DIFFERENTIAL UPTAKE OF TRANSITION ELEMENTS BY MESQUITE OBTAINED FROM PLANTS GROWN IN IMPACTED AND CLEAN SITES[1]

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Abstract: In this research, the uptake of Cu, Mo, Zn, As(III) and Cr(VI) by mesquite (*Prosopis spp.*) roots and shoots from two different sources was studied. The first source was seeds collected from plants grown in mine tailings in Arizona, USA (Seeds A) and the other source was seeds from a commercial vendor (Seeds B). In addition, the effects of above mentioned elements on plant growth and nutrient uptake were also investigated. Plants were grown for seven days in hydroponic media containing different concentrations of Cu, Mo, Zn, As(III) and Cr(VI) (0, 1, 5 and 10 mg L^{-1}). Plants grown from Seeds A grew faster and taller at a concentration of 1 mg L^{-1} of Cu, Mo, Zn, As(III) and Cr(VI) than at 0 mg L^{-1}; whereas plants grown from Seeds B had opposite response. This suggests that the seeds obtained from the mesquite plants grown in mine tailings have more phytotoxic tolerance than that of the Seeds B. However, 90% of the plants from both seeds grown in 5 and 10 mg L^{-1} of Cu, Mo, Zn, As(III) and Cr(VI) did not survive. This indicates that uptake of Cu, Mo, Zn, As(III) and Cr(VI) was influenced by its concentration in the growth medium. Uptake of Cu, Mo, Zn, As(III) and Cr(VI) by mesquite plants grown from Seeds A was 1.8 times greater than that of plants grown from Seeds B. At 1 mg L^{-1}, the root and shoot elongation of plants grown from Seeds A was significantly greater than that of plants grown from Seeds B. Plants grown from Seeds A absorbed micro and macronutrients to a lesser extent as compared to those grown from Seeds B. Morphological (xylem and phloem) changes inside the plants grown from both seeds are currently being investigated by infrared (IR) imaging and scanning electron microscope (SEM) techniques. The significant amount of Cu, Mo, Zn, As(III) and Cr(VI) concentrated in the plants grown from Seeds A, as well as the more extensive elongation of roots and shoots than that of Seeds B, indicate that mesquite seeds obtained from plants grown in mine tailings could be better adapted seeds for plants that could be potential hyperaccumulator of Cu, Mo, Zn, As and Cr in the mine tailings.

Additional Key Words: Uptake, Hyperaccumulator, Metals, Seed source, Mesquite.

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Introduction

Mine tailings have been recognized as a major environmental problem (Dudka and Adriano, 1997) to present and future generations. The highest environmental effects are due to the presence of low pH's (Wong et al., 1998), high concentration of heavy metals (Norland and Veith, 1995; Wong et al., 1998) and the high incidence of erosion agents (wind and water run-off). Release of metals from mine sites takes place mainly through acid mine drainage and erosion of waste dumps and tailings deposits (Salomons, 1995). These pollutant effects can reach local and, in some cases, regional scales (Rybicka, 1996) and affect urban or agricultural zones. Consequently, there is a risk of metal uptake by humans.

Therefore, proper reclamation of mine tailings is extremely important for the environment as well as for humans. However, their reclamation is often complicated and expensive due to their high heavy metal content and distribution (Ottenhof et al., 2007). In this sense, phytostabilization (Salt et al., 1998), one of the major branches of phytoremediation, can be used for in situ stabilization of mine tailings using plants. Significant research (US EPA, 2000; Meagher, 2000; Pulford and Watson, 2003) has been conducted on phytostabilization for mine tailings because of its multiple benefits; low-cost and environmentally friendly technology (Chaney et al. 1997, 2000; Baker et al., 1991), successful revegetation of the mine wastes (Norland and Veith, 1995) and bioextraction of metals (Pérez-de-Mora et al., 2006).

There are some plants which are tolerant to the unfavorable soil conditions of mine tailings and play a major role in reclamation of degraded mine soils (Freitas et al., 2003). In this study, such tolerant plants [desert broom (*Baccharis sarothroides*), mesquite (*Prosopis spp.*), desert willow (*Chilopsis linearis*), and whitethorn acacia (*Acacia constricta*)] were found to grow successfully at the copper mine tailings reclamation project (CMTRP) near Globe, AZ. When these plants are tolerant at this site, therefore, it is hypothesized that the seeds of these plants could also be very tolerant.

