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Effect of different sources of silicon on spinach grown in chromium contaminated soil

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Abstract
Green house experiment was conducted conducted to evaluate the effect of silicon in mitigating the heavy metals in soil and its uptake by spinach. Different silicon sources viz., rice hull biochar (RHB), diatomaceous earth (DE) and calcium silicate were used at different levels. Chromium concentration in spinach shoot after the crop harvest was found to be significantly lower in treatment that recorded highest Si concentration in shoot with the application of RDF + DE @ 500 kg Si ha^{-1}. In postharvest soil samples, lower concentration of Cr was obtained in T_9 with the application of RDF + DE @ 500 kg Si ha^{-1} and higher in treatment without silicon. Application of different sources of silicon was found to be effective in mitigation Cr contamination in plants and also in postharvest soil.

Keywords: Chromium, soil contamination, silicon, metal content, metal uptake

Introduction
Currently, anthropogenic inputs of metals exceeded due to increased urbanization and industrialization. The waste let into water bodies may contain suspended solids, plastics, metals, alkalies, acids, chromium, cyanide, cadmium, silver, oil, grease, organic wastes *etc*. This problem started long back but intensified during the last few decades, and now the situation has become alarming in many major cities of India (Girija *et al*. 2007) [1]. Wastewater irrigation may lead to the accumulation of heavy metals in agricultural soils and plants as noticed by Sharma *et al*. (2007) [2]. The physical and chemical properties also are affected by the irrigation of this water (Azad *et al*. 1986) [3]. Soil serves as both sink and source for heavy metals and monitoring the soil contamination levels provide a good indicator of environmental quality (Li *et al*., 2009) [4]. Heavy metals like Cu, Zn, Mn, Fe, Ni and Co are essential micronutrients for plant metabolism but when in excess of these metals and other non-essential elements such as Cr Cd, Pd, As and Hg become extremely toxic (Williams *et al*., 2000) [5]. Use of contaminated water for irrigation leads to heavy metal accumulation in soils followed by biomagnification in the food chain and that pose serious health hazards for human beings. Many studies have demonstrated the beneficial role of silicon in increasing plant resistance to abiotic and biotic stresses and also improve plant growth (Ratnayake, 2016; Pandey *et al.*, 2016) (6, 7). Hence, the study was formulated to know the effect of different silicon sources in mitigating heavy metal contamination in spinach and soil.

Material and Methods
Study area
A pot experiment was conducted to know the effect of silicon sources on mitigation of heavy metal toxicity in soil and crop plants. Bulk soil sample was collected from the agricultural land at N 13° 21'56" and E 77°43'37" of Hirandahalli village (Bangalore district) irrigated with Margondanahalli. Initial properties of soil are presented in table 1.

Experimental details
Experiment was conducted with 500 g soil per pot and spinach was taken up as the test crop. Silicon sources *viz*. RHB, DE and Calcium silicate ($CaSiO_3$) were finely powdered and the soils were treated uniformly at different doses *viz*., 100, 200, 300 and 500 kg Si ha^{-1}. Fifteen seeds of spinach were sown in each pot and the number of seedlings in each pot was reduced to eight a week after germination. Recommended dose of fertilizers applied for all the treatments uniformly @ 150:100:150 N: P_2O_5: K_2O kg ha^{-1}. Recommended dose of phosphorus and potassium were applied after 8 days after sowing along with 50 per cent

nitrogen and remaining nitrogen was applied after 15 days after sowing. The experiment was conducted with fourteen treatments replicated thrice and the pots were arranged with CRD design.

Treatment details
T_1: Control
T_2: RHB @ 100 kg Si ha^{-1}
T_3: RHB @ 200 kg Si ha^{-1}
T_4: RHB @ 300 kg Si ha^{-1}
T_5: RHB @ 500 kg Si ha^{-1}
T_6: DE @ 100 kg Si ha^{-1}
T_7: DE @ 200 kg Si ha^{-1}
T_8: DE @ 300 kg Si ha^{-1}
T_9: DE @ 500 kg Si ha^{-1}
T_{10}: $CaSiO_3$ @ 100 kg Si ha^{-1}
T_{11}:$CaSiO_3$@ 200 kg Si ha^{-1}
T_{12}: $CaSiO_3$@ 300 kg Si ha^{-1}
T_{13}:$CaSiO_3$@ 500 kg Si ha^{-1}
Initial and postharvest soil samples were analyzed for Cr and Si content. Spinach dry biomass per pot were recorded. Crop samples were analysed for Cr and Si content.

