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# Phytoremediation of soil polluted with lead and zinc by using some plant species around Ahangaran Lead and Zinc mine in Malayar city

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**ABSTRACT**

Soil contamination by heavy metals is one of the major environmental problems in the world. In such environments, especial species of plants have the ability to grow, adaptation and uptake the heavy metals. This study aimed to evaluate the absorption of lead and zinc present in soil in Ahangaran lead and zinc mine in Malayer city with plant species such as *Acantholimon olivieri* (Jaub. & Spach) Boiss., *Astragalus glaucacanthus* Fisch., *Ebenus stellata* Boiss., *Scariola orientalis* (Boiss) Sojak subsp Orientalis. and *Stipa barbata* Desf. For this purpose, in polluted sites and controlled site, sampling of aerial parts and underground parts was done with statistical method in completely randomized plot design with three replicates. Then lead content density in each sample was determined by Atomic absorption instrument model varian 220. Result indicated that Lead and zinc concentrations in aerial parts and underground parts plant in polluted sites was higher than the control site. In between the studied species, *Astragalus glaucacanthus* Fisch., has highest value of lead and zinc absorption in aerial parts respectively (118.6 and 190.69 mg/kg dry matter) and *Scariola orientalis* (Boiss) Sojak subsp Orientalis., has highest value of lead and zinc absorption in underground parts respectively (41.07 and 67.76 mg/kg dry matter). Also *Astragalus glaucacanthus* Fisch., *Acantholimon olivieri* (Jaub. & Spach) Boiss., and *Ebenus stellata* Boiss., species have the highest translocation factor of Lead (3.39, 3.19, 3.16) and Zinc (3.24, 3.06, 2.92) respectively.

**Key words:** Phytoremediation, Soil Pollution, Lead, Zinc, Plant Species, Ahangaran Mine, Malayer city.

## INTRODUCTION

Heavy metals are of the hazardous environmental pollutants which cause dangers to humans, plants and other organisms through entering the food chain. These metals can be entered into the biosphere through human activities such as incomplete combustion of fossil fuels, mining, refining rocks containing metals, urban waste water, agricultural pesticides and natural erosion of rocks (Samani Majd et al. 2008). The amount of heavy metals in soils is affected by different factors such as Parent rock, industrial pollution sources, chemical fertilizers used in agricultural and industrial and urban effluents which due to the physical and chemical properties of soil and environmental conditions, their aggregation and accumulation process is different in soil layers (EPA 2006). Soil type and age, the concentration in the atmosphere, precipitation, wind direction and speed, and type of vegetation are of the factors affecting on the distribution of these elements in the soil (Dabiri 2002). Some of these metals such as zinc, copper and nickel are essential in low amounts as micronutrients for plant growth and are absorbed from soil by the roots. Elements such as lead, although don't have a certain function in the physiological responses of plants, but due to the chemical similarity with essential elements, their absorption by the plants is possible (Ghorbanli et al, 1385). Evaluation of natural vegetation in the

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industrial and mining areas contaminated with heavy metals and determine the concentration of metallic elements in these areas is of the great importance in the terms of scientific and functional aspects and is taken into consideration as an biological approach in cleaning the contaminated environments and bio-tracking the contamination (Gao and Zhu 2003). Certain species of woody and pasture plants, fungi, lichens and algae, have the ability to absorb and accumulate large amounts of heavy metals present in soil without obvious toxic effects create in them (Rezvani et al. 2006). This species is called Super Accumulator and they can be used to refine the soil mineral and organic compounds (Yaron et al. 1996). This species is called hyper accumulator and they can be used to refine the soil mineral and organic compounds (Yaron et al. 1996). In order for a plant species to be recognized as Hyper accumulator of a heavy element, its concentration in aerial parts should be reached to the tolerance threshold which this amount for different elements depends on the type and amount of metals present in the soil, bioavailability of nutrients and plant species type (Akbarpoor et al. 2002). One of the methods of contaminated soils Bioremediation is called phytoremediation, in which by plant extracting, uptake and collecting the heavy metals in plant tissues will be done (McGrath et al. 2000). In this process parameters such as tolerance, vigorous root system, translocation factor, high growth rate and plant biomass are effective (Lasat 2000) and of its advantages over other methods can be mentioned to simply, low cost, environmentally friendly and taking widely advantage of it (Mattina et al. 2003). This study was conducted to evaluate phytoremediation potential of plant species including *Acantholimon olivieri* (Jaub. & Spach) Boiss., *Astragalus glaucacanthus* Fisch., *Ebenus stellata* Boiss., *Scariola orientalis* (Boiss) Sojak subsp Orientalis. and *Stipa barbata* Desf. around the Ahangaran lead and zinc mine located in malayer city in the terms of accumulation and tolerance of lead and zinc in soil so that the strong Hyperaccumulators species of these elements have been identified and used to purify contaminated soils in similar areas.