Therefore, the objectives of this research were to determine the phytotoxic tolerance of mesquite plants germinated from the seeds collected from the CMTRP site against the mesquite plants germinated from the seeds supplied by a commercial vendor. Plant growth and uptake of transition elements [Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$] were measured as an indicator for phytotoxic tolerance. In a previous study, higher concentrations of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ than the normal available range in the soil were found in the mine tailings near Globe, AZ. Therefore, Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ will be considered to verify the objectives of this research through hydroponic experiments.

Materials and Methods

Standards and reagents

All chemicals to prepare stock solutions and treatments were of analytical grade. All solutions were prepared with deionized water. All utensils and bottles utilized in the experiments were washed with 5 % nitric acid solution and rinsed with deionized water. The stock solution for Cu, Mo, Zn, As and Cr, 1000 ± 5 mg L^{-1}. Standards for calibration as well as experimental solutions (1 – 10 mg L^{-1}) for hydroponic experiments were prepared from the stock solution.

Seed collection

Seeds were collected from two different sources for this study. Many desert plants are growing in the numerous tailing impoundments containing Cu and other metals in the vicinity of Globe, Arizona, USA. Mesquite (*Prosopis spp.*) is one of the plants growing successfully at the CMTRP area. Seeds collected from the CMTRP area were the first source of seeds (Seeds A). The other source of seeds was from a commercial vendor (Seeds B).

Medium preparation and seed planting

Mesquite seeds from two different sources were sown in a metal free seedbed where the seedbed was watered regularly. After the germination of the seeds, approximately 20 plants from both sources were transferred into sterilized jars filled with media. A Hoagland modified nutrient solution previously described in literature (Peralta et al., 2001) was prepared as a media. Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ were added to the nutrient solution to obtain different concentrations (1, 5 and 10 mg L^{-1}), which were adjusted to pH 5.3. Plants were grown for 7 days after germination and each treatment was replicated three times for statistical purposes.

Effects of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ on plant growth from both seed sources

To determine the effect of transition elements on plant growth (evaluated as plant elongation), 10 plants/replicate/treatment were randomly selected, and the size of the roots and shoots were measured. Each plant was measured from the main apex of the root to the crown and from the crown to the main apex of the shoot. The plants were then washed for 5 minutes using a 5% HNO_3 solution to eliminate any external metals and then rinsed with DI water. Later, they were separated into roots and shoots, and oven dried at 64°C for 72 hours. The sample was analyzed to determine the concentrations of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$, and macronutrients.

Uptake of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$, and macronutrient in mesquite grown from both seed sources

The dried samples were digested on a microwave oven following the USEPA 3051 method (Kinston and Jassie, 1988). Later, samples were diluted and analyzed by inductively coupled plasma/optical emission spectrometry to determine the Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ concentration as well as the macronutrient (Ca, K, P, Mg) content.

Results

Effects of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ on Plant Growth from both seed sources

Elongation of roots and shoots of the plants at different concentration as well as the different seed sources are shown in Fig. 1. In general, Fig. 1 clearly shows that the elongation of the roots for plants grown from Seeds A were significantly larger than those roots grown from Seeds B. Similar results have been observed in the case of shoots. However, after 1 mg L^{-1} of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ concentration the plants grown from Seeds A or Seeds B, show the elongation of the shoots was dramatically decreased and most of the plants did not survive above the 5 mg L^{-1} concentration.

A strong negative relationship between shoot elongation and Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ concentration in tissues (when germinated from Seeds B) was observed (Pearson correlation coefficients -0.801, $P<0.01$). However, no significant relationship was found for these two variables in plants grown from Seeds A. The amount of root biomass accumulated from the plants grown from Seeds A (data not shown) was 15 times more than those of Seeds B. These results suggest that plants grown from Seeds A might have more phytotoxic tolerance than that of Seeds B.

