Phytoremediation of Arsenic Contaminated Soil by *Plumbago Zeylanica* L.

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1. Introduction – Arsenic is non-essential and generally considered toxic to plants. Arsenic in groundwater due to natural geological contamination has been recognized as an issue of great public health concern in many regions of the world, especially in South-East Asian countries. In Bangladesh, China, Hungary and India, As is found at high concentration in tapped groundwater resources [1,2]. Long term irrigation with the As contaminated groundwater is likely to increase its concentration in soil and crops [3, 4]. The maximum acceptable concentration of As in agricultural soil is 20 mg kg^{-1} in soil [5]. Arsenic absorbed by plants, including farm crops such as grains, vegetables and fruits, and their ingestion cause hazardous effects on human health, such as, cancer and cardiovascular or neurological disorders [6, 7] Bioavailability, uptake and phytotoxicity of As in plants are influenced by factors such as As concentration in soil, As species [8], soil properties, such as, redox potential, drainage conditions [9], pH and soil phosphorus content [10, 11].

Plants normally take up As predominantly in trivalent As(III) and pentavalent As(V) forms. Arsenite has high toxicity for radicular membranes because As(III) reacts with sulfhydryl groups of protein leading to cellular disfunction [12,13]. Arsenate is chemically similar to phosphate that uncouples the oxidative phosphorylation by displacing phosphate in ATP synthesis [11]. On exposure to excess As, plants show several toxic symptoms, such as, inhibition in seed germination [14], decrease in plant height [15, 16, 17, 18] reduction in root growth [8, 19], wilting and necrosis of leaf blade [16, 20], reduction in leaf area and inhibition of photosynthesis [9, 21], decrease in shoot growth [8, 19], and lower fruit and grain yield [15, 16, 17, 18]. Arsenic also impairs nutrient uptake and/or it may simply compete with essential plant nutrients, such as, phosphorus. Besides, As is known to reduce the production of bioactive constituents which are end products of plant secondary metabolism [22].

High levels of bioavailable metal concentrations in soil present enhanced toxicity in edible and phytotherapeutically effective parts of plants. This aspect is important for medicinal plants grown under natural conditions because some of them (e.g. *Matricaria recutita* L., *Hypericum perforatum* L.) can accumulate relatively high concentration of toxic metals in their shoots and could be considered for phytoremediation purposes [23]. The concept of phytoremediation was first proposed by [24] and involves the use of plant hyperaccumulators of heavy metals to remove pollutants from soils or waters.

On the basis extensive literature analysis, it has been observed that there is a paucity of research on *P. zeylanica* in relation to heavy metal toxicity. Because of its high therapeutic values, it is worthwhile to see the consequences of As toxicity in soil on physiological aspects as it grows widely in As polluted sites in India [25, 26]. The aim of this paper is to investigate the uptake mechanisms of As and its accumulation pattern in *P.*
zeylanica.

2. Experimental - ***Plant material*****:** Seeds of *P. zeylanica* were obtained from Medicinal Plant Garden, Rama Krishna Mission Asharam, Narendrapur, West Bengal (India). Seeds were surfaced sterilized with 0.1% $HgCl_2$ for 2 min, then washed thoroughly and soaked overnight in distilled water. Nursery was developed to raise the 6-8 cm long seedlings up to four-leaf stage. After that, seedlings were transplanted into earthen pots (one plant/ pot) containing 10 kg soil.
The experiments were arranged in single factorial design, consisting of three different As concentrations (50, 100 and 150 mg kg^{-1}, as $Na_2HAsO_4.7H_2O$) in five replicates. The soil without As supplementation served as the control set. The plants were grown under natural conditions of temperature, light and humidity during the growing season (March -November).

