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Phytoremediation of Soils Contaminated with Heavy Metals Resulting from Acidic Sludge of Eshtehard Industrial Town using Native Pasture Plants

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Abstract
Phytoremediation of heavy metals is considered as an inexpensive and practical technique for purifying contaminated soil, especially when eco-friendly native pasture plants of the contaminated area are used. In this study, heavy metals in soil including Cr, Zn, Cd, Pb, and Ni and native pasture plants around Eshtehard industrial town, which were contaminated due to the entry of acidic sludge, were examined. In this regard, the hyperaccumulation and phytostabilization potential of the plants in the study area were investigated. Accordingly, *A.tenuifolia* in Cd, Pb and Ni, *C.persica* in Zn, C. arenarius in Ni, *P. piptostigma* in Cr and Zn, *B. tectorum* in Cd and Zn, *S. hohenackeriana* in Cr, Pb and Cd, *P.aucheri* in Zn, and *P. harmala* L in Pb and Ni. Species with stabilizing potential include *A. tenuifolia* In Cd and Ni, *N. persica* in Zn, *A.tauschii* and *B. tectorum* in Ni, *P.aucheri* and *P.harmala* in Pb. Hyperaccumulating plants can be disposed of easily just like industrial wastes with heavy metals. Plants with stabilizing capacity can prevent the spread of heavy metal contamination to uncontaminated areas like the surrounding farms in addition to providing visual beauty for the region.
Keywords: acidic sludge, heavy metals, phytoremediation, Eshtehard industrial town

Introduction

Used oil was first purified and re-used by a German engineer in World War II (Arjmandi et al, 2010). Eshtehard industrial town is one of the largest centralized oil re-refining centers in Iran, which totally uses the acid-clay technology in order to refine used oil. In this method, acidic sludge with volumetric rate equal to one tenth of production rate of burned oil feed is produced (Khosro abadi, 1981). A large amount of the acidic sludge is discharged in the southern side of the industrial town. Discharge of this amount of burned oil, of which 30-50% is water-soluble (Arjmandi et al, 2010), results in contamination of soil and water (Nouri, 1992). Phytoremediation is one of the bioremediation methods used for remediating contaminated soil. The advantage of this method is its cost-effectiveness and possibility of using in large scale (Hartman, 1975). Phytoremediation refers to a variety of methods that apply green plants for removing contamination of soil, water (Branquinho et al, 2006) or even from air (Morikawa and Cem Erkin, 2003). In this study, two methods are examined as follows:

Phytoextraction: This heavy metal removal method depends on the natural ability of plants to uptake these metals. In this method, the best option to remove heavy metals is the use of hyperaccumulator plants (Zofen et al, 2013).

Phytostabilization: The efficiency of this method depends on the ability of plant roots to remove contaminants in soil and reduce their bioavailability. The objective of this method is to prevent movement of metal contaminants from soil and their entry to the food chain (Yoon and Zhou, 2006).

It is noteworthy that some physical and chemical properties of soil like pH, EC (Electrical conductivity), OC (Organic carbon), available phosphorus, CEC (Cation-exchange capacity), total concentration and exchangeable concentration of heavy metals in soil, humidity, sunny hours, rain, and type of soil can impact hyperaccumulation and/or phytostabilization of plants (Hassani et al, 2013).

According to Kabata-pendis and Pendis standard, if heavy metal concentration in soil ranges as follows for these heavy metals, soil contamination is critical: Cr 75-100 mg/kg, Zn 70-400 mg/kg, Cd 3-8 mg/kg, Pb100-400 mg/kg, Ni 100 mg/kg. Lower concentrations can be considered normal (kabata-Pendias, 1984). Furthermore, according to Standard Reference Plant, normal concentration of heavy metals in plants for Ni, Cr, Zn, Cd and Pb is 1.5 mg/kg, 1.5 mg/kg, 50 mg/kg, 0.05 mg/kg, and 1 mg/kg, respectively (van der Ent et al, 2012). According to standards, hyperaccumulator plants are defined as follows: first, Baker defined them as plants with heavy metal concentration in their shoots (leaves, stem and flower for Cd, Cr, Ni, Pb, and Zn as 100 mg/kg, 300 mg/kg, 1000 mg/kg, 1000 mg/kg, and 3000 mg/kg. According to EPA definition in 2000, hyperaccumulating plant is the plant that can concentrate the following amounts of heavy metals in their shoots and roots: 100 mg/kg of Cd, 1000 mg/kg of Cr and Pb, over 10000 mg/kg of Zn and Ni. It is noteworthy that this definition has removed the limitation of Baker's definition of considering only the plant's shoots. Furthermore, hyperaccumulation refers to plant's natural habitat and cannot be used for plants that grow under lab conditions (Lin et al, 2012). Plants suitable for phytostabilization also must have bioconcentration factor greater than one and translocation factor less than 1 (Yoon and Zhou, 2006).

