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Effect of silicon on reducing cadmium toxicity in durum wheat (*Triticum turgidum* L. cv. Claudio W.) grown in a soil with aged contamination

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ABSTRACT

Agricultural soil contamination and subsequently crops still require alternative solutions to reduce associated environmental risks. The effects of silica application on alleviating cadmium (Cd) phytotoxicity in wheat plants were investigated in a 71-day pot experiment conducted with a historically contaminated agricultural soil. We used amorphous silica (ASi) that had been extracted from a diatomite mine for Si distribution at 0, 1, 10 and 15 ton ASi ha^{-1}. ASi applications increased plant biomass and plant Si concentrations, reduced the available Cd in the soil and the Cd translocation to shoots, while Cd was more efficiently sequestrated in roots. But ASi is limiting for Si uptake by plants. We conclude that significant plant-available Si in soil contributes to decreased Cd concentrations in wheat shoots and could be implemented in a general scheme aiming at controlling Cd concentrations in wheat.

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1. Introduction

The productivity and biological efficiency of plants are limited by the presence of toxic elements in the soil. Among these toxins, cadmium (Cd) is highly toxic for both plants and animals [1,2]. It is widely used in household and industrial appliances, and domestic and industrial sewage sludge contains higher concentrations of Cd than other metals or metalloids. Cd enters the soil, whether intentionally or not, through atmospheric fallout, sewage sludge and fertiliser application [3]. Once in the soil, the bioavailability of Cd depends on a number of soil characteristics, soil-solution characteristics [4,5], interactions with other elements such as iron [6] and on plant species or populations that may have various capabilities of taking up metals.

Due to its high mobility and assimilability, Cd readily accumulates in the different plant parts and organs depending on the plant species [7]. Cadmium uptake by the roots and translocation to shoots has been characterised in a number of plant species, including wheat (*Triticum turgidum*) [8] and maize (*Zea mays*) [9]. After root absorption, Cd is translocated to the shoots mainly in an ionic form in xylem and phloem [10], either passively by transpiration rate [11] or actively through Fe transporters [12]. Cadmium accumulation in durum wheat grains may be due to the increase in Cd translocation from leaves and stalks to maturing grains [13] and may be related to phloem movement [14]. Similarly, Cd concentrations in grains correlate with Cd concentrations in shoots during the vegetative phase and the translocation rate from roots to shoots of plants both in wheat and rice (*Oryza sativa*) [15,16], indicating a direct effect of Cd uptake by plant and food chain contamination.

Concurrently, heavy metals contamination reduction or elimination from the soil and/or metal transfer reduction from soil to crops and further up the food chain has become a growing concern worldwide. Soil contamination is indeed considered one of the main threats to soil as identified in the EU soil communication [17], Cd being of great concern to human health along with mercury and lead. Foodstuffs are the main source of cadmium exposure for the non-smoking general population, and Cd is the only metal for which guide values have been published for food [18]. Different actions can be undertaken to reduce the absorption of Cd by plants, such as selecting metal-excluding plants [19–22], using soil amendments [e.g. 23] and/or applying an effective plant fertilisation [24]. However, these actions may not be efficient in all cases. There is still a need for a range of more efficient and economical approaches for coping with metal toxicity in plants that may occur in large areas.

Silicon, however, is not considered to be an essential element for higher plants, but it has been proved to be beneficial for the growth and development of many plants, particularly graminaceous plants such as rice and sugarcane [25,26]. Like most of the Poaceae, wheat is a Si accumulator, which implies that Si concentrations in shoots are generally over 10 mg g^{-1} and that the [Si]/[Ca] ratio is less than

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1 [27]. A number of studies show that Si is actively absorbed by wheat plants and transported to different plant organs [28]. Indeed, Si influx transporters have been identified in rice [29] and in maize [30] but not yet in wheat, although it has been suggested by Rains et al. [31]. The beneficial effects of Si are particularly distinct in plants exposed to biotic or abiotic stresses [32–38], and Si has been shown to alleviate the deleterious effects of metals in many plants [39–42]. There is an increasing body of literature showing that Si application to Cd-contaminated soils in hydroponic conditions enhances biomass production of many plant species such as maize [43–45] and strawberry [46]. Similarly, Si reduced toxicity symptoms in rice plants [47–49]. However, the role of Si in heavy metals tolerance in wheat and durum wheat has rarely been studied, despite the fact that wheat is the most produced cereal crop in Europe and the second most produced in the world, with 228 and 686 MT produced in 2009, respectively [50]. France is the second largest durum wheat producer in Europe. In addition, most of the studies related to the Si-mediated reduction of metal stress in plants are conducted on a short-term basis and in hydroponic cultures. In the few experiments conducted in soils, it was very difficult to distinguish between the soil factors and the root effects on Cd uptake [3] and thus to determine the consequences of Si addition to soil on Cd uptake. The possible mechanisms involved in minimising Cd accumulation in crops by improving silicon nutrition have been summarised by Sarwar et al. [24], and it appears that the mechanisms of the alleviating effects remain unclear and/or are probably multifaceted. Thus, we need longer term (as compared to hydroponic cultures), more realistic soil-based studies to fully understand the practical implications of Si-mediated metal tolerance so that successful field experiments can be conducted.

