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Effectiveness of *Amaranthus gangeticus* in Arsenic Extraction from Soil

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Abstract

Phytoremediation of heavy metal rich soil has become a practical approach nowadays. Though this method is very promising, it requires long time for complete remediation of contaminated soil. Assortment of appropriate plant for specific heavy metal is very important to decontaminate soil within short period of time. The present study was conducted on *Amaranthus gangeticus* to find out its potential to remove arsenic (As) from soil within short period of time. Phytoremediation trail was followed by growing plants in varying concentrations of As contaminated soil and subsequently one month of plant growing period it removed 72%-81% of the total soil As. This species accomplish maximum accumulation capacity of 17934 mg/Kg in shoots and store 72%-78% metal in aerial parts. Several parameters that have an influence on phytoremediation potential such as time, concentration, bioconcentration factor (BCF) and translocation factor (TF) were also calculated to investigate its appropriateness as effective hyperaccumulator.

Keywords: *Amaranthus gangeticus*; Arsenic (As) extraction; Heavy metal; hyperaccumulator.

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1. Introduction

Arsenic (As) existing in the environment from both natural and anthropogenic sources, have contaminated many sites worldwide [1]. During the last few decades higher concentration of As have been reported in groundwater of Bangladesh, Vietnam, Argentina, China, USA, Taiwan, Mexico, India, Thailand, Hungary, Chile and many other part of the world, where cultivation land is being polluted by As either by irrigation of As rich groundwater as well as by mining activities or past uses of arsenical agrochemicals [2-10]. Though various technologies are applied to remediate As contaminated soil and water, researchers working with phytoremediation appreciate that this emerging technology is most promising and environment friendly [11].

Phytoremediation, the noble strategy for removing contaminants from soil had generously attracted much attention as it is less costly and environment friendly process.

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The word "phytoremediation" comes from the Greek "phyto" = plant, and Latin "remedium" = remediation; refers to a diverse collection of plant-based technologies that use either naturally occurring or genetically engineered plants to remove pollutants from the environment, or to render them nontoxic [12-14]. Phytoremediation practice includes: phytoextraction, where plant uptake pollutants from soil and water, and translocate to and stored in the harvestable biomass of the plants [11]; phytodegradation, the degradation of organic pollutants by plants with the help of enzymes [15]; rhizofiltration, use of plant roots to absorb and precipitate heavy metals from polluted effluent [16]; phytostabilization, use of plants for stabilization of pollutes in polluted soil to eliminate the mobility and bioavailability of contaminants in environment, consequently preventing their migration to groundwater [17,18] and phytovolatilization, where pollutants move freely through plants, readily degrade and release in the atmosphere as less volatile material [19]; phytodesalination, refers to reclamation of salt-affected soils using halophytic plants [20]; Phytohydraulics, the ability of vegetation to evapotranspire sources of surface water and groundwater [21]; rhizodegradation; the breakdown of pollutants in the soil through the bioactivity that exists in the rhizosphere [21].

Plants that can accumulate high amounts of heavy metal without suffering phytotoxic effects are called hyperaccumulator [22]. The ideal plant for phytoremediation should grow rapidly, yield a high amount of biomass, and store high concentrations of metals in shoots [23]. Very few plants have capability to translocate high amount of metals from roots to shoots [11]. Extensive research demonstrated that plant remediate soils contaminated with organic chemicals through 'rhizosphere effect'- the zone around the root where there is an increase in microbial biomass and activity [24]. Considering practical approach based on hyper-accumulation threshold criteria, van der Ent *et al.* [25] recommended the following concentration standards for different metals and metalloids in dried foliage: 100 mg Kg^{-1} for Cd, Se and Tl; 300 mg Kg^{-1} for Co, Cu and Cr; 1000 mg Kg^{-1} for Ni, Pb and As; 3000 mg Kg^{-1} for Zn; 10000 mg Kg^{-1} for Mn, plants cultivated in natural habitats. Extensive number of *Pteris* species have been identified as potential As hyper-accumulator such as *Pteris vittata* up to 22,630 mg Kg^{-1} [26, 27], *Pteris longifolia* and *Pteris umbrosia* up to 7600 mg Kg^{-1} [27] in the shoot (frond). In addition, As-hyperaccumulating fern, *Pityrogramma calomelanos* (silver fern) are found capable of accumulating up to 8350 mg Kg^{-1} in the frond [28]. Investigation continued on some other plant species like *Corrigiola telephiifolia* accumulates up to 2110 mg Kg^{-1} [29], *Eleocharis acicularis* 1470 mg Kg^{-1} [30], and in recent days extensive research on several plant species are going on.

