

Reducing the Risk of Cd in Lettuce (*Lactuca sativa L.*) via Silicon Application

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CONTENT of Cd in fresh vegetables can be decreased by application of silicate fertilizers which decrease Cd concentration in the plant tissues. Lettuce plants (*Lactuca sativa L.*) which were evaluated in a pot experiment were grown on an artificially contaminated soil with Cd at 0, 5, 10 and 15 mg Cd kg⁻¹ applied as cadmium sulfate hydrate (3 CdSO₄· 8 H₂O). Three levels of Si (0, 100 and 150 mg Si kg⁻¹) were applied as calcium silicate (CaSiO₃) to assess Cd status in soil and plant. Si increased plant dry weight and the highest dry weight was given by the treatment of 5mg Cdkg⁻¹ and 150 mg Si kg⁻¹. Si-treated soil showed greater Cd than untreated one. All Cd-treated soils had considerably high contents of DTPA-extractable Cd in soils. Relationships between Cd and Si treated soils and Cd concentration in plant roots show that increased Si and Cd application leads to decreasing the Cd in the root after harvesting. The bio-concentration factor (BCF) was calculated to assess Si influences on Cd uptake. The BCF is a ratio of metal content in plant roots to its content in soil, in most cases the BCF < 1 and decreased gradually with the Si additions.

The treatment of 150 mg Si kg⁻¹ proved an effective level of Si that decreased Cd uptake in lettuce plant.

Keywords: Cadmium, Contaminated soil, Lettuce, Immobilization, Silicon nutrition.

Cadmium can cause an adverse effect to plants. Its sources are variable including various factories as smelters, car exhausts, plastic, and battery factories stations, heating systems, metal-working industries or urban traffic. It is widely used in pigments, plastic stabilizers and nickel-cadmium batteries (Sanita di Toppi and Gabrielli, 1999). Due to its easier solubility in water it is an extremely toxic element (Pinto *et al.*, 2004). Existence of Cd in edible plant parts is dangerous for human (Wagner, 1986). Chaney (1980) classified Cd along with Mo, Co and Se in one group of toxic elements. The other group includes Pb and Hg both groups accumulate in plants and cause phytotoxicity. Vegetables are vital

sources of vitamins and other beneficial nutrients. The leafy vegetables are mostly a major source of Cd along with other heavy metal (Mapanda *et al.*, 2007 and Wang *et al.*, 2007). John and Laerhoven (1976) reported that Cd can accumulate in lettuce and other vegetables. Also, rice can be a source of Cd intake for humans especially in high rate-rice consumption countries (Loutfy *et al.*, 2006 and Zheng *et al.*, 2007). It is very important to decrease Cd in plants that accumulate it in their leaves and grains to safe levels for protecting human health (Wagner & Yeargan, 1986 and Codex, 2006). Abd El Gawad *et al.* (2007) reported that Cd in Fayoum District soils showed indication of an increase to the maximum permissible limits. Application of P showed a tendency of increased Cd in plants (Pezzarossa *et al.*, 1993). Thus, increased Cd in the soil leads to increased Cd uptake in plants (Zarcinas *et al.*, 1999). High concentration of Cd in the soil is associated with high Cd content in various crops and distinctive plant parts. For example, Cd could be translocated from the rhizosphere to the aerial parts of tomato plants (Moral *et al.*, 1994). Also, Cd could be accumulated in leaves of lettuce content (Lehoczyk *et al.*, 1996).

Silicon is an abundant element in soils and is taken up by higher plants (Epstein, 1999 and Jarvis, 1987). It is important for plants growth (Epstein and Bloom, 2005). Si can help plants to maintain cell rigidity (Hossain *et al.*, 2002), and increases photosynthesis (Al-Aghabary *et al.*, 2004 and Liang *et al.*, 2007). Also, Si could remediate nutrient imbalance in plants (Gao *et al.*, 2006, Romero-Aranda *et al.*, 2006 and Liang *et al.*, 2007). Moreover, it could decrease Cd uptake by plant (Shi *et al.*, 2005, Rogalla & Romheld, 2002 and Liang *et al.*, 2007).

