Phytoremediation of arsenic contaminated soil and water

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Abstract
Arsenic-contamination of soil and water is widespread which poses a serious threat to plants, animals and humans. There has been growing interest in developing remediation of As-contaminated ecosystem. Studies were conducted to examine the uptake of Arsenic (III) and Arsenic (V) by mustard plants from water and soil. The results have shown that the As content and uptake by mustard plants were significantly affected due to varied levels of As (As III and As V) and P in soil and water. Increase in As concentration markedly increased the As uptake by crop and the effect was more pronounced on As (V) than As (III). Addition of P was found to inhibit the uptake and accumulation of As in plants. In general, roots accumulated large amount of As than shoots and flowers. Phosphate addition was found to decrease the bioavailability of As, particularly, As (III). The two soils (Egmont and Manawatu) significantly differed in influencing the bioavailability of As. However, at high rate (500 mg/kg) of P application was found to increase the bioavailability and thus As uptake by plants. The results provided evidence for P-induced As mobilization in contaminated soil at high rate of P addition. Introduction of earthworms to contaminated soil also increased the bioavailability of As.

Key Words
Arsenic, Phytoremediation, biotransformation, bioavailability, phosphate.

Introduction
Arsenic (As) is a toxic metalloid which enters terrestrial and aquatic ecosystems through both natural (geological) processes and anthropogenic (industrial and agricultural) activities. Arsenic-contamination of soil and water is widespread which poses a serious threat to plants, animals and humans. Arsenic is a unique carcinogen. Therefore, there is a growing interest in developing regulatory guidelines and remediation technologies for mitigating As-contaminated ecosystems. A range of technologies, including bioremediation, has been applied with varying levels of success either to remove As from the contaminated medium or to reduce its bio-toxicity. Plants are increasingly being used to enhance the removal of As. This technology is known as Phytoremediation and it attracts intensive research and commercial interests. The effectiveness of this technology is, however, variable and depends on several factors associated with soil and plant characteristics and the chemistry of As in the environment. Several soil amendments like lime, phosphate (P), compost etc., are used to enhance the effectiveness of phytoremediation. In the current study we examined the potential of mustard crop in hyper accumulating the As from soil and water and the impact of P on As uptake by crop.

Methods
Hydroponic experiment
A hydroponics experiment was conducted to examine the P-induced As uptake by Indian mustard crop (*Brassica juncea)*. The experimental set up consists of plastic containers (15 × 15 × 15 cm) which were placed in long PVC trays where the water was continuously circulated to prevent excessive evaporation of the nutrient solution. Provision was made for aerating the nutrient solution in the container by inserting a rubber scrubber and continuous aeration was done. Seeds of mustard plants were germinated separately in small pots filled with vermiculite as inert media. After ten days, seedlings of even size were carefully selected and transferred along with the vermiculite pots to plastic containers containing 1.75 litres of nutrient solutions. After a week of acclimatization of the plants in the hydroponics culture, the nutrient solution was replaced with fresh nutrient solution containing varied levels of As either As (III) or As(V), in combination with different concentration of P. There were three replicates of each treatment and the containers were placed randomly within a long plastic tray. After eight weeks the individual plants were harvested by carefully removing from the vermiculite-pots. The shoots, roots and flowers were separated, oven-dried at 60^{o}C for 72 hours and analysed for As by using a Graphite Furnace Atomic Absorption Spectrometry (GFAAS).

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Pot Experiment-I

A glass house pot experiment was conducted using two soils viz., Egmont (a high P fixing soil) and Manawatu, (silt loam, low P fixing soil) differ in chemical characteristics. Some important characteristics of soils are given in Table 1.

Table 1. Some important characteristics of Egmont and Manawatu soils

Characteristics	Egmont	Manawatu
pH	5.21	6.01
Organic C (g/kg)	78.5	29.1
P retention (%)	83	33
CEC (cmol/kg)	26.2	7.6
Dominant clay	Allophane	Mica / illite
Total As (mg/kg)	13.6	6.7
Acid oxalate Al (%)	3.54	0.09
Acid oxalate Fe (%)	1.63	0.31

