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Phytoremediation of barium, copper, zinc and arsenic contaminated soils by sunflower and alfalfa

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Abstract

The existing remediation techniques of heavy metal-contaminated soils are expensive, time consuming and environmentally destructive. Unlike organic compounds, metals cannot degrade, and thus effective cleanup requires their immobilization to reduce or remove toxicity. Phytoremediation is a developing technology for cleaning up contaminated sites, which is cost effective, and has aesthetic advantages and long term applicability. The main aim of this study was to screen the accumulation and distribution of barium (Ba), copper (Cu), zinc (Zn) and arsenic (As) in 30 cultivars of alfalfa (Medicago sativa L.) and giant sunflower (Helianthus giganteus) for their possible use in phytoremediation. Soil samples were collected from Saghand and Bandar Abbas which are closed to industrial complexes. Also, loam soil was used as a blank sample for comparing results. Among these species, alfalfa samples could not grow in these soils, hence, it was removed from experiments. Furthermore, influence of adding nitric acid, acetic acid, citric acid and oxalic acid on performance of sunflower was investigated. Results proved that sunflower could be used for phytoremediation, as, they showed the ability of toleration high concentration of heavy metals and they exhibited the capability of barium, copper, zinc and arsenic uptake. Moreover, outcomes from experiments ascertained that adding acid to soils increased bioavailability of heavy metals, since, adding acids to soils increased bioavailability of barium, copper, zinc and arsenic and these samples showed higher heavy metals uptake.

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Introduction

Human evolution has led to great scientific and technological progress. Nonetheless, global development raises new challenges, particularly in the field of environmental protection and conservation (Bennett *et al.*, 2003). Nearly every government advocates for an environment free from harmful contamination for their citizens. Nevertheless, the demand for a country's economic, agricultural and industrial development outweighs the demand for a safe, pure, and natural environmental. Ironically, it is the economic, agricultural and industrial progresses that are often related to polluting the environment (Ikhuoria and Okieimen, 2000).

Soil pollution has recently been attracting significant public attention since the extent of the problem in our soils calls for urgent action (Garbisu and Alkorta, 2003). Heavy metals are substantial environmental pollutants, and their toxicity is a problem of increasing importance for ecological, evolutionary, nutritional and environmental reasons. The term "heavy metals" refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech Water Treatment and Air Purification 2004). "Heavy metals" in a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3, or 5 times or more, greater than water (Hawkes 1997). Nevertheless, chemical properties of the heavy metals are the most influencing factors compared to their density.

Heavy metals make a major role to environmental pollution as a result of human activities such as mining, melting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping, and military operations. For instance, in agriculture Some heavy metals, e.g. Ba, Fe, Cu, Zn, Mn, As and Ni, are essential or beneficial micronutrients for microorganisms, plants and animals (Welch, 1995); others have no known biological or physiological function. Although, all heavy metals at high concentrations are often highly toxic and are an environmental risk. Based on their chemical and physical characteristics, three different molecular mechanisms of heavy metal toxicity can be identified: (i) production of reactive species by autooxidation and Fenton reaction (Fe, Cu), (ii) blocking of fundamental functional groups in biomolecules (Cd, Hg), and (iii) displacement of essential metal ions from biomolecules (Schutzendubel and Polle 2002).

For treatment of soils contaminated with heavy metals, different methodes are available (Martin and Bardos, 1996; BIO-WISE, 2000). Existing technologies resort to soil excavation and either landfilling or soil washing followed by physical or chemical separation of the contaminants. Although extremely variable and dependent on the contaminants of concern, soil properties, site conditions, and so on, the commonly enormous expenses associated with the deduction of metals from soils by methods of traditional physicochemical approaches explain why most companies incline to overlook the problem. Due to the fact that very often large areas are affected by heavy metal contamination, a removal is definitely tough. Hence, some methods are developed to retain the metals in the soil nonetheless decrease the threats connected to this presence (Diels *et al.* 2002).

