

Reuse of wastewater for irrigating tomato plants (*Lycopersicon esculentum* L.) through silicon supplementation

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ABSTRACT

When untreated wastewater is used for the irrigation of crops, dissolved heavy metals may impose negative impacts on plant growth and pose health risks. However, silicon nutrition may improve plant tolerance to metal toxicity through external and internal plant mechanisms. This work aims at investigating the effects of silicon on copper, nickel, manganese, cadmium and zinc detoxification in tomato plants. The plants were grown for 50 days by irrigating with tap water and wastewater and having silicon added at 25, 50 and 75 mg kg⁻¹ soil. Results revealed that wastewater irrigation caused an increase of 277–480% in copper, 178–233% nickel, 355–680% manganese, 500–900% cadmium and 117–337% zinc in tomato plants compared to tap water irrigation. The root:stem metal ratios showed that a major portion of absorbed metals was translocated to aerial plant parts when wastewater was applied without silicon. However, silicon supplementation precipitated the metals in soil and influenced their uptake and partitioning within the plant body. The shoot dry matter of tomato plants was negatively correlated with wastewater-induced stem metal concentrations. This study suggests that silicon-assisted metal tolerance of tomato plants was attributed to metal precipitation in soil, complexation in roots and, hence, reduction in their translocation to stems and leaves.

Key words | irrigation, metal toxicity, metal translocation, silicon, tomato, wastewater

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INTRODUCTION

Wastewater is a complex resource with both advantages and disadvantages for its use in irrigating crops. The main reasons for the use of wastewater are lack of alternative water resources, the reliability of wastewater supply, the nutrient value and proximity to urban markets. Rattan *et al.* (2005) reported that sewage water is a rich source of essential plant nutrients and organic matter, and hence it improves soil fertility and soil structure. Farming communities in semi-urban areas are using wastewater for irrigation due to its easy availability. However, there are many concerns about the environment, crop quality and soil sustainability in using such untreated wastewater. It may contain heavy metals which impose

negative impacts on plant growth and soil health (Bazai & Achakzai 2006).

According to Kashif *et al.* (2009), when untreated wastewater is used, the toxic substances are cycled into the soil and plant systems. Among these toxic substances, cadmium (Cd), zinc (Zn), manganese (Mn), copper (Cu) and nickel (Ni) are the major threats to plants, soil, the environment and human health. It has been reported that plants growing in a metal-polluted environment exhibit altered metabolism (Liang *et al.* 2007), growth reduction (Kashif *et al.* 2009), lower biomass production (Zhu *et al.* 2004), oxidative damage and metal accumulation (Shi *et al.* 2010). In this respect, there is a growing need to

manage metal ions of wastewater before its use in irrigating agricultural land.

There are a number of strategies available, including cementation, solvent extraction, evaporation, ion exchange, biological treatment and membrane processing, to reduce the hazardous elements in wastewater. However, these conventional technologies have raised the issues of efficacy when faced with low metal concentrations, high start-up or operating costs and low metal selectivity. Recently, it has been suggested that silicon (Si) may be used to ameliorate heavy metal toxicities in a wide variety of crops (Liang *et al.* 2005; Wang & Han 2007; Hashemi *et al.* 2010). Different scientists have proposed different mechanisms through which Si may ameliorate the toxicity of heavy metals in plants. For instance, Neumann & Nieten (2001) reported the accumulation of zinc silicate in the vacuole and cell wall of *Cardaminopsis halleri* leaves as a tolerance mechanism to cope with the metal toxicity. Rogalla & Römhild (2002) observed that cucumber (*Cucumis sativus*) plants treated with Si presented decreased Mn concentration in the cell wall. Okuda & Takahashi (1962) demonstrated that in rice plants Si reduced Mn uptake by promoting oxidizing power of the roots, while in bean plants Si did not reduce Mn uptake, but resulted in a homogeneous distribution of Mn in leaf blade (Williams & Vlamis 1957). Horst *et al.* (1999) found that Si modified the cation binding properties of the cell wall and led to a lower apoplasmic Mn concentration in cow pea. Iwasaki *et al.* (2002) demonstrated that Si maintained the reduced conditions that promoted the oxidation of excess Mn and its interaction with phenolics in the solution phase of apoplast. Similarly, it has been reported that the alleviation of Mn toxicity through Si application in cucumber plants was ascribed to a significant reduction in membrane lipid peroxidation (Shi *et al.* 2005).

Hammond *et al.* (1995) found that addition of Si reduced Al uptake into the roots. Cocker *et al.* (1998) reported that aluminosilicate or hydroxyaluminosilicate of low solubility is formed within the root cell wall which reduced the concentration of free toxic Al ions. Kidd *et al.* (2001) suggested that Si stimulates exudation of phenolics from maize roots which could chelate Al in rhizosphere and hence reduce its absorption. Neumann & Nieten (2001) reported that Zn co-existed with Si in cytoplasm. It was suggested that

the formation of Zn-silicate could be part of the mechanisms of Zn toxicity alleviation. Chen *et al.* (2000) reported that an increase in soil pH by the application of Si was dependable in reducing Cd uptake by plants. Shi *et al.* (2005) reported that heavy deposition of Si around the endodermis of rice roots physically blocked the apoplast bypass flow across the roots and restrained the apoplastic transport of Cd.

Previously, very little information was available regarding the role of Si in the management and reuse of wastewater having higher concentrations of metals for crop production, particularly vegetables, which were mostly irrigated with wastewater. Therefore, the objectives of this work were to investigate (1) the effect of wastewater on plant growth and metal accumulation in tomato plants and (2) the effect of Si on metal uptake and partitioning into different plant parts of tomato plants grown with wastewater irrigation.

MATERIALS AND METHODS

Plant culture

Twenty-five-day-old healthy and uniform seedlings of the tomato (*Lycopersicon esculentum* L.) variety 'Sahal' were transplanted into earthen pots, 28 cm in diameter and 30 cm deep, having a basal hole and filled with 12 kg of well-prepared soil in each pot. The soil was silt loam having electrical conductivity (EC) 0.97 dS m⁻¹, pH 7.9, sodium adsorption ratio (SAR) 9.8 (mmol L⁻¹)^{1/2}, organic matter 0.62%, Cu 12.9 mg kg⁻¹, Ni 13.1 mg kg⁻¹, Mn 52.9 mg kg⁻¹, Cd 0.96 mg kg⁻¹ and Zn 44.6 mg kg⁻¹. Initially, two plants were transplanted into each pot and then thinned to one after 15 days. A measured quantity (800 mL) of tap water and wastewater was applied to respective treatments at appropriate intervals. Analysis of tap water and wastewater is shown in Table 1. The wastewater irrigated plants were treated with 25, 50 and 75 mg Si kg⁻¹ soil supplied as sodium silicate. Recommended doses of nitrogen (110 mg N kg⁻¹ soil) as urea, phosphorus (75 mg P₂O₅ kg⁻¹ soil) as diammonium phosphate and potassium (60 mg K₂O kg⁻¹ soil) as potassium sulphate were applied. Additional N added via diammonium phosphate was subtracted from urea. During the experimental period,

