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Seed Growth and Heavy Metals Uptake of *Puccinellia distans* (Jacq.) Parl.

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Abstract

The use of plants to remediate contaminated soil has been the most rapidly developing component in environmental cleansing. Although total concentration of heavy metals in soil is used for regulatory review, it also is beneficial to assess the potential for ecosystem impact through a series of bioassays. One commonly used bioassay is seed germination. In current research study effect of contaminated soil with heavy metals (Pb, Zn, Cu, Fe) on germination and growth of *Puccinellia distans* were evaluated. Shoot and root concentrations of metals were also measured. Data obtained from the experiment indicated that the plant perform dose-dependent responses to the contaminated soils. Reduction in germination, root and shoot height and biomass were significantly ($P<0.05$) different when compared to the control. Analysis of tissue concentrations in the plant showed that heavy metals were mainly accumulated in the roots and also plant tissues demonstrated different affinity to take up heavy metals. In all the plant organs, the concentrations of heavy metals increased as the metals contents in the soil increased. **[Ebrahimi. Seed Growth and Heavy Metals Uptake of *Puccinellia distans* (Jacq.) Parl. International Journal of Agricultural Science, Research and Technology, 2012; 2(2):83-88].**

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1. Introduction

Heavy metals (HMs) contaminants are of environmental concern because of their adverse effects on human life (Ok et al., 2011). Many methods, including removal, incineration and removal followed by thermal desorption, have been used for the cleanup of contaminated soils (Joner and Leyval, 2001). In this way, phytoremediation, using plants to restore the deteriorated soils, is a promising technology in cleanup of polluted sites due to the characters of less destructive, low cost and environmentally friendly nature (Wang et al., 2012). To achieve maximum HMs reduction and to successfully establish stable vegetation cover, various criteria must be considered because unfavorable environmental conditions during seed growth and development in the field can reduce germination, vigor and processing quality of seed of plants (Egli et al., 2005;Gelin et al., 2006) therefore plants should be chosen carefully and they provide a maximum root surface area (Aprill and Sims, 1990).

In many studies, grasses and legumes have been used for their potential in this regard. Grass roots have the maximum root surface area compared to other plant types and may penetrate the soil to the depth of up to 3 m (Aprill and Sims, 1990). Researchers have observed that some plants species are endemic to contaminated soils and can tolerate greater than usual amounts of heavy metals or other toxic compounds (Peralta et al., 2000; Blaylock and Huang, 2000). Considering the cost of more extensive testing, germination tests have frequently been used in selecting species to be grown on contaminated sites and most of these studies have been conducted using seedlings or adult plants (Gratton et al., 2000; Pichtel et al., 2000). In a few studies, the seeds have been exposed to the contaminants (Claire et al., 1991; Xiong, 1998).

In this study, *Puccinellia distans* was planted in different concentrations of heavy metals contaminated soil. Heavy metals uptake and the effect of heavy metals on germination and growth were studied. Furthermore, using germination rate as an indicator of subsequent establishment and growth of the plant in contaminated soils was investigated.

2. Materials and methods

2.1. Soil Preparation

The soil was obtained from Takestan industrial city, an important industrial area of Qazvin (Northern Iran). The area is influenced by industrial wastewater then the soil is highly polluted with heavy metals. Soil sampling was obtained from the depth of 0–40 cm and mixed. All soil samples were sieved to 4mm and moisture contents were adjusted to 70% water-holding capacity. Uncontaminated soil was used as the control throughout the study. Selected physical and chemical properties of the contaminated and uncontaminated soil samples are listed in Table 1. The soil's texture (clay loam) was determined by hydrometer method (Day, 1982); CEC was measured by method of Bower and Hatcher (1966); pH was determined in a 1:5 soil to distilled water slurry after 1 hour of agitation using a digital pH-meter (Model 691, Metrohm AG Herisau Switzerland, Thomas, 1996); electrical conductivity (EC_e) using an EC-meter (Model Ohm-644, Metrohm AG Herisau Switzerland) (Rhoades, 1996); total soil N was analyzed colorimetrically with a continuous flow ion analyzer following wet digestion in sulfuric acid (Bremner, 1996); organic carbon was measured by the Walkley-Black method (Nelson and Sommers, 1996). The $CaCO_3$ equivalent was determined by neutralizing with HCl and back titration with NaOH (Black et al., 1965).