Among different technologies, in situ approach of phytoremediation is interesting as it offers site restoration, partial decontamination, maintenance of the biological activity and physical structure of soils, and is potentially inexpensive, aesthetically pleasing, and there is the probability of biorecovery of metals (Baker *et al.*, 1994 a, 1994 b). Phytoremediation is the name given to a set of remediation techniques that practice plants to clean or partly clean contaminated sites, or render the contaminants less toxic (Barkay and Schaefer, 2001; Duschenkov, 2003; EPA, 2004; Eapen *et al.*, 2007). The US phytoremediation market is expected to expand more than ten-fold between 1998 and 2005, to over $214 million (Evans 2002). In the last few years, some brilliant reviews have been published concentrating on various aspects of phytoremediation (Salt *et al.* 1995, 1998; Chaney *et al.* 1997; Raskin *et al.* 1997; Chaudhry *et al.* 1998; Wenzel *et al.* 1999; Meagher 2000; Navari- Izzo and Quartacci 2001; Lasat 2002; McGrath *et al.* 2002; McGrath and Zhao 2003; McIntyre 2003; Singh *et al.* 2003). The development of phytoremediation is being driven primarily by the high cost of many other soil remediation methods, as well as a desire to use a 'green', sustainable process.

Hence, phytoremediation has been reported in numerous studies. Phytoremediation has the best efficiency when the contaminants are dispersed in the soil at low concentration. Heavy metals contaminating soils commonly appear at low concentration, which has encouraged assessments of the decontamination potential of phytoremediation for heavy metals. Heavy metals are existing at several sites contaminated by these pollutants, and it has been verified regularly that plants can take them up from soil (Brooks, 1998; Baker *et al.*, 1989; Brown *et al.*, 1995; Ebbs and Kochian, 2002; Ban˜uelos and Ajwa, 1999; Huang *et al.*, 1997; Dickinson *et al.*, 1992; Dickinson and Lepp, 1997).

The main purpose of this research was to investigate the accumulation and distribution of heavy metals-contaminated soils in giant sunflower (Helianthus giganteus) and alfalfa (Medicago sativa L.). Furthermore, influence of adding different acids to soil samples was examined.

Materials and methods

Plants

In this study, two types of plants were investigated. Alfalfa (Medicago sativa L.) and giant sunflower (Helianthus giganteus) were used and each of these species were bought from a local seed market (TTSA SEED). These seeds were planted at depth of 2 cm at upper layer of soil.

Soils

Soils were obtained from two different regions of Iran. One batch of soil sample was attained from Saghand and the other batch was gained from Bandar Abbas. These places are closed to industrial complexes, therefore, it is expected that their soils contain high concentration of heavy metals. Table 1 shows characteristics of these two soils which were examined by doing X-ray fluorescence (XRF) analyzer. It illustrates that these soils have high concentration of heavy metals, therefore, they should be remediated.

Experimental design

20 plastic pots were prepared for experiments and their size were 30×30×30 cm and each of them contained 4 kg soil. 8 of them were filled with Saghand soil and 8 of them were filled with Bandar Abbas soil and rest of them were seeded by loam soil. These loam samples were used as an indicator to compare other samples.

In four pots which had Bandar Abbas soil seeds of sunflower and in four pots alfalfa seeds planted in depth of 2cm at the topsoil. This process was applied for Saghand soil, too. For the loam, alfalfa and sunflower seeds were planted in two pots each one, respectively. All pots were stored in the greenhouse and blue and red lamps were installed in order to simulate sunlight. The samples were in this condition for 18 hours. 150cc water added to each pot twice a day and temperature was controlled (20 to 25°C) with approximately 75% humidity. Soils have a low percentage of organic matter (less than half), hence, NPK fertilizer was added to pots after germination of seeds each 15 days. Characteristics of NPK is illustrated in table 2. Figure 1 elucidates pots after one week.

Trend of growth were investigated weekly and after one month it was delineated that alfalfa could not grow in these soils, as there was no germination sign and experiments were not applied on it. After one month, 100 cc citric acid, nitric acid, oxalic acid and acetic acid 0.5, 2.5 and 12.5 mM were added to 2 pots of Saghand soil, twice in one week. No acid was added to other pots as the goal was to evaluate effect of adding different acids on performance of plants. Figures 2 and 3 show samples after two and five weeks.