Table 1 | Chemical composition of tap water and wastewater used for irrigating tomato plants (*Lycopersicon esculentum* L.) along with maximum permissible limits (MPL) (Pescod 1992)

Constituents	Units	Tap water	Wastewater	MPL
Electrical conductivity (EC)	dS m ⁻¹	0.94	2.86	1.0
Sodium adsorption ratio (SAR)	(mmol L ⁻¹) ^{1/2}	8.45	12.65	8.0
Residual sodium carbonate (RSC)	Meq L ⁻¹	1.13	3.60	2.5
pH	–	7.1	7.3	6.5–8.4
Copper (Cu)	mg L ⁻¹	0.05	0.62	0.20
Nickel (Ni)	mg L ⁻¹	0.08	0.73	0.20
Manganese (Mn)	mg L ⁻¹	0.01	0.86	0.20
Cadmium (Cd)	mg L ⁻¹	0.01	0.32	0.01
Zinc (Zn)	mg L ⁻¹	0.09	3.25	2.0

the maximum temperature ranged from 7 to 35 °C and the minimum from 5 to 25 °C while relative humidity dropped from 95 to 68%.

Metal determination

Fifty days after transplanting, the plants were harvested, washed with distilled water and separated into leaves, stems and roots, and air dried. The plant samples were then dried at 70 °C in an oven (EYELA WFO-600ND; Tokyo Rikaikai Co., Ltd, Tokyo, Japan) for 48 hours to provide dry matter yield. Oven-dried plant samples were finely ground in a grinder fitted with stainless steel blades and chamber (MF 10 IKA-WERKE, GMBH & CO. KG, Germany). A 0.5-g portion of oven-dried plant samples was digested in a mixture of HNO₃ and HClO₄ (2:1) at 250 °C (Miller 1998). Digested plant samples were analysed for Cu, Ni, Mn, Cd and Zn on atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan). Post-harvest soil samples were collected and analysed for Cu, Ni, Mn, Cd and Zn on atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) according to Havlin & Soltanpour (1981).

Statistical analysis

The statistical analysis was conducted using M stat-C. The experiment was designed and analysed based on a completely randomized design with five replications. Data were subjected to analysis of variance (ANOVA) to compare the effects of treatments. The differences between the means

were compared using the least significant difference test (LSD, $P \leq 0.05$) (Steel *et al.* 1997).

RESULTS

Biomass accumulation and partitioning

Wastewater-induced metal toxicity produced a significant ($P \leq 0.05$) effect not only on biomass accumulation but also on its partitioning to shoots and roots of tomato plants (Table 2). The results revealed a reduction of 60% in shoot dry matter, 32% in root dry matter and 56% in total dry matter accumulation with untreated wastewater irrigation compared to the tap water control irrigation. However, when Si was used with wastewater, it interacted with metals, reduced their uptake and improved biomass accumulation. The shoot dry matter was increased by 98% with the application of 25 mg Si kg⁻¹ soil, 122% with 50 mg Si kg⁻¹ soil and 71% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. In comparison with wastewater irrigation having zero Si, the root biomass of Si-treated plants irrigated with wastewater was increased by 4.5, 29 and 13% with 25, 50 and 75 mg Si kg⁻¹ soil, respectively. Total dry matter was improved by 80, 103 and 54% with 25, 50 and 75 mg Si kg⁻¹ soil, respectively, as compared to wastewater irrigation without added Si. The present study exhibited a consistent increase in dry matter production with the increase of Si concentration up to 50 mg kg⁻¹ soil, beyond which it did not further increase by increasing Si to 75 mg kg⁻¹ soil

Table 2 | Biomass accumulation and partitioning in tomato plants (*Lycopersicon esculentum* L.) grown with tap water and wastewater irrigation by supplying different levels of silicon (values are the mean of five replications)

Treatments	Shoot dry matter (g plant ⁻¹)	Root dry matter (g plant ⁻¹)	Total dry matter (g plant ⁻¹)	Root:shoot ratio
Control (Tap water)	24.58a	3.60a	28.18a	0.146b
Wastewater	9.88d	2.44bc	12.32d	0.245a
Wastewater + 25 mg Si kg ⁻¹ soil	19.60bc	2.55bc	22.15c	0.130c
Wastewater + 50 mg Si kg ⁻¹ soil	21.94b	3.16b	25.1b	0.144b
Wastewater + 75 mg Si kg ⁻¹ soil	16.94c	2.11c	19.05cd	0.124c

Values followed by the same letter in a column were not significantly different at $P \leq 0.05$.

(Table 2). The root:shoot ratios were 67% higher in wastewater irrigation compared to tap water control irrigation. However, when Si was added to wastewater irrigation, the root:shoot ratios were decreased by 47, 41 and 49% with 25, 50 and 75 mg Si kg⁻¹ soil, respectively, compared to wastewater irrigation having zero Si.

Copper accumulation and partitioning

Wastewater irrigation caused a significant ($P \leq 0.05$) increase in Cu concentration of tomato plants grown with untreated wastewater compared to the tapwater control irrigation (Table 3). The increase in Cu concentration also significantly ($P \leq 0.05$) varied in different plant parts.

The concentration of Cu was increased by 277% in leaves, 480% in stems and 372% in roots with the irrigation of wastewater compared to tap water irrigation. Silicon nutrition produced a significant ($P \leq 0.05$) effect not only on Cu accumulation but also on its distribution among leaves, stems and roots. Addition of Si decreased Cu concentration in tomato leaves by 36, 55 and 63% with 25, 50 and 75 mg Si kg⁻¹ soil, respectively, compared to wastewater irrigation having zero Si. Similarly, the Cu concentration in the tomato stems was decreased by 41, 58 and 65% with the application of 25, 50 and 75 mg Si kg⁻¹ soil, respectively, as compared to wastewater irrigated tomato plants without supplemental Si. The Cu concentration in tomato roots, however, was increased by 17% with the application of

Table 3 | Metals concentration and partitioning in tomato plants (*Lycopersicon esculentum* L.) grown with tap water and wastewater using different levels of Si (values are the mean of five replications)