We conducted a greenhouse experiment to investigate the beneficial effects of Si on durum wheat plants grown in pots using a soil with aged contamination. The main objectives of the study were as follows: (1) to investigate the effect of amorphous silica (ASi) application on metal-stressed wheat plants grown in contaminated soil; (2) to highlight the effect of amorphous silica application on soil available metal pool and on metal transfer to wheat plants with an emphasis on Cd; and (3) to identify the possible mechanisms responsible for the reduction of metal toxicity and accumulation in plants.

2. Materials and methods

2.1. Materials

The soil was collected in Rafz (414 m above sea level, Switzerland) from the surface (0–20 cm) of an agricultural soil that had been polluted twenty five years ago by municipal and industrial sewage sludge and, as a result, is contaminated by a range of heavy metals including cadmium (Cd), copper (Cu), zinc (Zn) and lead (Pb). It has been used since then for crop production. According to the FAO taxonomy, it is a sandy loam haplic luvisol (US-taxonomy: typic hapludalf). The soil is neutral to slightly alkaline and the soil is virtually free of carbonate. The carbon content is in the normal range usually for agricultural soils (Table 1). More information on the soil characteristics can be found in Krebs et al. [53].

In France, durum wheat is cultivated on 400 000 ha, mostly in the South, and yielded a production of 2 Mton in 2009. *T. turgidum* L. cv. Claudio W. was grown in the Rafz soil with and without Si applications. We applied a natural ASi powder (siliceous fossil meal, Clarcel 78®) that had been extracted from a diatomite mine exploited by CECA (ARKEMA Group) from Saint-Bauzile (France), which contains 87% SiO_2 and makes it interesting because it is rather concentrated. The remaining 13% include Al, Fe, Ca, Mg and K. ASi is an opal A-like mineral that was chosen because it has been shown to dissolve easily [54].

Table 1
Initial physicochemical properties of the Rafz soil used in the pot experiment.

Physicochemical properties	
pH (1/2.5 soil to water ratio)	6.8
Organic C ($mg\,g^{-1}$)	16.0
Total N ($mg\,g^{-1}$)	8.0
Olsen P ($mg\,g^{-1}$)	0.163
Sand ($mg\,g^{-1}$)	501
Silt ($mg\,g^{-1}$)	335
Clay ($mg\,g^{-1}$)	164
Cation exchange capacity (cobaltihexamine; $cmol^+\,kg^{-1}$)	10.8
Si Tamm[a] ($mg\,g^{-1}$)	0.33
Si CBD[b] ($mg\,g^{-1}$)	1.25
Total Cd ($mg\,kg^{-1}$)	0.7
Total Zn ($mg\,kg^{-1}$)	495
Total Cu ($mg\,kg^{-1}$)	48.5
Total Pb ($mg\,kg^{-1}$)	309

[a] Tamm extraction [51].
[b] CBD citrate bicarbonate dithionite extraction [52].