Bulk soil samples were air-dried and crushed to pass through 5-mm screen to ensure homogeneity of soil before use in the glasshouse pot study. A sub-sample of 1000 g of soil was weighed into heavy grade plastic bags. The soils were mixed with graded levels of As (0, 50, 100 and 200 mg/kg) as arsenite [As(III)] or arsenate [As(V] and thoroughly mixed. The moisture content was brought to approximately 75 per cent of field capacity and the As-spiked soils were incubated at 25^{o}C for 60 days. At the end of 60 days phosphate solutions at a rate equivalent to 0, 100, 250, 500 mg P/kg as KH_2PO_4 were added to soil and the incubation was continued for further 60 days. At the end of 60 days the soils were transferred into 9-inch plastic pots (1725 cm^3). The soil in each pot was slightly compacted to a bulk density of approximately 1 g/cm^3 and the moisture content was brought to field capacity with distilled water. Approximately 10 to 15 seeds of Indian mustard (*Brassica juncea)* were sprinkled on soil surface and gently covered with soil. After germination the plants were thinned and only three plants per pot were allowed to grow. After eight weeks the individual plants were harvested and analysed for As. The dynamics and bio-availability of As in soils were examined by determining the amount of readily labile As extracted by 0.05 *M* $(NH_4)_2SO_4$. The speciation of As in soils spiked with As(III) and As(V) was determined for selected treatments as per the fractionation procedure outlined by Wenzel *et al.* (2001).

Pot Experiment-II

The As-contaminated soil samples were collected from an abandoned sheep dip site in Hamilton, New Zealand. About 1500 g of air-dried soil was placed in heavy grade polyethylene bags. Phosphate solutions as KH_2PO_4 were added to soil at a rate equivalent to 0, 100, 250 and 500 mg P/kg and the moisture content was adjusted to field capacity with distilled water. The soil and P solutions were thoroughly mixed in polyethylene bags and incubated at 25^{o}C for 60 days. At the end of 60 days the soil was transferred into 9-inch plastic pots (1725 cm^3). The soil in each pot was slightly compacted to a bulk density of approximately 1 g/cm^3 and the moisture content was brought to field capacity with distilled water. Approximately 10 to 15 seeds of Indian mustard (*Brassica juncea*) were sprinkled on soil surface and gently covered with soil. Pots were randomised on the bench and their positions were changed weekly to minimize variations in microenvironment. After germination the plants were thinned and only three plants per pot were allowed to grow. Initially for two weeks 100 ml of nutrient solution was added to all pots. At alternate days the plants were watered and grown for eight weeks. After eight weeks the individual plants were harvested and As content was determined.

In another set of treatment, approximately 1500 g of soil was placed in heavy grade polyethylene bags and added with different rates of P (0, 100, 250 and 500 mg P/kg). The soil and P solutions were thoroughly mixed in polyethylene bags and incubated at 25^{o}C for 60 days. There were four treatments replicated four times. At weekly intervals the soil was mixed and moisture content was adjusted for any weight loss. At the end of 60 days the soil was transferred into 9-inch plastic pots (1725 cm^3). The pots were kept as such without growing any plants. At weekly intervals the soil was mixed and moisture content was adjusted for any weight loss. The effect of earthworms on the bioavailability of As in the contaminated soil was examined by incubating the contaminated soil (500 g) with 10 medium sized earthworms, which were collected from the contaminated soil. This treatment was replicated three times. This incubation was conducted for 180 days. The soil samples were analysed for various fractions of As. The plant samples were dried and As was determined using a Graphite Furnace Atomic Absorption Spectrometry (GFAAS). The

dynamics and bio-availability of As in soils were examined by determining the amount of readily labile As extracted by 0.05 *M* $(NH_4)_2SO_4$.

Results

Arsenic uptake from water

The presence of P in nutrient solution significantly decreased both As(III) and As(V) uptake by crop. The crop removal of As from solution ranged from 252 to 876 mg/kg. Significant difference was observed due to different levels of As and P. In general, crop removal of As(V) was relatively higher than As(III). Addition of P markedly reduced the plant uptake of As (Table 2).

Table 2. Effect of As and P on As uptake by mustard plants

Treatments	Arsenic (mg/kg)		
	P0	P (0.005 mmol)	P (0.025 mmol)
1. No As(III)	0.0	0.0	0.0
2. As (III) (0.005 mmol)	270.2	416.5	291.2
3. As(III) (0.025 mmol)	623.7	467.0	513.1
4. No As(V)	0.0	0.0	0.0
5. As (V) (0.005 mmol)	252.0	254.0	456.0
6. As(V) (0.025 mmol)	876.0	472.7	603.0
LSD (0.05) As:78.8,		P:39.7, As × P : NS	

The relative distribution of As in plants shows that Brassica sp. accumulated As mainly in the roots followed by shoots and flower. On a dry weight basis, the roots contained the highest mean As concentration being several fold higher than As in shoots and flowers. Only a small amount of As was found accumulated in flowers. Upon uptake mustard plants accumulated about 69 to 75% of As in roots. The As concentration in roots, shoots and flowers were highly influenced by both As and P levels in solution. Both As and P addition markedly reduced the As accumulation in roots. Arsenic concentrations in shoots of plants have been reported to depend mainly on the root system activity (Carbonell-Barrachina *et al.* 1997).

