Bioremediation potential of spinach (*Spinacia oleracea* L.) for decontamination of cadmium in soil

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Greenhouse pot culture studies were conducted to evaluate the bioremediation potential of spinach for removal of cadmium. The results indicated that spinach was able to take up cadmium in substantial quantity in the shoot. There were no visual cadmium toxicity effects on spinach and no significant reduction in the dry matter yield up to 20 μg g^{-1} cadmium. Significant growth retardation of fenugreek crop grown after spinach was observed at all the levels of cadmium, with no cadmium uptake in the shoot. This study revealed that spinach is a cadmium-tolerant crop and can be used for phytoremediation purpose.

Keywords: Bioremediation, cadmium, *Spinacia oleracea*, toxicity, *Trigonella foenum*.

ENVIRONMENTAL contamination by heavy metals is a serious problem throughout the world. Cadmium is one of the most toxic heavy metals which threaten the food chain. It accumulates in soil due to usage of industrial effluents, phosphatic fertilizers, municipal sewage sludge and application of city compost for crop production[1,2]. Cadmium uptake by agriculturally important crops has resulted in the inhibition of plant growth and nutrient uptake[3]. Cadmium is not an essential element in the metabolic processes in plants and animals. In most of the plants it is taken up from contaminated soils through the process of passive uptake. It is ingested by human beings and animals with the edible parts of agricultural and horticultural crops or derived products. Excessive human intake of cadmium is of concern as this element accumulates over a time in the body. It may cause impairment of kidney function and bone diseases. The International Agency for Research on Cancer has classified cadmium as a human carcinogen (group I)[4]. The vegetable foods contribute more than 70% of cadmium intake in humans[5].

The residence time of cadmium in soil is over 1000 years[6]. Phytoremediation is a novel technique to clean up contaminated soils using green plants, which offers the benefit of being *in situ*, low cost and environmentally sustainable[7,8]. Soil clean-up techniques such as isolation and containment, mechanical separation, chemical treatment or soil flushing have proven to be effective in small areas[9]. These methods require special equipment and intensive labour. Furthermore, they are not only costly but also cause soil disturbances, and are not readily accepted by the communities. Phytoremediation, the use of plants for removing soil contaminants, has recently become a tangible alternative to traditional methodologies[10,11]. Phytoextraction is one of the phytoremediation strategies based on the use of green plants to remove pollutants (i.e. heavy metals) from soil. Its efficiency depends on the chemical properties of the element removed and its uptake, translocation and accumulation by plants. Cadmium is more mobile than any other heavy metals due to its weak affinity for soil colloids and hence is easily transferred to the crops[6]. Some varieties of *Thlaspi* spp. and ecotypes of *Silene vulgaris* have been found to be cadmium accumulators[12,13]. *Brassica juncia* is highly capable of tolerating and accumulating cadmium[14].

In general, cadmium toxicity symptoms in plants are leaf chlorosis, necrosis and reduction in plant growth. Toxicity symptoms result from disturbance of many important physiological processes and interference in the uptake and transport of mineral nutrients[15]. Spinach and fenugreek are economically important vegetable crops and popular in many parts of the world. Ingestion of contaminated plant parts can lead to serious health consequences.

The present study was conducted to determine the uptake and accumulation of cadmium in vegetable crops, spinach (*Spinacia oleracea* L.) and fenugreek (*Trigonella foenum* L.). Levels of different mineral nutrients, namely Cu, Fe, Mn and Zn in the edible plant parts were also studied to assess any synergistic or antagonistic effects of accumulation of cadmium in the plant tissue on the uptake of these essential metals.

A greenhouse pot culture experiment was conducted using a neutral soil from Trombay experimental field. The physico-chemical properties of the experimental soil are listed in Table 1. All the soil properties were analysed by standard analytical techniques[16,17]. The soil was

Table 1. Physico-chemical properties of the experimental soil

Soil characteristics	Value
pH (soil–water 1 : 2)	7.6
Electrical conductivity (μSm^{-1})	0.8
Cation exchange capacity (cmol (p^{+}) kg^{-1})	28
Water-holding capacity (%)	57
Organic carbon (%)	0.48
Available P (μg g^{-1})	35
Total N (%)	0.14
DTPA extract* (μg g^{-1})	
Cd	2.6
Cu	11
Fe	120
Mn	12.3
Zn	50.7

*Diethylene triamine pentaacetic acid.

