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Effect of Na_2SiO_3 on heavy metal uptake by field grown *Basella alba* L. in Matara, Sri Lanka

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Abstract. In this study, we investigated heavy metal uptake and the effects of Na_2SiO_3 on heavy metal absorption by field grown *Basella alba* L (Basellaceae). The concentrations of Fe, Cr, Pb and Cd in the field soils were 29755.30 ± 292.02, 32.99 ± 0.97, 26.01 ± 1.02, 0.13 ± 0.004 µg/g, respectively. These concentrations are significantly below the maximum permissible limits reported by FAO/WHO. Although Fe, Cr, Pb and Cd were present in the soil, only Fe was absorbed by *B. alba*; the tissue concentrations of other heavy metals were below the detection limit. The distribution of Fe from soil to different plant parts was investigated by calculating transfer factors. Low transfer factors indicated low absorption and translocation of Fe from soil to plant tissue. We also investigated the effects of Na_2SiO_3 on metal absorption by applying two different concentrations of Na_2SiO_3 (Si-100 mg/L and Si-50 mg/L) alongside a control. There was a significant reduction of Fe absorption in *B. alba* treated with Si-100mg/L of Na_2SiO_3 compared to that of plants treated with Si-50 mg/L of Na_2SiO_3 and the control.

Keywords. Heavy metal uptake, maximum permissible limits, silicon

1 Introduction

Farmers use synthetic and organic fertilizers as well as pesticides at several stages during the cultivation of vegetables. These fertilizers and pesticides enhance the growth of crops by providing essential nutrients and by controlling pests but some of these cause soil contamination via the release of heavy metals (McLaughlin *et al.* 2000). Heavy metals can be toxic even under low concentrations and can have lasting impacts on human and ecosystem health (Gall *et al.* 2015). Most farmers apply fertilizers 2-8 times in excess than the dosage recommended by the Department of Agriculture, Sri Lanka (Jayathilaka *et al.* 1989). Although several studies have been carried out on soil contamination by heavy metals in the up country region (elevation 1000 m above the sea level) of Sri Lanka (Jayathilaka *et al.* 1981, Premarathna *et al.* 2005), limited research has been conducted to date on heavy metal contamination in the low country region of Sri Lanka (Premarathne *et al.* 2011).

Farmers in the low country region of Sri Lanka mainly use animal fertilizers such as cattle and poultry manure during the cultivation of green leafy vegetables, including *Basella alba* L (Premarathna *et al.* 2011). These natural fertilizers contain relatively high concentrations of Zn, Se, Mn, Co, As and Fe (Bolan *et al.* 2010). Consumption of heavy metal contaminated vegetables is one of the direct pathways of heavy metal entry to the food chain (Sharma *et al.* 2009; Chen *et al.* 2014). World Health Organization (WHO) has proposed maximum permissible limits for different heavy metals in soil and vegetables (Chiroma *et al*. 2014). Leafy vegetables are known to accumulate heavy metals (Neilson and Rajakaruna 2014); several studies have been carried out to assess heavy metals in vegetables (Guptha *et al*. 2010; Abah *et al*. 2014, Kananke *et al.* 2014; Rajapakshe *et al*. 2011; Premarathna *et al.* 2005), including those grown in Sri Lanka. Results of studies conducted in Sri Lanka have shown that heavy metals present in several leafy vegetables grown in certain areas of the country are above the maximum permissible limits set by WHO (Guptha *et al*. 2010; Abah *et al.* 2014; Kananke *et al.* 2014; Rajapakshe *et al.* 2011; Premarathna *et al.* 2005; Jayasinghe *et al.* 2005; Rathnayake *et al.* 2004).