Amaranth is a rapid growth-universal crop, remarkable for its capacity of biosynthesis and low rate of photorespiration [31]. *Amaranthus gangeticus* are beautiful red edible leaves used as leafy vegetable in some countries. Limited effort needed to cultivate this plant in large ground made it a satisfactory choice for present phytoremediation study. In addition, this annually grown plant species have ability to tolerate drought, high heat.

In this study, *Amaranthus gangeticus* was tested to find its accumulation efficiency in As contaminated soil. Various effects such as time, concentration, BCF, TF are evaluated to ascertain this plant as excellent as hyperaccumulator.

2. Experimental

2.1. *Soil-contamination*

To carry out present study, top soil (0-20 cm) was collected from agricultural land, air dried and sieved to pass through a 2 mm sieve for removal of foreign bodies and coarse particles. Particle size distribution of the soil was 90% sand, 6% silt and 4% clay. Soil weighed and analyzed for As. Dry density of the soil was 1.75 gm/cm^3. Equal (2 Kg) amount of soil placed in several identical pots of 10 cm depth. Soil was contaminated with known concentration (0.1 mg/L, 0.2 mg/L, 0.3 mg/L, 0.5 mg/L, 0.7 mg/L, 0.9 mg/L) of As solution in such a way that the pots contain 57.14 mg Kg^{-1}, 114.29 mg Kg^{-1}, 171.42 mg Kg^{-1}, 285.71 mg Kg^{-1}, 400 mg Kg^{-1}, and 514.29 mg Kg^{-1} of As. Contaminated soils were mixed properly to make it homogenous and allowed to adjust for 7 days before seeding.

2.2. *Plant*

Amaranth seeds were collected from local market and then equal seeding was done for each pot. Distilled water was added periodically as required to maintain moisture content of soil at field capacity during growing period of plants. The pots were randomly arranged outside laboratory to ensure natural condition of sunlight and air. Distilled water was added every 2 days.

2.3. *Sample collection*

Collection of sample soil was done by keeping several days of interval at two different depths (2.5 and 5 cm). Sample collections are made from different places in following days of test. Plants were grown for 4 weeks and at the end of the experiment plants along with roots were harvested. The plant divided into roots and above ground parts. Roots were washed with tap water and distilled water and oven dried at 105°C for 24 h. Shoots were further separated as stem and leaves, oven dried at 105° C for 24 h and total dry weight was recorded. Then the samples were ground into fine powder with mortar; acid digested with 2.5 mL HNO_3 and 7.5 mL HCl for one day prior to As test.

2.4. *Arsenic testing*

Amount of As in plant tissue and soil was determined by silver diethyldithiocarbamate (SDDC) method. The process involved reduction of As^{+5} to As^{+3} by Zn. Firstly, sample was taken into a clean generator bottle then 5 mL concentrated HCl, 2 mL of KI solution, and 0.4 mL of stannous chloride solution were added successively. Sample was allowed to

stand 15 min for the reduction of As from pentavalent state to trivalent state. Lead acetate solution was imported in glass wool and scrubber introduced with this. 4 mL SDDC solution was added to the adsorber assembly. After addition of 3 g Zn dust to generator bottle it is placed for 30 min for evolution of As. Solution from adsorber was collected and tested with Atomic Absorption Spectrophotometer (AAS).

2.5. *Bioconcentration factor and translocation factor*

Bioconcentration factor indicates the appropriateness of a plant in accumulating a metal into its harvested tissues from the surrounding environment [32]. It is calculated by following equation [33,34].

$$\text{Bioconcentration Factor (BCF)} = \frac{\text{Concentration of metal in the } harvested\ plant\ tissue}{Concentraion\ of\ metal\ in\ soil}$$

Translocation factor (TF) is a useful parameter to evaluate the capability of plant to (a) accumulate the metal [35]. It is calculated by the relation: the ratio of concentration of metal in the shoot to the concentration of metal in the roots [34,36].

$$\text{Translocation factor} = \frac{\{Concentration\ of\ metals\}\ shoot}{\{Concentration\ of\ metals\}\ root}$$

The results were presented as mean with standard deviation and three replicate measurements were made on each sample. The data obtained in three replications were statistically analyzed using IBM SPSS at a statistical significance as p value less than 0.05.