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passed through a 2 mm sieve. Plastic pots were filled with 3 kg of sieved soil. The soil was treated with 0, 5, 10, 20 and 50 μg g^{-1} concentrations of cadmium applied as $CdCl_2 \cdot 2½ H_2O$ salt. Each treatment was replicated for four times. After application of cadmium to soils, the pots were incubated for one week during which the moisture levels in the soil were maintained at 60% water-holding capacity, which was predetermined in earlier experiments.

Spinach (variety – local) seeds and fenugreek (variety–local) seeds were used for sowing after sterilization (0.1% $HgCl_2$ for 1 min, further washed with running tap water 3–4 times and then distilled water, and dried on a blotting paper). For the first crop, 20 seeds of spinach were sown in the pots. The first spinach crop was grown for eight weeks and harvested in two stages. Ten plants from every pot were harvested after 3 weeks and the remaining plants were harvested after eight weeks. Plant shoot samples were washed thoroughly with distilled water and dried. Fenugreek plants were sown after harvest of spinach to study the residual effect of cadmium. After eight weeks of growth the plant shoot samples were harvested, washed thoroughly in distilled water and dried. After harvesting the fenugreek crop, spinach was grown as the third crop for a period of eight weeks and harvested. Plant samples were processed similarly as for the previous two crops.

All the plant shoot samples were dried in a hot-air oven at 70°C till the constant weight and dry matter yield (DMY) were recorded. For heavy-metal analysis, all the dried shoot samples were wet-digested using concentrated HNO_3–$HClO_4$ (5 : 1) (AR – S.D. Fine Chemicals, India) di-acid mixture[18]. Cadmium and various micronutrients, namely Cu, Fe, Mn and Zn in the plant extract were analysed using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES-JY 48-Polychromator). The detection limit of all the elements estimated was in the range 0.2–10 μg ml^{-1}.

Analysis of variance was carried out and least significance difference (LSD) ($P = 0.05$) was used for multiple comparisons between the treatment means (IRRISTAT-ANOVA).

Cadmium is a common contaminant in soils. Its uptake was studied in a cropping sequence of spinach–fenugreek–spinach. Data on the uptake of cadmium in both first and third spinach crops (Table 2) revealed that as the spike concentration of cadmium in soil increased, the uptake of cadmium by the shoot also increased significantly compared to control (Table 2). At 50 μg g^{-1} of cadmium treatment, uptake of cadmium reached a maximum of approximately 192 μg g^{-1} dry wt of the plant. Spinach plants did not show toxicity effects like chlorosis and necrosis due to various doses of cadmium at any stage of growth in both the first and third spinach crops in the sequence.

During the first three and eight weeks of growth in the first crop, no significant difference in DMY was observed at all the levels of cadmium treatment compared to control plants (Table 3). However, in the cropping sequence when spinach was grown as the third crop to study the residual effect of cadmium, a significant decrease in DMY was observed at 50 μg g^{-1} level.

The uptake of Cu, Mn, Zn and Fe in spinach was influenced differentially by cadmium treatment. Plant uptake of Cu, Zn, Mn and Fe significantly decreased during all the cadmium treatments compared to the control during the first three weeks of plant growth (Table 4). However, uptake of these elements slowly showed an increase and after eight weeks a significant increase in uptake was recorded in all the micronutrients, especially at higher cadmium treatment levels (Table 4). In the residual spinach crop significant increase in uptake was observed in the case of all the micronutrients compared to control plants (Table 5).

Fenugreek was the second crop in the cropping sequence. The plants which received 50 μg g^{-1} cadmium in soil were stunted and sick in appearance. Toxicity was also observed at 5, 10 and 20 μg g^{-1} cadmium levels. Cadmium at 5, 10 and 20 μg g^{-1} levels reduced DMY by about 50% whereas 80% reduction was observed at 50 μg g^{-1} level of cadmium (Figure 1). However, surprisingly, the uptake of cadmium in fenugreek plants at all levels of cadmium treatment was found to be below detectable limits. The uptake of iron was not significantly

Table 2. Uptake of cadmium by spinach

Treatment (Cd, μg g^{-1} soil)	Cadmium (μg g^{-1} dry wt) First crop spinach Harvest 1	Harvest 2	Residual (third) crop spinach
0	8.21	4.68	4.5
5	33.83*	48.68*	30.8*
10	42.34*	55.39*	58.1*
20	45.06*	66.24*	85.9*
50	102.4*	191.5*	106.4*
LSD ($P = 0.05$)	20.06	37.46	22.85

*Significant increase in uptake.