Heavy metals analysis

After five weeks, plants were removed from pots and stored in the greenhouse for drying. For dry weight calculation, the samples were kept in oven at 70 °C for 24 hours. Then, they were retained at 550 °C for 3 hours and their ashes were collected. Afterwards, 20 cc of solution which contained 3:1 nitric acid to hydrochloric acid and 1 mg ash of each sample were solved in this solution. For enhancing solving process, this experiment implemented on heater for one hour and this method continued till acids were evaporated. Eventually, concentration of heavy metals was measured by plasma atomic emission spectrophotometry (ICP-AES).

Results

Growth rate

All samples were examined weekly and the plants size were measured during weeks. Figure 4 demonstrates length of stems belonged to sunflower specie in various soils. It shows that growth of sunflower in Bandar Abass soil was fairly less than Saghand soil.

It can be inferred that presence of heavy metals did not have a significant influence on growth of sunflowers, since, growth rate in Saghand soil after five weeks even is higher than loam soil.

Table 1. Concentration of metals in Saghand and Bandar Abbas soil.

Elements	Saghand Soil	Bandar Abbas Soil	Unit
Zinc	260	200	ppm
Arsenic	93	102	ppm
Copper	166	94	ppm
Barium	116	133	ppm

Table 2. Characteristics of NPK fertilizer.

Components	Percentage of Composition
Nitrogen	20
Phosphor	5.6
Potassium	4
Urea	10.4
Phosphorus in aqueous solution	20
Potassium in aqueous solution	20
Boron in aqueous solution	0.02
Copper	0.005
Iron	0.07
Manganese	0.03
Zinc	0.01

Plasma Atomic Emission Spectrophotometry

For evaluating concentrate on of heavy metals in plants, plasma atomic emission spectrophotometry model Optima 7300 DV was used. Afterwards, 100 mg citric acid, nitric acid, oxalic acid and acetic acid was added to sunflower sampels which planted in Saghand soil. Figure 5 shows barium uptake by sunflower in Saghand soil in different concentration of acids. By comparing results, it can be deduced that highest uptake has occurred in 12.5 mM citric acid concentration and increasing content of nitric acid and acetic acid had a significant negative influence on affecting barium by the plant, since, it decreased less than a sample with 0.5 mM citric acid. Copper uptake by sunflower in Saghand soil is illustrated in figure 6. It shows that adding citric acid had the best affect on copper sunflower uptake, since, it demonstrates the highest uptake. It can be inferred that adding acids to the soil had a proper influence on copper uptake, hence, all samples with these acids show higher uptake than samples without acids.

Fig. 1. seeds after one-week planting.

Fig. 2. Seeds after two weeks.

Furthermore, figures 7 and 8 display zinc and arsenic uptake by sunflower in Saghand soil. For sunflower, rising concentration of acids till 2.5 mM had a proper affect on zinc uptake efficiency, however, increasing from 2.5 to 12.5 mM had a negative influence in all samples. Among various acids, highest zinc concentration is detected in a sample which had 2.5 mM oxalic acid. At 0.5 mM oxalic acid, zinc uptake was 122 ppm and it increased till 168.71 ppm at 2.5 mM oxalic acid, then, it decreased to 150 ppm at 12.5 mM oxalic acid concentration. This trend was repeated among other acids. For instance, at 12.5 mM citric acid, zinc uptake was less than 2.5 mM citric acid. Therefore, it should be considered that excessive acids had a negative influence on zinc uptake, since, at 12.5 mM acids zinc uptake was less than samples without acids.

Moreover, outcomes of arsenic uptake by sunflower in Saghand soil is presented in figure 8. For arsenic uptake, adding oxalic and citric acids to samples had similar effect and they were more effective than inserting acetic and nitric acid. At 12.5 mM citric acid and oxalic acid highest uptake took place which showed they were eally effetive. However, results illustrate that samples whitout acid were not proper for arsenic uptake as they were only 5.5, 5, 6 and 7 ppm for nitric acid, acetic acid, citric acid and oxalic acid, respectively. These results were much less than adding 2.5 mM and 12.5 mM acids.

