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Uptake and Effects of Five Heavy Metals on Seed Germination and Plant Growth in Alfalfa (*Medicago sativa* L.)

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Heavy metal contamination and the problems that it poses to the biota have been well documented (Raskin and Ensley, 2000; Meagher, 2000). In some soils, such as those found in certain areas of El Paso, Texas, there are sites that have lead and copper concentrations as high as 5,067 mg/kg and 4,955, respectively. These are concentrations that are above the permissible levels established by the U.S. EPA and furthermore, may pose a serious health concern (Gardea-Torresdey et al., 1996). Because traditional cleanup processes of heavy metal contaminated soils are expensive and practical only in small areas (Moffat, 1995), researchers have looked for new cost effective technologies that include the use of microorganisms, biomass, and live plants (Miller, 1996; Ebbs and Kochian, 1998; Gardea-Torresdey et al., 1996)

Some heavy metals in higher doses 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 have the ability to grow in sites where soils contain greater than usual amounts of heavy metals or other toxic compounds (Banuelos et al., 1997; Raskin and Ensley, 2000). Several other studies have been conducted in order to determine the capability and the amount of heavy metals that different plant species can uptake (Thompson et al., 1997; Raskin et al., 1999). Most of these studies have been conducted in adult or already germinated plant seeds (Flores et al., 1999; Lee et al., 1999; Chatterjee and Chatterjee, 2000; Öncel et al., 2000). In a few of these studies, the seeds have been exposed to the contaminants (Claire et al., 1991; Vojtechova and Leblova, 1991; Xiong, 1998). The present paper reports data concerning the ability of alfalfa seeds (*M. sativa* L.) 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, NM. Thirty seeds were immersed in 3% v/v formaldehyde for 5 minutes to avoid fungal contamination, washed with deionized water and placed in Mason jars of one-pint capacity.

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Each jar contained 250 mL of the following nutrient medium: $Ca(NO_3)_2 4H_2O$, $3.57x10^{-4}$ M; H_3BO_3, $2.31x10^{-5}$ M; $Ca(Cl_2)$ $2H_2O$, $2.14x10^{-3}$ 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; $MnSO_4H_2O$, $7.69x10^{-6}$ M; MoO_3, $1x10^{-5}$ M, and agar-agar, 1% w/v. The heavy metals Cd(II), Cr(VI), Cu(II), Ni(II), and Zn(II) were obtained from the salts $Cd(NO_3)_2$ $4H_2O$; $K_2Cr_2O_7$; $CuSO_4$ $5H_2O$; $Ni(NO_3)_2$; and Zn $(NO_3)_2$ $6H_2O$.

The concentrations of each heavy metal used in this study were 0, 5, 10, 20, and 40 ppm. For each treatment the pH was adjusted to 5.3. Each treatment consisted of three replicates for statistical purposes. The seeds were set under 12/12 hr light/dark cycle and temperatures of 25 °C during the day and 18 °C during the night. The seedlings were harvested after two weeks. The roots were washed with deionized water and the germination rate, root and shoot length were recorded. The plants were then separated into roots and shoots, dried in an oven at 60°C for three days, and the dry weight of biomass was determined.

Dried samples were digested using the EPA 200.3 method (McDaniel, 1991) in which the final volume was adjusted to 10 mL. Reagent blanks were prepared in each digestion batch. Heavy metals content of the extracts was determined with flame atomic absorption spectrometry (FAAS) (Perkin-Elmer 3130). The data were analyzed through one-way analysis of variance (ANOVA) to determine the effect of treatments, and least significant difference (LSD) proofs were performed to test the statistical significance of the differences between means of treatments.

RESULTS AND DISCUSSION

The effects of concentrations of Cd(II), Cr(VI), Cu(II), Ni(II), and Zn(II) on seed germination, root and shoot length in alfalfa plants of cultivar Malone are shown in Table 1. In general, the data show a reduction in seed germination, root and shoot elongation as metal concentration in the growing media increases. The 10 ppm-dose of Cd(II) and Cr(VI), and the 20 ppm-dose of Cu(II) and Ni(II), significantly reduced the seed germination ($P< 0.01$). At a concentration of 40 ppm, Cd(II) and Cr(VI) reduced the seed germination by 50.0%, and those seed which germinated died by the end of the second week. Likewise Cu(II) and Ni(II) at 40 ppm reduced the seed germination by about 40.0% and 25.0%, respectively. Similar results were obtained in a study using nickel (Claire et al., 1991). However, Zn(II) did not significantly reduce the seed germination even at a concentration of 40 ppm ($P< 0.01$).

