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Application of Nano-Particles of *Euphorbia Macroclada* for Bioremediation of Heavy Metal Polluted Environments

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Abstract. Environmental pollution with heavy metals is a common problem in all countries. A field study was conducted in a dried waste pool of a Lead mine in Arak (Iran) to find the native accumulator plant(s). Concentrations of heavy metals were determined both in the soil and the plants that were grown in a dried waste pool by using flame atomic absorption method. The concentration of total Cu, Zn, Pb and Ni were found to be higher than the natural soil and the toxic levels. The results showed that six dominant vegetations namely, *Scariola orientalis*, *Rreseda lutea*, *Centaurea virgata*, *Gundelia tournefortii*, *Euphorbia macroclada* and *Eleagnum angustifolia* accumulated heavy metals. Based on the results, it was concluded that *Euphorbia macroclada* belonging to Euphorbiaceae is the best Pb accumulator and also a good accumulator for Zn, Cu and Ni. The Bioaccumulation ability of nano-particles prepared from *E. macroclada* was evaluated in experimental water containers. The study showed that the amount of heavy metals in polluted media decreased several times during two weeks bioremediation. Based on the obtained data, *E. macroclada* is an effective accumulator plant and its nano-particles are useful for watery media detoxification and bioremediation in critical conditions.

Keywords: Heavy metal pollution, Detoxification, Metal accumulator plants, Phytoremediation.

1. Introduction

Heavy metals are released into the environment as a result of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping, and melting operations [1]. All the heavy metals at high concentrations have strong toxic effects and are regarded as environmental pollutants [2]. Proper management of plants in such areas may significantly contribute to restoring the natural environment. Numerous efforts have been undertaken recently to find methods of removing heavy metals from soil, such as phytoremediation [1, 3]. Perhaps, not surprisingly, phytoremediation was initially proposed as an environmental cleanup technology for the remediation of metal-contaminated soil [4]. The phytoremediation of heavy metals, a cost-effective green technology based on the use of metal-accumulating plants, is a technique used to remove toxic metals from the soil and water. The identification of metal hyperaccumulators, plants capable of accumulating extraordinarily high metal levels, demonstrates that plants have the genetic potential to clean up contaminated soil. Phytoremediation has recently become a subject of public and scientific interest and a topic of many recent researches [1, 3, 5]. The aim of this research was to find new, native accumulator plants and to evaluate its nano-particles ability regarding bioremediation of polluted water sources.

2. Studied area

A dried waste pool of the mines should be considered as high concentrated metal sources because the sedimentations of wastewater resulted from washing processes of mining were stored in ponds and then dried.

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An artificial old dried waste pool of a Lead mine, located between Arak and Malayer in Iran, was studied as a polluted area in this research. The dried sediments are similar to natural soil but with a high metal concentration. The Plants grown in the dried old waste pool were collected and their scientific names and characteristics were determined. The concentration of heavy metals was determined in the waste pool soil and was compared with the natural soil. The amount of heavy metals was determined in different parts (shoots and leaves) of the plants. The plants with high concentration of heavy metals were chosen as accumulators.

3. Heavy metal determination

Heavy metals were determined in soil samples of the sediment of the waste pool that was regarded as a polluted soil and the soil samples of 5Km further than the mine site were regarded as natural soil. At each subjected plot, 10-15 samples of the soil (depth 10-15 cm) were taken and sieved through a 1 cm sieve. To estimate the total heavy metals in the plants, samples (shoots and leaves) were dried at 105 ˚C for 24h in acid-washed and reweighed volumetric 100 ml Pyrex conical flasks. The content (about 1 g) was digested in 20 ml of boiling concentrated (65%) nitric acid (especially made pure for spectroscopy). The solution was boiled in a hot plate until light fumes were given off. Next, the samples were cooled down and the digests were filled up to 100 ml with deionized water and left overnight to allow the remaining soil particles to settle out of the suspension. Finally, 20 ml of each sample solution was used for heavy metal concentration measurements, using the flame atomic absorption method for Pb, Cu, and Zn and graphite furnace technique for Cd measurement (Aanalyst 800, Perkin-Elmer). The accumulator plants were identified regarding the concentration of heavy metals in the subjected plants.