## MATERIALS AND METHOD

### The study area

Ahangaran lead and zinc mine is located in the protected area Lashgardar in the geographic area of 48˚59´35˝ east longitude and 34˚11´8˝ north latitude, with an area of approximately 25 sq km in southeastern of Hamadan province and 22 km northeast of malayer. The mine altitude from sea level is fluctuating from the 2045 m in the south to 2750 m in the north. In this study, all meteorological data were obtained over a 10 year period (2002-1382) from the synoptic meteorological station in Malayer city (Iran Meteorological Organization, 2012). Accordingly, the average annual precipitation is 133.77 mm, the average annual temperature is 13.65 °C, the area climate is cold semi-arid and the number of dry months is 6 months (Fig. 1).

### Survey Method

In the study area, based on the field observations, four sites (three sites with pollution and one control and without pollution site) were considered in order to sampling of plant species and soil. In the site selection it has been tried that they have the same soil series, altitude and slope. Then the plant species of *Acantholimon olivieri* (Jaub. & Spach) Boiss., *Astragalus glaucacanthus* Fisch., *Ebenus stellata* Boiss., *Scariola orientalis* (Boiss) Sojak subsp *Orientalis*. and *Stipa barbata* Desf. which exist in the form of dominant and prevalent among the sites were selected as the studied plant species (Table 1). At each site in summer, sampling from aerial and underground parts of plant was conducted in completely randomized plot design with 3 replications. In regard to the treatments under study, 240 samples were prepared, which after the coding were transferred to the laboratory. Then samples were washed by hydrochloric acid, 0.01 normal and distilled water and after the separation of aerial and underground parts, they were dried in a ventilated oven for 48 h at 75 °C. The dried samples were powdered by an electric mill and for extraction the method of digestion by nitric acid, normal 4 was used at temperature of 95 °C. After filtering the extracts, the concentration amount of lead and zinc in each plant samples was measured using a Atomic absorption instrument model varian 220 (James and Wells, 1990).

In each site, sampling from soil was conducted in the active area of the root and the depth of 0-10 and 10-20 cm. In the laboratory, soil samples were dried at room temperature and beaten by a plastic hammer and passed through a 2 mm sieve and the experiments including the soil texture, pH, electrical conductivity, organic carbon, available potassium, phosphorus, total nitrogen and total and absorbable lead and zinc concentrations in the samples were measured according to standard methods (Klute, 1986).

Statistical analyzes was performed using Spss software. For this purpose, two-way analysis of variance test was used for judging the significance of the treatments effect on the parameters under investigation and Duncan test was used to compare the average of component at 95% confidence level.