2.2. Plant growth and Germination

Seeds were obtained commercially and about 1 kg of soil samples was added to plastic pots (diameter 20×diameter 15×height 35 cm). A filter of paper was placed at the bottom of each pot to prevent soil escaping from the drainage holes. 25 seeds of the plant were buried evenly throughout each pot at least 1 to 2 cm from the edge. The pots were watered from the top during the germination period so that, soil moisture was kept near 70% field capacity. The necessary light for the growth of the plants was obtained from the sun. The samples were put behind the glass windows of the greenhouse and received the solar light during the experiment. The minimum and maximum temperature was 18 and 25C◦, respectively. For each treatment, germination was monitored closely over 14 days of the trials. The number of germinated seeds in each pot was recorded and expressed as a percentage of the number of seeds added. Germination rate was derived by taking the germination count at daily intervals up to 14 days and computed according to the method proposed by Maguire (1962).

For determination of the plant biomass, root and shoot length at the end of the experiment (6 weeks after germination period in the greenhouse), the plants were uprooted carefully from the soil media and thoroughly washed in deionized water to dislodge excess soil adhering to the roots.

2.3. Heavy metals extraction

Plant samples were preliminarily dissected in roots and shoot to recognize the different bioaccumulation capability. Plant organs were washed before analysis. As a second step, samples were dried at 70 °C to a constant weight for approximately 48 h and ground into fine powder in an agate mortar. Metals were analyzed after mineralization of dry shoot material in a microwave oven with 5 ml of nitric acid (69% v/v), 5 ml deionized water and 2 ml H2O2 (30% v/v). The digest was made to 25 ml final volume with deionized water, filtered (0.45 mm, Millipore) and then analyzed for Pb, Zn, Cu and Fe using ICP/OES (Model GBC, 2004). Dried soil samples were passed through a 2mm diameter sieve. About 100 mg dry sediment was digested with HNO3 and HCl (3:1) in a microwave oven. After mineralization, the samples were diluted, filtered and analyzed. Metal concentrations (Pb, Zn, Cu, Fe) were measured as described for the plant samples. Soil samples were divided into 4 groups considering metal concentration each group with 5 pots (replicates).

2.4. Data Analysis

All data were checked for their normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene test), and where necessary, data were log-transformed before statistical analysis. The statistical processing was mainly conducted by analysis of variance (ANOVA). Duncan test post hoc analysis was performed to define which specific mean pairs were significantly different. A probability of 0.05 or lower was considered as significant.

3. Results and discussion

3.1. Soil Characteristics

Soils from the contaminated and uncontaminated sites had clay loam textures and Electrical conductivity of the soils ranged between 2.30-2.66 dSm^{-1}. pH varied between 8.3-8.5 (Table 1). The results show that the soils are similar in term of physical and chemical characteristics. Concentration of heavy metals are listed in Table 2, the highest concentrations were measured for treatment 4 and the lowest heavy metals concentration was found at treatment one.

3.2. Effects of heavy metals on seed germination, root and shoot growth

Effects of different concentrations of Pb, Zn, Cu, and Fe on seed germination and growth of *Pu. distans* are listed in table 3. In general, there was a reduction in seed germination as metals concentration in the soils increased. Significant decreases ($P<0.05$) of germination rate and percentage with the increasing heavy metals concentration in the solution have been observed. These parameters at treatment one are the same with those of the control and at treatment four, the parameters are as high as 41.29% and 32.10%of the control respectively. At treatment one, the root length is different from the control, decreasing to 8.13 of the control. At treatment four, the root length is only as long as one third of the control.

Although response to heavy metals concentration varied between roots and shoots length, it demonstrated an overall dose dependent response to the contaminated soil and a decrease in root and shoot growth was evident as compared to the control treatment. At treatment one (the lowest concentration) reduction in root and shoot size was about 8.40 % and 5.53% respectively as compared to root and shoot size of the control. According to the length difference of the root and shoot between the lowest heavy metals concentration and control, root seems to be more sensitive to heavy metals than the shoot. The data corresponding to the biomass with various treatments are presented in Table 3. Data reveal that root and shoot dry biomass decreased as a function of metal concentrations in tissues. In fact, in treatment four (the highest concentration of heavy metals) the mean biomass significantly decreased into 2.66% of the control and reached its minimum around 27.52mg/kg.

3.3. The seedling heavy metals uptake

The heavy metals concentration in the roots and shoot of *Pu. distans* are shown in Table 4. Concentrations of heavy metals in shoots and roots increased significantly as a function of the metal concentration in soil compared to control. It is interesting to note that the response of *Pu. distans* to the heavy metals, seems to be a combination of some stimulating and inhibiting effects, and heavy metals concentration in the plant is significantly affected by both content supplied in soil and the plant tissue as well as by the interaction between the two factors (Table 4).