4. Evaluation of Metal Removing

Euphorbia macroclada, used as an accumulator plant for this study, belongs to *Euphorbiaceae* and is a common and native plant in the main area. This species occurred more than others, especially in the central region of the studied pool. Its roots and shoots were blended until fine particles formation. Nano-particles of the powder were collected by passing through a mesh with pores of 0.2-2 µm.

Ten pots were selected and filled with 15 kg water containing the heavy metals with concentration the same of originally polluted waste pool. *Euphorbian* plants nano-particles were mixed with the water. Each pot contained 500 g *Euphorbian* plant nano-particles.

Euphorbian plant nano-particles were removed by using a double layer of Watman filter paper after two weeks. Heavy metals were determined in the water of the pots, before starting the study and also after two weeks.

5. Results of Heavy Metal Accumulator Recognition

This research studied the flora of Arak Lead Mine. Plants that were more popular and could grow at the waste pool of the mine were collected and analyzed for their scientific name and classification. The determinations of the heavy metals in plant shoots showed that some of them acted as accumulators and are illustrated in table 1. Results showed that the amounts of Cadmium in some plants, including *Marrubium vulgar*, *Onosma kotschyi, Hultemia persica, Stipa lessingiana, Salix excelsa, Centaurea virgata* and *Reseda lutea*, were more than others (Table 1). *Marrubium vulgar* is, however, the best Cd accumulator plant (9 ppm). The study indicated that the best Cu accumulator plant was *Euphorbia macroclada (*65 ppm) but some species including *Centaurea virgata, Scariola orientalis* and *Cirsium congestum*_also accumulated Cu considerably. Analyzing the amount of Fe in the experimental plants showed that we can consider *Reseda lutea* as the best Fe accumulator (5490 ppm) but the amount of Fe in *Euphorbia macroclada, Centaurea virgata* and *Gundelia tourneforti* was also more than the other studied plants (Table 1).

The amount of Ni in most of the studied plants was lower than the toxic level and was lower than that of the natural soil as well. Results showed that the best Zn accumulator overall was *Euphorbia macroclada* (1873 ppm), while *Reseda lutea, Salix excelsa, Scariola orientalis* and *Cynedon dactylon* were other accumulators. Among the studied plants the best Pb accumulator was *Euphorbia macroclada* (1138 ppm), meanwhile *Centaurea virgata, Scariora orientalis and Cardaria draba* also accumulated Pb relatively (Table 1).

Table 1: Concentration of some heavy metals (ppm) in shoots and leaves of studied plants. Data indicate that Euphorbia macroclada is the best accumulator for Pb and also for Zn, Cu and Ni. The species that accumulated high amount of Fe is Reseda lutea and Ni is Marobium vulgar. Each data represent means ± SE of 10-15 samples. Bolded data represent species that accumulated the highest and significant amount of each metal (*P<0. 01, **P<0.05) .

Species	Cd	Cu	Fe	Ni	Pb	Zn
Achillea filipendalina	2.00±0.18	40.00±6.2	1479±324	6.00±0.8	135.00±32	38.00±6.1
Biebersteinia multifida	7.00±0.9	20.00±4.5	480±91	4.00±0.8	23.00±3.5	ND
Cardaria draba	2.40±0.2	26.40±3.6	1324±245	8.40±1.2	776.00±105	1600.00±185
Centaurea virgata	2.20±0.2	36.65±4.5	944±186	6.30±0.9	590.00±121	1262.00±145
Cydonia oblonga	1.80±0.2	22.93±4.2	460±74	7.40±1.2	310.67±45	1428.00±240
Dendrostellaria lessertii	2.00±0.17	28.00±5.7	800±214	7.00±1.7	353.00±65	139.00±22
Eleagnus angustifolia	0.80±0.06	9.60±1.6	148±38	5.20±0.65	404.00±78	980.00±156
Euphorbia macroclada	2.35±0.25	****65.00±9.5**	1040±128	**14.25±2.4**	***1138.00±195**	***1873.00±22.7**
Gundelia tournefortii	2.30±0.25	24.00±3.8	1952±316	8.40±1.3	652.00±86	820.00±14.3
Hultemia persica	3.00±0.5	23.00±2.5	580±67	8.00±1.5	62.00±9.5	48.00±7.5
Juglans regia	1.60±0.4	22.20±2.9	340±46	11.93±1.9	214.33±35	598.67±106
Leutea petidaris	3.00±0.25	19.00±2.5	246±39	4.00±0.7	25.00±4	--
Marrubium vulgare.	**9.00±1.2**	34.00±3.1	540±95	4.00±0.65	78.00±12	58.00±9.4
Myostis caespitosa	2.00±0.16	24.00±3.1	80±34	5.00±0.6	61.00±9.5	62.00±10.5
Onosma kotschyi	3.00±0.5	47.00±7.6	160±48	6.00±1.1	39.00±6	151.00±25
Reseda lutea	5.50±1.1	57.50±8.5	***5490±980**	7.00±1.3	371.50±57	233.00±36
Salix excels	3.93±0.9	35.13±4.8	1891±344	9.13±1.6	404.00±65	685.67±95
Scariola orientalis	2.60±0.4	43.40±5.9	1000±245	8.60±1.4	884.00±141	1468.00±160
Smymium cordifolium	3.00±0.5	24.90±3.5	400±67	7.00±0.9	4.00±0.5	20.00±3.2
Stipa lessingiana	ND	22.50±3.5	673.5±148	7.50±1.2	68.00±11	39.50±6.5
Ziziphora tenuior	4.00±0.6	50.00±6.8	1060±168	7.00±0.9	228.00±35	55.00±9.5