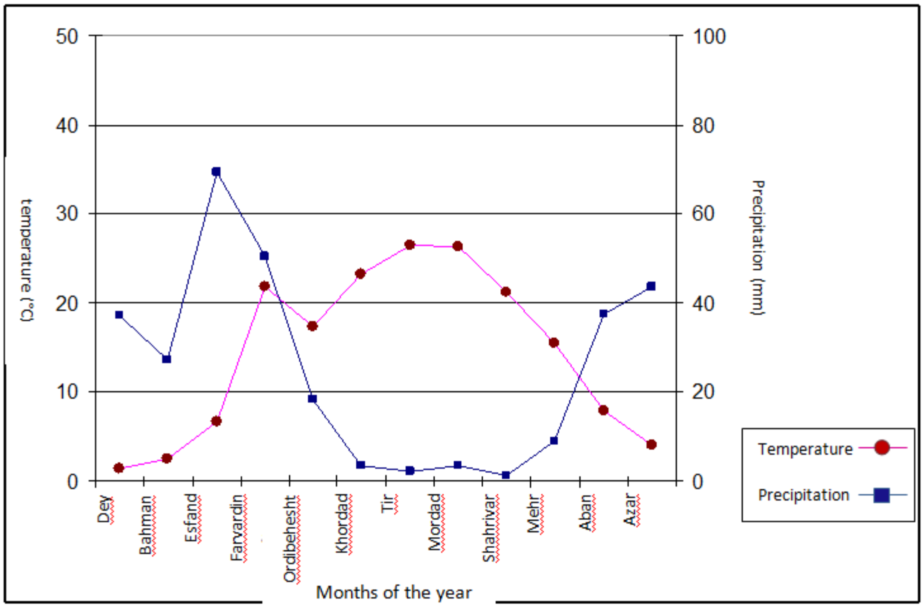


**Fig. 1.** Embrothermic curve of Malayer city.

**Table 1.** Some features of plant species sampled in the study area

| Family | Scientific name | Growth form | Usage |
|---|---|---|---|
| Plumbaginaceae | *Acantholimon olivieri* (Jaub. & Spach) Boiss. | bush | Conservation |
| Papilionaceae | *Astragalus glaucacanthus* Fisch. | bush | Conservation - Pasture |
| Papilionaceae | *Ebenus stellata* Boiss. | bush | Pasture - Conservation |
| Asteraceae | *Scariola orientalis* (Boiss) Sojak subsp Orientalis. | Herbaceous Perennial | Forage |
| Poaceae | *Stipa barbata* Desf. | Herbaceous Perennial | Pasture |

## RESULTS

### Concentration of Lead and Zinc in soil

Results indicate that the study area soil is of the alkaline type with the average pH of 7.38, with medium textured, sandy – loam, and a small percentage of organic ingredients (Table 2). Because this area is a mining area, variation range of high lead and zinc concentration in soil sampling locations is large so that the minimum and maximum lead concentration (6750 and 417.5 mg/kg) and zinc concentration (11812.5 and 688.78 mg/kg) is related to A and D (control) sites. The results showed that there is a significant difference between the amount of absorbable lead and zinc in polluted sites and control site (Table 3).

### Lead and zinc concentrations in aerial parts (leaves)

The results showed that the highest concentration of lead and zinc is in the leaves of Astragalus glaucacanthus Fisch. and A site, respectively, with 118.6 and 190.69 mg per kg, and the lowest is in the leaves of Stipa barbata Desf. and D site (control), respectively, with 8.3 and 10.94 mg per

Table 2. Physical and chemical characteristics of the soil samples in studied sites.

| Phosphorus (ppm) | Total nitrogen ( %) | Potassium (ppm) | Organic carbon ( %) | ganic matter content | Lime percent | Acidity | Salinity | Sand ( %) | Silt ( %) | Clay ( %) | Texture | Depth (cm) | site | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8.8 | 0.22 | 265 | 0.76 | 0.51 | 43.3 | 7.26 | 0.46 | 55.6 | 25.9 | 18.5 | sandy – loam | **0-10** | **A** | **Contaminated** |
| 9 | 0.27 | 280 | 0.91 | 0.63 | 50.1 | 7.35 | 0.39 | 52.1 | 28 | 19.9 | sandy – loam | **10-20** | | |
| 7.9 | 0.16 | 205 | 0.68 | 0.45 | 50 | 7.37 | 0.47 | 63.2 | 19.6 | 17.2 | sandy – loam | **0-10** | **B** | |
| 8.5 | 0.2 | 220 | 0.79 | 0.49 | 63.5 | 7.42 | 0.4 | 59.8 | 21.8 | 18.4 | sandy – loam | **10-20** | | |
| 6.1 | 0.21 | 170 | 0.53 | 0.43 | 47.3 | 7.48 | 0.39 | 65.4 | 17.9 | 16.7 | sandy – loam | **0-10** | **C** | |
| 6.6 | 0.24 | 190 | 0.59 | 0.52 | 58.1 | 7.59 | 0.34 | 12.5 | 69.9 | 17.6 | Loamy | **10-20** | | |
| 10.2 | 0.27 | 195 | 0.8 | 0.56 | 39.5 | 7.48 | 0.5 | 70.0 | 14.9 | 15.1 | sandy – loam | **0-10** | **D** | **Control** |
| 11 | 0.33 | 225 | 1.05 | 0.67 | 51.1 | 7.76 | 0.54 | 8.8 | 75.2 | 16 | Loamy | **10-20** | | |