2.2. Protocol of the experiment

2.2.1. Experimental design

The pot experiment was conducted in a greenhouse at 18–25 °C and 60% humidity. Silica was added at doses of 0, 1, 10 and 15 ton ASi ha^{-1} to each soil batch treatment and then thoroughly mixed by hand. The effective doses that were applied to the pots were calculated by taking a 20-cm depth of soil with a density of 1.49 $g/cm^3\,ha^{-1}$, assuming that the diatomite was 87% pure. Each batch was then divided to fill the replicated pots. All treatments were performed in three replicates without plants and in four replicates with plants. Pots were initially seeded (mid February 2009, immediately after mixing with ASi) at a density of six seeds per pot, then thinned to four individuals per pot after five days of germination. Each pot was fertilised with a 30-ml solution containing 130 $mg\,L^{-1}$ N (as NH_4NO_3), 130 $mg\,L^{-1}$ P (as Ca $(H_2PO_4)_2$), 180 $mg\,L^{-1}$ K (as 50% KCl and 50% K_2SO_4) and 40 $mg\,L^{-1}$ Mg (as $MgSO_4{\cdot}7H_2O$). Pots were regularly watered and randomly rotated. Weeds were removed regularly when present. Two Rhizon soil moisture samplers (SMS, Rhizon®, Rhizosphere Research Products, NL) were installed per pot. They were first dipped into a 10% HNO_3 p.a. solution overnight and then washed with distilled water until the pH returned to 7. The SMS were then inserted horizontally in the wet soil two days after sowing. All plastic and glass wares were rinsed with 10% HNO_3.

2.2.2. Plant sampling and analysis

Plants were harvested after 71 days, just before booting stage, by cutting the shoots approximately one centimetre above the soil surface. Plant samples were washed with distilled water and oven dried at 70 °C until a constant weight was reached. Roots were separated from soil by the following procedure: rinsing with distilled water, followed by a second rinsing with 0.02 M ethylenediaminetetraacetic (EDTA) solution and three other rinsing steps with distilled water, before oven drying at 80 °C until a constant weight was reached. Total dry weights of the roots and shoots were measured. All shoot and root samples were finely ground before digesting them with concentrated HNO_3 (70%) in a water bath at 95 °C for 6 h. Solutions were filtered on 0.22 μm membrane before analysis (modified from Keller et al. [55]). Silicon was extracted according to Guntzer et al. [56].

2.2.3. Soil solution, soil sampling and analysis

Soil solution samples were collected at different intervals of time (47, 57, 64 and 71 days after sowing), analysed for pH, acidified with 70% sp HNO_3 and measured for Si, Cu, Zn, Cd and Pb concentrations. Soil samples were taken at the beginning (just after mixing) and at the end of the experiment. The samples were oven dried at 40 °C until constant weight and sieved at 2 mm before analysis. Soil pH was measured (soil/water ratio of 1/2.5) and DTPA-TEA extractions were performed according to a modified Lindsay and Norvell [57] procedure. We used 0.005 M DTPA, 0.1 M TEA and 0.01 M $CaCl_2$ at pH 7.3; the soil/solution ratio was 2/20; the solutions were horizontally shaken for 120 min and centrifuged and filtrated through a 0.22 μm cellulose acetate membrane.

Cadmium and the other elements in the soil extractions and plant digests were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES Jobin-Yvon, Ultima-C). Metals from soil solution samples were also measured by ICP-MS (Perkin-Elmer Elan 6000, LMTG Toulouse. Si concentrations in soil solutions were measured by the molybdate blue colorimetric method [81] with a spectrophotometer (Jasco V650).

2.3. Statistical treatment

Student's *t*-test was performed to test whether the average concentrations in the treated pots differed from those of controlled pots. Results that were significantly different were marked by (*) for $p \leq 0.05$, (**) for $p \leq 0.01$ and (***) for $p \leq 0.001$. Statistics were performed using the XLStat package (Addinsoft, Paris, F).

3. Results

3.1. Effect of Si treatments on plant parameters

3.1.1. Plant growth and biomass

The shoot length of wheat plants (Fig. 1), as well as the dry weight (DW) of both shoots and roots (Fig. 2), increased with increasing doses of supplemented Si. Shoot dry weights of the 10- and 15-ton ASi ha^{-1} pots were respectively 10% and 42% larger than those of the control pots. The maximum significant increase in root dry weight (approximately 43% of the control) was also observed in the 15-ton ASi ha^{-1} pots.

3.1.2. Silicon uptake and accumulation

The Si concentration in both shoots and roots did not increase significantly with increasing Si doses, and Si concentrations in shoots were slightly larger than those in roots (Table 2). The shoot to root ratio was not significantly different, but the 15-ton ASi ha^{-1} treatment was the largest.

Fig. 1. Wheat plants grown in pots from a historically contaminated soil treated with increasing doses of amorphous silica (ASi): 0 (control), 1, 10 and 15 ton ASi ha^{-1}. The effect of silicon on wheat growth can be seen in differences of the length of the above-ground parts in each treatment, especially between the control and the 15 ton ha^{-1} ASi treatment.