The root of the plants exposed to the 5-ppm dose of Cd(II), Cr(VI), Cu(II), Ni(II), and Zn(II) grew more than the root of the control treatment by 22%, 166.0%, 156.0%, 63.0%, and 105.0%, respectively (Table 1 and Figures 1 and 2). In addition, the 10 ppm concentration of Cr(VI), Cu(II), and Ni(II) still increased the root size over the control root size for about 37.0%, 54.0%, and 37.0%, respectively. However, at the same dose, Cd(II) reduced the root size by 6.0%, as compared to the control root elongation. A concentration-dependent inhibition of root growth at the dose of 20 and 40 ppm was seen in the treatments using Cr(VI), Cu(II), and Ni(II). This phenomenon is known as hormesis, where small doses increase response while greater doses diminish the response (Calabrese and Baldwin, 1999). Cd(II) produced similar effects in wheat seedlings (Öncel et al., 2000). All Zn(II) concentrations increased the root length by more than 100.0% of the control.

The effect of the treatments using these heavy metals on shoot elongation was a little different (Table 1). Exposures of 5 ppm-dose of Cd(II) reduced the shoot elongation by 17.0%, compared with the shoot length of the control plant. A dose of 5 ppm of Cr(VI), Cu(II), Ni(II), and Zn(II) increased the shoot length by 14.0%, 60.0%, 36.0% and 7.7%, as compared to the control, respectively. However, Cd(II) and Cr(VI) at a 10 ppm-dose significantly reduced the shoot elongation ($P< 0.01$) and their effect was already evident. When the concentration of these two metals was increased to 40 ppm, the shoot size diminished by 80.0% and 76.0% respectively. Furthermore, these metals at a concentration of 40 ppm led to the death of most of the seedlings by the end of the second week. These data correspond with that of Öncel et al. (2000), who found that Cd(II) reduces the chlorophyll *a* and *b* in wheat, whereas Chatterjee and Chatterjee (2000) found that Cu(II) and Cr(VI) significantly decreased the water potential and Fe(II) concentration in cauliflower. The detrimental effects of Cu(II) and Ni(II) were significant at the dose of 40 ppm, causing an elongation reduction of 76.0% and 58.0% respectively. However, Zn(II) showed a positive effect in shoot elongation (10.0% of the control) even at 40 ppm.

The Cd(II), Cr(VI), Cu(II), Ni(II), and Zn(II) concentrations in root and shoot of the alfalfa plants (cultivar Malone), which were grown in agar-based media, are listed in Table 2. In general, the metal concentration in the plant was significantly affected by the dose of the metal in the media and by the different plant tissues ($P< 0.01$). Metal concentration found within the roots and shoots showed that an increase in heavy metal content strongly correlated with the heavy metal content in the media. Several studies have demonstrated that the metal concentration in the plant tissue is a function of the heavy metal content in the growing environment (Flores Tana et al., 1999; Lee et al., 2000; Xiong, 1998).

In general, the ratio of the amount of metal in the shoots to the amount of metal in the roots increased with the dose. This plays an important role in determining the feasibility of phytoremediation because it indicates the amount of heavy metal that could be removed using the aerial portion of the plants. In this study the ratio (%) of Cd(II) present in the shoot to that of the root, for 5 ppm, 10 ppm, and 20 ppm in the media was of 9.6%, 41.3%, and 61.8, respectively. The corresponding ratios for Cr(VI) were 27.3%, 18.4%, and 43.1%, respectively. It was not possible to determine the metal uptake of Cd(II) and Cr(VI) at a concentration of 40 ppm due to the death of most of the plants by the end of the second week. Cu(II) presented a consistent increasing ratio from 15.2%, 52.9%, 61.8% and 58.1% within the plant for the respective dose in the medium of 5, 10, 20 and 40 ppm. The ratio of Ni(II) oscillated between 25.0% and 47.0%. However, a lower ratio of metal in shoot to root was found for Zn(II). For this metal the ratios were 6.7%, 5.2%, 10.0%, and 17.8%, respectively. According to Raskin and Ensley (2000), for most toxic metals, the rate of translocation from root to shoot is much lower compared with the rate of uptake.

Based on these results, we conclude that the 5 ppm-dose of the heavy metals studied here stimulated the root and shoot elongation of the alfalfa plant (cultivar Malone). The dose of 40 ppm of Cd(II), Cr(VI), Cu(II), and Ni(II), significantly

Table 1. Seed germination, root and shoot length of alfalfa (cultivar Malone) after two weeks of exposure to heavy metals.