Metal element (mg kg ⁻¹)	Plant part	Tap water (control)	Wastewater	Wastewater + 25 mg Si kg ⁻¹ soil	Wastewater + 50 mg Si kg ⁻¹ soil	Wastewater + 75 mg Si kg ⁻¹ soil
Copper (Cu)	Root	0.11e	0.52b	0.61ab	0.68a	0.70a
	Stem	0.10e	0.58b	0.34c	0.24cd	0.20cd
	Leaf	0.13e	0.49bc	0.31c	0.22cd	0.18d
Nickel (Ni)	Root	0.12f	0.40c	0.50b	0.82a	0.83a
	Stem	0.16f	0.47b	0.21d	0.17de	0.13e
	Leaf	0.14f	0.39c	0.17de	0.13e	0.14e
Manganese (Mn)	Root	0.18 g	0.82d	1.6c	2.6a	1.98b
	Stem	0.13 g	1.22d	0.56e	0.24f	0.39ef
	Leaf	0.15 g	1.17d	0.64e	0.29ef	0.28f
Cadmium (Cd)	Root	0.01f	0.06d	0.08c	0.12a	0.10b
	Stem	0.01f	0.10b	0.05d	0.04e	0.07c
	Leaf	0.01f	0.10b	0.04e	0.04e	0.06d
Zinc (Zn)	Root	0.69d	1.5c	2.7b	3.59a	3.6a
	Stem	0.66d	2.89b	1.2c	0.8cd	1.0c
	Leaf	0.8d	3.3a	2.7b	1.36c	1.15c

Values in a column followed by the same letter are not significantly different at $P \leq 0.05$.

25 mg Si kg⁻¹ soil, 30% with 50 mg Si kg⁻¹ soil and 34% with 75 mg Si kg⁻¹ soil as compared to wastewater treatment without supplemental Si, indicating that Si complexed Cu and increased its retention in the roots rather than in the stems and leaves.

The root:stem Cu ratio was decreased by 18% in wastewater irrigation compared to the tap water control irrigation, indicating that roots retained less Cu and translocated more to stems under metal toxicity (Figure 1). Addition of Si to wastewater irrigation retained more Cu in roots and increased root:stem Cu ratio by 99% with the application of 25 mg Si kg⁻¹ soil, 214% with 50 mg Si kg⁻¹ soil and 289% with 75 mg Si kg⁻¹ soil as compared to wastewater irrigation without supplemental Si. Similarly, the root:leaf Cu ratio was increased by 86% with the application of 25 mg Si kg⁻¹ soil, 191% with 50 mg Si kg⁻¹ soil and 267% with 75 mg Si kg⁻¹ soil as compared to wastewater irrigation without supplemental Si. In contrast, stem:leaf Cu ratio was decreased by 7% with the application of 25 mg Si kg⁻¹ soil, 8% with 50 mg Si kg⁻¹ soil and 6% with 75 mg Si kg⁻¹ compared to wastewater irrigation without supplemental Si.

Nickel accumulation and partitioning

Nickel is a trace element but is also included in the top 20 toxins (Christopher 2000) and its high accumulation in agricultural soils and plants leads to phytotoxicity (Shi *et al.* 2010). Higher concentration of Ni depresses yield and disturbs the pattern of nutrient transport (Wang *et al.* 2004). Results revealed that Ni accumulation and partitioning were significantly ($P \leq 0.05$) affected in tomato plants irrigated with wastewater as compared to tap water irrigation (Table 3). The concentration of Ni was increased by 233% in roots, 193% in stems and 178% in leaves of tomato plants grown with wastewater irrigation compared to tap water control. Addition of Si retained Ni in roots and its accumulation in roots was increased by 25% with the application of 25 mg Si kg⁻¹ soil, 105% with 50 mg Si kg⁻¹ soil and 107% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. In contrast to roots, the Ni concentration in tomato stems was decreased by 55% with the application of 25 mg Si kg⁻¹ soil, 64% with 50 mg Si kg⁻¹ soil and 72% with 75 mg Si kg⁻¹ soil compared to wastewater

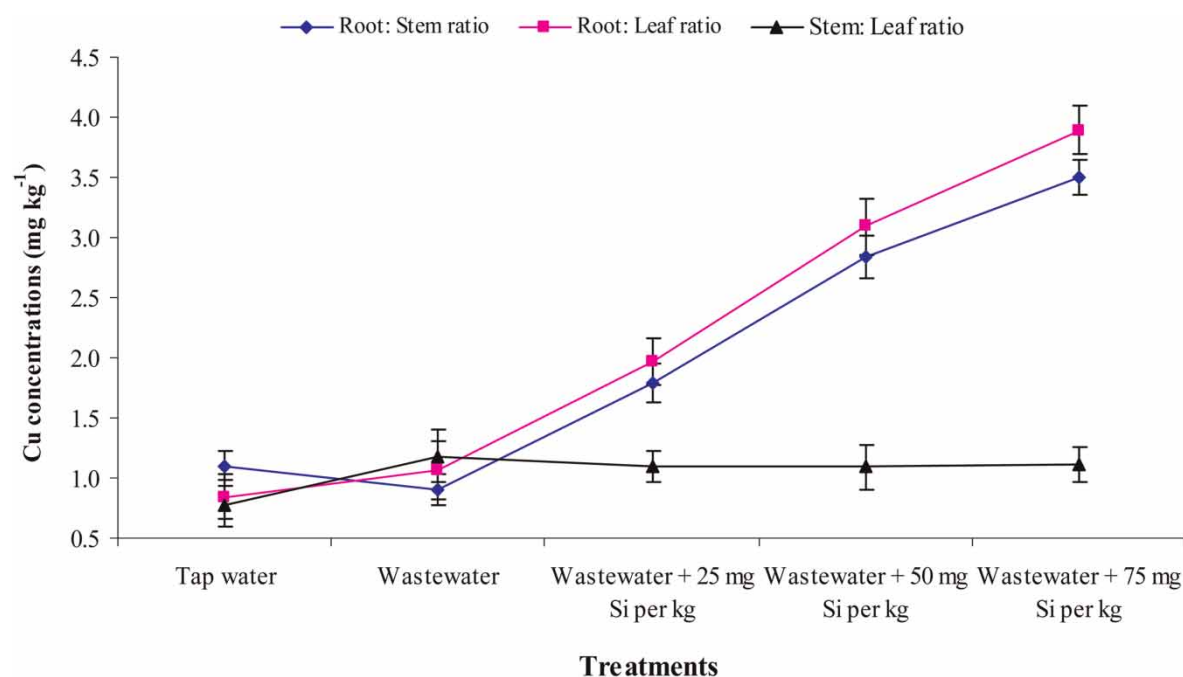


Figure 1 | Copper (Cu) partitioning in tomato plants (*Lycopersicon esculentum* L.) grown with tap water (control) and wastewater using different Si concentrations (values are the mean of five replications \pm SE).

irrigation without supplemental Si. Similarly, leaf Ni concentration was reduced by 56% with the application of 25 mg Si kg⁻¹ soil, 66% with 50 mg Si kg⁻¹ soil and 64% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si.