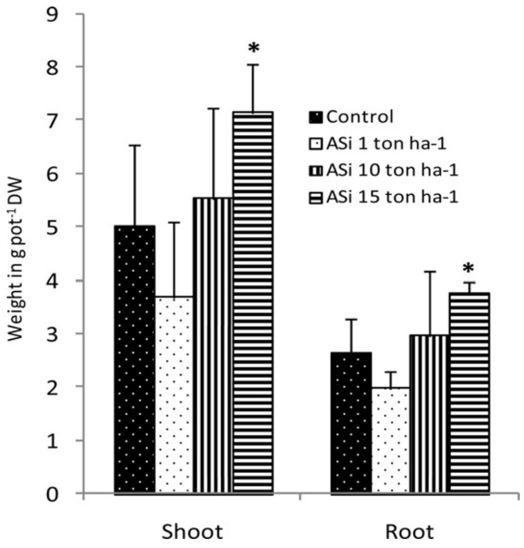

Fig. 2. Dry weight of wheat plant shoots and roots grown in a historically contaminated soil treated with increasing doses of ASi (0, 1, 10 and 15 ton Si ha^{-1}). Bars represent SD of four replicates. (*) denotes significance relative to the respective control as determined by Student's *t*-test at a $p < 0.05$ and (**) at $p < 0.01$.

Table 2
Si concentration (g kg^{-1} DW) in shoot and root as well as the shoot-to-root ratio of wheat plants grown in a historically metal-contaminated soil treated with increasing doses of ASi. Values are mean ± SD ($n = 4$).

ASi application (ton ha^{-1})	Shoot (g kg^{-1} DW)	Root (g kg^{-1} DW)	Shoot/root
0	3.98 ± 0.81	3.21 ± 0.81	1.24
1	4.27 ± 0.66	3.27 ± 0.68	1.31
10	4.66 ± 0.69	4.65 ± 1.74	1.00
15	4.43 ± 0.25	3.19 ± 0.20	1.39

Plant uptake was calculated using Si concentrations multiplied by the biomass production (Fig. 3). Shoot uptake increased significantly in applications greater than 10-ton ASi ha^{-1}, being 63% larger in the 15-ton Si ha^{-1} treatment than in the control pots. However, this effect was not significant for roots.

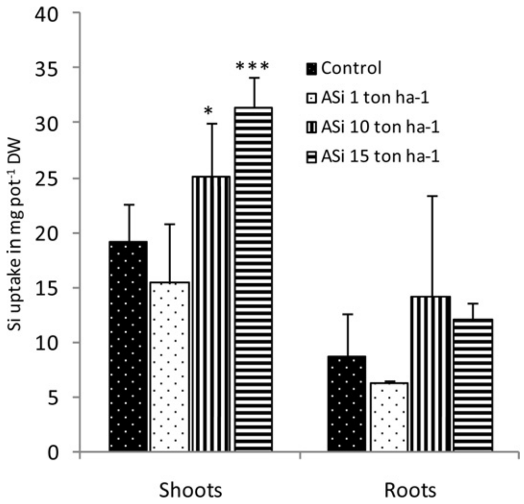

Fig. 3. Total Si uptake in mg pot^{-1} of wheat shoots and roots grown in a historically contaminated soil treated with increasing doses of ASi. Bars represent SD of four replicates. (*) denotes significance relative to the respective control as determined by Student's *t*-test at a $p < 0.05$ and (***) at $p < 0.001$.