Arsenic accumulation and multi-element uptake

Microwave assisted extraction technique was used for complete extraction of As and multi element (Na, K, Ca, Mg, Mn, Fe, Cu, Zn and P) from the plant sample. According to Balarama Krishna (2012), 0.1 g of dried plant samples, were put into a 15 ml PP tube to which 3 ml of extractant mixture (1ml of sub-boiled HNO_3, 1 ml of H_2O_2 and 1 ml H_2O) was added. Then the tubes were vortexed to ensure that the entire sample was completely wetted. The vessel was placed inside the microwave cavity (CEM Corporation, USA) and the temperature was ramped up to 200 ± 5 °C in 5 min at 1200 W power and held for 5 min. The vessels were opened after cooling to room temperature and the sample digests were diluted with high purity water to the required volume for multi- element and As analysis by ICP-OES and ICP-MS, respectively, using an external calibration method. Appropriate blanks prepared similarly were also analyzed. The quality assessment of the results, optimization of present MAE procedure and validation of process was done by analyzing the certified reference material namely sea lettuce BCR-279 which has a certified As concentration of 3.2 μg g^{-1}.

Arsenic translocation and bioaccumulation

The translocation factor (TF) reflects an ability of a plant in translocating As from root to shoot and is expressed as [27, 28, 29] TF = $[As]_{SHOOTS}/[As]_{ROOTS}$

The bioconcentration factor (BF) reflects the effectiveness of a plant in concentrating As into its biomass as expressed as [30].

BCF= $[As]_{PLANT\ TISSUE}/[As]_{SOIL}$

3. Results– ***Distribution of macronutrients in shoots and roots***

Phosphorus (P) uptake was elevated in the presence of As in soil, and shoot contained more P than roots. Arsenic addition had significant influence on P accumulation in the shoot (r = 0.982**). Phosphorous content was elevated to 2.3 and 31% in the shoot at 50 and 100 mg kg^{-1}As, respectively, compared to the control. However, slight drop was observed at 150 mg kg^{-1} as compared to 100 mg kg^{-1}As treated plants. In roots, P uptake increased (11.31 g $plant^{-1}$) at low dose of As (50 mg kg^{-1}), whereas it decreased by 11.59 and 36.07% at higher concentrations of As (100 and 150 mg kg^{-1} respectively) (r = -0.703^{NS}). In the absence of As, the P translocation factor (P concentration ratio of the shoot to the root) was 40.03. However, in the presence of As, the ratio increased to 82.04. The above ground biomass was positively correlated to P accumulation in the shoot (0.966*).

Calcium (Ca) uptake was positively influenced by As accumulation in plants (r = 0.951*). Shoots of treated plants contained significant (P< 0.05) amount of Ca, i.e. 33.35, 64.61 and 98.39 % more than in the control plants. However, in roots, Ca accumulation only increased by 17.17% at 50 mg kg^{-1} As, but then decreased at greater As doses (100 and 150 mg kg^{-1} As) by 17.49 and 41.56% respectively (r = -0.772^{NS}).

The accumulation of Magnesium (Mg) was similar to Ca in both shoots and roots. There was a positive correlation between As and Mg accumulation (r= 0.980*). Arsenic addition (50, 100 and 150 mg kg^{-1}) to soil increased Mg uptake in shoots by 10.81, 59.53 and 80.67%, respectively, but decreased in roots similar to Ca (r = 0.892^{NS}).

Potassium (K) uptake in shoot increased significantly (P < 0.05) in the presence of high doses of As (100 and 150 mg kg^{-1}) by 23.79 and 45.10%, respectively (r = 0.897^{NS}). However, in root K accumulation decreased exponentially by 8.15, 31.27 and 48.47% with increasing concentrations of As in soil (r = -0.959*).

Distribution of micronutrients in shoots and roots

Zinc (Zn) uptake in the shoots and roots was significantly affected (P< 0.05) by As, ranging from 50 to 150 mg kg^{-1} in soil. In shoots, Zn accumulation increased at 50 and 100 mg kg^{-1} As by 0.50 and 64.52%, respectively, but it decreased at the highest As concentration (150 mg kg^{-1}) by 40.87% (r = 0.917*). In roots, Zn accumulation declined at higher concentrations of As (100 and 150 mg kg^{-1}) by 16.8 and 44%, respectively (r = 0.734^{NS}).

Iron (Fe) uptake in shoot displayed a positive trend (P< 0.05) with increasing concentration of As (50 to 150 mg kg^{-1}), rising by 37.84, 44.69 and 68.83% (r = 0.897^{NS}), respectively. In roots, Fe uptake decreased at higher concentrations of As (100 and 150 mg kg^{-1}) (r = -0.882^{NS}), and showed similar pattern of reduced accumulation as in case of Zn.