**Table 3.** Total and absorbable lead and zinc concentrations (mg/kg) in soil samples.

| **site** | | | | |
|---|---|---|---|---|
| **Control** | **Contaminated** | | | |
| D | C | B | A | |
| 417.5 | 1560 | 3563.5 | 6750 | Total Lead |
| 688.78 | 2729.02 | 6236.13 | 11812.5 | Total Zinc |
| 11.3 | 74 | 81.5 | 147 | Absorbable Lead |
| 19.09 | 128.76 | 140.18 | 239.9 | Absorbable Zinc |

**Table 4.** Lead concentrations (mg/kg) on dry weight of aerial parts of plant species.

| **Plants sites** | | ***Acantholimon olivieri* (Jaub. & Spach) Boiss.** | ***Astragalus glaucacanthus* Fisch.** | ***Ebenus stellata* Boiss.** | ***Scariola orientalis* (Boiss) Sojak subsp Orientalis.** | ***Stipa barbata* Desf** |
|---|---|---|---|---|---|---|
| Conta-minated | A | 52.6 C | 47.3 C | 98.1 D | 118.6 D | 86.2 D |
| | B | 35.06 B | 30.5 B | 65.4 C | 79.06 C | 57.4 C |
| | C | 27.1 B | 24.2 B | 50.3 B | 60.8 B | 44.1 B |
| Control | D | 9.2 A | 8.3 A | 17.3 A | 20.9 A | 15.2 A |

kg (Tables 4 and 6). The ANOVA showed that the effect of site on the accumulation of lead and zinc concentration in the leaves of the plant species has a significant difference in 95% confidence level (Table 5 and 7), so that the concentrations of these elements in the leaves of studied plants in A site have the highest and in D site (control) have the lowest rate.

**Lead and zinc concentrations in underground parts (roots)**

Results of studies showed that the highest concentrations of lead and zinc is in roots of Scariola orientalis (Boiss) Sojak subsp Orientalis., and in A site, respectively, with 41.07 and 67.76 mg/kg, and the lowest is in the roots of Acantholimon olivieri (Jaub. & Spach) Boiss., and D site (control), respectively, with 5.41 and 8.92 mg/kg (Tables 8 and 10). ANOVA also indicated that the effect of sites on the accumulation of lead and zinc concentration in the root of plant species at the 95% confidence level has a significant difference (Tables 9 and 11), so that the concentration of these elements in the roots of studied plants has the highest level in site A and lowest level in site D (control).

Given the importance of translocation ratio of elements from underground to aerial parts or translocation factor (the ratio of the element concentration in aerial parts to concentration of the same element in the underground parts), the results show that species of Astragalus glaucacanthus Fisch., Acantholimon olivieri (Jaub. & Spach) Boiss., and Ebenus stellata Boiss. have highest translocation coefficient of lead (3.39, 3.19 and 3.16) and zinc (3.24, 3.06 and 2.92) from underground parts to aerial parts, and somehow have been able to transfer these elements to their aerial and have the lowest accumulation of these elements in their underground parts (Table 12).

## DISCUSSION AND CONCLUSION

Lead and zinc concentrations in contaminated sites in aerial and underground parts of plants is more than the control site, and respectively, D, C, B and A show increase. The existence of significant differences between the concentrations of these elements in different sites can be associated to the atmospheric concentrations rate and changes in physical and chemical properties of soil such as clay particles, pH, total concentration and absorbable nutrients in the soil. Marry et al. (1986) have noted proportional of the concentration of elements in the environment (soil and atmosphere) to their absorption capability by plants. Also Samani Majd et al. (2008) stated that clay colloids present in the soil, by absorbing heavy metals prevent leaching and their translocation to the lower layers of soil and therefore increase the rate of nutrient uptake by plants.