The heavy metals in the studied burned oil enter soil from various sources: Cr from bearings, Pb from petroleum, Ni from valves, Cd from plating engine parts, and Zn from oil additives (Rauckytea et al, 2006). The burned oil with concentration of 50% in soil inhibits plant germination (Rahati Asiabar and Gandom kar, 2010). Therefore, plants resistant to acidic sludge can grow in soils with about 15% acidic sludge. There are many records of phytoremediation of soils contaminated with crude oil; however, there is no record of phytoremediation of soils contaminated with acidic sludge. In the present study, concentration of heavy metals in soil and plants of the area, hyperaccumulator plants, and phytostabilizer plants will be introduced. Among these, *Bromus tectorum* L. has been recognized as a Cd hyperaccumulator for the first time. Furthermore, all phytostabilizers have been studied and introduced for the first time as phytostabilizers.

Materials and Methods

The study site

This study was performed in areas contaminated with acidic sludge of Eshtehard industrial town with the longitude of 35˚ 41´ 38 ˝N and latitude of 50 ˚ 17 ´14 ˝E. The area is at the mean altitude of 1200 m above sea level. It has rather dry weather with the mean annual rainfall of 220 mm, mean annual relative humidity of 55.3%, a total of 3023 sunny hours per year, mean annual frost hours of 1872, and prevailing wind from North West and West with the speed of 4 to 6 knots (Van Reeuwijk, 2002). (Fig. 1)