Table 3. Effect of cadmium on dry matter yield of spinach

Treatment (Cd, μg g^{-1} soil)	Dry matter yield (DMY; g pot^{-1}) First crop spinach Harvest 1	Harvest 2	Residual (third) crop spinach
0	0.286	0.489	0.230
5	0.283	0.547	0.264
10	0.295	0.542	0.258
20	0.282	0.527	0.198
50	0.296	0.462	0.157*
LSD ($P = 0.05$)	NS	NS	0.069

*Significant reduction in DMY.

Table 4. Micronutrient uptake (μg g^{-1} dry wt) by spinach at different levels of cadmium (1st crop)

	Harvest 1 (3 weeks)				Harvest 1 (8 weeks)			
Treatment (Cd, μg g^{-1} soil)	Cu	Fe	Mn	Zn	Cu	Fe	Mn	Zn
0	16.2	314	83.8	64.69	22.67	242	141.4	82.6
5	6.40*	256*	78.6	38.69	21.89	245	130.8	83.8
10	4.47*	186*	74.5	27.96*	20.53	254	170.4**	92.5
20	3.72*	156*	69.1*	24.8*	19.56	246	153.7	97.9**
50	1.48*	117*	65.1*	20.5*	10.37*	528**	172.2**	70.8
LSD (P = 0.05)	1.26	30.4	7.6	21.45	3.7914	46.07	22.89	10.5

*Significant reduction in uptake. **Significant increase in uptake.

Table 5. Micronutrient uptake (μg g^{-1} dry wt) by spinach at different levels of cadmium (second crop)

Treatment	Cu	Fe	Mn	Zn
0	34.67	403	42.8	30.4
5	35.44	439	40.2	30.7
10	41.82	483*	40.7	42.8*
20	49.46*	693*	50.1	42.3*
50	49.33*	906*	58.7*	43.4*
LSD (P = 0.05)	4.6	34	5.9	7.8

*Significant increase in uptake.

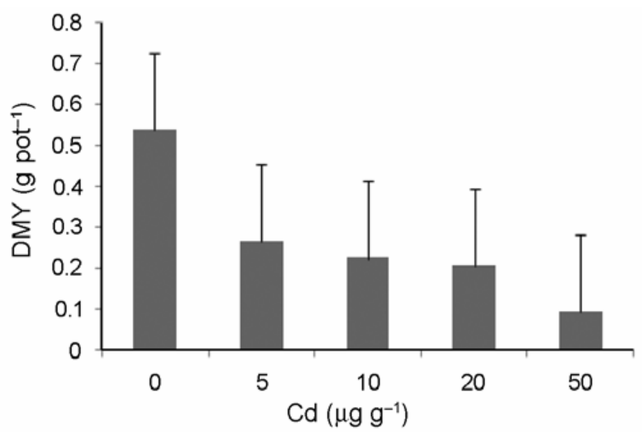

Figure 1. Dry matter yield (DMY) of fenugreek as affected by different levels of cadmium.

affected at 5 μg g^{-1} cadmium-treated pots. However, significant reduction in iron uptake was observed at 10, 20 and 50 μg g^{-1} cadmium-treated pots. Maximum reduction in iron uptake (90%) was observed at 50 μg g^{-1} level cadmium-treated pot (Figure 2). The uptake of zinc was significantly decreased at all levels of cadmium treatment and maximum reduction (80%) was noticed at 50 μg g^{-1} level (Figure 3). The uptake of copper and manganese was completely affected in pots treated with different levels of cadmium.

We report cadmium interaction with two different vegetable crops grown sequentially. Cadmium is not an essential micronutrient for plants; however, its passive uptake in highly contaminated soil could be a distinct possibility. We wanted to ascertain whether such passive uptake causes any toxicity. These studies are relevant as many of the vegetable crops in metro cities are grown near railway tracks where high levels of contamination are likely due to indiscriminate dumping of batteries and plastics.