The results showed that the lead and zinc concentration in aerial parts (leaves) of plant species has been greater than that of the underground parts (roots). Increased concentration of these elements in aerial parts can be connected to high concentrations of lead and zinc in soil and their ability to absorb and transfer from root to leaf. Asgari et al. (2011) and Lasat (2000) in phytoremediation process were mentioned parameters such strong root system and elements translocation factor from underground to aerial parts to be very important.

The results showed that the species of Astragalus glaucacanthus Fisch., has the maximum adsorption of lead and zinc in aerial parts, respectively (118.6 and 190.69 mg per kg of plant dry matter) and Scariola orientalis (Boiss) Sojak subsp Orientalis., has the maximum adsorption of lead and zinc in the underground parts, respectively with (41.07 and 67.76 mg per kg of plant dry matter). Given the importance of translocation ratio of elements from underground to aerial parts or translocation factor (the ratio of the element concentration in aerial parts to concentration of the same element in the underground parts), our results indicate that species of Astragalus glaucacanthus Fisch., Acantholimon olivieri (Jaub. & Spach) Boiss., and Ebenus stellata Boiss., have the highest ability to transport lead and zinc from underground parts to aerial ones. Lasat (2000) stated that the elements translocation factor from underground to aerial parts can be used to identify the Hyperaccumulators species. Brooks (1998) stated that plants with the lowest concentrations of heavy metals in underground parts can transfer these elements to their aerial parts. Some researchers have also used the metal concentration ratio in aerial parts to its concentration in root in order to describe the resistance and plant response to the presence of high levels of metals in soil, which this ratio in accumulator plants is greater than 1 and in repellent plants is less than 1 (Marry et al. 1986; McGrath et al. 2000).

Due to the high potential of mineral deposits in the country and expanding metallurgical industries, it is necessary to control environmental pollution caused by them. The results showed that the lead and zinc concentration in plant samples in the study area is high which represents the ability of these plants to absorb and accumulate heavy elements. Accordingly, the species of *Astragalus glaucacanthus* Fisch., *Acantholimon olivieri* (Jaub. & Spach) Boiss., and *Ebenus stellata* Boiss., are

**Table 5.** Analysis of variance of lead concentration in aerial parts of plant species affected by site.

| Source of variation | Degrees of freedom | sum of squares | Mean square | F | P |
|---|---|---|---|---|---|
| Site | 3 | 2482.79 | 827.59 | 75235.45 | 0.001* |
| Species | 4 | 385.12 | 96.28 | 8752.72 | 0.000* |
| Site × Species | 12 | 43.88 | 3.65 | 331.81 | 0.012* |
| Error | 101 | 1.20 | 0.011 | | |
| Total | 120 | 2912.99 | | | |

* Significant at 5% probability level.

**Table 6.** Zinc concentrations (mg/kg) in the dry weight of aerial parts of the plant species.

| ***Stipa barbata*** **Desf.** | ***Scariola orientalis*** **(Boiss) Sojak subsp Orientalis.** | ***Ebenus stellata*** **Boiss.** | ***Astragalus glaucacanthus*** **Fisch.** | ***Acantholimon olivieri*** **(Jaub. & Spach) Boiss.** | **species** / **Plants sites** | |
|---|---|---|---|---|---|---|
| 70.78 C | 80.12 C | 137.23 D | 190.69 D | 154.86 D | A | Contaminated |
| 41.58 B | 51.69 B | 84.54 C | 120.44 C | 102.91 C | B | |
| 33.75 B | 39.27 B | 65.79 B | 95.32 B | 81.89 B | C | |
| 10.94 A | 11.52 A | 21.71 A | 34.48 A | 26.54 A | D | Control |

**Table 7.** analysis of variance of zinc concentration in aerial parts of plant species affected by site.

| Source of variation | Degrees of freedom | sum of squares | Mean square | F | P |
|---|---|---|---|---|---|
| Site | 3 | 4096.40 | 1365.53 | 75862.77 | 0.000* |
| Species | 4 | 635.44 | 158.86 | 8825.55 | 0.002* |
| Site × Species | 12 | 72.41 | 6.04 | 335.55 | 0.008* |
| Error | 101 | 1.89 | 0.018 | | |
| Total | 120 | 4806.34 | | | |

* Significant at 5% probability level.