Silicon may halt Cd accumulation in cereals and legumes through a multiple processes. Stabilization of Cd may occur upon adding Si in soluble forms of silicate to soil (Nwugo and Huerta, 2008, Zhang *et al.*, 2008, Shi *et al.*, 2005 and Feng *et al.*, 2010) or upon applying industrial by-product materials containing silicon in many forms. Such materials include the basic slag by-product of the steel industry. Increased plant tolerance to existence of high contents of Cd in the rhizosphere may be augmented by adding Si to the root media (Nwugo & Huerta, 2008 and Vaculik *et al.*, 2012)

The purpose of the current study was to assess the effect of Si application on alleviation of Cd accumulation and toxicity in lettuce.

Materials and Methods

A pot experiment was conducted; a randomized complete block design, factorial (2 factors) to assess the possible alleviation effect of Si additions to Cd-contaminated soil on lettuce (*Lactuca sativa* L.) Cd-uptake. The treated sandy loam soil was contaminated with Cd and then treated with different Si additions (see Table 1 for main soil properties). Factors of the experiment and their

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treatments were as follows: i) Si additions of 0, 100 and 150 mg Si kg⁻¹.ii) Cd induced contamination by 4 treatments of 0, 5, 10 and 15 mg Cd kg⁻¹.The treatments were replicated three times. Therefore, the total number of pots was 36 (3 Si X 4 Cd X 3 replicates). A surface soil (0-30 cm) was collected from an arable field in Kafr El-Hamam Research Station of the Agriculture Research Center, El Sharkia Governorate, Egypt.

Lettuce seeds were washed then soaked in distilled water at 30°C overnight , then sterilized with sodium hypochlorite solution (1%) for 15 min followed by thorough washing with distilled water. PVC pots of 8.0 kg soil were used. Five seeds were sown per pot; then when seedlings were 2cm height, they were thinned to 2 plants per pot. Pots were irrigated using tap water (of 0.51 dSm⁻¹ and pH of 7.35 and contents of 1 µg Cd L⁻¹) to keep the moisture content at about 80% of the water holding capacity. Si and Cd were added as calcium silicate (CaSiO₃), and cadmium sulfate hydrate (3 CdSO₄. 8 H₂O), respectively.Plants were allowed to grow for 65 days after which they were removed from the soil, and were weighed. Collected plants were dried in oven at 70°C overnight, and then dry weight was recorded. Soil and plant samples were taken at the end of the experiment for analyses.Soil was analyzed according to Page *et al.* (1982) and Klute (1986). Plant samples were analyzed for Cd according to Stewart (1989) and measured by atomic absorption spectrophotometer. Extractable Cd was extracted from the soil using a mixture of 0.005 M DTPA (DiethethyleneTriamine Penta Acetic acid) + 0.1 M TEA (Triethanolamine) + 0.01 M CaCl₂ at pH 7.3 according to Lindsay and Norvell (1978).

TABLE1. Main properties of experimental soil

pH*	EC* dS m ⁻¹	CaCO ₃ (gkg ⁻¹)	O.M. (gkg ⁻¹)	Particle size distribution %			
				Sand	Silt	Clay	Texture *
7.6	1.16	16.77	2.4	74.14	8.06	17.8	sandy loam

*: pH in 1:2.5 soil: water suspension; EC: of paste extract; Texture: according to the International Soil Texture Triangle (Farshad, 1984).