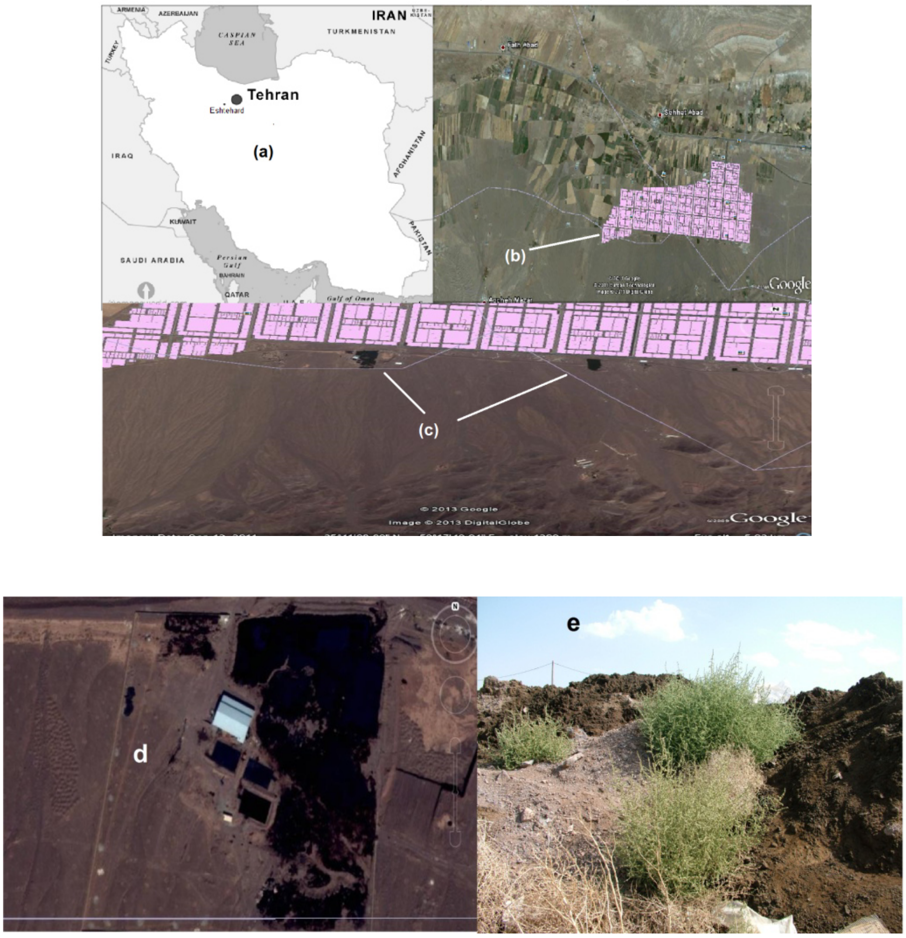

Fig.1 Location of the study area; Map of Iran (a), map of Eshtehard industrial town (b), maps and a picture of acid sludge polluted areas (c) & (d) & (e).

Sampling

The samples were collected from June to September in 2011_2013. Sampling from the mature plants in two areas, contaminated and control, was performed in a way that all shoots and roots were removed from the soil. Due to the sparse vegetation of the contaminated areas, samples were removed using systematic-random method (plotless and by direct observation) (Neishabori, 2011). The samples were identified after being delivered to the herbarium laboratory of Islamic Azad University of Science and Research of Tehran. Soil samples were removed separately from the site each plant was sampled, at the depth of 0-20 cm (The Rhizosphere area).

Measuring concentration of heavy metals in samples

The acid digestion method was used to determine total concentration of the heavy metals in the soil samples (Sposito and Change 1982; Khorasani et all, 2010). An amount of 2 g of each soil sample was poured in a screw cap Erlenmeyer flask and 15 ml of 4N nitric acid was added to it. After that, the flasks were placed in a warm bath for 12 hours at 80°C. Then, the samples were passed through a filter and the concentration of the heavy metals was determined using the atomic absorption spectrophotometer (Spectraa 200 Varian, Australia). The physicochemical properties of the soil were examined using standard methods (Van Reeuwijk, 2002).

The dry ash extraction method was used to determine total concentration of the heavy metals in the plant samples (Khorasani et all, 2010 ; Shaw, 1989). To do so, 2 g of each plant sample was put in a porcelain crucible. The samples were placed in an oven for 2 hours at 55°C. After that, 5 ml of 2N hydrochloric acid was added to the samples. Then, the samples were passed through the filter and the concentration of the heavy metals was determined using the atomic absorption spectrophotometer (Spectraa 200 Varian, Australia).