Fig. 3. Plants after five weeks.

Disscussion

The increase of heavy metal contamination in soils worldwide causes important risk to human, animal and plant health. Cleaning up the contaminated soils through phytoremediation has gained increasing interests, since it is more cost-effective with fewer side effects than physical and chemical techniques. In this study we tested the ability of giant sunflower (Helianthus giganteus) and alfalfa (Medicago sativa L.) to phytoremediate soils contaminated by heavy metals. These plants can produce large biomass and were selected for this study. Hence, the large biomass can hold big amount of heavy metals per plant.

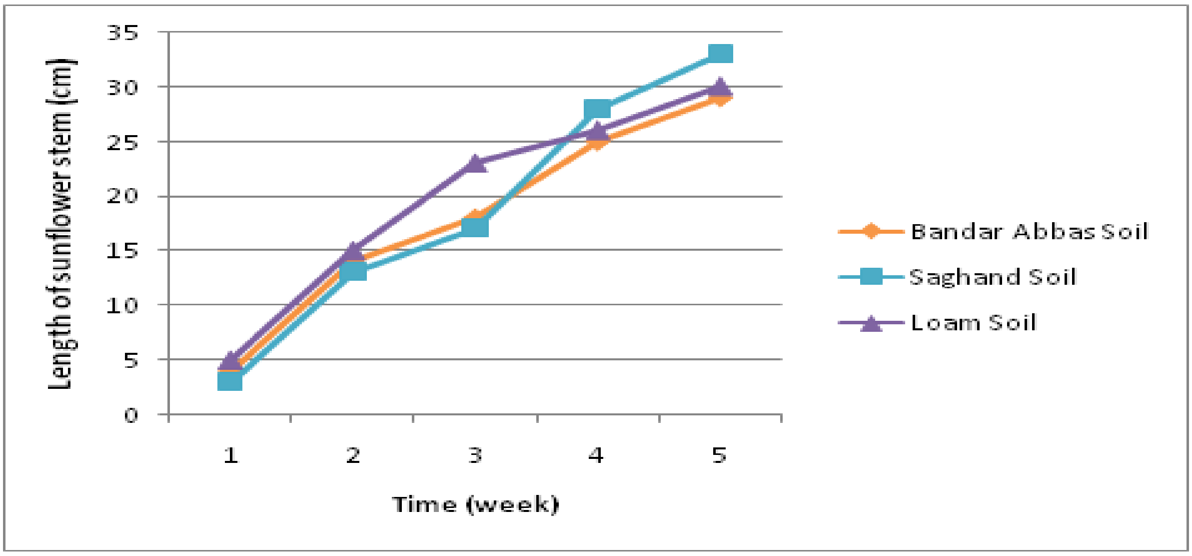

Fig. 4. Growth rate of sunflower in different soils.