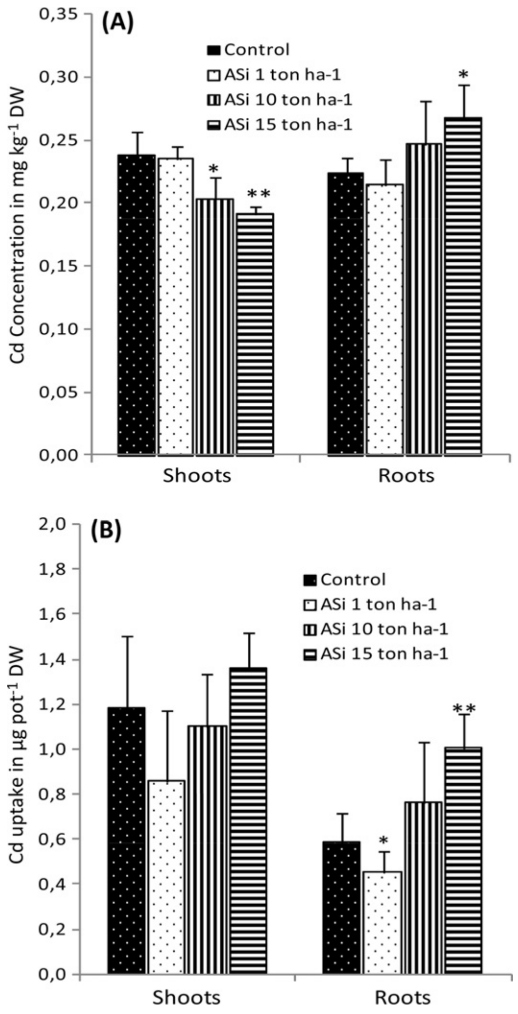

Fig. 4. Cadmium concentration in mg kg^{-1} (A) and Cd total uptake in mg pot^{-1} (B) in shoots and roots of wheat plants grown in a historically contaminated soil treated with increasing doses of ASi. Bars represent SD of four replicates. (*) denotes significance relative to the respective control as determined by Student's *t*-test at a $p < 0.05$ and (**) at $p < 0.01$.

3.1.3. Metal distribution in plants

Cadmium concentrations in the plant shoots significantly decreased with increasing ASi supplementation (Fig. 4A), while the opposite trend was observed for roots. However, the shoot to root ratio remained constant for all treatments (Table 2). Contrarily, when the total Cd content from both shoots and roots was calculated (Fig. 4B), the Cd amount in shoots was similar to that of control plants, while the root total uptake was still significantly larger with the largest Si application. The other metal concentrations (Zn, Cu and Pb) were larger in roots than in shoots (Table 3). Zinc concentrations increased in both shoots and roots with Si treatments, while Cu and Pb concentrations did not increase significantly in both shoots and roots throughout the Si treatments.

Table 4
Soil pH measured after crop harvest in a historically metal-contaminated soil sown or unsown with wheat plants and treated with increasing doses of ASi. Values are mean ± SD ($n = 3$ without plants, and $n = 4$ with plants).

ASi applications (ton ha^{-1})	Without plant	With plant
0	6.09 ± 0.15	6.42 ± 0.10
1	6.11 ± 0.10	6.57 ± 0.13
10	5.98 ± 0.13	6.52 ± 0.15
15	6.01 ± 0.14	6.50 ± 0.09

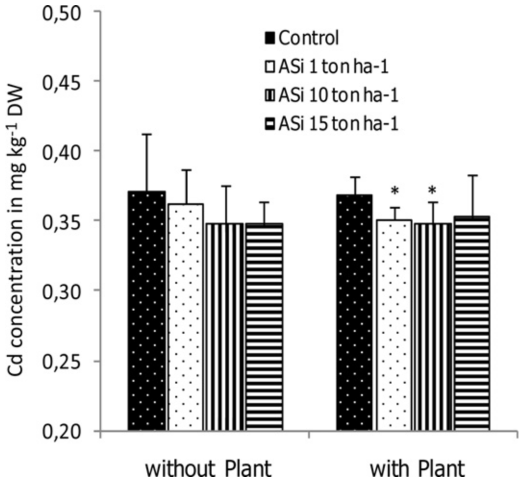

Fig. 5. Effect of ASi application on DTPA-TEA extractable Cd concentration measured in a historically contaminated soil sown or not with wheat plants and treated with increasing doses of ASi. Concentration in mg kg^{-1} is measured after harvest. Bars represent SD of four replicates. (*) denotes significance relative to the respective control as determined by Student's *t*-test at a $p < 0.05$.

3.2. Effect of Si treatments on soil characteristics

Soil pH in pots with plants was larger than in pots without plants (Table 4). The pH slightly decreased in the pots without plants treated at 10 and 15 ton ASi ha^{-1}. No pH changes were observed along the various treatments with plants. Soil pH in pots with plants was larger than in pots without plants (Table 4). In non-sown pots, pH was slightly lower (not significantly) after 71 days in the pots that had been treated with 10 and 15 ton ASi ha^{-1} than in both the control and the 1-ton ASi ha^{-1} pots. In sown pots, pH did not change significantly with ASi treatments as compared to the control pots.

Fig. 5 presents the results of the DTPA-TEA extractions performed on the soil collected from the pots after wheat harvest. In pots both with and without plants, DTPA-extractable Cd concentrations slightly decreased in all ASi treatments, but this decrease

Table 3
Zinc, Cu and Pb concentrations (mg kg^{-1} DW) in shoots and roots of wheat plants grown in a historically metal-contaminated soil treated with increasing doses of ASi. Values are means ± SD ($n = 4$).