Silicon (Si) is present as silicate minerals in the Earth's crust and these minerals undergo chemical and physical withering, finally getting incorporated in to the soil. In the soil solution, Si is present as uncharged monomeric orthosilicic acid (H_4SiO_4) with concentration in the range of 0.1-0.6 mM (Epstein *et al.* 1994; Ma *et al*. 2002). Although Si is the second most abundant element on the Earth's crust, plants can absorb Si only in the form of orthosilicic acid which is quickly precipitated as amorphous Si after absorption (Lux *et al.* 2003). Therefore, amorphous Si is the only form of Si present in plants (Ding *et al.* 2008). Amorphous Si particles that are precipitated in plant cells are called phytoliths but the locations and the proportions can vary with the plant species as well as the age of plants (Ponzi *et al.* 2003; Sangster *et al.* 2001). Number of studies has shown that metal toxicity can be alleviated with the application of small quantities of Si (Neumann *et al.* 2001; Ma *et al.* 2002; Rogalla *et al.* 2002; Liu *et al.* 2009; Ma *et al*. 2008). Ma and Takahashi (2002) have showed that after the application of Si, the oxidizing capacity of roots increases so that ferrous ions oxidize to ferric ions, preventing the uptake of Fe. Manganese toxicity is also reduced with the application of Si because Mn binds to the cell wall, limiting cytoplasmic concentrations (Rogalla *et al.* 2002). Neumann and zur Nieden (2001) showed that with the application of Si, Zn can be co-precipitated with Si in cell walls, resulting in less soluble Zn in plants. It was also shown that silicic acid has the ability to decrease As accumulation (Ma *et al.* 2008).

As heavy metals have persistent and accumulative nature, they have the ability to concentrate through the food chain and reach lethal doses to humans (Sharma *et al.* 2009; Gall *et al.* 2005). Therefore, it is important to analyze heavy metals present in field grown vegetables such as *B. alba* to determine whether they comply with the permissible limits proposed by WHO. *Basella alba* is a green leafy vegetable with important mineral nutrients; people frequently include this leafy vegetable in their diet. We conducted the present study to investigate heavy metal absorption by *B. alba* and the effect of Si on heavy metal absorption by the plant.

2. Materials and Methods

2.1 Study site

The study was conducted at Sulthanagoda, Matara District, Southern Province of Sri Lanka. Average annual temperature and the annual rain fall of this area are 26.7 ^{0}C and 2327 mm, respectively.

2.2 Experimental design

Experiment was conducted using three different concentrations of Na_2SiO_3 (Si- 0 mg/L-control, Si- 50 mg/L and 100 mg/L). Each treatment was composed of three replicate beds (1m x 2m × 2m) arranged in a Randomized Complete Block Design (RCBD). Basal fertilizer (NPK) application was done as recommended by the Department of Agriculture, Sri Lanka (Bolan *et al.* 2010). Cattle and poultry manure were also applied as basal fertilizer. We commenced this work on 15.01.2015.

2.3 Plant material

Seeds of *B. alba* were from one mother plant of the farmer’s field at Sulthanagoda and they were sawn in a nursery and maintained for one month, and then seedlings were transferred into beds.

2.4 Preparation of plant and soil samples prior to the analysis of heavy metals (Before the application of Na_2SiO_3)

After one month of planting, three plants were pulled off randomly from each bed and washed with tap water, followed by three separate washes with deionized water and air dried. Then, roots, stems and leaves were separated from each plant, cut into small pieces, freeze dried for 5 days in separately labeled zip lock bags and stored at -4 ^{0}C until analyses were carried out.

10 g of three soil samples were taken from the middle of each bed up to 1 feet depth from the rhizosphere of the harvested plants, using a stainless steel spatula, mixed well and air dried for two days followed by oven drying at 70 ^{o}C for three days. Soil samples were kept in labeled zip lock bags until further analysis.

2.5 Application of Na_2SiO_3 and preparation of plant and soil samples prior to the analysis of heavy metals

Three different concentrations of liquid Na_2SiO_3 (Si - 100 mg/L, 50 mg/L and 0 mg/L) were added to the rooting zone of the plants in the three replicate beds as a spray application on a weekly basis for two months. After one month of Na_2SiO_3 application, three plants from each bed were pulled out and washed with tap water followed by three washes with deionized water and air dried. Then the roots, stems and leaves were separated and cut into small pieces and freeze dried for five days. Labeled samples were stored at -4 ^{o}C until analyses were carried out.