Silicon supplementation also produced a significant ($P \leq 0.05$) effect on Ni partitioning into roots, stems and leaves under wastewater irrigation. In the present study, the root:stem Ni ratio was increased by 180% with the application of 25 mg Si kg⁻¹ soil, 467% with 50 mg Si kg⁻¹ soil and 650% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. Similarly, the root:leaf Ni ratio was increased by 185% with the application of 25 mg Si kg⁻¹ soil, 512% with 50 mg Si kg⁻¹ soil and 475% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. However, the stem:leaf Ni ratio was relatively less affected by applied Si and increased by only 2% with the application of 25 mg Si kg⁻¹ soil and 8% with 50 mg Si kg⁻¹ soil, while, when Si was applied at 75 mg kg⁻¹ soil, the stem:leaf Ni ratio was decreased by 25% compared to wastewater irrigation without supplemental Si (Figure 2).

Manganese accumulation and partitioning

Manganese is also a trace element, but its high quantity causes deleterious effects on the health of plants. The present study indicates that Mn concentration was significantly ($P \leq 0.05$) increased in tomato plants irrigated with wastewater compared to the tap water control (Table 3). Manganese concentration was increased by 355% in roots, 384% in stems and 680% in leaves of tomato plants grown with wastewater compared to control (tap water-irrigated plants). Addition of Si to growth medium not only influenced the Mn uptake and accumulation in plant organs but also its partitioning into different plant tissues. Manganese concentration was increased in tomato roots by 95% with the application of 25 mg Si kg⁻¹ soil, 217% with 50 mg Si kg⁻¹ soil and 141% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. In tomato stems, Mn concentration was decreased by 54% with the application of 25 mg Si kg⁻¹ soil, 80% with 50 mg Si kg⁻¹ soil and 68% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. Similarly, in tomato leaves, Mn concentration was decreased by 45% with the application of 25 mg Si kg⁻¹

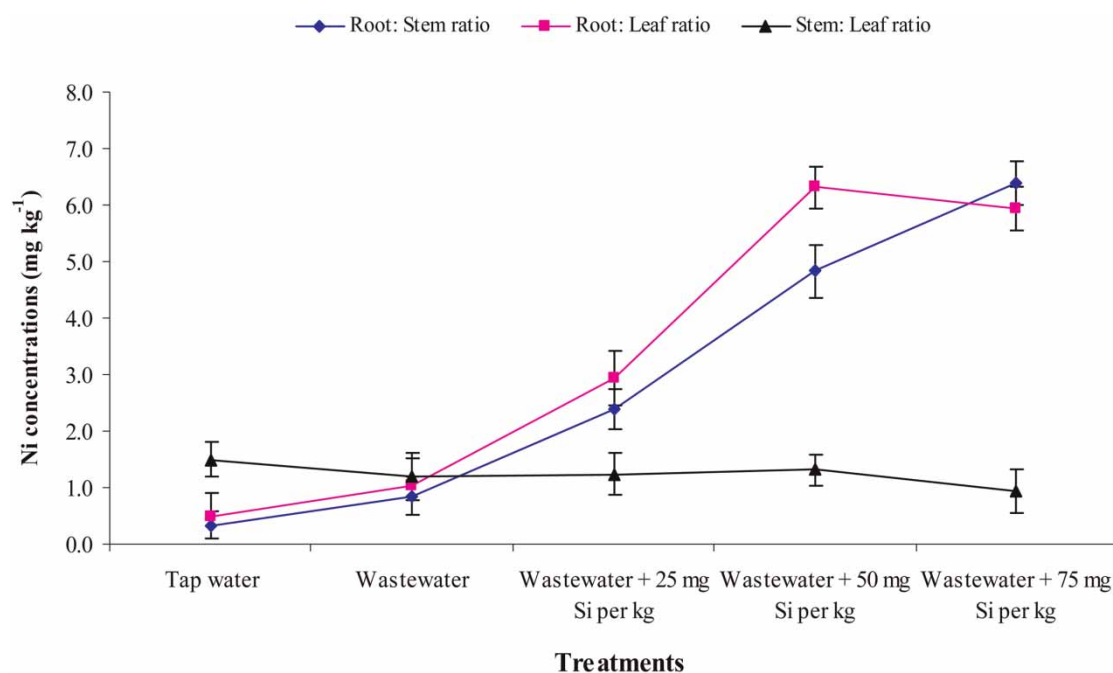


Figure 2 | Nickel (Ni) partitioning in tomato plants (*Lycopersicon esculentum* L.) grown with tap water (control) and wastewater using different Si concentrations (values are the mean of five replications \pm SE).

soil, 75% with 50 mg Si kg⁻¹ soil and 76% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si.

The root:stem Mn ratio was increased by 327% with the application of 25 mg Si kg⁻¹ soil, 1,516% with 50 mg Si kg⁻¹ soil and 658% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. These results indicated that added Si bound Mn in the roots and reduced its movement to aerial plant parts. Similarly, the root:leaf Mn ratio was increased by 257% with the application of 25 mg Si kg⁻¹ soil, 1,181% with 50 mg Si kg⁻¹ soil and 910% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. In contrast, the stem:leaf Mn ratio was relatively less affected and decreased by 15% with the application of 25 mg Si kg⁻¹ soil and 20% with 50 mg Si kg⁻¹ soil, while the stem:leaf Mn ratio was increased by 33% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si (Figure 3).

