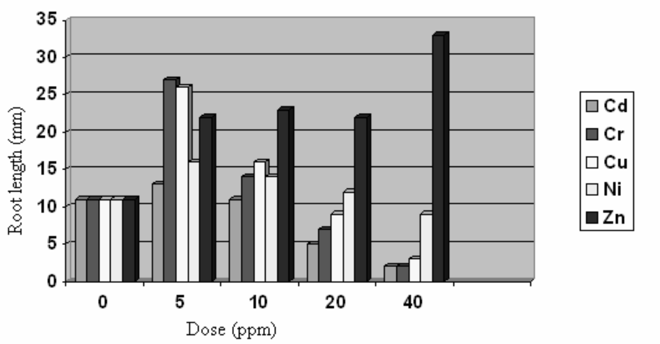

Fig. 2. Root length after two weeks of exposure to heavy metal-enriched media

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 as seen in Figure 3. Cd^{+2} values reduced the shoot size by about 16.0% as compared with shoot size of the control group at 5 ppm dose. The dose of 5 ppm of Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2} increased the shoot length in 13.0%, 59.0%, 35.0%, and 6.6%, respectively, related to the growth of the control treatment. However, 10 ppm dose of Cd^{+2} and Cr^{+6}, significantly reduced the shoot growth as shown in the control plants ($P<1\%$). When the concentration of Cd^{+2} and Cr^{+6} were increased to 20 ppm, the shoot size diminished by 62.0% and 65.0%, respectively. However, these metals showed lethal effects over the alfalfa plants at 40 ppm concentration. These data corresponded with those of Oncel et al. (2000), who found that Cd^{+2} reduces the chlorophyll a and b in wheat, whereas Chatterje and Chatterjee (2000) found that Cu^{+2} and Cr^{+6} significantly decreased the water potential and Fe^{+2} concentration in cauliflower. Cu^{+2} and Ni^{+2} exert detrimental effects at the dose of 40 ppm, causing a shoot elongation reduction of 69.0% and 57.0%, respectively.

However, at 40 ppm dose of Zn^{+2} produced a positive effect in shoot growth (10% over control group). The results indicated that low concentrations of Cr^{+6}, Cu^{+2}, and Ni^{+2} had micronutrient-like effects on the alfalfa. According to obtained data, the Zn^{+2} have positive effects on the growth of the alfalfa, even at moderately high concentrations in this research.

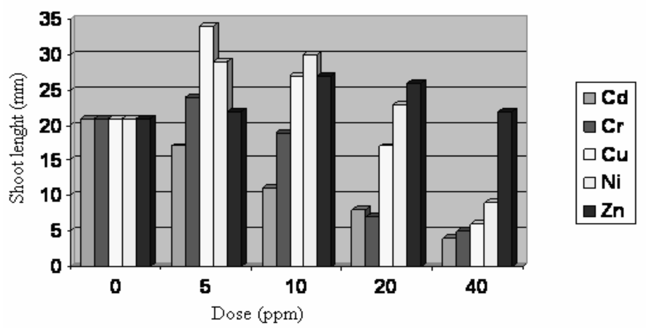

Fig. 3. Shoot length after two weeks of exposure to heavy metals

Conclusion

According to the results, the seed germination of the alfalfa plant is seriously affected by 20 ppm of Cd^{+2}, Cr^{+6}, and by 40 ppm of Cu^{+2}, Ni^{+2}. The root and shoot growth are stimulated by 5 ppm of Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2}. Alfalfa plants did not show any capabilities to germinate and grow in a medium containing 20 ppm of Cd^{+2} and Cr^{+6}, and 40 ppm of Cu^{+2} and Ni^{+2}. However, alfalfa was able to germinate and grow efficiently at any Zn^{+2} concentration evaluated in this research. This research indicates that the alfalfa plant may be grown directly in soils individually contaminated with moderate amounts of Cd^{+2}, Cr^{+6}, Cu^{+2}, and Ni^{+2}. Detailed studies need to be done in order to establish the maximum amount of Zn^{+2} 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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The seed germination and plant growth were significantly affected by Cd^{+2} and Cr^{+6} at 10 ppm, as well as by Cu^{+2} and Ni^{+2} at 20 ppm and higher concentrations. Zn^{+2} did not affect seed germination. The plant roots were exposed to 5 ppm-dose of Cd^{+2}, 5 and 10 ppmdose of Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2}.Â The seed germination was significantly affected by Zn^{2+} , Pb^{2+} and Cu^{2+} at 10mg/l. Mean germination index (GI) values of Jatropha curcas in soil spiked with Zn^{2+} , Pb^{2+} and Cu^{2+} ions were 107-118, 54-88 and 98-99 respectively. Also, increase in Cu^{2+} in the growing media reduced the shoot length while Zn^{2+} and Pb^{2+} increased the shoot length. Root lengths were significantly promoted by Pb^{2+} and insignificantly affected by Zn^{2+} and Cu^{2+}. Although plants require certain heavy metals for their growth and upkeep, excessive amounts of these metals can become toxic to plants. The ability of plants to accumulate essential metals equally enables them to acquire other nonessential metals [27].Â The effect of heavy metal toxicity on the growth of plants varies according to the particular heavy metal involved in the process. Table 1 shows a summary of the toxic effects of specific metals on growth, biochemistry, and physiology of various plants.Â Rice (Oryza sativa). Reduction in seed germination; decrease in seedling height; reduced leaf area and dry matter production. [35, 36]. Tomato (Lycopersicon esculentum). The effects of heavy metals on seed germination and plant growth on alfalfa plant (medicago sativa). C. AYDINALP1 and S. MARINOVA2 1 Uludag University, Faculty of Agriculture, Department of Soil Science, Bursa 16059, Turkey 2 Nikola Poushkarov Institute of Soil Science and Agroecology, BGâ€“1080 Sofia, Bulgaria. Abstract. C. AYDINALP, C. and S. MARINOVA, 2009. The effects of heavy metals on seed germination and plant growth on alfalfa plant (Medicago sativa). Bulg. J. Agric. Sci., 15: 347-350. The effects of Cd^{+2}, Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2} on Alfalfa Plant (Medicago sativa) were studied in this... The effects of heavy metals toxicity on medicinal plants used in traditional medicines has been reported worldwide, since the accumulation of heavy. Rev. Chim., 71 (7), 2020, 16-36.Â For this reason, a number of heavy metals should be considered a potential threat to medicinal plants and human health [54]. Metals can be absorbed and transported from polluted soil into plants through several mechanisms: phytoextraction, phytostabilization, phytovolatilization, rhizofiltration and produce some changes in plants (Figure 2). Figure 1. Sources of heavy metals, and foliar, root uptake of heavy metals in plants (from Hasan et al. [51], according to the provisions MDPI â€œThis is an open access article.