**Table 8.** Lead concentrations (mg/kg) in dry weight of the underground parts of plant species.

| ***Stipa barbata*** **Desf.** | ***Scariola orientalis*** **(Boiss) Sojak subsp Orientalis.** | ***Ebenus stellata*** **Boiss.** | ***Astragalus glaucacanthus*** **Fisch.** | ***Acantholimon olivieri*** **(Jaub. & Spach) Boiss.** | **species** / **Plants sites** | |
|---|---|---|---|---|---|---|
| 36.95 C | 41.07 C | 26.93 D | 37.06 D | 30.65 D | A | Contaminated |
| 24.6 B | 28.37 B | 17.65 C | 20.70 C | 20.43 C | B | |
| 17.07 B | 19.12 B | 13.78 B | 18.5 B | 15.91 B | C | |
| 6.47 A | 9.67 A | 5.75 A | 6.03 A | 5.41 A | D | Control |

**Table 9.** Analysis of variance of the lead concentration in underground parts of plant species affected by site.

| Source of variation | Degrees of freedom | sum of squares | Mean square | F | P |
|---|---|---|---|---|---|
| Site | 3 | 1657.96 | 552.65 | 184216.66 | 0.002* |
| Species | 4 | 266.77 | 66.69 | 22230 | 0.043* |
| Site × Species | 12 | 32.73 | 2.72 | 906.66 | 0.001* |
| Error | 101 | 0.83 | 0.003 | | |
| Total | 120 | 1958.29 | | | |

* Significant at 5% probability level.

**Table 10.** Zinc concentrations (mg/kg) in dry weight of underground parts of plant species

| *Stipa barbata* Desf. | *Scariola orientalis* (Boiss) Sojak subsp Orientalis. | *Ebenus stellata* Boiss. | *Astragalus glaucacanthus* Fisch. | *Acantholimon olivieri* (Jaub. & Spach) Boiss. | species / Plants sites | |
|---|---|---|---|---|---|---|
| 60.83 C | 67.76 C | 44.43 D | 61.14 D | 50.57 D | A | Contaminated |
| 40.59 B | 46.81 B | 29.12 C | 34.15 C | 33.70 C | B | |
| 27.13 B | 30.54 B | 22.73 B | 30.53 B | 26.25 B | C | |
| 10.67 A | 15.08 A | 9.48 A | 9.94 A | 8.92 A | D | Control |

**Table 11.** Analysis of variance of zinc concentrations in underground parts of plant species affected by site.

| Source of variation | Degrees of freedom | sum of squares | Mean square | F | P |
|---|---|---|---|---|---|
| Site | 3 | 2735.63 | 911.87 | 0.000* | 70143.84 |
| Species | 4 | 440.17 | 110.06 | 0.012* | 8466.15 |
| Site × Species | 12 | 54.01 | 4.51 | 0.001* | 346.92 |
| Error | 101 | 1.36 | 0.013 | | |
| Total | 120 | 3231.17 | | | |

* Significant at 5% probability level.

**Table 12.** Lead and zinc concentrations ratio in aerial to underground parts in plant species

| Source of variation | Translocation factor (TF) | |
|---|---|---|
| | Lead | Zinc |
| *Acantholimon olivieri* (Jaub. & Spach) Boiss. | 3.19 | 3.06 |
| *Astragalus glaucacanthus* Fisch. | 3.39 | 3.24 |
| *Ebenus stellata* Boiss. | 3.16 | 2.92 |
| *Scariola orientalis* (Boiss) Sojak subsp Orientalis. | 1.26 | 1.14 |
| *Stipa barbata* Desf. | 1.29 | 1.12 |

suitable species for refining the soils contaminated with lead and zinc which also can recommended at the same areas.

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