Cadmium accumulation and partitioning

Cadmium is a highly toxic trace element and has been ranked number seven among the top 20 toxins

(Christopher 2000). Wastewater irrigation produced a significant ($P \leq 0.05$) effect on Cd accumulation and partitioning in tomato plants (Table 3). Cadmium concentration was increased by 500% in roots, 900% in stems and 900% in leaves of tomato plants irrigated with wastewater compared to control (tap water-irrigated plants). Application of Si increased Cd concentration in tomato roots by 33% with 25 mg Si kg⁻¹ soil, 100% with 50 mg Si kg⁻¹ soil and 66% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. In contrast to root, stem Cd concentration was reduced by 100% with the application of 25 mg Si kg⁻¹ soil, 60% with 50 mg Si kg⁻¹ soil and 30% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. Similarly, leaf Cd concentration was decreased by 60% with the application of 25 mg Si kg⁻¹ soil, 60% with 50 mg Si kg⁻¹ soil and 40% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. Added Si trapped Cd in roots and increased the root:stem Cd ratio by 166% with the application of 25 mg Si kg⁻¹ soil, 400% with 50 mg Si kg⁻¹ soil and 138% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. Similarly, the root:leaf Cd ratio was

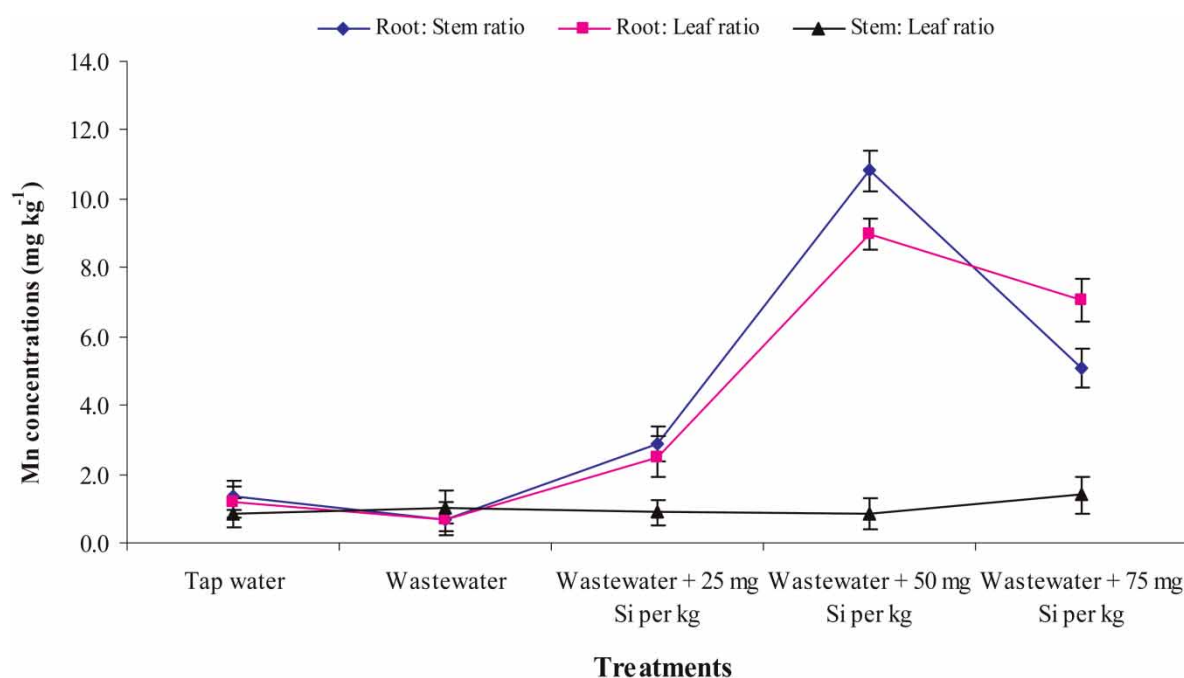


Figure 3 | Manganese (Mn) partitioning in tomato plants (*Lycopersicon esculentum* L.) grown with tap water (control) and wastewater using different Si concentrations (values are the mean of five replications \pm SE).

increased by 233% with the application of 25 mg Si kg⁻¹ soil, 400% with 50 mg Si kg⁻¹ soil and 178% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. The stem:leaf Cd ratio was increased by 25% with the application of 25 mg Si kg⁻¹ soil and 17% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si (Figure 4).

Zinc accumulation and partitioning

Like other metals, excess Zn adversely affects plant growth by disturbing plant metabolism (Wang *et al.* 2004). Results revealed that Zn concentration was significantly ($P \leq 0.05$) increased in tomato plants irrigated with wastewater compared to the tap water control (Table 3). Zinc concentration was increased by 117% in roots, 337% in stems and 312% in leaves of tomato plants grown with wastewater compared to control (tap water-irrigated plants). Silicon supplementation increased root Zn concentration by 80% with the application of 25 mg Si kg⁻¹ soil, 139% with 50 mg Si kg⁻¹ soil and 140% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without

supplemental Si. In contrast to root, stem Zn concentration was decreased by 58% with the application of 25 mg Si kg⁻¹ soil, 72% with 50 mg Si kg⁻¹ soil and 65% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation having zero Si. Similarly, Zn concentration in tomato leaves was reduced by 18% with the application of 25 mg Si kg⁻¹ soil, 58% with 50 mg Si kg⁻¹ soil and 65% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. The root:stem Zn ratio was increased by 332% with the application of 25 mg Si kg⁻¹ soil, 763% with 50 mg Si kg⁻¹ soil and 592% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. These results indicated that added Si trapped Zn in the roots and reduced its movement to aerial plant parts. Similarly, the root:leaf Zn ratio was increased by 122% with the application of 25 mg Si kg⁻¹ soil, 486% with 50 mg Si kg⁻¹ soil and 595% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. Conversely, the stem:leaf Zn ratio was relatively less affected and decreased by 50% with the application of 25 mg Si kg⁻¹ soil, 33% with 50 mg Si kg⁻¹ soil and only 1% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si (Figure 5).