Metal	Dose ($mg\ L^{-1}$)	Germination rate (%)	Root length (mm)	Shoot length (mm)
Cd	0	$100 \pm 0.0_{a}$	$10.5 \pm 4.8_{efgh}$	$21.0 \pm 7.0_{bcd}$
	5	$100 \pm 0.0_{a}$	$12.8 \pm 5.5_{efg}$	$17.9 \pm 5.0_{cde}$
	10	$70 \pm 1.0_{ef}$	$9.9 \pm 3.6_{efgh}$	$11.1 \pm 4.0_{f}$
	20	$70 \pm 2.6_{ef}$	$4.9 \pm 2.0_{hij}$	$7.8 \pm 3.3_{g}$
	40	$55 \pm 2.9_{gh}$	$1.7 \pm 0.7_{j}$	$4.1 \pm 2.19_{g}$
Cr	5	$100 \pm 0.0_{a}$	$27.9 \pm 7.4_{ab}$	$24.0 \pm 6.3_{bcd}$
	10	$85 \pm 2.1_{bcd}$	$14.4 \pm 4.3_{def}$	$18.9 \pm 5.1_{cde}$
	20	$80 \pm 0.0_{cde}$	$6.0 \pm 2.0_{ghij}$	$7.1 \pm 3.3_{g}$
	40	$45 \pm 2.1_{h}$	$2.2 \pm 1.2_{ij}$	$5.0 \pm 2.2_{g}$
Cu	5	$100 \pm 0.0_{a}$	$26.9 \pm 7.6_{ab}$	$33.6 \pm 6.5_{a}$
	10	$100 \pm 0.0_{a}$	$16.2 \pm 4.6_{cdef}$	$27.8 \pm 4.8_{ab}$
	20	$80 \pm 1.7_{cde}$	$7.8 \pm 3.0_{fghi}$	$16.7 \pm 4.9_{def}$
	40	$60 \pm 1.0_{fg}$	$3.0 \pm 1.6_{hij}$	$6.3 \pm 2.8_{g}$
Ni	5	$100 \pm 0.0_{a}$	$17.1 \pm 5.9_{bcde}$	$28.5 \pm 5.5_{ab}$
	10	$90 \pm 2.3_{abc}$	$14.4 \pm 4.8_{def}$	$29.5 \pm 8.2_{ab}$
	20	$80 \pm 2.9_{cde}$	$10.3 \pm 3.2_{efgh}$	$22.8 \pm 6.8_{bcd}$
	40	$75 \pm 1.2_{de}$	$9.0 \pm 3.3_{efgh}$	$8.8 \pm 2.2_{f}$
Zn	5	$100 \pm 0.0_{a}$	$21.5 \pm 7.6_{bcd}$	$22.6 \pm 7.9_{bcd}$
	10	$100 \pm 0.0_{a}$	$23.0 \pm 7.9_{bc}$	$28.3 \pm 6.6_{ab}$
	20	$95 \pm 0.6_{ab}$	$21.9 \pm 6.6_{bcd}$	$26.0 \pm 5.5_{abc}$
	40	$95 \pm 0.6_{ab}$	$33.6 \pm 9.0_{a}$	$23.1 \pm 6.8_{bcd}$
F ratio		15.65**	190.68**	176.84**

Results are means ± SD. Means with different letters are significantly different from each other ($p < 0.05$) according to LSD-test (LSD = 8.6 for shoots, 8.2 for roots, and 2.3 for seed germination). ** = Significant at 0.01.

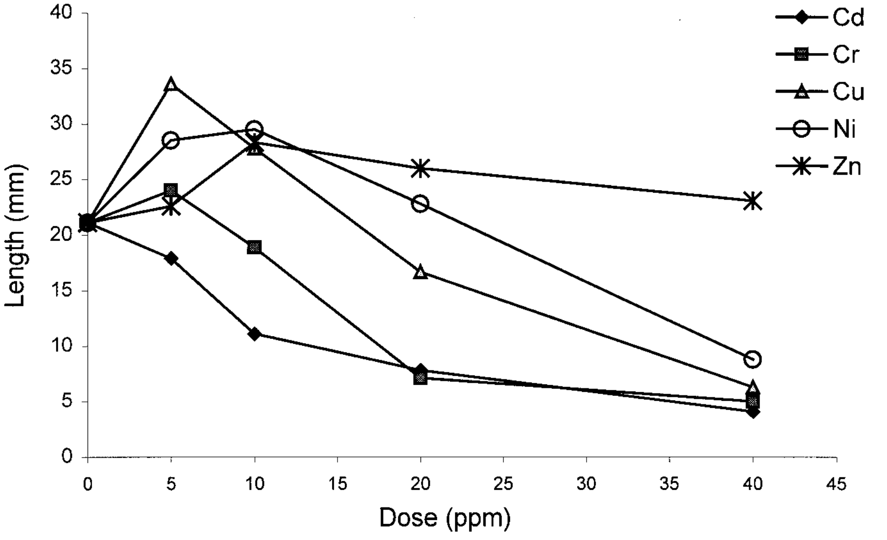

Figure 1. Shoot length of alfalfa plants (cultivar Malone) after two weeks of culture in heavy metal enriched media.