Results and Discussion

Plant growth

Generally, plant (root + shoot) dry biomass weight was decreased by the addition of Cd with and without Si treatments. Specifically, the lowest plant growth was associated with the highest Cd addition under conditions of no Si addition (Table 2). A considerable decrease of biomass by 68.4% was observed as an indicative of a severe adverse effect of Cd on lettuce growth. The biomass decrease due to Cd was advanced with increased addition of Cd averaging of 20.2, 40.8 and 54.5 % under 5, 10 and 15 mg Cd kg⁻¹ application, respectively. This biomass decrease was particularly major when no Si was added, *i.e.* 29.7, 50.7 and 68.4 compared with lower decreases under conditions of Si at its high rate , *i.e.* 7.9 , 31.3 and

73.1 % caused by the low, medium and high Cd rate, respectively. Sani-Ahmed *et al.* (2015) observed severe adverse effect of lettuce plants grown in nutrient solutions containing 3 to 12 mg Cd L⁻¹. Also, Chiang *et al.* (2006) noted biomass decrease in plants under presence of Cd. Indirect toxicity to plants could occur through occupation of Cd in place of useful plant nutrients (Taiz and Zeiger, 2002). Inhibition of translocation of plant nutrients and some organic acids can occur due to the existence of Cd in plant or in its root vicinity (Jadia and Fulekar, 2008). Results of the experiment show that the adverse effect of Cd was alleviated by the application of Si. The increases of plant growth due to Si were averaged as 20.7 and 38.8% by the low and high Si rates, respectively. Although such increase was slight under no application of Cd giving no more than 10% , it was considerably greater in presence of the Cd-high rate and amounted to 59.7 and 97.7% under the low and high Si additions, respectively. The positive response to Si additions under presence of Cd was reported by Horst *et al.* (1978) on beans, Cunha *et al.*, (2009) and Dresler *et al.* (2015) on maize who presented different hypotheses of Cd and Si interaction.

TABLE 2. Dry Weight (g plant⁻¹) of lettuce plants as affected by Cd and Si added to the soil

Cd addition (mg kg ⁻¹ soil)	Si addition(mg kg ⁻¹ soil)			Mean
	0	100	150	
0	30.74	33.12	33.72	32.53
5	21.62	25.16	31.07	25.95
10	15.14	19.42	23.18	19.25
15	9.70	15.50	19.18	14.79
Mean	19.30	23.30	26.79	
LSD 0.05 : Cd = 2.27 Si = 1.11 Cd×Si = 2.21				

Cadmium concentrations in plant (shoot + root)

Under no Cd addition, Cd concentration in lettuce did not exceed 2 mgkg⁻¹, whereas under presence of Cd it was notably high (Table 3) Cd concentration of 17.33 mgkg⁻¹ was associated with the addition of 100 mg Si to the soil of 5 mg Cd kg⁻¹. The positive effect of Si on decreasing accumulation of Cd in plant is well demonstrated. Low Cd accumulation in plant was most noticed under the soil which is contaminated by the high rate of Cd. Decrease of Cd accumulation in plants due to Si additions to the Cd-contaminated soils was reported for maize (Cunha *et al.*, 2009 and Dresler *et al.*, 2015), and beans (Horst *et al.*, 1978). Zhang *et al.* (2008) stated that Si hinders Cd translocation in plants. Van-der- Vorm (1980) found that Si was taken up by plants that underwent gradual transition and metabolic absorption was depending on its concentration in the root media for soybean, sunflower, wheat, sugarcane and rice. Cunha and Nascimento (2009) reported that the taken up of Si by plant was not retained in the roots but was translocated to above-ground plant parts under the effect of Si. The alleviation effect of Si on the uptake of the toxic heavy metals was attributed to anti-oxidative reactions occurring in plant tissues. Naeem *et al.* (2015) noted a retardation of Cd transportation from roots to shoots of wheat under the effect of Si application to the soil.

TABLE 3. Cd content in lettuce plants (mg kg^{-1} dry weight) as affected by Cd and Si additions to soil

Cd addition (mg kg^{-1} soil)	Si addition(mg kg^{-1} soil)			Mean
	0	100	150	
0	1.61	0.85	0.68	1.05
5	35.73	19.42	17.33	24.16
10	53.66	36.99	29.47	40.04
15	69.11	52.09	40.49	53.90
Mean	40.03	27.34	21.99	
LSD 0.05 : Cd = 1.76 Si = 2.22 Cd×Si = 4.44				

