Bioremediation of Heavy Metal Pollution by Nano-Particles of *Noaea Mucronata*

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***Abstract*—Environmental pollution with heavy metals is a common problem in many countries. Great efforts have been made in the last two decades to reduce pollution sources and remedy the polluted soil and water resources. A field study was conducted in a dried waste pool of a Lead mine in Zanjan (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 that natural soil and the toxic levels. The results showed that six dominant vegetation namely *Centaurea virgata*, *Gundelia tournefortii*, *Scariola orientalis*, *Rreseda lutea*, *Noaea mucronata* and *Eleagnum angustifolia* accumulated heavy metals. Based on the results, it was concluded that *Noaea mucronata* belonging to Chenopodiaceae is the best Pb accumulator and also a good accumulator for Zn, Cu and Ni, but the best Fe accumulator is *Reseda lutea* and the best one for Cd is *Marrobium vulgare*. The bioaccumulation ability of nano-particles prepared from *N. mucronata* was evaluated in experimental water containers. The study showed that the amount of heavy metals in polluted water decreased several times during three days bioremediation. Based on the obtained data, *N. mucronata* is an effective accumulator plant and its nano-particles are useful for watery media detoxification and bioremediation in critical conditions.**

***Index Terms*—Heavy metal pollution, detoxification, metal accumulator plants, phytoremediation.**

I. 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].

Land and water are precious natural resources on which rely the sustainability of agriculture and the civilization of mankind. Unfortunately, they have been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities. The pollution includes point sources such as emission, effluents and solid discharge from industries, vehicle exhaustion and metals from smelting and mining, and nonpoint sources such as soluble salts (natural and artificial), use of insecticides/pesticides, disposal of industrial and municipal wastes in agriculture, and excessive use of fertilizers [2-4]. 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. Sawidis [5] showed that heavy metals have toxic effect on the pollen growth and pollen tube growth and cause to be a range of strong morphological abnormalities, characterized by uneven or aberrant growth, including apical branching or swelling at the tip of the pollen tube. Several researchers also showed toxic effects of heavy metals on different organisms [6-8].

Numerous efforts have been undertaken recently to find methods of removing heavy metals from soil, water and other environmemts, such as phytoremediation [9, 10]. Plant vegetation plays an increasingly important ecological and sanitary role for chemically polluted lands [9]. Proper management of plants in such areas may significantly contribute to restoring the natural environment. Perhaps, not surprisingly, phytoremediation was initially proposed as an environmental cleanup technology for the remediation of metal-contaminated water and soil [11- 14]. The identification of metal hyperaccumulators, plants capable of accumulating extraordinar 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 research [1, 9, 15]. The Ability of selecting species of plants, which are either resistant to heavy metals, or can accumulate great amounts of them, would certainly facilitate reclamation of contaminated areas [16 - 21].

Phytoremediation is a cost-effective technology for environmental cleaning. We need new and variable accumulator plants for phytoremediation in different climates and conditions, so new studies are still necessary to find new accumulator plants for using in different conditions. With this idea, the aim of this study was to elucidate or verify accumulating ability of plants that were grown in the polluted sites of a lead and zinc mine (Angouran, Iran) and to evaluate their phytoremediation ability for waste water refining.

II. Material and Methods

A. Studied Area

The 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. An artificial old dried waste pool of a Lead mine, located in Angouran, Zanjan 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 this area were collected and their scientific names and characteristics were determined. The concentration of heavy metals was

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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.

B. 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.

C. Evaluation of Metal Removing

Noaea mucronata, used as an accumulator plant for this study, belongs to *Chenpodiaceae* and because it is common the main area and its accumolatng ability is considerable. 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 and used for heavy matals removing from watery metal-polluted media.

Fifty pots were selected and filled with 15 kg water. They were devided in to five groups and each group was chosen for a metal removing experiment. In ten pots (group A) water contained Cadmim with the concenteration samed with the waste water (54 ppm). In ten pots (group B) Cupper was added in the concentration of 123 ppm. In three other groups (C, D, E) the concentrations of Ni, Pb and Zn were 98, 14800 and 2950 ppm respectively. In each pot *Noaea mucronata* plant nano-particles (500 g) was added. After three days, the particles were removed by using a double layer of Watman filter paper after No. 4. Heavy metals were determined in the water of the pots, before the beginning of the study and also after three days treatment with the above mentioned particles.