6. The Bioremediation Results

Euphorbia macroclada was chosen as an accumulator in this study because it is a common plant in the studied polluted area and can effectively accumulate most of the studied heavy metals (Table 1). Its nano-particles were used for bioremediation of heavy metal polluted soils. The data showed that the concentrations of all the heavy metals decreased considerably after the experiment. The decrease in Pb, Cd, Ni, Cu and Zn are illustrated in Table 2. The data showed that the decrease of Pb in experimental pots was more than the other metals (%98). Meanwhile, the decrease of other heavy metals was also considerable (Cd, %72.04; Zn, %79.03; Ni, %33.61; Cu, %73.38).

Table 2: Comparison between concentration of some heavy metals (ppm) in the polluted water before and after bioremediation by nano-particles of Euphorbia macroclada. Data indicate that amounts of all studied heavy metals were decreased due to bioremediation. Decreasing of metal concentration in experimental groups are significant (*P<0.01, ** P<0.05). Each data represent the means ± SE of 10 samples in experimental group and 3 samples in control group.

Metals (ppm) Samples	Cd	Cu	Ni	Pb	Zn
Waste water before bioremediation	54.10±6.8	123±9.5	98.20±14.80	14800±1750	2950±155
Waste water after bioremediation	*14.50±4.2	*28.5±3.5	**61.2±8.9	*244.50±31.5	*496.67±38.9

7. Discussion

Heavy metals contamination of arable soil showed several problems, including phytotoxic effects of certain elements such as Cd, Pb, Zn and Cu which are well known as micronutrients and cause several phytotoxicities if critical endogenous levels are exceeded [2, 6]. All heavy metals at high concentrations have strong toxic effects and are regarded as environmental pollutants [7].

According to the results of the following study, the plants mentioned below can be regarded as heavy metal accumulators while they are different regarding their accumulating ability: *Euphorbia macroclada*, *Reseda lutea, Salix excelsa, Scariola orientalis, Cydonia oblonga,* and *Centaurea virgata* (Table 2). To conclude, *E. macroclada* should be considered as the best and the right Pb accumulator plant (according to [8, 9]) because it accumulated more than 1000 ppm (1138 ppm).

E. macroclada was selected as a good metal accumulator especially a good Pb accumulator, and then it was chosen for metal remediation. Its ability to remove heavy metals from polluted water has been evaluated as well. The nano-particles provided from *E. macrocladan* plants were kept in experimental pots for two weeks and then the amount of their heavy metals were compared with that of the control pots. The data showed that the concentrations of all the subjected metals decreased (Pb, 92%; Zn, 76.05%; Cu, 74.66%; Cd, 69.08%; Ni, 31.50%) among which Pb showed the highest decrease (Table 2). Nano-particles of *E. macroclada* is suggested for removing and detoxification of heavy metals, (especially Pb, Cd, Cu and Zn), from polluted environments [10, 11].

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