3. Results and Discussions

Fig. 1 (a-f) shows that the As concentrations in soil come down with time. They demonstrate that the plants take up metal gradually from soil, but the uptake rate is much higher in the earlier period of plant life (experimental time) and there is no linear relationship with increasing concentration [37]. Soil samples collected from just beneath the surface (2.5 cm) show much high reduction in As and it is because of their high adsorption on roots as they sprinkle from the top soil.

Soil sample containing initial As concentration of 57.14 mg Kg^{-1} for the sample of 2.5 cm depth from the soil surface decreased to 20.81 mg Kg^{-1} in 10 days. The As concentration was 21.03 mg Kg^{-1} at the same treatment period for the sample of 5 cm depth. The final concentrations of metal were 11.5 and 10.17 mg Kg^{-1} for sample of 2.5 and 5 cm depth, respectively, which give an average 81% treatment efficiency at the end of the treatment (Fig. 1a).

Fig. 1b represents decrease of initial concentration of 114.29 mg Kg^{-1} for the samples of 2.5 and 5 cm depths, the metal concentration in soil decreased to 49.67 and 74.67 mg Kg^{-1}, respectively, just in 10 days. The average treatment efficiency of 72% is achieved at the end of the experimental period of 30 days.

The As concentrations in the soil sample of 2.5 and 5 cm depths reduced to 50.16 and 100.83 mg Kg^{-1}, respectively, in 10 days from an initial of 171.42 mg Kg^{-1} (Fig. 1c). Soil sample containing As concentration of 285.71 mg Kg^{-1} shows an an average treatment of 75% at the end of the experiment (Fig. 1d).

Fig. 1e demonstrates that initial As concentration of 400 mg Kg^{-1} is reduced to 245.01 and 269.17 mg Kg^{-1} for the sample of 2.5 and 5 cm depths, respectively, in merely 10 days.

Fig. 1f depicts the changes for the soil sample containing initial As concentration of 514.29 mg Kg^{-1}. The metal concentration decreased to 178.17 and 318 mg Kg^{-1} for the sample of 2.5 and 5 cm depths, respectively, after 10 days which shows a final average treatment efficiency of 80.33%.

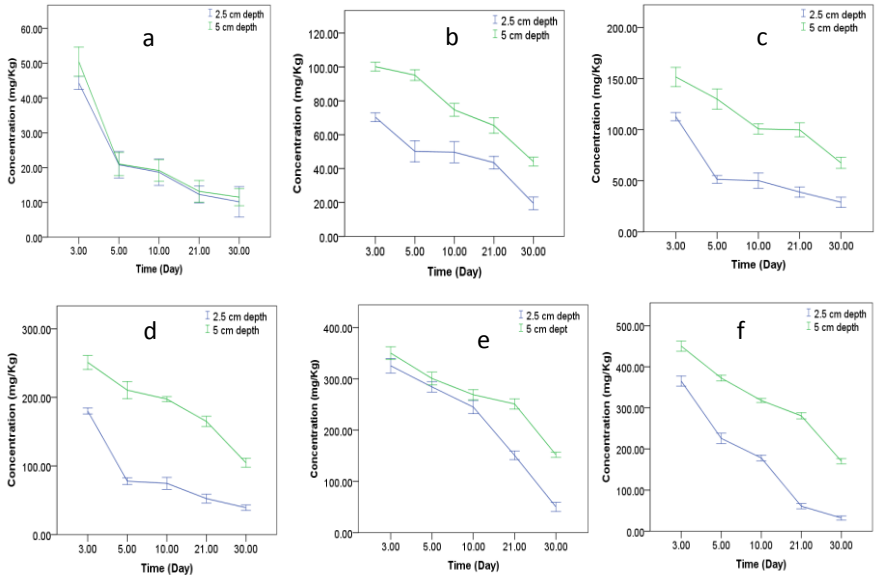

Fig. 1. As concentration in soil vs time (a-initial 57.14 mg Kg^{-1}, b- initial 114.29 mg Kg^{-1}, c- initial 171.43 mg Kg^{-1}, d- initial 285.71 mg Kg^{-1}, e- initial 400 mg Kg^{-1}, f- initial 514.29 mg Kg^{-1}).