DTPA- extractable Cd in the soil after harvest

All Cd-treated soils had considerably high contents of DTPA-extractable Cd. While the non-Cd-treated soils showed very low contents that is not exceeding $0.002 \text{ mg Cd kg}^{-1}$, the Cd-contaminated soils exhibited higher concentrations that were exceeding those by 175 folds up to as much as 768 folds. Increasing of the applied Cd-rates to the soil elevated the extractable Cd contents (Table 4). Also, results revealed that soils which received Si exhibited greater extractable Cd than non-Si-additions (Table 4). Shi *et al.* (2005) attributed the increase of Cd content in the rhizosphere of the Si-treated soils to the effect of Si in increasing Cd contents in the root system of plant (wheat). Kirkham (2006) reported that the applied Si to soil undergoes a variety of reactions leading to alleviation of Cd negative effect to soil and plant. On the other hand, Liang *et al.* (2005) noted no effect of Si on availability of Cd in a soil that is treated with 50 mg Si kg^{-1} .

TABLE 4. DTPA-extractable Cd fromsoil (mg kg^{-1}) after termination of lettuce growth as affected by Cd and Si addition to the soil

Cd addition (mg kg^{-1} soil)	Si addition(mg kg^{-1} soil)			Mean
	0	100	150	
0	0.001	0.001	0.002	0.001
5	0.176	0.365	0.437	0.326
10	0.401	0.549	0.606	0.519
15	0.525	0.730	0.769	0.675
Mean	0.278	0.414	0.457	
LSD 0.05 : Cd = 0.33 Si = 0.20 Cd×Si = 0.34				

Bio-concentration factor (BCF) of cadmium

The bio-concentration factor (BCF) is the ratio of metal content in the plant roots to that in soil (Malik *et al.*, 2010). Values of BCF for Cd in the current experiment varied by 0.26 to 1.52 (Fig. 1). The lowest BCF values were recorded by the treatment receiving the low rate of Cd and the high rate of Si addition, while the highest BCF values were obtained by treatment receiving the low rate of Cd with no Si applied. The second highest of 1.41 was that receiving neither Cd nor Si. Arifin *et al.* (2012) reported that BCF for Cd was decreased with increasing the Cd concentration in the growth media indicating a restriction in its translocation from soil to plant. Fitz and Wenzel (2002) found that plants exhibiting BCF values less than one are unsuitable for phytoremediation. Yoon *et al.* (2016) reported that wire grass (*Gentiana pennelliana* L.) plants accumulate high amounts of heavy metals in their roots whereas, most of the other plants such as nut grass (*Cyperus esculentus* L.) and *Bigodesbania* (*sesbania herbacea* L.) have low BCF indicating low ability to accumulate heavy metals.

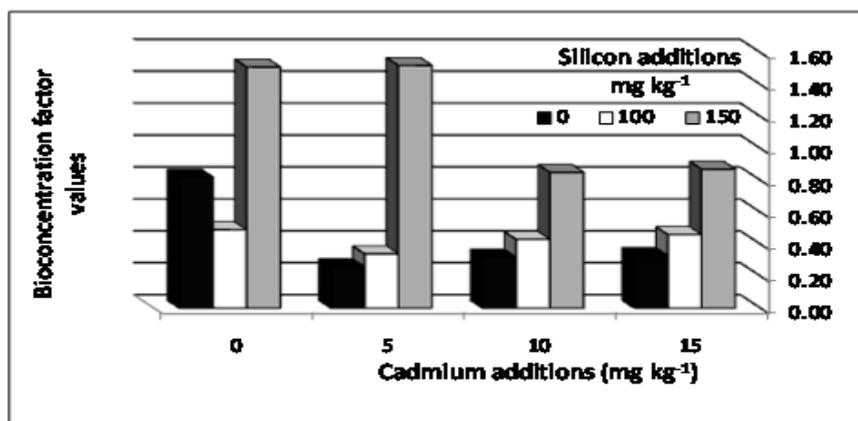


Fig.1. Bioconcentration factor as influenced by different Cd concentrations including (no Cd addition, 5, 10 and 15 mg Cd kg⁻¹) and different Si concentrations include (no Si addition, 100 and 150 mg Si kg⁻¹).