10 g of three soil samples from each bed were collected, air dried, and then oven dried for three days at 70 ^{o}C. Dried soil samples were kept in labeled zip lock bags. Same procedure was repeated for the samples collected after second month.

2.6 Analysis for heavy metals

Soil Samples:
Soil samples were crushed, sieved (less than 2 mm pore size) and mixed to obtain homogenized mixtures. Approximately 4 g of soil sample was ashed using a muffle furnace for 6-8 h by controlling the temperature within the range of 490-500 ^{0}C. Subsequently, the sample was cooled down to room temperature and about 10 mL of analytical grade HCl: HNO_3 (1:3) mixture was added and the resultant sample, filtered using 0.45µm filter paper,

transferred in to a 50 mL volumetric flask and diluted up to the mark with deionized water.

GF-AAS was calibrated by using Fischer Scientific calibration standards and the results were obtained from Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS) [model GBC 932+, Australia].

Plant Materials:
Plant materials were ground and mixed well to get homogenized mixtures. The procedure used for soil samples was followed for acid digestion of plant materials, but, only 10 mL of HNO_3 was added instead of $HCl:HNO_3$ (1:3) mixture. Concentration of heavy metal ions were obtained from Graphite Furnace Atomic Absorption Spectrophotometer after calibration.

2.7 Transfer factors

In order to understand the translocation of Fe into different plant parts, transfer factors among different plant parts:soil were calculated.

$$\text{Fe transfer factor of leaves:soil} = \frac{\text{Concentration of Fe in leaves}}{\text{Concentration of Fe in soil}}$$

3 Results and Discussion

3.1 Heavy metals present in soil

As shown in Table 1, higher concentrations of Fe, Cr and Pb were detected compared to Cd in all the soil samples tested. Concentrations of Fe, Cr, Pb and Cd in field soils were 29755.30 ± 292.02, 32.99 ± 0.97, 26.01 ± 1.02, 0.13 ± 0.004 µg/g, respectively. All the concentrations were below the maximum permissible limits set by WHO (Chiroma *et al.* 2014).

Premarathna *et al.* (2011) have reported heavy metal concentrations in certain crops and soil in up-country and some parts of low-country regions of Sri Lanka. They have observed much higher concentrations of Cd and Pb in soil samples than those observed in the present study. According to their results, concentration of Cd in Sedawatta in Colombo district, Sri Lanka has been in the range of 0.61-3.28 µg/g whereas those in Kandapola in Nuwara Eliya District, Sri Lanka has been in the range of 0.39-1.96 µg/g. Concentrations of Pb were reported to be 39-118 and 27-97 µg/g respectively. Some of these reported concentrations in up- and low-country soils of Sri

Lanka are higher than the maximum permissible limits. In contrast, the soil samples analyzed in the present study showed negligible amount of Cd and considerably lower amount of Pb when compared to the maximum permissible limits (Chiroma *et al.* 2014).

Table 1. Heavy metals present in soil after the Silicon (Si) treatments.

	Metal concentration in soil (µg/g)		
	Before spraying Na_2SiO_3	**1 month after spraying Na_2SiO_3**	**2 months after spraying Na_2SiO_3**
Fe			
Si (100 mg/L)	28582.31 ± 290.45 [a]	27877.96 ± 290.45 [d]	27240.80 ± 296.86 [g]
Si (50 mg/L)	27362.71 ± 287.40 [b]	25188.91 ± 298.52 [e]	27171.35 ± 286.38 [g]
Si (0 mg/L)	29755.30 ± 292.02 [c]	29445.89 ± 294.41 [f]	29523.45 ± 293.61 [h]
Cd			
Si 100 mg/L	0.13 ± 0.004 [a]	0.11 ± 0.004 [c]	0.11 ± 0.004 [d]
Si 50 mg/L	0.00002 ± 0.004 [b]	0.00012 ± 0.004 [b]	0.00002 ± 0.004 [e]
Si 0 mg/L	0.13 ± 0.004 [a]	0.11 ± 0.004 [c]	0.00002 ± 0.004 [d]
Cr			
Si 100 mg/L	35.56 ± 1.02 [a]	30.89 ± 1.03 [cd]	34.56 ± 1.06 [f]
Si 50 mg/L	29.19 ± 1.00 [b]	29.52 ± 0.98 [ce]	29.54 ± 1.04 [g]
Si 0 mg/L	32.99 ± 0.97 [a]	30.91 ± 1.01 [de]	32.56 ± 1.06 [fg]
Pb			
Si 100 mg/L	28.31 ± 1.11 [ab]	27.90 ± 1.15 [d]	28.10 ± 1.29 [f']
Si 50 mg/L	25.50 ± 1.13 [ac]	21.15 ± 1.27 [e]	23.79 ± 1.53 [g]
Silicon 0 mg/L	26.01 ± 1.02 [bc]	24.43 ± 1.13 [de]	21.59 ± 1.22 [g]