ASi applications (ton ha^{-1})	Zn		Cu		Pb	
	Shoot (mg kg^{-1} DW)	Root (mg kg^{-1} DW)	Shoot (mg kg^{-1} DW)	Root (mg kg^{-1} DW)	Shoot (mg kg^{-1} DW)	Root (mg kg^{-1} DW)
0	57.5 ± 4.2	94.8 ± 2.3	8.1 ± 0.3	14.0 ± 0.6	1.1 ± 0.1	29.7 ± 3.5
1	77 .4 ± 3.5[***]	106.7 ± 6.6[**]	9.3 ± 0.7[**]	13.2 ± 0.3[*]	1.3 ± 0.1[**]	28.1 ± 0.9
10	60.1 ± 13.4	108.6 ± 17.9	7.9 ± 1.4	14.2 ± 1.4	1.1 ± 0.3	30.5 ± 5.1
15	58.7 ± 6.3	108.7 ± 9.7[**]	8.6 ± 0.6	13.3 ± 0.5	1.2 ± 0.1	29.2 ± 1.0

[*] Significance relative to the respective control as determined by Student's *t*-test at a $p < 0.05$.
[**] Significance relative to the respective control as determined by Student's *t*-test at a $p < 0.01$.
[***] Significance relative to the respective control as determined by Student's *t*-test at a $p < 0.001$.

Table 5
DTPA-TEA extractable Zn, Cu and Pb (mg kg^{-1} DW) concentrations measured after crop harvest in a historically metal-contaminated soil sown or unsown with wheat plants and treated with increasing doses of ASi. Values are means ± SD ($n = 4$).

ASi application (ton ha^{-1})	Zn (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)		Pb (mg kg^{-1} DW)	
	Without plant	With plant	Without plant	With plant	Without plant	With plant
0	121.2 ± 11.5	101.1 ± 7.1	15.5 ± 0.2	15.6 ± 0.3	80.5 ± 1.2	93.9 ± 4.3
1	116.7 ± 2.6	98.7* ± 4.2	15.5 ± 0.3	15.3 ± 0.4	76.5* ± 2.9	86.9* ± 1.9
10	116.8 ± 0.7	98.3 ± 2.3	15.2 ± 0.3	15.2* ± 0.1	80.2 ± 1.5	86.8* ± 2.6
15	113.1 ± 5.3	93.2* ± 1.1	14.9* ± 0.3	15.3 ± 0.7	78.2 ± 4.7	92.2 ± 5.5

* Significance relative to the respective control as determined by Student's t-test at a $p < 0.05$.

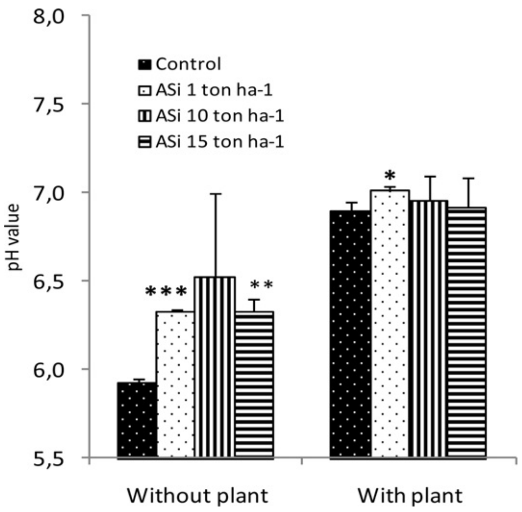

Fig. 6. pH measured in the soil solution collected by Rhizon® in pots of a historically metal-contaminated soil sown or unsown with wheat plants and treated with increasing doses of ASi. Sampling was performed 71 days after sowing, just before harvesting. (*) denotes significance relative to the respective control as determined by Student's t-test at a $p < 0.05$, (**) at $p < 0.01$ and, (***) at $p < 0.001$.

was only statistically significant in sown pots. A decreasing trend was observed for Zn, but not for Cu and Pb concentrations (Table 5) for pots both with and without plants.

3.3. *Effect of Si treatment on the soil solution*

3.3.1. *pH*

The pH of the soil solution collected from pots with plants was larger than the pH of soil solution of pots without plants (Fig. 6). In pots without plants, pH significantly increased with each Si treatment. The pH remained almost the same in soil solutions collected from pots with plants.