Correlations between application rates of Si and Cd contents in plant roots

Cadmium concentrations in lettuce's roots were presented in Fig. 2. Silicon application decreased Cd concentrations in roots as compared to the same Cd treatments without Si application similar results reported by Rizwan *et al.* (2016). Ma *et al.* (2001) proposed that Si deposition may decrease the transpiration in the surface layers of rice. Such a reduction in transpiration would reduce Cd translocation to shoots. Other mechanisms associated to the decrease in Cd toxicity when plants are supplied with Si could be (i) enhanced apoplasmic binding of Cd as proposed for maize (Vaculík *et al.*, 2012) and rice (Wang *et al.*, 2000 and Liu *et al.*, 2009), (ii) altered Cd subcellular distribution and (iii) reduced Cd content in cell organelle fractions of leaves as identified for peanuts (Shi *et al.*, 2010).

In addition relationships between Si application rate and Cd contents in roots are calculated in Fig. 3. After harvest, the Cd contents in lettuce showed significant negative correlations with the

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increasing rate of Cd application. The trend was most prominent with the high rate application of Si (150 mg kg⁻¹).

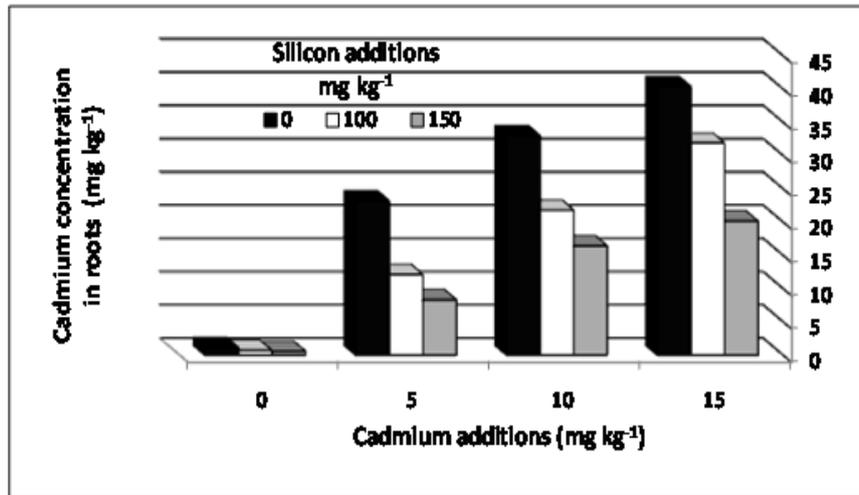


Fig.2. Cadmium concentrations in lettuce's roots in (mg kg⁻¹) after harvest under application of (no Cd addition, 10 and 15 mg Cd kg⁻¹ and silicon applications (no Si addition, 100 and 150 mg Si kg⁻¹))