3.1. *Mechanism of As uptake, accumulation and translocation*

Plants uptake heavy metals into their roots, where they either store them or translocate them to the shoots [15]. Plants develop a series of mechanism in response to heavy metal stress. A number of plants cope with heavy metals by binding them in complexes with phytochelatins (PCs) and sequestering the complexes inside their cells [38]. PCs have been shown to be involved in the detoxification of As in plants [38-42]. *Amaranthus gangeticus* withdraws As by distributing it in its roots, stem and leaf. Previous studies show that As hyperaccumulator plants uptake As via phosphate transport systems [43,44]. It is also demonstrated that As transferred from root to shoot as arsenite [45]. Root

pressure and leaf transpiration are supposed to be responsible for translocation of As ions from root to shoot [14,46-47].

Amaranthus gangeticus take up and store metal in root, stem and leaf as presented in Table 1. The plants accumulate larger portion of the metal in aerial parts rather than in roots at all initial concentrations of the metal in the soil. Good hyperaccumulator accumulates a metal more in shoots than in roots and so *Amaranthus gangeticus* is a good As-hyperaccumulator. Maximum accumulation found in aerial part of the plant tissue is 17934 mg Kg^{-1} for soil contaminated with the highest concentration of the metal (514.29 mg Kg^{-1}). So *Amaranthus gangeticus* can be a successful alternative of *Pteris vittata*, ever found highest As accumulator [48], where nutrient requirement of soil and climatic condition are more suitable for *Amaranthus gangeticus* than for *Pteris vittata***.**

Table 1 outlines the distribution of metals in plant tissue at different concentrations of the metal.

Table 1. Comparison of As accumulation in different parts of plant (mean ±SD, n=3) at different concentrations of the metal in soil

Concentration of As in soil	Accumulation of As in		
	Root	Stem	Leaf
	Weight (mg)		
57.14	1273 ± 16	2055 ± 23	2559 ± 42
114.29	2562 ± 79	2675 ± 47	6376 ± 56
171.43	4392 ± 144	5283 ± 99	7939 ± 107
285.71	5947 ± 42	6297 ± 34	8660 ± 141
400	5193 ± 92	8646 ± 85	9180 ± 153
514.29	4850 ± 147	8821 ± 107	9110 ± 130

Bioconcentration factor is more important measure than translocation factor (TF) for considering the potential of a species for phytoextraction. According to Yoon *et al.* [50], only plant species with both BCF and TF greater than 1 have the potential to be used for phytoextraction. BCF and TF values are given in Table 2 for different initial concentrations, where the highest BCF is 574.74 obtained for contaminated soil with 57.14 mg Kg^{-1} As and the lowest BCF is 225.3 obtained for 514.29 mg Kg^{-1}. An interesting feature of Table 2 is that BCF values decreased with the increase of concentration. Early studies on phytoremediation showed that high concentrations of metals in soil can lead to decreasing BCF values [15,25].

Translocation factor greater than 1 indicates the translocation of the metal from root to aerial part [51]. In the present study, TF is greater than 1, as shown in Table 2, for all pots with different initial concentration of As.

Table 2. Bioconcentration and Translocation factor.

Concentration of As in soil (mg/kg)	57.14	11.29	171.43	285.71	400	514.29
BCF	547.74	362.94	361.35	290.54	226.25	225.3
TF	3.62	3.62	3.01	2.52	3.433	3.69

4. Conclusion

Present study clearly demonstrates the efficiency of *Amaranthus gangeticus* in extracting As from soil. Percentage of reduction (72%-81%) of the metal is outstanding just in one month. In addition, BCF and TF values were favorable for the studied plant (BCF>>1 and TF>1). Effect of As accumulation in presence of higher amount of other heavy metals in soil should be investigated in future study. Proper management of heavy metal prone plants is needed to keep the environment safe and sound. However, disposal of arsenic affected plants can be made by a process such as incineration away from any agricultural land.

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