Processing of soil samples
Soil samples collected were shade dried, were powdered and sieved through 2 mm mesh size sieve. One gram of 2 mm size soil was ground and sieved through 0.2 mm sieve and stored separately for digestion and analysis of total elemental content.

Total Cr content in the soil samples
0.05 g of 0.2 mm sieved soil samples were predigested with 8 ml HNO_3 (70 %) and 2 ml H_2O_2 (30 %). All the samples were replicated twice. Later, the samples were digested using a microwave digester (Milestone- START D) at 150 ^{0}C with following steps: 1200 w for 15 minutes, 1200 w for 10 minutes and venting for 10 minutes. The digested sample was stored in clean plastic tubes of 50 ml capacity, after making up the volume using double distilled water and the Cr was analysed using ICP-OES (Thermo fisher-model ICAP 7000 series) with the help of multi nutrient standard solution (Dospatliev *et al*., 2012) [8].

Available silicon in the soil samples
Soil was extracted with 0.1M Calcium chloride and the Si was determined by using UV- visible spectrophotometer at 820nm as given by Haysom and Chapmen (1975) [9] and also soil was extracted with 0.5 M Acetic acid and the Si was determined by using UV- visible spectrophotometer at 630nm (Korndorfer *et al*., 2001) [10]

Total chromium and silicon content in plant samples
The shoot and root samples were separated and washed with running tap water followed by double distilled water to remove surface contaminants. The samples were dried in the hot air oven at 70 ^{0}C for 72 hours, powdered and analyzed for their heavy metals content by following the same method as in soil.
The Si concentration in the plant samples was determined as described by Ma and Takahashi (2002) [11].

Chromium and silicon uptake by plant samples
Heavy metal and silicon uptake by crop was computed from their respective elemental concentration and expressed in mg pot^{-1} using following formula.

Heavy metal uptake = Heavy metal content (mg kg^{-1}) X dry weight of crop (g pot^{-1})

(µg pot^{-1})

$$\text{Silicon uptake} = \frac{\text{Silicon content (\%) X dry weight of crop (gpot}^{-1})}{10}$$

(mg pot^{-1})

The analyses and interpretation of the data was done using the Fisher's method of analysis and variance technique as given by Panse and Sukhatme (1967) [12].

Table 1: Initial physico-chemical properties and elemental content of soil

Parameters	
Texture	Sandy loam
Maximum water holding capacity (%)	23.3
pH	7.40
Electrical conductivity (dS m^{-1})	0.24
Available N (kg ha^{-1})	260.66
Available P_2O_5 (kg ha^{-1})	42.41
Available K_2O (kg ha^{-1})	285.21
Total elemental content(mg kg^{-1})	
Cr	110.3
Cd	5.03
Ni	8.88
Pb	7.51
Available Silicon (mg kg^{-1})	
CC-Si	28.42
AA-Si	70.54

Results
Dry biomass of spinach
Significantly highest dry shoot yields was observed with the application of RDF + DE @ 500 kg Si ha^{-1}. The lowest dry root biomass of 1.66 g pot^{-1} was obtained in the treatment that did not receive any silicon sources (Fig 1). Lowest yield with the application of RDF alone may be due to presence of Cr in the control pots and application of RDF alone that may not be sufficient to overcome the extent of toxic effect of Cr and to improve yields of spinach.

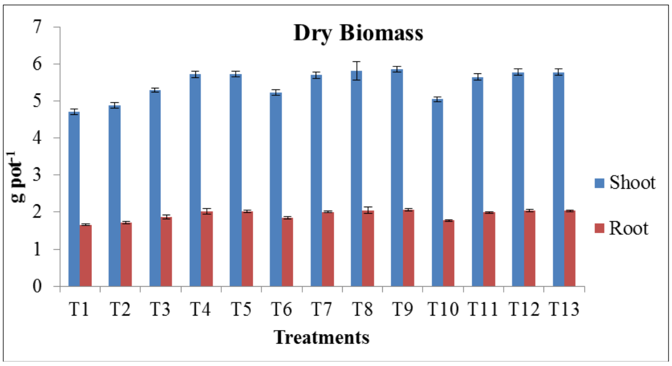

Fig 1: Effect of different silicon sources on dry biomass of spinach at harvest grown in chromium contaminated soil
Content and uptake of chromium in spinach

The concentration of Cr in spinach shoot was reduced with application of different levels of Si. Significantly lower concentration of 10.10 mg kg^{-1} was recorded in treatment which received DE @ 500 kg Si ha^{-1}. In root samples, the concentration of Cr ranged from 1.88 to 2.45 and higher Cr in root was observed with the application of RHB @ 200 kg Si ha^{-1} and on par with RHB @ 100 kg Si ha^{-1}. The concentration of Cr in shoot samples was higher than the permissible limits of EU for Cr in vegetables (0.3 mg kg^{-1}). Application of Si was able to reduce the Cr to some extent but not lower than the EU limits.