Methods for determining phytoremediation factors

To measure the bioconcentration factor, concentration of the heavy metal in the plant roots was divided by concentration of the heavy metal in soil, and to determine the translocation factor, concentration of the heavy metal in the shoots was divided by concentration of the heavy metal in the roots (Tukura et all, 2012).

Results

Heavy metal content in soil

Total concentration of the heavy metals was measured in soil samples of the contaminated and control areas during three years. The control area was selected regarding similarities between the physicochemical properties of the control area and the contaminated area. Table 1 shows that total concentration of the heavy metals with mean three replicates in the area contaminated with acidic sludge. It is noteworthy that sampling sites were labeled according to the plant grown in them for easy distinction.

Table 1: Concentration of the heavy metals in soil in mg/kg and standard deviation

Soil sampling site (plant's name)	Total concentration of heavy metals in soil				
	Cr	Zn	Cd	Pb	Ni
Achillea tenuifolia Lam.	556.7±39.1	922.6±44.5	42.7±5.1	1304.7±21.5	890.5±74.8
Centaurea persica Boiss.	190.0±52.3	1807.7±92.3	11.8±1.9	255.3±11.5	916.7±89.6
Cousinia congesta Bunge	169.2±38.1	1580.2±79.6	36.1±3.0	119.0±8.5	845.6±73.3
Nonnea persica Boiss.	185.3±42.2	1749.3±94.1	43.7±8.4	1347.1±50.6	1522.4±150.1
Ceratocarpus arenarius L.	234.8±79.0	644.3±34.8	39.3±4.8	1224.9±35.2	875.5±9.8
Alhagi camelerum Fisch.	174.7±19.6	2341.3±121.8	39.2±3.9	1236.7±11.1	1361.4±55.2
Papaver piptostigma Bienert ex Fedde	187.2±33.0	1761.9±99.6	19.5±2.0	743.2±10.0	907.7±121.8
Aegilops tauschii Cosson .	179.2±5.9	1681.9±81.2	41.9±5.8	129.3±7.3	89.2±0.8
Bromus tectorum L.	441.3±32.6	1850.9±114.5	47.0±5.1	1425.4±62.3	93.5±8.7
Stipa hohenackeriana trin. & Ruper	175.1±37.0	1644.1±92.5	39.0±4.1	1246.7±79.0	876.4±47.9
Pteropyrum aucheri Jaub. & Spach	331.2±21.3	1727.0±86.2	42.5±5.9	1328.3±8.4	908.0±56.1
Peganum harmala L.	178.2±4.9	2231.6±148.9	88.2±9.9	1271.9±89.6	1321.1±142.2