The values which share the same letter have no significant difference. Comparisons were carried out for each metal separately.

Based on a one-way ANOVA, a significant difference ($p<0.05$) was noted for soil Fe among the treatments (100 mg/L, 50 mg/ L, and the control) during both application stages: before spraying (28,582.31 µg/g, 27,362.71 µg/g, 29,755.30 µg/g, respectively) and one month after spraying (27,877.96 µg/g, 25,188.91 µg/g, 29,445.89 µg/g, respectively). Two months after spraying, a significant difference was noted in both treatments, including 100 mg/L (27,240.80 µg/g) and 50 mg/L (27,171.35 µg/g) compared to the control (29,523.45 µg/g). Furthermore, there was no significant difference in the Fe concentrations in soil among three application stages.

3.2 Fe accumulation in different plant parts of *Basella alba*

Table 2 summarizes Fe transfer factors of different plant parts:soil. All transfer factors are very small thus only small portion of Fe present in soil has been translocated to plant tissue. Transfer factors decreased in the order of root:soil > stems:soil > leaves:soil. In other words, translocation of Fe decreased from the bottom to the top of the plant. About one thousandth of Fe in soil was transferred to leaves of *B. alba*.

Table 2. Fe transfer factors of different plant parts:soil of *B. alba*.

Plant part: Soil	Fe Transfer factors
Leaves: Soil	1.30×10^{-3}
Stems:Soil	1.90×10^{-3}
Roots:Soil	9.70×10^{-3}

Although heavy metals were present in the soil, only Fe was absorbed by *B. alba*. All the other heavy metals were below the detection limit. Kananke *et al.* (2014) have reported heavy metal accumulation in some leafy vegetables including *B. alba* collected from open market sites in Piliyandala area in Colombo district, Sri Lanka. According to their report, concentrations of Ni, Cd, Cr and Pb in *B. alba* were above the maximum permissible limits set by FAO/WHO (Chiroma *et al.* 2014).

Si-mediated heavy metal absorption has been observed in many plants (Rogalla *et al*. 2002; Neumann *et al*. 2001; Liu *et al*. 2009; Ma *et al*.2008; Wang *et al.* 2000). Wang *et al.* have (2000) reported a reduction of Cd uptake in rice with the application of Si (Wang *et al.* 2000). Similarly, Si mediated Cd uptake has been observed in other plants such as strawberry, cucumber, and maize (Nwachukwu *et al.* 2007; Chiroma *et al.* 2014; Wijewardena *et al.* 2004).

Table 3 shows that the Fe content in different plant tissues are significantly different among two treatments of Na_2SiO_3 and the control, indicating differences in the capacity for Fe uptake. Increase in accumulation of Fe was observed during the first month despite the Na_2SiO_3 treatment. It may be due to increase in accumulation of Fe with time. However, after two months of Na_2SiO_3 application, Fe accumulation was decreased. Fe concentrations of leaves after two months of Si applications (both in Si-100mg/L and Si-50 mg/L) are less than before treatments. For example, Fe concentrations in leaves treated with Si-100 mg/L before and after application are 55.02 ± 2.05 μg/g and 16.27 ± 2.30 μg/g respectively. Further, Table 3 shows that the application of higher concentration of Si lowers the capacity for Fe absorption. For example, Fe concentrations of leaves treated with Si – 100 mg/L before and after the application and the leaves treated with Si-50 mg/L before and after the application are 55.02 ± 2.05 μg/g, 16.27 ± 2.30 μg/g respectively, and 24.13 ± 1.99, 13.27 ± 2.09 μg/g, respectively. There is no significant difference in leaves treated with Si-0 mg/L (control) before and after the application.