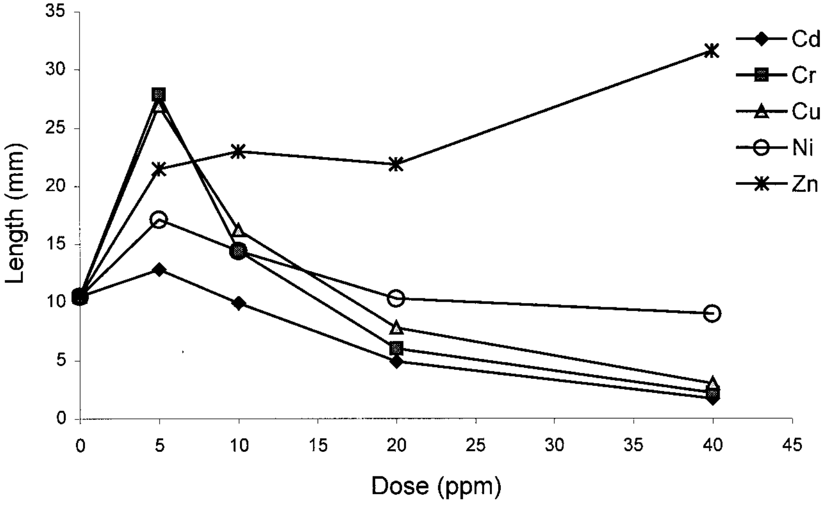

Figure 2. Root length of alfalfa plants (cultivar Malone) after two weeks of culture in heavy metal enriched media.

Table 2. Metal concentration in roots and shoots and uptake: translocation ratio of alfalfa plants (cultivar Malone) after two weeks of growth in heavy metals enriched media.

Metal	Dose ($mg\ L^{-1}$)	Metal uptake ($mg\ kg^{-1}$) Shoots	Roots	Ratio (%) Shoot: Root
	5	$589 \pm 106_{efgh}$	$6122 \pm 246_{bcd}$	9.6
Cd	10	$2427 \pm 392_{bc}$	$5876 \pm 606_{bcd}$	41.3
	20	$4145 \pm 708_{a}$	$6710 \pm 2058_{bcd}$	61.8
	40	D*	D*	___
	5	$438 \pm 116_{fgh}$	$1605 \pm 266_{de}$	27.3
	10	$909 \pm 308_{egh}$	$4940 \pm 1577_{cde}$	18.4
Cr	20	$1882 \pm 962_{bcd}$	$4362 \pm 405_{cde}$	43.1
	40	$1476 \pm 463_{cde}$	D*	___
	5	$498 \pm 33_{fgh}$	$3269 \pm 527_{cde}$	15.2
	10	$757 \pm 37_{eg}$	$1432 \pm 414_{de}$	52.9
Cu	20	$4145 \pm 708_{bch}$	$6710 \pm 2058_{bc}$	61.8
	40	$4791 \pm 848_{a}$	$8208 \pm 2423_{bc}$	58.4
	5	$267 \pm 22_{fgh}$	$1046 \pm 284_{de}$	25.5
	10	$713 \pm 71_{egh}$	$1397 \pm 304_{de}$	51.0
Ni	20	$1023 \pm 663_{defg}$	$1936 \pm 188_{de}$	52.8
	4	$2755 \pm 377_{b}$	$5841 \pm 1754_{bcd}$	47.2
	5	$740 \pm 81_{egh}$	$11091 \pm 2315_{b}$	6.7
	10	$1254 \pm 365_{ef}$	$24310 \pm 9004_{a}$	5.2
Zn	20	$2293 \pm 228_{bc}$	$23013 \pm 5677_{a}$	10.0
	40	$4036 \pm 215_{a}$	$22648 \pm 1071_{a}$	17.8
F ratio		132**	12.7**	

Results are means ± SD. Means with different letters are significantly different from each other ($p < 0.05$) according to LSD-test (LSD = 898 for shoots and 5895 for roots). n= 20, r = 3. D* = Seeds germinated but most of them die. ** = Significant at 0.01.

reduced the ability of the seed to germinate and grow in the contaminated medium. However, alfalfa was able to germinate and grow efficiently at any Zn(II) concentration evaluated in this study. The amount of metal untaken by the plants was significantly affected by the dose of the metal in the media and by the tissue of the plant ($P < 0.01$). The heavy metals were untaken in the following order: Zn(II)>Cu(II)>Cd(II)>Ni(II)>Cr(VI). The ratio (%) of the amount of metal in shoot to the amount of metal in root for Cd(II) and Cu(II) was about 62.0%; for Ni(II), Cr(VI), and for Zn(II), it was 53.0%, 43.0%, and 18.0%, respectively. These data indicate that the alfalfa plant may be grown directly in soils moderately contaminated with Cd(II), Cr(VI), Cu(II), and Ni(II). Further studies need to be done 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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