III. RESULTS

A. Determination of Heavy Letals in the Soil

This research studied the flora of waste pool of Angouran mine that is the largest lead and zinc mine in Iran located in Zanjan province (Fig. 1). The evaluation of heavy metal in the soil of the waste pool and the natural soil (5 km away from the mine) showed that the amount of some heavy metals in the waste pool of the mine were several times higher than the natural areas (Table 1).

TABLE I: CONCENTRATION OF SOME HEAVY METALS (PPM) IN WASTE POOL OF ANGOURAN LEAD AND ZINC MINE AND NATURAL SOIL (OUT OF MINE). THE DATE SHOWED THAT AMOUNTS OF MOST STUDIED METALS IN WASTE POOL SOILS ARE SEVERAL TIMES HIGHER THAN NATURAL SOILS. EACH DATA REPRESENTED THE MEANS ±SE OF 12 SAMPLES. DIFFERENTIATIONS BETWEEN WASTE POOL AND NATURAL SOILS ARE SIGNIFICANT (×P<0.01, ××P<0.05).

*The samples prepared out of the mine site

	Cd	**Cu**	**Fe**	**Ni**	**Pb**	**Zn**
Waste pool soil	××81.10±6.8	×162±9.5	1730±95	1730±95	×16700±2750	×2950±155
***Natural soil**	0.20±0.18	10.00±1.80	80±12.4	78.00±9.70	67±24	181±37

B. Results of Heavy Metal Accumulator Recognition

This research studied the flora of a Lead Mine located in Angouran, Zanjan, Iran. 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 2.

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 2). *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 2).

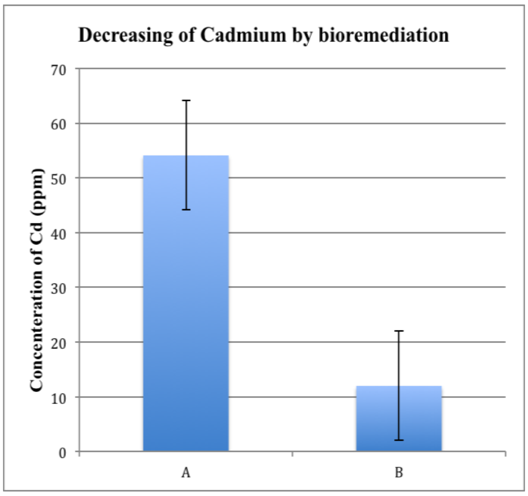
The amount of Ni in most of the studied plants was lower than the toxic level and was lower than that of the natural soil as well. Results showed that the best Zn accumulator overall was *Euphorbia macroclada* (1873 ppm), while *Reseda lutea, Salix excelsa, Scariola orientalis* and *Cynedon dactylon* 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 2).

TABLE II: CONCENTRATION OF SOME HEAVY METALS (PPM) IN SHOOTS AND LEAVES OF STUDIED PLANTS. DATA INDICATE THAT NOAEA MUCRONATA 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 REPRESENTED THE 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±3.5	1324±245	8.40±1.2	776.00±105	1600.00±185
Cenecio glaocus	5.50±0.6	16.50±4.6	1324±245	8.40±1.2	776.00±105	1600.00±185
Centaurea virgate	2.20±0.2	36.65±4.5	944±186	6.30±0.9	590.00±121	1262.00±145
Chenopodium album	1.60±0.4	22.20±2.9	340±46	11.93±1.9	214.33±35	598.67±106
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
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
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
Noaea mucronata	7.25±0.5	****72.00±8.5**	11230±142	**12.50±3.5**	***1056.00±164**	***1645.00±114**
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

C. The Bioremediation Results

Noaea mucronata 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 2). Its nano-particles were used for bioremediation of heavy metal polluted water. The data showed that the concentrations of all the heavy metals decreased considerably after three days remediation. The decrease in Pb, Cd, Ni, Cu and Zn are illustrated in figures 1-5. 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).