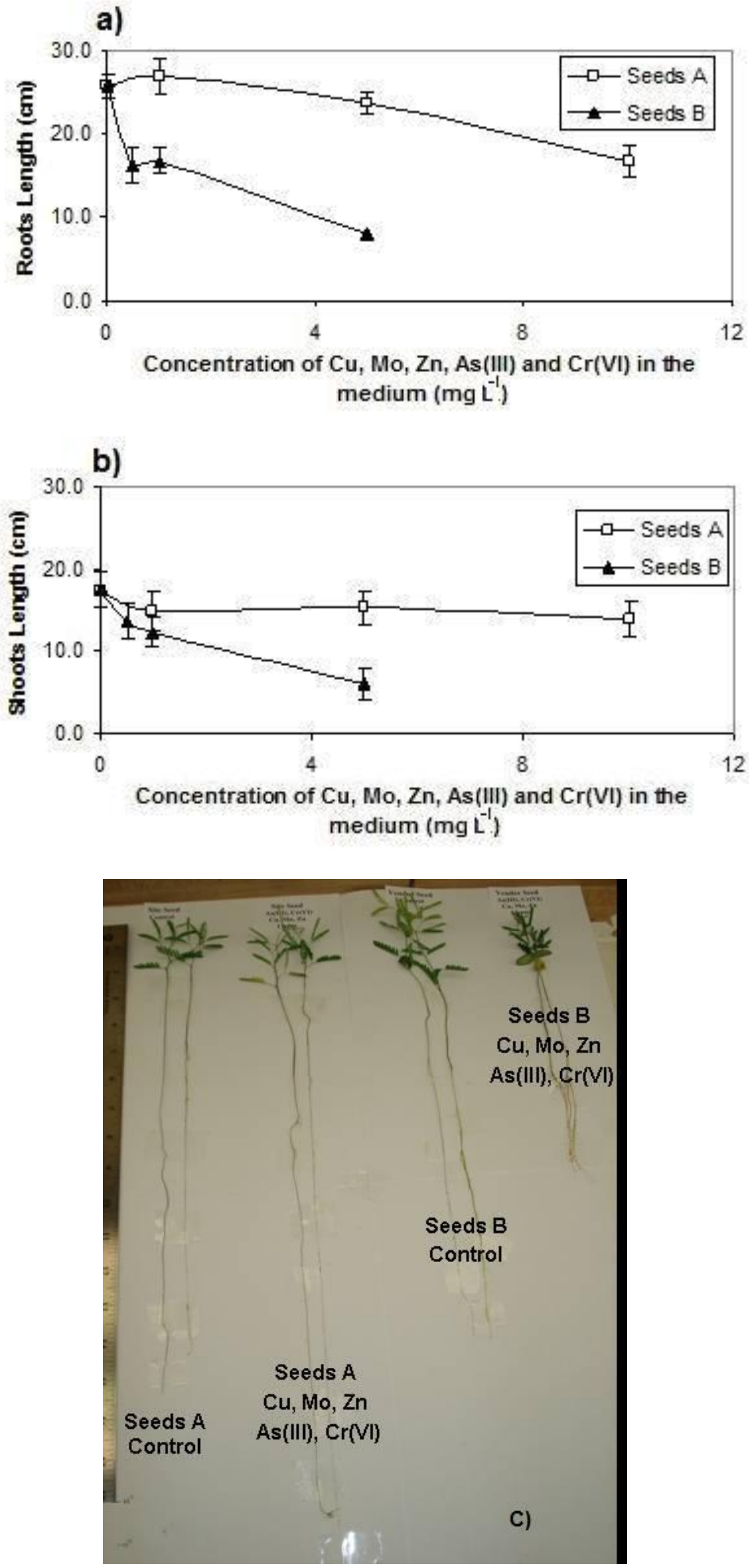

Figure.1: (a) Length of mesquite roots and (b) shoots after 7 days of growth either from Seeds A (□) or Seeds B (▲) in hydroponic media containing different concentrations of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ in the media. (c) Plants growth in hydroponics from Seeds A and B. The different treatments are written under the plants. Data represent average of 20 plants ± SE.