Manganese (Mn) uptake was significantly affected by As (50 to 150 mg kg^{-1}), the increase varying from 2.02 to 3.68 g $plant^{-1}$ in shoots (r = 0.848^{NS}) and 0.08 to 0.03 g $plant^{-1}$ in roots (r = -0.828^{NS}). Mn accumulation was significantly higher in shoots than in roots and increased linearly by 20.23, 46.42 and 119.04% with 50, 100 and 150 mg kg^{-1}As, respectively.

The range of accumulation of copper (Cu) in shoots and roots was 0.67 to 1.14 g plant^{-1} (r = 0.841^{NS}) and 0.04 to 0.02 g plant^{-1} (r = -0.592^{NS}), respectively, under the influence of As stress. Cu accumulation in shoots and roots of treated plants was found to be less as compared to Zn, Fe and Mn.

Total As Uptake and Distribution

After 32 weeks of growth, As uptake in the plants, increased with higher concentrations of As in soil. It is interesting to note that As accumulation in shoots was much higher than in roots, with an estimated >95% of As taken up by the plant accumulating in the above ground biomass. Maximum accumulation of 4450.53 and 4719.00 mg plant^{-1} was observed in the shoots of plants grown in soil with 100 and 150 mg kg^{-1} As, respectively

Translocation factors (TFs) were between 2.2- 13 indicating that there is an efficient translocation of As from root to shoot. Arsenic concentration of 150 mg kg^{-1} in soil reduced the As translocation from root to shoot.

Bioconcentration factors (BCFs) increased significantly with increasing supplements of As. The BCF values ranged from 0.9 - 2.0 in shoots of treated plants which showes that *P. zeylanica* accumulates As efficiently in its biomass and can be considered as an As hyperaccumulator.

Discussion- *P. zeylanica*, accumulated relatively high levels 43.5- 300 mg kg^{-1} of As on a dry matter basis in its shoots, while As in the soil was between 50- 150 mg kg^{-1}. Arsenic level in the shoot was more than those in the soil, which clearly demonstrated As accumulation capability of *P. zeylanica*. Arsenic accumulation depends on plant species [31], its concentration in soil [32], physical and chemical properties of soil, such as, pH and clay content [33] and the presence of other ions [34].

Typical of a hyperaccumulator, *P. zeylanica* concentrated >95% of the As in its shoots. Efficient transport of As to the above ground biomass is important for efficient As detoxification. Translocation factor (TF) has been used as an index to measure the effectiveness of plant metal translocation from roots to shoot [35]. In this study, the TFs (ratio of As in shoot to roots) of *P. zeylanica* were from 2.2- 13. This suggests that the plant can actively uptake As from soil and store it in above ground parts. The trait of effective translocation of As to the shoot from the roots has been observed particularly in hyperaccumulators [36]. In addition to plant biomass and As concentrations, bioconcentration factor (BF) (the ratio of As concentration in the plant tissue to that in the soil), has been used to characterize the effectiveness of plants as As accumulator [37]. Like all hyperaccumulators, BFs in shoots of *P. zeylanica*, were greater than one (0.9 to 2), indicating the ability of the plant to bioconcentrate As from soil.

4. **Conclusions -** Arsenic uptake was significantly enhanced with increasing soil As concentrations, and accumulated more in shoots than roots. Thus, it appears to be promising for phytoremediation which is an emerging cost-effective, non-intrusive, and aesthetically pleasing technology. However, the practical use of *P. zeylanica* in the remediation of soils is still to be demonstrated. Along with increased As uptake in *P. zeylanica*, there was enhanced uptake of macro and micro plant nutrients especially P. The transport of As did not affect the phosphorus transport in the shoot of *P. zeylanica*. Because arsenate and phosphate are chemical analogues, they may compete chemically. The ability of *P. zeylanica* to allow As transport without interfering with phosphate transport may be important in allowing the plant to hyperaccumulate As without sacrificing its health.