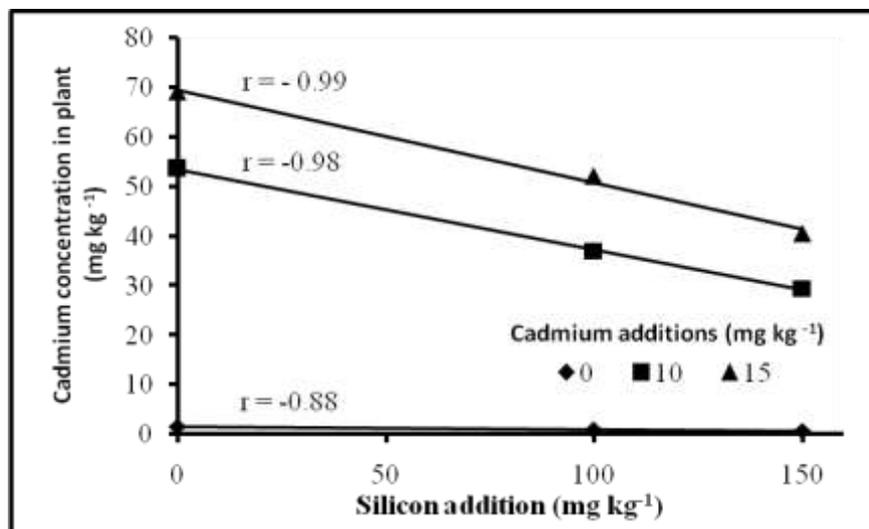


Fig. 3. Relationships between the cadmium applications (no Cd addition, 10 and 15 mg Cd kg⁻¹ and silicon applications (no Si addition, 100 and 150 mg Si kg⁻¹)) on Cd concentration in plant

Conclusion

Silicon has a positive effect on the growth of lettuce. As well, Si application gave high plant biomass and decreased Cd accumulation in plant. The higher Si application was more effective than the low rate. The bio-concentration factor (BCF) was below 1. Therefore, Si can be used as a soil amendment to maintain the plant health and minimize the accumulation of Cd plant.

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(Received: 26/6/2016;
accepted: 9/10/2016)

خَفْضُ خَطَرِ زِيَادَةِ إِمْتِصَاصِ الكَادَمِيُومِ فِي نَبَاتِ الخَسِّ (*Lactuca sativa*) عَنِ طَرِيقِ السَّلِيلِيكُونِ

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محتوى الكادميوم في الخضروات الطازجة قد يقل من خلال الإضافات المفيدة والمغذيات النباتية مثل السليكون حيث يؤدي الى مسك الكادميوم في التربة وكذلك تخفض من الكادميوم الممتص في انسجة النبات . وقد تم تقييم صنف من الخس (خس البركة L). في تجربة اصص ونمت على كادميوم مضاف للتربة تحت مستويات (صفر ، 5 ، 10 ، 15 ملجم كادميوم/كجم تربة) مضاف من هيدرات سلفات الكادميوم و ثلاثة مستويات من السليكون (صفر ، 100 ، 150 ملجم سليكون/كجم تربة) مضاف من سليكات الكالسيوم $CaSiO_3$ لدراسة حالة الكادميوم في التربة والنبات. وأضافة السليكون أدت لتحسين الوزن الجاف للنبات و أعلى وزن جاف للنبات كانت مع معاملة الكادميوم 5 ملجم كادميوم/كجم + وسليكون 150 ملجم سليكون/كجم و زيادة الاضافة من السليكون تؤدي الى زيادة الوزن الجاف للنبات. ومن ناحية أخرى الاضافة من السليكون تؤدي الى زيادة تركيز الكادميوم في التربة و سجلت مع المعاملة 15 ملجم كادميوم /كجم تربة + 150 ملجم سليكون/كجم تربة. التربة التي اخذت اظهرت ارتفاع في محتواها من الكادميوم عن التي لم تاخذ. كل معاملات التربة بالكادميوم اعطت ارتفاع في الكادميوم المستخلص بال DTPA. العلاقة بين الكادميوم والسليكون المضاف ومحتوى جذور النبات أظهرت ان الزيادة في السليكون والكادميوم المضاف ادت الى انخفاض تركيز الكادميوم في جذور الخس بعد الحصاد. من أجل تقييم تطبيق السليكون لتقليل و علاج النباتات، و تم استخدام مؤشر و هو معامل التركيز الاحيائي (نسبه تركيز المعادن في الجذور النباتية لتركيزها في التربة) حيث كان معامل التركيز الاحيائي > 1 في معظم الحالات و القيم أنخفضت تدريجيا في الاضافات المرتفعه بأضافة السليكون. أضافة السليكون أدت الى انخفاض تركيز الكادميوم في صنف الخس عن طريق في حدوث انخفاض تركيز الكادميوم في النبات و حركة الكادميوم من التربة للنبات. أضافة السليكون في مستوى 150 ملجم سليكون/كجم تربة أثبتت فعالية كبيرة في تقليل تركيز الكادميوم في الخس.