Uptake of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ and macronutrient content in mesquite grown from both seed sources

Uptake of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ in mesquite plants grown from both seed sources after 7 days of treatment are shown in Table 1. It can be easily seen that the more Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ was absorbed by the mesquite plants grown from Seeds A. At 1 mg L^{-1} concentration, the roots of plants grown from Seeds A absorbed 1.8 times more Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ than the roots of plants grown from Seeds B.

Table 1: Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ and macronutrient concentrations in mesquite roots and shoots after 7 days of growth in hydroponic media from both seed sources at a concentration of 1 mg L^{-1} of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$.

	Concentration (mg kg^{-1} of dry weight)			
Element	**Seeds A**		**Seeds B**	
	Root	Shoot	Root	Shoot
Cu	947.9 ± 16.3	2117.4 ± 41.1	527.2 ± 6.3	1080.3 ± 40.2
Mo	323.0 ± 5.6	3716.5 ± 52.8	179.6 ± 2.9	1896.2 ± 25.8
Zn	3797.2 ± 89.1	8548.2 ± 89.8	2111.9 ± 45.2	4361.3 ± 49.8
As(III)	254.2 ± 3.9	332.9 ± 2.9	141.4 ± 1.8	169.8 ± 2.6
Cr(VI)	676.9 ± 9.1	1245.1 ± 4.5	376.5 ± 3.8	635.3 ± 8.9
Al	1037.3 ± 45.2	1357.8 ± 19.8	2814.4 ± 58.2	3581.1 ± 77.9
Ca	112038.0 ± 598	155950.8 ±7128	401494.0 ±5612	125898.1 ±4215
Mg	14179.5 ± 412.1	9310.7 ± 527.3	52334.2 ± 897.2	7209.2 ± 89.6
K	89736.0 ± 714.2	284004.0 ±1987	65784.9 ± 1124	192345.9 ±1202

The effect of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ on the uptake of Fe, Ca, Mg, K, and P by the two mesquite progenies is presented in Table 1. As seen in this table, the roots and shoots of plants grown from VS at 1 and 5 mg/l treatments concentrated more Ca, Mg, K and P than plants grown from site seeds except for Fe. It is possible that in mesquite Cu, Mo, Zn, As and Cr use the same uptake channels as Ca, Mg, K and P. Thus in the progeny of site seed plants, the higher uptake of Cu, Mo, Zn, and Cr, interfere with the uptake of Ca, Mg, K and P. Furthermore, it is possible that plants grew in the site require higher amounts of Cu, Mo, and Zn for enzymatic reactions as a mechanism of adaptation towards contaminated mine tailings. Or it is possible that these plants used these elements as major nutrients. While plants grown from vendor seeds could not use Cu, Mo, Zn at the same level as plants grown from site seeds because the higher intracellular concentrations of these ions might cause toxicity. To deal with this potential stress, plants grown from vendor seeds might have evolved several mechanisms to control the homeostasis of intracellular ions. Such mechanisms include regulation of ion influx (stimulation of transporter activity at low intracellular ion supply, and inhibition at high concentrations), and extrusion of intracellular ions back into the external solution. (Krämer et al., 1997).

Therefore, it can be concluded that mesquite plants grown from Seeds A have more uptake capacity than that of Seeds B. Seeds A collected from the mesquite plants growing in the contaminated area might use Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ as a nutrient towards their growth and survival. The amount of transition element accumulated in the mesquite plants grown from Seeds A indicated that plants grown from Seeds A could be a better option for phytostabilization or reclamation of metal contaminated tailings in Arizona, USA.

Conclusions

The results of this study clearly demonstrate that the uptake of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ by mesquite plants was dependent on the sources of the seed collection. There were significant changes in the roots elongation between the plants grown from Seeds A and Seeds B at a concentration of 1 mg L^{-1} of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$. Approximately 1.8 times higher accumulation of Cu, Mo, Zn, $As^{(III)}$ and $Cr^{(VI)}$ was found in mesquite plants from Seeds A than that of Seeds B. The movements of the nutrients in the plant are greatly affected by the collected Seeds sources as well. Finally, this research shows that mesquite (*Prosopis spp.)* seeds collected from the contaminated mine tailings area could be a better source of seeds for mine reclamation or phytostabilization in Arizona, USA than the commercially obtained seed.

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