There were significant variations in the uptake of cadmium and micronutrients as well as DMY of all these three crops. In the first and third crops of spinach, cadmium uptake increased with increasing cadmium concentration in soil. However, the dry matter was not significantly affected, except during the third crop at 50 μg g^{-1} cadmium level. Various levels of cadmium significantly affected the uptake of micronutrients, viz. Fe, Zn and Mn in the first spinach crop. However, the effect tapered-off at the second harvest and third crop of spinach. There was significant increase in the uptake of all these microelements at higher levels of cadmium treatment in spinach harvested as the third crop. DMY of spinach is closely related to the metabolism. Higher uptake of cadmium was observed at the initial stages, but DMY remained unaffected during harvests 1 and 2 of the first and third spinach crops. These results suggest that spinach is tolerant to cadmium at 5, 10 and 20 μg g^{-1} cadmium levels.

Plants vary widely in tolerance to excess cadmium in soils. In several plant species, these differences are genetically controlled through different biochemical pathways. Cadmium-tolerant plants must be able to prevent the absorption of excess cadmium or detoxify the cadmium after it has been absorbed; however, the exact physiological mechanisms of cadmium toxicity or tolerance are still debated[3].

Cadmium hyperaccumulator is defined as the plant species capable of accumulating more than 100 μg g^{-1} cadmium in the shoot dry weight[19]. The present study reveals that the concentration of cadmium accumulation in both the spinach crops at 50 μg g^{-1} cadmium treatment in soil was more than 100 μg g^{-1}. It has been reported that at 100 μg g^{-1} cadmium levels in soil, the spinach crop could accumulate 52 μg g^{-1} cadmium in 5 weeks and 68 μg g^{-1} cadmium in 10 weeks[20]. In most plant species, cadmium concentration varies from 3 to 20 μg g^{-1} dry wt of the plant; however, it may reach more than 20 μg g^{-1} in the cadmium-contaminated soils. *Ipomoea aquatica* (water spinach – a potential aquatic plant to remediate cadmium-contaminated wastewater) in hydroponics solution could accumumulate 138 μg g^{-1} dry wt of cadmium at 14 days of growth[21].

Reports on the effects of cadmium on DMY and its toxicity in different plant species are at variance. The results from the present study showed that spinach could tolerate cadmium up to 20 μg g^{-1} in soil, as seen in terms of the growth response. The plants showed no visual metal-induced toxicity like chlorosis, necrosis and leaf burn even after being exposed for 8 weeks up to 50 μg g^{-1} cadmium level in soil.

Uptake of Cu, Fe, Mn and Zn was influenced differentially in spinach. Zinc uptake by the first spinach crop (3 weeks) was significantly decreased at 10, 20 and 50 μg g^{-1} of cadmium in soil, but after 8 weeks Zn uptake was significantly increased at 20 μg g^{-1} cadmium in soil; there was no significant difference in other treatments compared to the control. In the third spinach crop there was significant increase in Zn uptake above 5 μg g^{-1} cadmium treatment in the soil compared to the control. These results are in accordance with the earlier work where at low cadmium concentration of 0.1 μg ml^{-1}, zinc uptake by *Brassica* leaves was increased over the control[22]. The mobility of zinc (an essential element) within the plant could have an effect on the greater movement of cadmium to the leaves, since both zinc and cadmium are chemically similar. Iron concentration in the shoot decreased significantly at 5, 10, 20 and 50 μg g^{-1} cadmium treatment in soil compared to the control, but in the following third spinach crop reversal effect was observed where Fe uptake was significantly increased at 10, 20 and 50 μg g^{-1} cadmium in the soil compared to the control soil (no cadmium). Studies on *Brassica chinesis* showed that with increasing cadmium concentration in the solution (10 μg ml^{-1}) the uptake of iron in the stem and roots was also increased[22]. Further, these results suggest that cadmium may be responsible for increasing the uptake of micronutrients after an initial reduction. The increased uptake of micronutrients resulted in increase in DMY. These results are in agreement with the studies on *Sedum alrfedii* Hance, a new cadmium-tolerant hyperaccumulator plant species, which showed differential uptake and also increase in the uptake of various nutrients under cadmium stress[23].