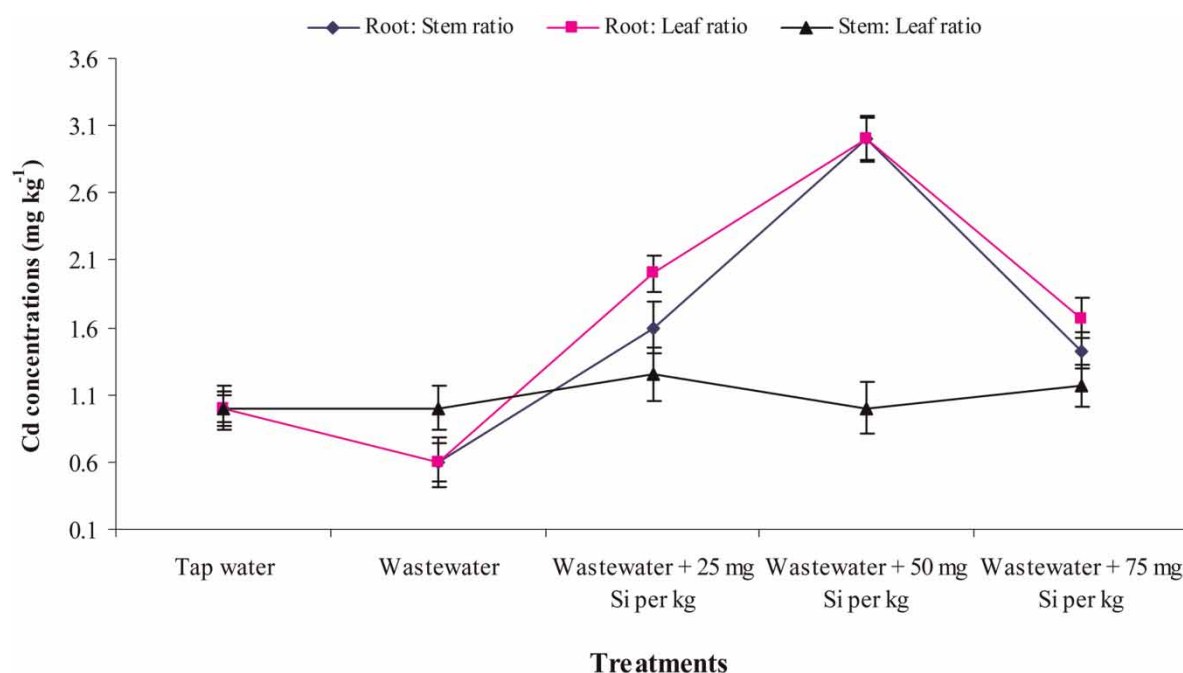


Figure 4 | Cadmium (Cd) partitioning in tomato plants (*Lycopersicon esculentum* L.) grown with tap water (control) and wastewater using different Si concentrations (values are the mean of five replications \pm SE).

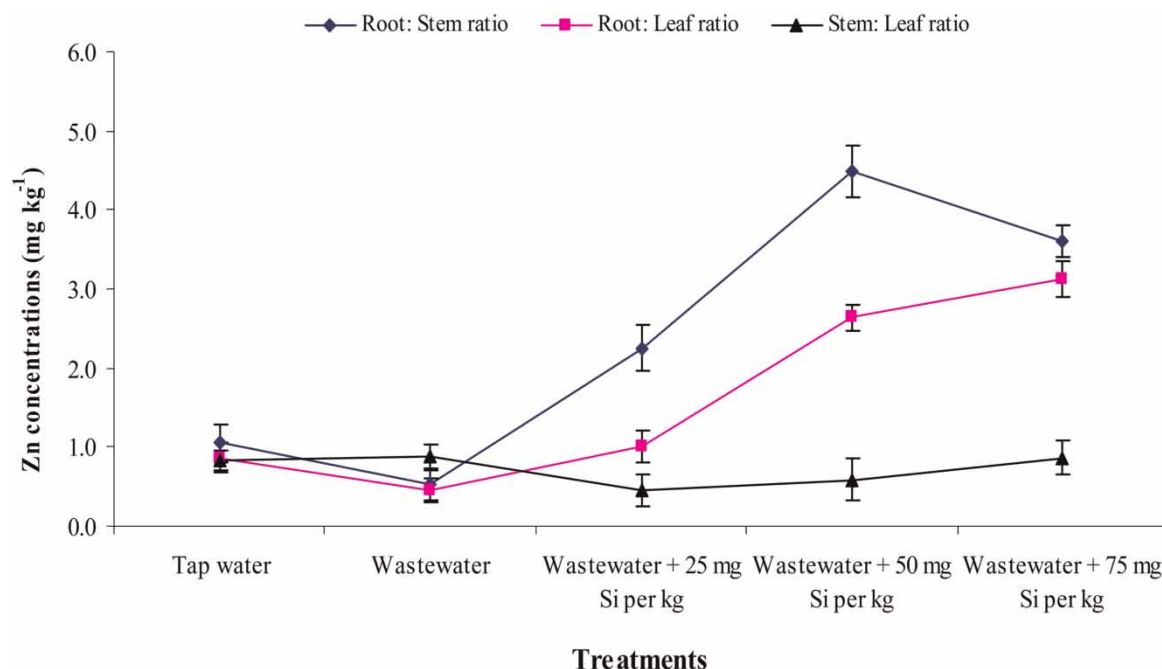


Figure 5 | Zinc (Zn) partitioning in tomato plants (*Lycopersicon esculentum* L.) grown with tap water (control) and wastewater using different Si concentrations (values are the mean of five replications \pm SE).

Metal accumulation in soil

The concentrations of heavy metals in soil irrigated with tap water and wastewater with different levels of Si are shown in Table 4. The study revealed that application of wastewater increased Cu concentration by 24.6%, Ni 11.4%, Mn 12.2%, Cd 19.3% and Zn 33.3% as compared to tap water control irrigation. Addition of Si to wastewater irrigation increased the soil Cu concentration by 7.1% with the application of 25 mg Si kg⁻¹ soil, 14.3% with 50 mg Si kg⁻¹ soil and 18.5% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. Similarly, the soil Ni concentration was

increased by 13.1% with the application of 25 mg Si kg⁻¹ soil, 16% with 50 mg Si kg⁻¹ soil and 14.2% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. The soil Mn was relatively less affected and increased by only 3% with the application of 25 mg Si kg⁻¹ soil, 4.3% with 50 mg Si kg⁻¹ soil and 1.4% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. The Cd concentration in soil was increased by 11.1% with the application of 25 mg Si kg⁻¹ soil, 11.9% with 50 mg Si kg⁻¹ soil and 15.3% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si. Likewise, the soil Zn concentration was increased by 7.7%

Table 4 | Metals accumulation in soil irrigated with tap water and wastewater using different Si levels (values are the mean of five replications \pm SE)

Metal element (mg kg ⁻¹)	Tap water control	Wastewater	Wastewater + 25 mg Si kg ⁻¹ soil	Wastewater + 50 mg Si kg ⁻¹ soil	Wastewater + 75 mg Si kg ⁻¹ soil
Copper (Cu)	13.4 \pm 0.49	16.7 \pm 0.85	17.9 \pm 0.80	19.1 \pm 1.50	19.8 \pm 0.75
Nickel (Ni)	15.7 \pm 0.80	17.5 \pm 1.15	19.8 \pm 2.15	20.3 \pm 2.15	20.0 \pm 1.80
Manganese (Mn)	61.8 \pm 3.10	69.4 \pm 1.25	71.5 \pm 1.70	72.4 \pm 3.45	70.4 \pm 2.60
Cadmium (Cd)	0.98 \pm 0.20	1.17 \pm 0.35	1.30 \pm 0.45	1.31 \pm 0.65	1.35 \pm 0.96
Zinc (Zn)	53.4 \pm 1.85	71.2 \pm 1.60	76.7 \pm 2.70	77.5 \pm 1.70	77.9 \pm 1.25

Table 5 | Correlations among shoot dry matter (SDM) and stem metal concentrations in tomato plants (*Lycopersicon esculentum* L.) grown with wastewater irrigation

	SDM	Cu	Ni	Mn	Cd	Zn
SDM	1					
Cu	-0.88393	1				
Ni	-0.82426	0.930254	1			
Mn	-0.94255	0.97206	0.943436	1		
Cd	-0.97988	0.845816	0.722863	0.88355	1	
Zn	-0.92918	0.951194	0.974266	0.987391	0.85238	1

with the application of 25 mg Si kg⁻¹ soil, 8.4% with 50 mg Si kg⁻¹ soil and 9.4% with 75 mg Si kg⁻¹ soil compared to wastewater irrigation without supplemental Si.