Arsenic uptake by mustard and relative distribution in different parts

The uptake of As by mustard was significantly influenced by varied levels of As both in the presence and absence of P. Increase in As(III) markedly increased the As uptake. The highest value was observed at an application rate of 200 mg/kg. Increase in P levels up to 250 mg/kg notably increased the As uptake and at 500 mg/kg the As uptake was found decreased. This trend was observed with both As(III) and As(V) application. Arsenic uptake was relatively higher in Manawatu soil than Egmont soil. The As uptake varied between 29.5 and 209 mg/kg for As(III), whereas it was from 7.42 and 283.4 mg/kg for As(V). Such variation could be due to differential chemical and mineralogical properties of these two soils. While P application at 100 mg/kg remarkably increased the As uptake, further increase in P levels significantly reduced the As uptake. The highest uptake was due to the combined application of 200 mg As/kg and 100 mg P/kg.

Relative distribution of As in different parts

The relative distribution of As in different parts of mustard is depicted in Figure1. The results showed that the mustard plants accumulated As primarily in the roots. Only relatively low quantities of As were found translocated to the shoot and flowers. On a dry weight basis, the roots contained the highest mean As concentration being several fold higher than As in shoots and flowers. Only a small amount of As was found accumulated in flowers with the application of As(V). Upon uptake brassica accumulated about >70% of As in roots. The As concentration in roots, shoots and flowers were highly influenced by both the As and P levels in solution. Both As and P addition had markedly reduced the As accumulation in roots and shoots.

Dynamic, bioavailability and speciation of As in soil

Phosphate treatment was found to decrease the bioavailability of As in soil, particularly when added as As(III). Consistently up to 4 months, P application remarkably increased the $(NH_4)_2SO_4$-As during incubation. This could be due to P induced mobility of As in soil. However, the soils collected after the harvest of crops have shown marked reduction in the $(NH_4)_2SO_4$-As due to P applications. A comparison of the data on $(NH_4)_2SO_4$-As in Egmont and Manawatu soils after the harvest of crops showed that the soils differed markedly with regard to P effect. In Egmont soil, increase in P levels appeared to decreased the bioavailability of As(III) and As(V), whereas in Manawatu soil, increase in P levels increased the bioavailability of both As(III) and As(V).

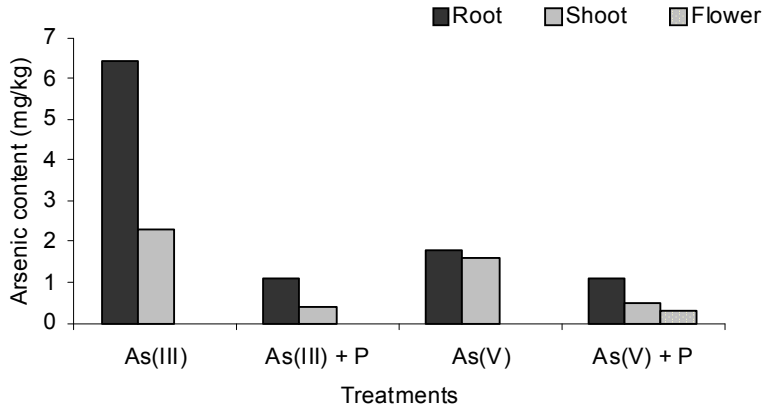

Figure 1. Relative distribution of As in plant parts

The data on the bioavailability of As in the contaminated soil as measured by the 0.05 *M* $(NH_4)_2SO_4$ extractable As showed that the addition of P remarkably increased the bioavailability of As in the contaminated soil. Initially, the concentrations of $(NH_4)_2SO_4$-As in soil was only 171 µg/kg which was markedly increased due to P application. This trend was also observed in contaminated soil incubated with P but without any plants. Further, P application remarkably decreased the specifically-sorbed As [as extracted in 0.05 *M* $(NH_4)H_2PO_4$] in soil. This provides clear evidence for the P induced mobility of As in soil. It is interesting to observe that the bioavailability of As in the contaminated soil was tremendously increased due to the introduction of earthworms. Earthworms also appeared to have enhanced the adsorption of As in soil as the data have shown high values for sorbed-As [as extracted in 0.05 *M* $(NH_4)H_2PO_4$). Large amounts of As (25 mg/kg) was also found accumulated in the earthworm tissues.

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