Concentration and phytoremediation factors in plants

All the studied plants were sampled in three replicates during three years, and concentration of the heavy metals was measured separately in two parts: the plants' shoots including stems, leaves, and seeds, and the plants' roots including root and rhizome. The results of these measurements are shown in figure 2.

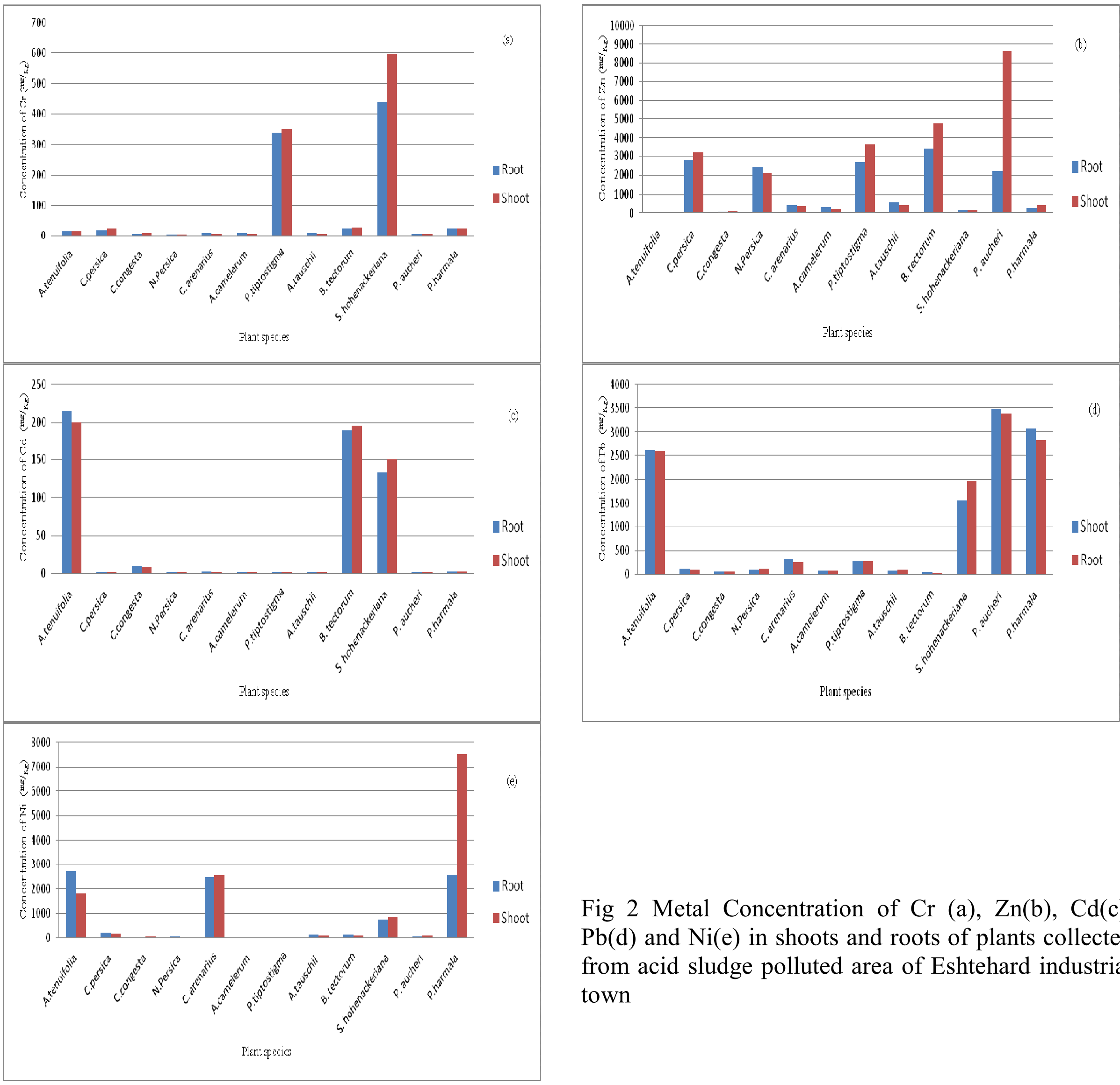

Fig 2 Metal Concentration of Cr (a), Zn(b), Cd(c), Pb(d) and Ni(e) in shoots and roots of plants collected from acid sludge polluted area of Eshtehard industrial town

Based on measurements in shoots and roots of the each plant and in soil, concentration of heavy metals are shown in table 2.

Table 2: Phytoremediation factors including the translocation factor (TF) and the bioconcentration factor (BCF).

Plant name	Cr		Zn		Cd		Pb		Ni	
	TF	BCF	TF	BCF	TF	BCF	TF	BCF	TF	BCF
Achillea tenuifolia Lam.	1.01	0.03	0.88	0.06	0.92	5.06	0.99	2.00	0.67	3.06
Centaurea persica Boiss.	1.22	0.10	1.16	1.54	1.26	0.12	0.87	0.46	0.96	0.21
Cousinia congesta Bunge	1.19	0.05	1.05	0.07	0.84	0.27	1.02	0.54	1.37	0.05
Nonnea persica Boiss.	0.98	0.02	0.85	1.41	0.75	0.02	1.20	0.07	0.76	0.03
Ceratocarpus arenarius L.	0.58	0.05	0.94	0.63	0.85	0.06	0.79	0.26	1.03	2.84
Alhagi camelerum Fisch.	0.81	0.06	0.72	0.15	0.81	0.03	0.84	0.07	1.20	0.03
Papaver piptostigma Bienert ex Fedde	1.04	1.81	1.37	1.51	2.67	0.01	0.93	0.40	0.69	0.00
Aegilops tauschii Cosson .	0.73	0.06	0.75	0.33	1.64	0.02	1.32	0.59	0.85	1.51
Bromus tectorum L.	1.24	0.05	1.40	1.83	1.03	4.04	0.78	0.03	0.77	1.54
Stipa hohenackeriana trin. & Ruper	1.36	2.51	0.90	0.11	1.13	3.41	1.26	1.25	1.14	0.88
Pteropyrum aucheri Jaub. & Spach	0.86	0.02	3.93	1.27	0.79	0.01	0.98	2.62	1.29	0.08
Peganum harmala L.	1.14	0.13	1.43	0.13	1.25	0.03	0.92	2.42	2.91	1.96

Soil pollution source

Based on the results in Table 1, the level of Cr, Zn, Cd, Pb, and Ni in all samples of the contaminated area was higher than the standard level provided by Pendis and Kabata-Pendis. Having been in the critical range, the level of these metals showed severe contamination of the soil with heavy metals in the contaminated area (kabata-Pendias, 2004). The samples removed from the control area were within the allowed standard concentration. Therefore, it can be concluded the source of contamination of the soil in the contaminated area was the acidic sludge left in the soil.