DISCUSSION

Wastewater-induced metal toxicity caused a significant ($P \leq 0.05$) reduction in plant growth and development (Kaya *et al.* 2006) and such reduction generally occurred differently in different plant parts (Liang *et al.* 2007). For instance, in the present experiment, although both root and shoot dry matter were markedly reduced with wastewater irrigation, root dry matter was relatively less affected than shoot dry matter and this led to higher root:shoot ratios when tomato plants were grown with wastewater irrigation (Table 2). The higher root:shoot ratios in wastewater irrigation could be associated with the translocation of photosynthates from leaves to roots where they were trapped and utilized to produce higher root biomass. Furthermore, a major part of toxic metals absorbed by roots was transferred to shoots when plants were grown in a metal-polluted environment. Past studies also demonstrated that plants irrigated with wastewater demonstrated altered metabolism, reduced growth and biomass accumulation as well as higher root biomass production (Liang *et al.* 2007; Ashraf *et al.* 2009).

Wastewater irrigation produced marked effects not only on biomass production but also on heavy metal deposition in soil, as well as their uptake, accumulation and distribution in different plant parts. Sushant & Scott (2010) indicated that, due to long-term use of sewage sludge, higher values of metals were built up in the soil, which influenced their uptake and accumulation within the plant body

and led to phytotoxicity. The reduction in root:stem metal ratios under wastewater irrigation compared to tap water control indicated that roots retained less and translocated more metals to stems and leaves to adapt to the stress conditions. Previous studies also demonstrated that, in stress conditions, most of the toxic ions absorbed by roots are either exuded back to the soil or translocated to aerial parts (Greenway & Munns 1980). The mitigation of metal toxicity by added Si could be attributed to external (growth media) and internal plant mechanisms (Cocker *et al.* 1998; Wang *et al.* 2004). This hypothesis was confirmed in the present study where Si interacted with wastewater-induced metal ions, retained them in soil and, consequently, reduced their uptake and accumulation within the plant body. Earlier studies also reported that the external plant mechanisms of Si's ameliorative effects on toxic metals were mainly due to the decrease of metal phytoavailability by increasing soil pH and forming metal silicate precipitates (Ma *et al.* 1997; Cocker *et al.* 1998; da Cunha *et al.* 2008; da Cunha & Nascimento 2009; Song *et al.* 2009). Moreover, the root:stem and root:leaf metal ratios were markedly reduced by increasing Si from 0 to 75 mg kg⁻¹ soil under wastewater irrigation, indicating that Si application retained more metals in roots and reduced their translocation to stems and leaves. Oliva *et al.* (2011) reported that Si mitigated metal toxicity in plants by retaining more metals in roots and translocating less to stems and leaves. Liang *et al.* (2005) demonstrated that deposition of Si in the endodermis of roots provided binding sites for heavy metals that restricted their movement to stems and leaves. Rogalla & Römhild (2002) indicated that the addition of Si decreased Mn in symplast and more was bound to the cell wall compared with non-Si-treated plants. Shi *et al.* (2005) also reported that Si alleviated Cd stress in plants by depositing

high concentrations of Si in cell walls of the endodermis and epidermis to restrict transport of the metal in bypass flow from roots to shoots. The results revealed that improvement in biomass accumulation was linearly increased by Si addition to wastewater irrigation up to 50 mg kg⁻¹ soil and beyond that it started to decline. This could be due to polymerization of soluble Si to colloidal salicic acid and finally to silica gel when the concentration of soluble salicic acid exceeded 2 mM (Kaya et al. 2006). These results were in contrast to da Cunha & Nascimento (2009) who reported a linear increase in dry matter by increasing Si from 0 to 200 mg kg⁻¹. There was a very high negative relationship between shoot dry matter and stem metal concentrations, while there was a positive relationship among different metals with each other (Table 5).

CONCLUSION

Wastewater irrigation markedly influenced metals' deposition in soil as well as their uptake, accumulation and distribution among plant tissues in tomato plants. When wastewater was used without supplemental Si, most of the absorbed metals were translocated to aerial plant parts, reducing shoot dry matter more, and led to higher root: shoot ratios. However, Si supplementation ameliorated wastewater-induced metal toxicity by precipitating metals in soil as well as retaining more metals in roots, reducing their translocation to stems and leaves and, consequently, improving biomass accumulation. Although all levels of applied Si were significantly effective in mitigating metal toxicity, 50 mg Si kg⁻¹ soil gave relatively better results compared to the others. This study indicates that wastewater can safely be used to irrigate crops without accumulating toxic concentration of heavy metals, particularly in aerial plant parts, using Si supplementation.