Table 3. Fe absorption capacities of different plant parts of *B. alba* after treating with different concentrations of Na_2SiO_3 at different time periods.

	Fe concentration in plant samples (μg/g)		
	Na_2SiO_3 (Si-100 mg/L)	**Na_2SiO_3 (Si - 50 mg/L)**	**Control (Si -0 mg/L)**
Before spraying Na_2SiO_3			
Leaves	55.02 ± 2.05 [a]	24.13 ± 1.99 [d]	32.90 ± 2.26 [g]
Stem	67.96 ± 2.01 [b]	60.95 ± 2.15 [e]	69.04 ± 2.30 [h]
Root	394.73 ± 2.11 [c]	233.24 ± 2.19 [f]	198.53 ± 1.87 [i]
1 month after spraying Na_2SiO_3			
Leaves	114.33 ± 1.77 [a]	74.21 ± 2.79 [d]	37.62 ± 2.70 [g]
Stem	363.73 ± 1.77 [b]	133.93 ± 2.82 [e]	123.56 ± 3.16 [h]
Root	472.32 ± 1.77 [c]	545.04 ± 2.81 [f]	462.57 ± 2.75 [i]
2 months after spraying Na_2SiO_3			
Leaves	16.27 ± 2.30 [a]	13.27 ± 2.09 [d]	34.54 ± 2.33 [g]
Stem	84.89 ± 2.31 [b]	129.91 ± 2.35 [e]	82.46 ± 2.53 [h]
Root	285.22 ± 2.14 [c]	741.99 ± 2.16 [f]	341.49 ± 2.29 [i]

The values which share the same superscript letters have no significant ($p<0.05$) difference. Comparisons were made separately for different spraying stages.

Our results show a significant difference in Fe accumulation among different plant tissues including stem, leaves and roots collected from the plants exposed to different treatments. Our results also show that Fe accumulation in different plant parts is significantly different ($p<0.05$) within treatments. When comparing the accumulation of Fe in a particular plant part within different treatments, significant differences were noted in all the plant parts. Also, when comparing the accumulation of Fe among different treatments under different time periods a significant difference was shown in all the plant parts.

We document that the highest Fe concentrations are found in the roots and the lowest concentrations are found in the leaves in all three treatments under all three application stages.

4 Conclusion

Concentrations of Fe, Cr, Pb and Cd in the soils tested were 29755.30 ± 292.02, 32.99 ± 0.97, 26.01 ± 1.02, 0.13 ± 0.004 µg/g, respectively. Concentration of Cd in the tested soil is much less, compared to other heavy metals. All these concentrations are below the maximum permissible limit reported by FAO/WHO. Therefore, the soil samples from our study site were not heavily contaminated by the studied heavy metals. Among the metals studied, only Fe was absorbed by *Basella alba*. Transfer factors calculated for Fe among different parts of the plant:soil revealed that translocation of Fe is different among individual parts of the plant. Our experimental results (we had to limit up to three replicates due to high cost of analysis) also showed that application of Na_2SiO_3 reduces Fe absorption capacity of *B. alba*. There was a significant difference in Fe accumulation in different plant tissue treated with different treatments of Na_2SiO_3. Fe accumulation in different plant parts was significantly different within treatments and among different treatments from three different application stages. Between the two concentrations of Na_2SiO_3 used, Si-100 mg/L reduced the absorption capacity more than that of Si-50 mg/L. Therefore, we can conclude that Fe absorption in field grown *B. alba* can be decreased by treating the plants with Si-100 mg/L of Na_2SiO_3 than treating with Si-50mg/L of Na_2SiO_3.

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