Introduction of plant species with the potential for phytoremediation

A. tenuifolia is recognized as hyperaccumulator for Pb and Cd according to both definitions with concentrations of 2585 mg/kg and 5192 mg/kg of Pb, 199.8 mg/kg and 415.9 mg/kg of Cd in shoots and whole plant, respectively. It is also recognized as Ni hyperaccumulator with adsorption of 1836 mg/kg according to Baker's definition. Mashhoor Roodi (Mashhoor Roodi et all, 2012) and Mohsenzadeh (Mohsenzadeh and Chehregani Rad, 2011) reached the same results. *C. persica* is recognized as Zn hyperaccumulator with adsorption of 3228 mg/kg of Zn in shoots according to Baker's definition. Chehregani (Chehregani and Malayer 2007) found the same results. *C. arenarius* is recognized as Ni hyperaccumulator with adsorption of 2559 mg/kg in its shoots according to Baker's definition. Xiao-yong (Xiao-yong, 2007) found the same results. *P. piptostigma* is according to Baker's definition a hyperaccumulator in its shoots for Cr and Zn by adsorbing 351.5 mg/kg and 3648 mg/kg of these metals respectively. Ghaderian (Ghaderian et all, 2009) and Hajiboland (Hajiboland and Mirmanafi, 2007) reached similar results. *B. tectorum* adsorbed 195.9 mg/kg and 358.8 mg/kg of Cd in its shoots and whole plant, respectively, so it is recognized as hyperaccumulator for Cd according to both definitions. It also adsorbed 4760 mg/kg of Zn in its shoots and is recognized as Zn hyperaccumulator according to Baker's definition. Sinegani (Sinegani and Dastjerdi, 2009) found similar results for hyperaccumulation of Zn and Ni. but the current study is the first one to examine and prove this quality for Cd. *S. hohenacheriana* could adsorb 150.8 mg/kg and 283.7 mg/kg of Cd, 596.2 mg/kg and 1036 mg/kg of Cr, and 1967 mg/kg and 3531 mg/kg of Pb in its shoots and whole plant, so it is hyperaccumulator of these metals according to both definitions. Mohsenzadeh (Mohsenzadeh and Chehregani Rad, 2011) and Solhi (Solhi et all, 2012) reached similar results. *P. aucheri* accumulated 8634 mg/kg and 10834 mg/kg of Zn, and 3393 mg/kg and 6868 mg/kg of Pb in its shoots and whole plant, so it is recognized as hyperaccumulator for them according to both definitions. Ghaderian (Ghaderian et all, 2009) found the same results relative to contamination. *P. harmala* could accumulate 7510 mg/kg and 10095 mg/kg of Ni, and 2830 mg/kg and 5903 mg/kg of Pb, and is recognized as hyperaccumulator for both of them according to both definitions. Zamani (Zamani et all, 2013) reached the same results relative to contamination.

Introducing plant species with phytostabilization potential

No plants with phytostabilization quality for Cr were found in the region. *N. persica* (TF:0.85, BCF:1.41) is a good choice for Zn phytostabilization. *A. tenuifolia* (TF: 0.92, BCF: 5.06) is suitable for Cd phytostabilization. *A. tenuifolia* (TF: 0.99, BCF: 2.0) and *P.aucheri* (TF:0.98, BCF:2.62) and *P. harmala* (TF:0.92, BCF:2.42) are appropriate for Pb phytostabilization. *A. tenuifolia* (TF:0.67, BCF:3.06) and *A. tauschii* (TF:0.85, BCF:1.51) and *B. tectorum* (TF:0.77, BCF:1.54) are appropriate for Ni phytostabilization. It is noteworthy that these species have been recognized as phytostabilizers for the first time.

Discussion

Among the study plants, *A. tenuifolia* is hyperaccumulator for Cd, Pb and Ni, *C. persica* is hyperaccumulator for Zn, *C. arenarius* is hyperaccumulator for Ni, *P. piptostigma* is hyperaccumulator for Cr and Zn, *B. tectorum* is hyperaccumulator for Cd and Zn, *S. hohenackeriana* is hyperaccumulator for Cr, Cd and Pb, *P. aucheri* is hyperaccumulator for Zn and *P. harmala* is hyperaccumulator for Pb and Ni. The following are recognized as phytostabilizers: *A. tenuifolia*. for Cd and Ni, *N. persica* for Zn, *A. tauschii* and *B. tectorum* for Ni, *P. aucheri* and *P. harmala* for Pb. Among these, *B. tectorum* has been introduced for the first time as a Cd hyperaccumulator. Furthermore, all phytostabilizers have been studied and recognized so for the first time. Hyperaccumulating plants can be disposed easily after adsorbing heavy metals. Phytostabilizers give the contaminated regions a visual beauty in addition to stabilizing the heavy metals and preventing their spread in the surrounding farm lands.

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