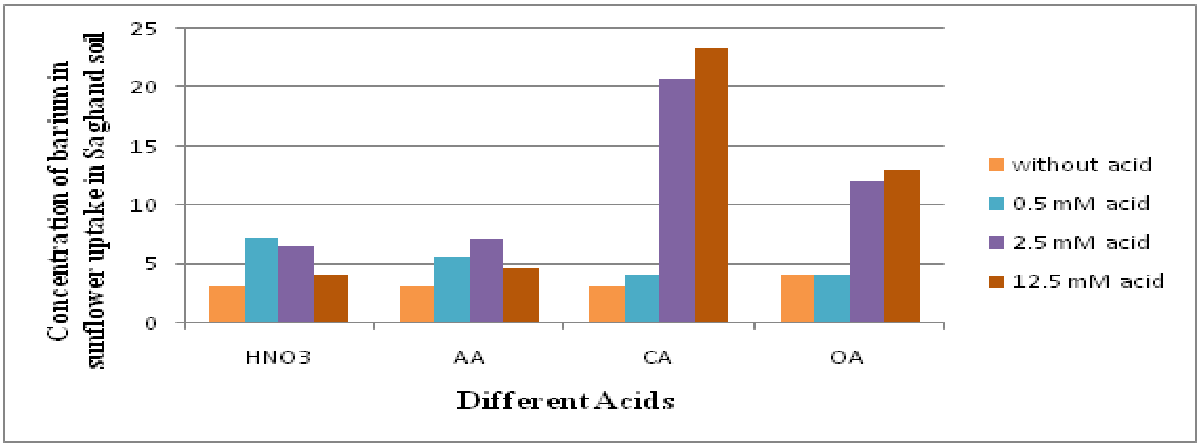

Fig. 5. Concentration of barium uptake of sunflower in Saghand soil.

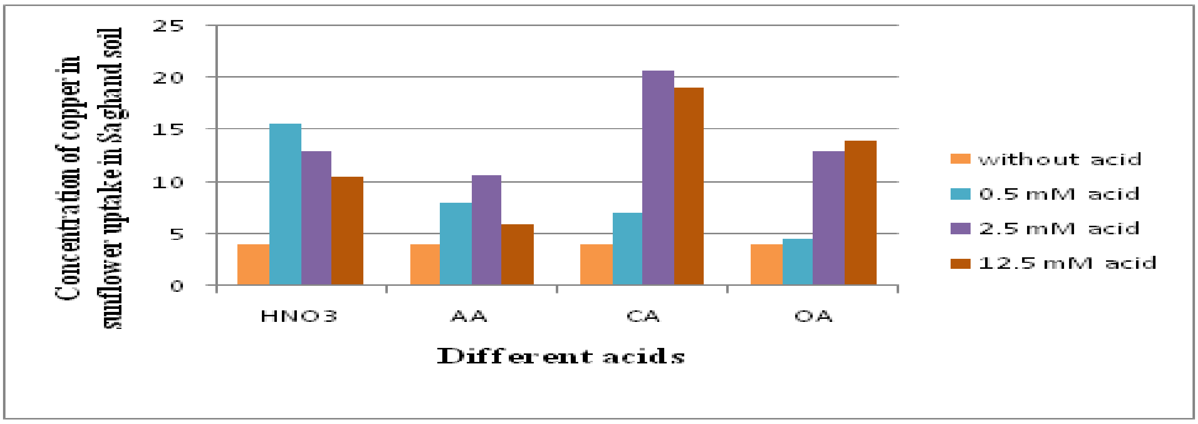

Fig. 6. Concentration of copper uptake of sunflower in Saghand soil.

Accumulation of selected metals varied greatly among plants species Uptake of elements by a plant is primarily dependent on the plant species, its inherent controls, and the soil quality. Our results demonstrate that giant sunflower (Helianthus giganteus) have an unusual ability to take up heavy metals from soil and to transport and concentrate these metals in their shoots. Nevertheless, alfalfa (Medicago sativa L.) samples could not stand soils properties and they were not considered in experiments.