REFERENCES

- Ashraf, M., Rahmatullah, Maqsood, M. A., Kanwal, S., Tahir, M. A. & Ali, L. 2009 Growth responses of wheat cultivars to rock phosphate in hydroponics. *Pedosphere* **19**, 398–402.
- Bazai, Z. A. & Achakzai, A. K. K. 2006 Effect of wastewater from Quetta City on the germination and seedling growth of lettuce (*Lactuca sativa* L.). *J. Appl. Sci.* **6**, 380–382.
- Chen, H. M., Zheng, C. R., Tu, C. & Shen, Z. G. 2000 Chemical methods and phytoremediation of soil contaminated with heavy metals. *Chemosphere* **41**, 229–234.
- Christopher, S. C. 2000 Phytochelatins and their roles in heavy metal detoxification. *J. Plant Physiol.* **123**, 825–832.
- Cocker, K. M., Evans, D. E. & Hodson, M. J. 1998 The amelioration of aluminum toxicity by silicon in higher plants: solution chemistry or an in planta mechanism? *J. Plant Physiol.* **104**, 608–614.
- da Cunha, K. P. V. & do Nascimento, C. W. A. 2009 Silicon effects on metal tolerance and structural changes in maize (*Zea mays* L.) grown on Cd and Zn enriched soil. *Water Air Soil Pollut.* **197**, 323–330.
- da Cunha, K. P. V., do Nascimento, C. W. A. & da Silva, A. J. 2008 Silicon alleviates the toxicity of cadmium and zinc for maize (*Zea mays* L.) grown on a contaminated soil. *J. Plant Nut. Soil Sci.* **171**, 849–853.
- Greenway, H. & Munns, R. 1980 Mechanisms of salts tolerance in non-halophytes. *Am. Rev. Plant Physiol.* **31**, 149–190.
- Hammond, K. E., Evans, D. E. & Hodson, M. J. 1995 Aluminum/silicon interaction in barley (*Hordeum vulgare* L.) seedlings. *Plant Soil* **173**, 89–95.
- Hashemi, A., Abdolzadeh, A. & Sadeghipour, H. R. 2010 Beneficial effects of silicon nutrition in alleviating salinity stress in hydroponically grown canola (*Brassica napus* L.) plants. *Plant Nut. Soil Sci.* **56**, 244–253.
- Havlin, J. L. & Soltanpour, P. N. 1981 Evaluation of the AB-DTPA soil test for Fe, Zn, Mn, Cu. *Soil Sci. Soc. Am. J.* **45**, 55–70.
- Horst, W. J., Maier, P., Fecht, M., Naumann, A. & Wissemeier, A. H. 1999 Physiology of manganese toxicity and tolerance in *Vigna unguiculata* (L.) Walp. *Plant Nut. Soil Sci.* **162**, 263–274.
- Iwasaki, K., Maier, P., Fecht, M. & Horst, W. J. 2002 Effects of silicon supply on apoplastic manganese concentrations in leaves and their relation to manganese tolerance in cowpea (*Vigna unguiculata* L.). *Plant Soil* **238**, 281–288.
- Kashif, S. R., Akram, M., Yaseen, M. & Ali, S. 2009 Studies on heavy metals status and uptake by vegetables in adjoining areas of Hudiara drain in Lahore. *Soil Environ.* **28**, 7–12.
- Kaya, C., Tuna, L. & Higgs, D. 2006 Effect of silicon on plant growth and mineral nutrition of maize grown under water stress conditions. *J. Plant Nut.* **29**, 1469–1480.
- Kidd, P. S., Llugany, M., Poschenrieder, C., Guns, B. & Barcel, J. 2001 The role of root exudates in aluminum resistance and silicon-induced amelioration of aluminum toxicity in three varieties of maize (*Zea mays* L.). *J. Exp. Bot.* **52**, 1339–1352.
- Liang, Y. C., Sun, W. C., Zhu, Y. G. & Christie, P. 2007 Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. *Environ. Pollut.* **147**, 422–428.
- Liang, Y., Wong, J. W. C. & Wei, L. 2005 Silicon-mediated enhancement of cadmium tolerance in maize (*Zea mays* L.) grown in cadmium contaminated soil. *Chemosphere* **58**, 475–483.

- Ma, J. F., Sasaki, M. & Matsumoto, H. 1997 [Al-induced inhibition of root elongation in corn, *Zea mays* L. is overcome by Si addition](#). *Plant Soil* **188**, 171–176.
- Miller, R. O. 1998 Nitric-perchloric wet digestion in an open vessel. In: *Handbook of Reference Methods for Plant Analysis* (Y. P. Kalra, ed.). Soil Plant Analysis Council Inc. and CRC Press, Washington, DC, USA, pp. 57–62.
- Neumann, D. & Niesen, U. Z. 2001 [Silicon and heavy metal tolerance of higher plants](#). *Phytochemistry* **56**, 685–692.
- Okuda, A. & Takahashi, E. 1962 Effect of silicon supply on the injuries due to excessive amounts of Fe, Mn, Cu, As, Al and Co in barley and rice plants. *J. Soil Sci. Plant Nut.* **33**, 1–8.
- Oliva, S. R., Mingorance, M. D. & Leidi, E. O. 2011 [Effects of silicon on copper toxicity in *Erica andevalensis* Cabezedo and Rivera: a potential species to remediate contaminated soils](#). *J. Environ. Monitor.* **13**, 591–596.
- Pescod, M. B. 1992 *Wastewater Treatment and Use in Agriculture*. FAO Irrigation and Drainage Paper 47, FAO, Rome, pp. 125.
- Rattan, R. K., Datta, S. P., Chonkar, P. K., Suribabu, K. & Sing, A. K. 2005 [Long term impact of irrigation with sewage effluents on heavy metal content in soils, crops and ground water – a case study](#). *Agric. Ecosyst. Environ.* **109**, 310–322.
- Rogalla, H. & Römhild, V. 2002 [Role of leaf apoplast in silicon-mediated manganese tolerance of *Cucumis sativus* L.](#) *Plant Cell Environ.* **25**, 549–555.
- Shi, G., Cai, Q., Liu, C. & Wu, L. 2010 [Silicon alleviates cadmium toxicity in peanut plants in relation to cadmium distribution and stimulation of antioxidative enzymes](#). *Plant Growth Regul.* **16**, 45–52.
- Shi, X. H., Zhang, C. C., Wang, H. & Zhang, F. S. 2005 [Effect of Si on the distribution of Cd in rice seedlings](#). *Plant Soil* **272**, 53–60.
- Song, A., Li, Z., Zhang, J., Xue, G., Fan, F. & Liang, J. 2009 [Silicon-enhanced resistance to cadmium toxicity in *Brassica chinensis* L. is attributed to Si-suppressed cadmium uptake and transport and Si-enhanced antioxidant defense capacity](#). *J. Hazard. Mater.* **172**, 74–83.
- Steel, R. G. D., Torrie, J. H. & Dicky, D. A. 1997 *Principles and Procedures of Statistics: A Biometrical Approach*. 2nd edn, McGraw-Hill, New York, USA.
- Sushant, K. & Scott, L. 2010 Soluble silicon modulates expression of *Arabidopsis thaliana* genes involved in copper stress. *J. Plant Physiol.* **10**, 1016–1022.
- Wang, X. S. & Han, J. G. 2007 [Effects of NaCl and silicon on ions distribution in the roots, shoots and leaves of two alfalfa cultivars with different salt tolerance](#). *J. Soil Sci. Plant Nut.* **53**, 278–285.
- Wang, Y. X., Stass, A. & Horst, W. J. 2004 [Apoplastic binding of aluminum is involved in silicon-induced amelioration of aluminum toxicity in maize](#). *Plant Physiol.* **136**, 3762–3770.
- Williams, D. E. & Vlamis, J. 1957 [The effect of silicon on yield and manganese-54 uptake and distribution in leaves of barley grown in culture solutions](#). *J. Plant Physiol.* **32**, 404–409.
- Zhu, J. K., Wei, G. Q., Li, J., Qian, Q. Q. & Yu, J. Q. 2004 [Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber \(*Cucumis sativus* L.\)](#). *Plant Sci.* **167**, 527–533.

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