Significantly lower uptake of Cr was recorded in treatment which received DE @500 kg Si ha^{-1} (59.17 µ pot^{-1}) and higher uptake was with the application of RHB @ 200 kg Si ha^{-1} (T_3) (81.71 µ pot^{-1}) (Table 2).

Table 2: Effect of different silicon sources on chromium content and uptake in spinach

Treatments	Cr content (mg kg^{-1})		Cr uptake (µg pot^{-1})	
	Shoot	Root	Shoot	Root
T_1: Control	16.20 ±0.81 a	2.45 ±0.034 a	76.33 ±4.7 ab	5.69 ±0.16 de
T_2: RHB @ 100 kg Si ha^{-1}	15.80 ±0.79 a	2.33 ±0.033 b	77.06 ±4.8 ab	5.72 ±0.16 cde
T_3: RHB @ 200 kg Si ha^{-1}	15.40 ±0.74 ab	2.22 ±0.031 c	81.71 ±11 a	6.01 ±0.17 abc
T_4: RHB @ 300 kg Si ha^{-1}	14.54 ±0.68 b	1.94 ±0.027 f	83.18 ±15 ab	6.30 ±0.18 a
T_5: RHB @ 500 kg Si ha^{-1}	10.70 ±0.54 fg	1.95 ±0.027 ef	61.37 ±3.8 b	6.10 ±0.17 ab
T_6: DE @ 100 kg Si ha^{-1}	12.20 ±0.61 cd	1.90 ±0.027 g	63.84 ±4.0 ab	5.51 ±0.15 e
T_7: DE @ 200 kg Si ha^{-1}	11.20 ±0.56 def	1.88 ±0.026 g	63.90 ±4.0 b	5.97 ±0.17 bcd
T_8: DE @ 300 kg Si ha^{-1}	10.20 ±0.51 fg	2.11 ±0.029 d	59.40 ±5.0 b	5.84 ±0.33 bcd
T_9: DE @ 500 kg Si ha^{-1}	10.10 ±0.50 g	2.12 ±0.030 d	59.17 ±3.7 b	5.67 ±0.16 de
$T_{10:}$ $CaSiO_3$ @ 100 kg Si ha^{-1}	12.60 ±0.63 c	1.85 ±0.026 h	63.69 ±3.9 ab	4.71 ±0.13 fg
T_{11}:$CaSiO_3$@ 200 kg Si ha^{-1}	11.80 ±0.59cde	1.83 ±0.026 h	66.67 ±4.1 ab	5.01 ±0.14 f
T_{12}: $CaSiO_3$@ 300 kg Si ha^{-1}	10.50 ±0.53 fg	1.98 ±0.028 e	60.69 ±3.8 b	4.64 ±0.34 g
T_{13}:$CaSiO_3$@ 500 kg Si ha^{-1}	10.30 ±0.52 fg	1.98 ±0.028 e	59.57 ±3.7 b	4.72 ±0.13 fg

Data are expressed as mean (n =3) ±SD. Significant differences between treatments (p< 0.05) are indicated by different letters.

Concentration and uptake of silicon in spinach

Application of silicon as different sources increased the shoot silicon concentration and uptake ranged from 1.0 to 1.33 per cent and 47.71 and 77.10 mg kg^{-1}. Highest Si concentration was observed with the application of DE @ 500 kg Si kg ha^{-1} followed by DE @ 300 kg Si kg ha^{-1} (Table 3). Highest Si concentration in roots was also recorded in the same treatment. The lower concentration and uptake of Si was recorded in control treatment.

Table 3: Effect of different silicon sources on silicon content and uptake in spinach