3.3.2. *Silicon concentration*

The silicon concentration was always larger in the soil solutions collected from pots without plants than in soil solutions from pots with plants (Fig. 7). Si concentrations changed with time: in soil solutions from pots without plants, Si concentrations increased between the 41st and the 57th day after sowing and then decreased till harvest in all Si treatments. In pots with plants, Si concentrations slightly decreased over time for all treatments, including controls.

3.3.3. *Trace element concentrations*

Cadmium, Zn, Cu and Pb concentrations were measured once after 57 days of sowing because this was when Si concentrations exhibited the largest differences between treatments, and we assumed the potential effect on trace elements would be the largest as well. Cadmium concentrations were lower in all soil solutions from sown pots than in solutions from unsown pots for all ASi treatments (Fig. 8), and Si showed no observable effect.

As for Cd, differences between treatments were not significant for Zn, Pb and Cu. Zn concentrations in soil solutions increased unevenly in all pots (without and with plants), with ASi treatments (Table 6), while Cu concentrations decreased in pots without plants in all ASi treatments. Zinc, Cu and Pb concentrations were higher in both soil solutions without and with plants in the 15 ton ASi ha^{-1}.

4. Discussion

This study indicates that ASi supply increased the biomass of both shoots and roots of durum wheat (Fig. 2), even at low doses. This positive effect of Si on growth and biomass has also been observed in experiments with maize plants in both soil and hydroponic conditions [41,43–45] and in strawberries grown in

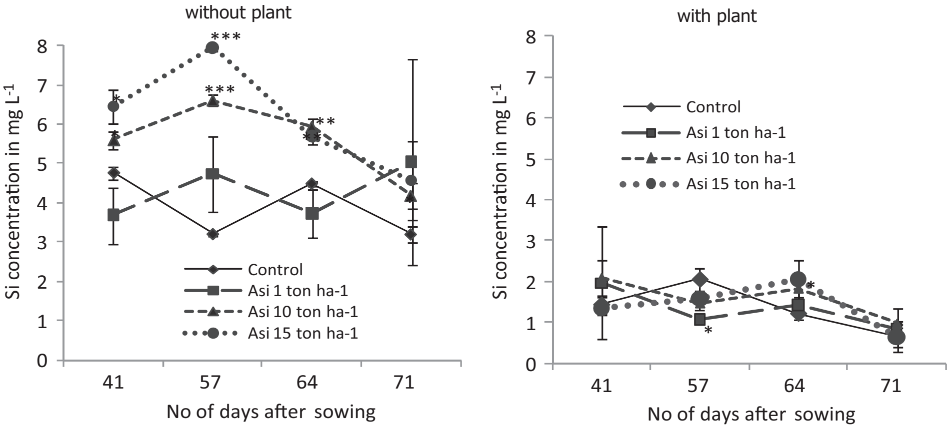

Fig. 7. Evolution of Si concentration through time in the soil solution collected by Rhizon® in pots of a historically metal-contaminated soil sown or unsown with wheat plants and treated with increasing doses of ASi. Bars represent SD of two replicates. (*) denotes significance relative to the respective control as determined by Student's t-test at a $p < 0.05$, (**) at $p < 0.01$ and, (***) at $p < 0.001$.

Table 6
Zinc, Cu and Pd concentration in $\mu g\,L^{-1}$ measured in the soil solution collected 57 days after sowing. Values are means ± SD ($n = 2$).

ASi applications (ton ha^{-1})	Zn ($\mu g\,L^{-1}$)		Cu ($\mu g\,L^{-1}$)		Pb ($\mu g\,L^{-1}$)	
	Without plant	With plant	Without plant	With plant	Without plant	With plant
0	nd	29.1 ± 4.5	18.4 ± 2.0	20.3 ± 6.6	0.72 ± 0.01	0.74 ± 0.54
1	84.9 ± 4.8	30 .9 ± 5.8	15.8 ± 1.0	19.2 ± 7.1	0.73 ± 0.08	0.85 ± 0.28
10	88.1 ± 17.4	26.9 ± 0.2	10.2 ± 2.1*	19.0 ± 1.3	0.56 ± 0.12*	0.56 ± 0.07
15	120.3 ± 0.1	34.6 ± 1.2	16.2 ± 1.3	29.2 ± 2.5	0.95 ± 0.11	1.13 ± 0.12

nd: no data available.

* Significance relative to the respective control as determined by Student's *t*-test at a $p < 0.05$.

contaminated soil [46]. Here, the increase in biomass was concomitant with a decrease in Cd concentration in shoots (Fig. 4A), indicating either a dilution effect (same uptake but larger biomass) or a decrease in Cd uptake leading to less toxicity and thus healthier plants (see Fig. 1).