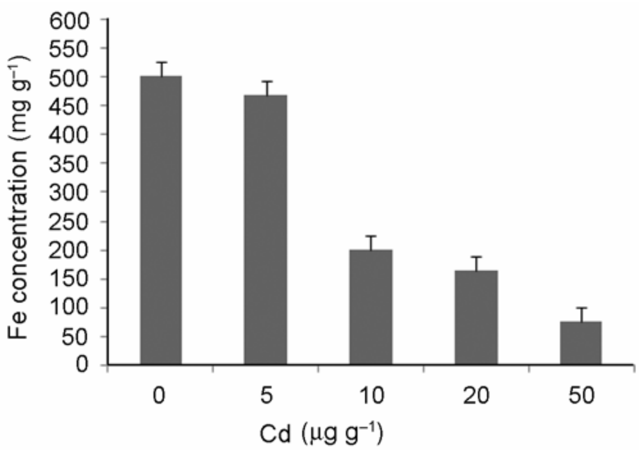

Figure 2. Fe content of fenugreek as affected by different levels of cadmium.

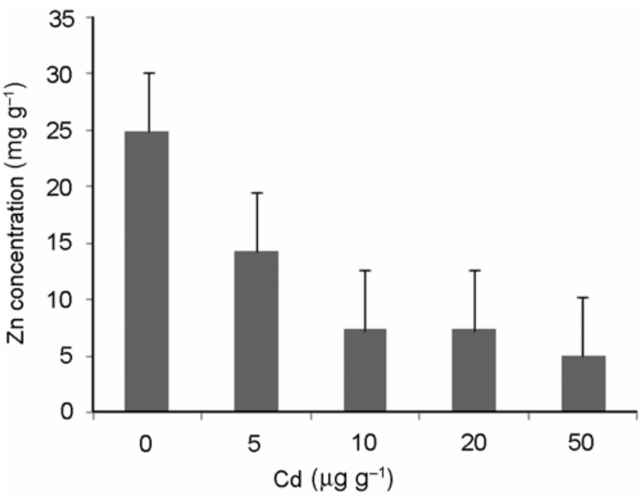

Figure 3. Zn content of fenugreek as affected by different levels of cadmium.

Fenugreek is a cadmium-sensitive crop. Visual signs of phytotoxicity such as yield reduction and chlorosis were observed even at 5 μg g^{-1} cadmium treatment. DMY of fenugreek was significantly reduced at 5, 10, 20 and 50 μg g^{-1} cadmium in soil compared to the control. Reduction in biomass of fenugreek was also reported earlier at more than 10 μg g^{-1} cadmium in soil[24]. Rye grass and cabbage shoot DMY decreased about 10–20% compared to that of control, and maize and white clover shoot DMY decreased ~ 50% compared to that of control when plants were grown with up to 14 μM cadmium[25]. Reduction in dry biomass due to cadmium stress was also reported for *Zea mays*[26]. Fe uptake was not reduced significantly at 5 μg g^{-1} cadmium treatment compared to the control. Fe and Zn contents in fenugreek were significantly reduced under cadmium stress above 10 μg g^{-1}. Presence of cadmium in the growing medium has shown to suppress iron uptake by the plants[27]. In general, cadmium has been shown to interfere with the uptake and transport of several elements and water. Inhibitory effects of cadmium on the uptake and accumulation of Cu, Fe, Mn and Zn were noted in conventional crop plants also[25].

Spinach is an important vegetable crop consumed worldwide. A plant concentration of more than 100 μg g^{-1} cadmium may be regarded as exceptional; even on a cadmium-contaminated site[28]. The present findings indicate that spinach can be an useful plant material for phytoremediation of cadmium-contaminated soils. Many agricultural lands in our country are often polluted by industrial wastewater; therefore, cadmium content in the spinach leaves should be monitored regularly. Efforts must be taken for bioremediation of cadmium in such soils. Spinach definitely could be such a bioremediation measure.

Spinach is a common vegetable crop grown all round the year. Our studies have shown that it can take up cadmium from soil to a significant level. It is an easily cultivable crop, with minimum cultivation practices. The biomass generated is easy to harvest. Hence it would be a good candidate for bioremediation of the cadmium-contaminated soils. The generated biomass can be either subjected to biomethanation or composting to reduce the volume and then processed for recycling of cadmium.

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