Treatments	Si Content (%)		Si uptake (mg pot^{-1})	
	Shoot	Root	Shoot	Root
T_1: Control	1.00 ±0.04 d	0.13 ±0.04 d	47.11 ±2.3 g	2.19±0.65e
T_2: RHB @ 100 kg Si ha^{-1}	1.09 ±0.03 cd	0.14 ±0.04 d	53.16 ±2.5 fg	2.33±0.67e
T_3: RHB @ 200 kg Si ha^{-1}	1.10 ±0.04 cd	0.14 ±0.04 d	58.21 ±2.8 ef	2.44±0.73de
T_4: RHB @ 300 kg Si ha^{-1}	1.10 ±0.03 cd	0.17 ±0.07 d	62.99 ±3.0 de	3.17±0.81de
T_5: RHB @ 500 kg Si ha^{-1}	1.25 ±0.04 ab	0.19 ±0.08 d	71.40 ±3.2 abc	3.37±0.82d
T_6: DE @ 100 kg Si ha^{-1}	1.15 ±0.04 bc	0.15 ±0.07 d	59.91 ±2.8 ef	2.54±0.73de
T_7: DE @ 200 kg Si ha^{-1}	1.20 ±0.04 abc	0.16 ±0.03 d	68.58 ±3.1 bcd	2.88±0.86de
T_8: DE @ 300 kg Si ha^{-1}	1.32 ±0.04 a	0.33 ±0.09 ab	77.10 ±5.6 a	6.23±1.31b
T_9: DE @ 500 kg Si ha^{-1}	1.33 ±0.04 a	0.39 ±0.04 a	77.97 ±3.4 a	7.64±0.96a
$T_{10:}$ $CaSiO_3$ @ 100 kg Si ha^{-1}	1.12 ±0.03 bcd	0.14 ±0.15 d	56.66 ±2.7 ef	2.48±1.45e
T_{11}:$CaSiO_3$@ 200 kg Si ha^{-1}	1.20 ±0.04 abc	0.14 ±0.07 d	67.69 ±3.1 cd	2.58±0.78de
T_{12}: $CaSiO_3$@ 300 kg Si ha^{-1}	1.30 ±0.04 a	0.25±0.13 c	75.25 ±3.3 abc	4.72±1.99c
T_{13}:$CaSiO_3$@ 500 kg Si ha^{-1}	1.31 ±0.04 a	0.30 ±0.04 bc	75.88 ±3.4 ab	5.65±0.89bc

Data are expressed as mean (n =3) ±SD. Significant differences between treatments (p < 0.05) are indicated by different letters.

Silicon and chromium content in postharvest soils

Total Cr content in soil after harvest of spinach varied in all treatments due to application of different sources and levels of silicon. Irrespective of silicon sources Cr content was reduced with increasing levels of Si as compared to control. Significantly higher content of total Cr was noticed in treatment without Si application (104.9 mg kg^{-1}) however, lowest total Cr content was recorded in treatment which received $CaSiO_3$ @ 500 kg Si ha^{-1} (96.3 mg kg^{-1}).

The amount of AA-Si (Acetic acid extractable silicon) with the application of different sources and levels of silicon ranged from 72.45 to 85.00 mg kg^{-1}. The AA-Si content was higher with the application of $CaSiO_3$ @ 500 kg Si ha^{-1}. The $CaCl_2$-Si (calcium chloride extractable silicon) content with the application of different sources and levels of Si different soils varied from 33.63 to 38.45 mg kg^{-1} (Table 4).

Table 4: Effect of different silicon sources on total chromium, CC-Si and AA-Si content of post-harvest soil

Treatments	Cr	CCSi	AASi
	(mg kg^{-1})		
T_1: Control	104.9 ±1.47 a	31.33 ±1.15 i	72.45 ±1.01 i
T_2: RHB @ 100 kg Si ha^{-1}	104.5 ±1.46 ab	34.00 ±0.62 g	75.98 ±1.06 g
T_3: RHB @ 200 kg Si ha^{-1}	103.9 ±1.45 abc	37.10 ±0.66de	77.41 ±1.08 f
T_4: RHB @ 300 kg Si ha^{-1}	101.5 ±1.42 defg	37.65 ±0.67 cd	78.65 ±1.10 e
T_5: RHB @ 500 kg Si ha^{-1}	98.7 ±1.38 hi	40.32 ±0.70 a	81.45 ±1.14 c
T_6: DE @ 100 kg Si ha^{-1}	103.1 ±1.44 abcd	33.00 ±0.60 h	73.80 ±1.03 h
T_7: DE @ 200 kg Si ha^{-1}	103.0 ±1.44 abcd	36.36 ±0.65 e	80.21 ±1.12 d
T_8: DE @ 300 kg Si ha^{-1}	100.7 ±1.41efgh	39.21 ±0.69 b	82.98 ±1.16 b
T_9: DE @ 500 kg Si ha^{-1}	97.1 ±1.36 ij	39.63 ±0.69 ab	83.41 ±1.17 b
$T_{10:}$ $CaSiO_3$ @ 100 kg Si ha^{-1}	102.6 ±1.44 bcde	32.00 ±0.59 i	76.41 ±1.07 g
T_{11}:$CaSiO_3$@ 200 kg Si ha^{-1}	100.0 ± 1.40 fgh	36.20 ±0.65 e	79.63 ±1.11 d
T_{12}: $CaSiO_3$@ 300 kg Si ha^{-1}	99.5 ±1.39 gh	38.02 ±2.64 cd	84.63 ±3.13 a
T_{13}:$CaSiO_3$@ 500 kg Si ha^{-1}	96.3 ±1.35 j	38.25 ±0.68 c	85.00 ±1.19 a