Heavy metal concentrations were significantly higher in root tissue than in aboveground components (leaf and stem), and plant organs demonstrated different affinity to take up heavy metals. Mean values for organs showed the maximum accumulation at treatment 4 that showed significant accumulation ($P<0.05$) comparing with other treatments. Although data demonstrated an increase in concentration of heavy metals, there was no significant difference between content of heavy metals in root and shoot among treatment two and three.

Table 1. Physical and chemical characteristics of soil samples were collected for the greenhouse treatments.

Site	Texture	CEC(meq/100gr)	N%	OC%	EC(dS m^{-1})	pH	$CaCO_3$ (%)
1	Clay loam	37.00	0.13	0.12	2.55	8.5	12.21
2	Clay loam	34.20	0.12	0.15	2.50	8.4	12.00
3	Clay loam	34.30	0.14	0.12	2.66	8.3	12.50
4	Clay loam	33.60	0.15	0.14	2.30	8.5	13.60
Control	Clay loam	35.40	0.10	0.15	2.40	8.5	15.30

Soils were sampled from 0 to 40 cm depth with a 5.5-cm-diameter hand-driven corer.

Table 2. Total concentration of heavy metals (mgkg^{-1}) for contaminated treatments based on soil collected from the industrial area.

Treatment	Pb	Zn	Cu	Fe
1	737.12±14.00^{a}	523.11±15.33^{a}	20.84±4.21^{a}	239.00±45.12^{a}
2	1090.00±95.21^{b}	1623.42±25.21^{b}	55.33±3.44^{b}	984.68±55.13^{b}
3	2504.02±94.50^{c}	2216.03±30.21^{c}	71.22±5.57^{b}	1588.45±135.05^{c}
4	5071.15±76.22^{d}	2829.17±45.23^{c}	123.02±79.76^{c}	2592.26±169.73^{d}
Control	ND	ND	ND	ND

Results are Mean ± SE. Different letters indicate significant difference between treatments (Duncan's test).
ND= NOT Detected/Below detectable range.

Table 3. Germination rate, germination percentage, (growth after 14 d of exposure), biomass (DW), roots and shoots length of *Pu. distans* (at the end of growing trial).

Treatment	Germination Rate (%)	Germination (%)	Root Length(mm)	Shoot Length(mm)	Biomass(mg)
1	96.54±0.00^{a}	95.00±1.46^{a}	42.90±3.10^{a}	39.00±1.30^{a}	66.22±1.23^{a}
2	75.20±1.80^{b}	73.36±1.17^{b}	24.61±2.42^{b}	33.42±1.12^{b}	41.31±1.23^{b}
3	64.75±1.30^{c}	45.54±1.22^{c}	18.72±1.17^{bc}	29.27±1.10b^{c}	32.04±1.32^{c}
4	41.29±1.24^{d}	32.10±1.32^{d}	17.70±1.04^{c}	16.73±1.22^{d}	27.52±1.23^{d}
Control	100.00±0.00^{a}	100.00±1.30^{a}	51.03±2.11^{a}	44.53±1.22^{a}	72.00±1.39^{a}

Results are Mean±SE. Different letters indicate significant difference between treatments (Duncan's test).

Table 4. Heavy metal concentration (mg/kg) in seedling roots and shoots tissues of *Pu. distans* at the end of growing trial.

Treatment	Pb Root	Shoot	Zn Root	Shoot	Cu Root	Shoot	Fe Root	Shoot
1	879.21±10.93^{A-a}	188.00±4.00^{A-b}	623.10±9.12^{A-a}	267.30±7.05^{A-b}	85.12±4.50^{A-a}	72.07±8.00^{A-b}	121.10±14.00^{A-a}	102.00±1.14^{A-b}
2	4501.28±23.07^{B-a}	721.17±9.33^{B-b}	1975.33±16.29^{B-a}	712.27±6.44^{B-b}	265.19±6.00^{B-a}	125.25±7.00^{B-b}	1311.22±14.10^{B-a}	557.22±5.00^{B-b}
3	4611.18±35.15^{B-a}	1000.27±9.00^{B-b}	2289.52±24.61^{B-a}	996.12±11.21^{B-b}	276.00±12.10^{B-a}	133.36±7.63^{B-b}	2234.13±16.00^{C-a}	977.12±9.23^{B-b}
4	5233.00±36.00^{C-a}	1911.14±13.20^{C-b}	35200.42±43.92^{C-a}	1987.14±17.00^{C-b}	812.33±14.24^{C-a}	507.62±11.20^{C-b}	3000.11±16.10^{D-a}	1023.09±13.10^{C-b}
Control	ND	ND	ND	ND	ND	ND	ND	ND

Results are Mean ± SE. Different capital letters in each column indicate significant differences between treatments. Different lower case letters in each row indicate significant differences between organs (Duncan's test). ND= NOT Detected/Below detectable range.