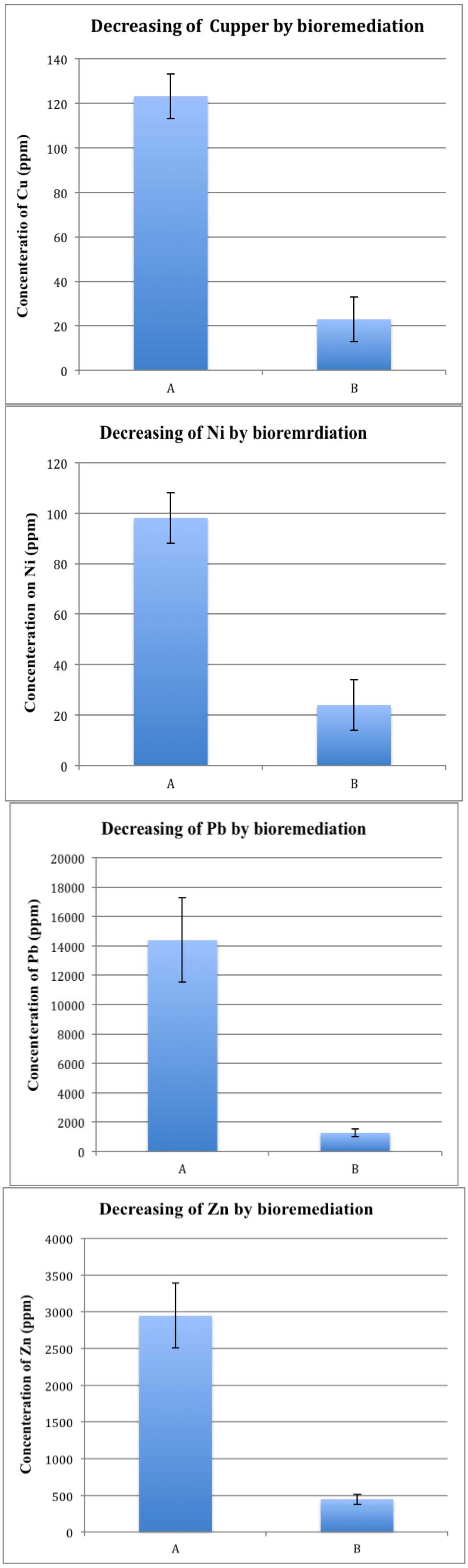


Fig. 1. Comparison between concentration of some heavy metals (ppm) in the polluted water before (A) and after bioremediation (B) by nano-particles of *Noaea mucronata*. Data indicate that amounts of all studied heavy metals were decreased due to bioremediation. Decreasing of metal concentration in experimental groups is significant (P<0.01). Each data represent the means ±SE of 10 samples.

IV. Disscusion

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 [6, 7, 8].

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: *Noaea mucronata*, *Reseda lutea*, *Salix excelsa*, *Scariola orientalis*, *Chenopodium album*, *Cydonia oblonga*, and *Centaurea virgata* (Table2). To conclude, *N. mucronata* should be considered as the best and the right Pb accumulator plant (according to Baker et al. [12, 13]) because it accumulated more than 1000 ppm.

N. mucronata 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 *N. mucronata* plants were kept in experimental pots for three days and then the particles were removed by filteration and the amount of the heavy metals were compared with the prior bioremediation. 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 (Figure 1). Nano-particles of *E. macroclada* is suggested for removing and detoxification of heavy metals, (especially Pb, Cd, Cu and Zn), from polluted environments [20, 21].

V. Conclusion

The results of this research work showed that some plants were able to accumolate heavy metals considerably. Such plant species are interested for bioremediation of heavy metal-polluted areas and media. Nano-particles provided fron *Noaea mucronata*, as the most effective accumolator plant, were used for removing some heavy metals from polluted water. Results indicated that the nano-particles of *N. mucronata* are useful for watery media detoxification and bioremediation in critical conditions.

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