Data are expressed as mean (n =3) ±SD. Significant differences between treatments (p < 0.05) are indicated by different letters.
CC-Si: Calcium chloride extractable silicon, AA-Si: Acetic acid extractable silicon

Discussion

Significantly highest dry shoot yields was observed in treatment with the application of DE @ 500 kg Si ha^{-1}. Application of DE was found to be better in improving fresh yields compared to other sources (Fig 1). Application of $CaSiO_3$ was found to be on par with DE. The results are in line with the findings of Tabar (2013) [13]. The presence of Cr in the soil would have decreased growth, photosynthetic pigments and protein as in control and was also supported by Kumar *et al.* (2010) [14]. Application of DE was found to be better compared to other treatments in reducing the Cr content in spinach shoot might be due to higher solubility and the presence of other nutrients in this material, that has helped in the physiological growth and development of crop and the tolerance against heavy metal stress has been improved. Increase in content and uptake of Si in both shoot and root may be due to higher availability of Si with the applied DE material. Among different treatments, application of DE recorded higher Si content in postharvest soils than other sources (Fig 2). Significant increase in AA-Si and $CaCl_2$-Si content may be attributed to increase in pH of soil which in turn increased the ionization and also the dissolution of soil Si and mainly contributed by applied DE material and the same was observed by Sandhya (2016) [15].

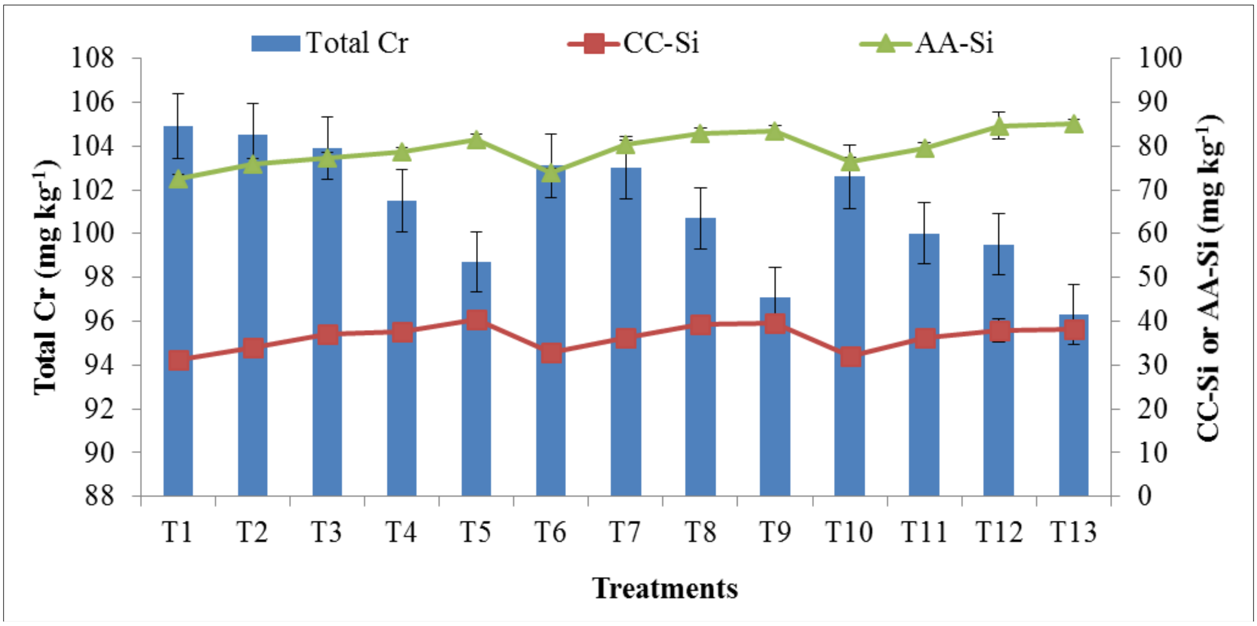

Fig 2: Effect of different silicon sources on total chromium and CC-Si and AA-Si content (mg kg^{-1}) of post-harvest soil

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