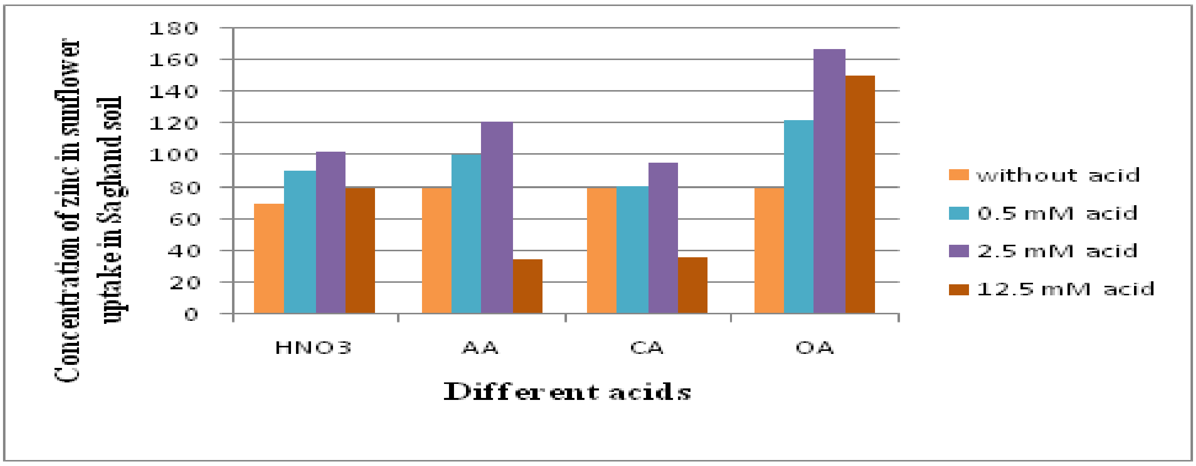

Fig. 7. Concentration of zinc uptake of sunflower in Saghand soil

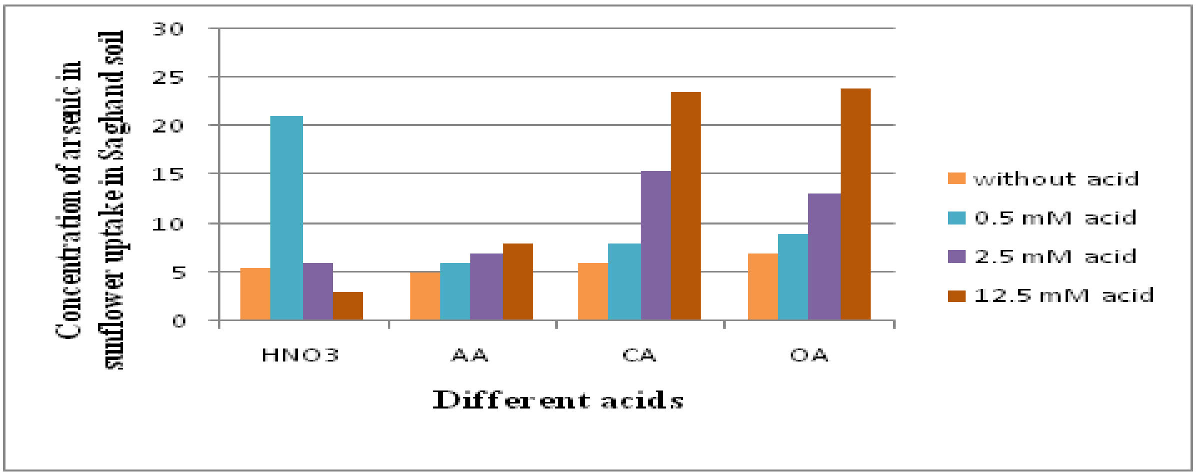

Fig. 8. Concentration of arsenic uptake of sunflower in Saghand soil.

Conclusion

In this research, potential effect of phytoremediation on heavy metals contaminated soil was investigated. In this regard, alfalfa (Medicago sativa L.) and giant sunflower (Helianthus giganteus) seeds were planted in Bandar Abbas and Saghand soils. These soils have a high concentration of heavy metals. According to literature, adding acids could increase bioavailability of heavy metals uptake. Therefore, nitric acid, acetic acid, citric acid and oxalic acid in different concentrations were added to some samples and in this way, significance of adding acids was investigated. It can be concluded that sunflower have an ability to be planted in heavy metals contaminated soils. Results showed that sunflower could uptake zinc, copper, arsenic and barium.

However, alfalfa samples could not grow in these soils so they were removed from experiments. Also, results verified that adding citric acid has a significant effect on barium, copper and arsenic uptake, nevertheless, it should be considered that excessive citric acid had a negative influence on zinc and copper uptake. However, the best result for barium uptake was detected at 12.5 mM citric acid. For copper uptake, 2.5 mM citric acid showed the best result. The highest zinc concentration is detected in a sample which had 2.5 mM oxalic acid. Thus, it can be concluded that oxalic acid had the best effect on zinc uptake of sunflower. Amongst other acids concentrations, 2.5 mM exhibited the highest effect on zinc uptake for all acids. Arsenic uptake of sunflower was significantly affected by adding acids to samples. 12.5 mM citric acid and oxalic acid showed the highest uptake.

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