Discussion

This article demonstrates effects of heavy metals on the plant emergence and the growth of *Pu. distans*. Results obtained from current experiment show a reduction in seed germination and growth as metals concentration in the soils increased. The results are similar to those obtained by Claire et al. (1991). These authors have carried out an experiment using nickel and other heavy metals on cabbage, lettuce, millet, radish, turnip, and wheat. Similar results have also been observed in *Brassica pekinensis* (Xiong, 1998), lettuce and radish (Nwosu et al., 1995), *Medicago sativa* (Peralta et al., 2000).

Decrease in root and shoot growth was evident as compared to the control treatment. Arduini et al. (1994) found the similar result in a research on morphology of *Pinus pinea* and *Pinus pinaster* using cadmium and copper. Peralta et al. (2004) reported that reduction in chlorophyll could diminish aboveground organs growth. In current study, the data revealed that the root seems to be more sensitive to heavy metals than the shoot, because increase in heavy metals concentrations of soil, causing a root elongation reduction more than the shoot. One of the explanations for roots to be more responsive to contaminants in environment might be that roots were the specialized absorptive parts so that they were affected earlier and subjected to accumulation of more toxic elements than any of the other parts. This could also be the main reason that root length was usually used as a measure for determining heavy metal-tolerant ability of plants. Xiong (1998) found similar effect using lead in *B. pekinensis* seed.

The highest total dry biomass (roots plus shoots) was observed in control followed by treatment one and the lowest total dry biomass was seen in treatment four (the highest heavy metals concentration). Toxic metals decrease water absorption in plant tissues causing undesirable impacts in plant growth (Fuentes et al., 2006). Similar results have also been reported in study of Papazoglou et al. (2005) and Peralta et al. (2004).

There are many studies on heavy metal uptake and accumulation by plants. For example, greenhouse experiments using cereal crops such as barley (*Hordeum vulgare*), maize (*Zea mays*), and wheat (*Triticum aestivum*) grown in a sandy soil spiked with various levels of Cd, Cu, Pb, and Zn, showed that Cd, Cu, and Zn content of plant tissues was significantly increased (Chlopecka, 1996). Regarding concentration of metals to aerial tissues, root had more important role and absorption of metals in the root was higher. Therefore, the metals investigated in root of the plant showed a significant increase and relationship between plant root and metals accumulation was clearly visible. So, the roots of the plant could be considered as an index of Pb, Zn, Cu and Fe absorption.

The relatively low accumulation of heavy metals in aboveground organs was probably due to the need of plants to prevent toxicity to the photosynthetic apparatus as suggested by other authors (Stoltz and Greger, 2002; Bragato et al., 2006). In all the tissues (root and shoot), the concentrations of heavy metals increased without exception as the metals contents in the soil increased.

Published data revealed that heavy metal content in plant is a function of heavy metal concentration in the soil. Kabata-Pendias and Pendias (1984) reported that the plants would take up the metal via root system when the soil is contaminated with heavy metals. In this study, root and shoot are directly subjected to heavy metals. Thus, the heavy metal concentration in the tissues is more closely related to that of the soil.

4. Conclusion

The present study compared the effect of Pb, Zn, Cu and Fe to *Pu. distans* using two types of soil (contaminated and uncontaminated soil). The seed germination declined with increase in heavy metals concentrations showing significant reduction in seed germination and growth of *Pu. distans* indicates higher potential of germination at the low concentration of heavy metals than high concentrations. Shoot and root growth was the more sensitive endpoint, and consequently biomass would be a good parameter to assess heavy metals toxicity in soil. Metal accumulation in tissues depends on metal concentration in soil and plant section (shoots or roots). The heavy metals accumulation observed in this study using to *Pu. distans* showed that Pb, Zn, Cu and Fe are mainly accumulated by roots and are partially translocated to shoots.

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