Concurrently, plants treated with ASi accumulated significantly more Si in shoots than in roots (Fig. 3). This activity where accumulation increases in shoots when plants are supplied with available Si is typical for Si-accumulating plants, such as rice [26], and indicates an active uptake [29,58]. Active uptake has also been suggested for wheat [31]. Silicon is generally taken up as silicic acid from the soil solution [25,32]. It is then precipitated primarily as amorphous silica (Opal A; $SiO_2{\cdot}nH_2O$) in cell walls, lumens and in the intercellular spaces as phytoliths [59,60] where water evaporates from the plants [42,61]. However, Si concentrations in shoots and roots did not increase with increased ASi supplementation in the soil, even though Si concentrations in the soil solution were initially larger (Fig. 7). The increase in biomass probably contributed to a dilution effect.

Cadmium concentrations in shoots and roots of wheat plants did not follow the same trend as shoot concentrations decreased while root concentrations increased with increasing Si application doses (Fig. 4A). Similar results were also observed in rice treated with foliar Si [47] or in rice grown hydroponically [49,62,63], in *Brassica chinensis* [64], in peanut [65] and in cucumber [33]; but our results disagree with authors who found both an increase in root and shoot concentrations following Si application [45] or a decrease in both roots and shoots Cd concentrations [48]. In wheat, a decrease in both root and shoot Cu concentrations was also observed in wheat grown hydroponically [66], but in our case no decrease was observed for the other metal concentrations in either roots or shoots. This increased Cd retention in roots could be due to Si deposition in the roots as found by several authors. For example, Zhang et al. [63] reported that Cd was mainly deposited in the vicinity of the endodermis and epidermis in rice roots, while Shi et al. [49] observed that Si was mainly deposited in the cell walls of the endodermis. However, in our study Si concentrations were not significantly increased in roots, while Cd concentrations increased. In general, mineral nutrients that reach the endodermis must be actively taken up to be uploaded in the xylem. The increased Cd amount measured in wheat root could have been immobilised at the endodermis as a way of reducing metal toxicity by isolating the metals into metabolically inactive parts, which is also one function of the apoplast [37,67]. Lux et al. [68] concluded that the development of apoplastic barriers against Cd movement to the xylem, including maturation of the endodermis, was stimulated by high external Cd. However external Cd concentrations, as measured in the soil solution after 57 days, were not significantly different between the ASi treatments.

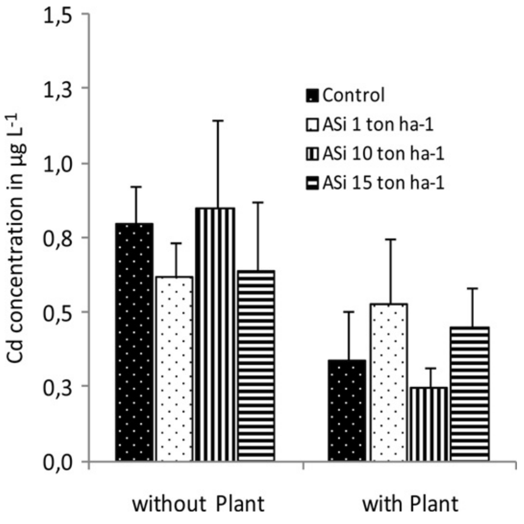

Fig. 8. Cd concentration in $\mu g\,L^{-1}$ measured in the soil solution collected by Rhizon® 57 days after sowing in pots of a historically metal-contaminated soil sown or unsown with wheat plants and treated with increasing doses of ASi. Bars represent SD of four replicates (with plant) or two replicates (without plant).

Contrarily, soluble Si concentrations in plants, especially apoplastic soluble Si, might be more representative of the "efficient" Si concentrations in plants than the total Si concentration, as suggested by Iwasaki et al. [69]. In our case, treated plants were continuously exposed to enhanced Si concentrations in the soil solution (Fig. 7). Consequently, although Si concentrations in roots did not increase with Si applications, soluble apoplastic Si concentrations might have been larger than in untreated plants and constant over the whole growth period. This possibility was suggested by an increased total Si output in leaves and thus might have stimulated the maturation of the endodermis and increased Cd concentrations in roots. Vaculik et al. [45] indeed suggested that Si stimulated the development of endodermal suberin lamellae in maize plants grown hydroponically and exposed to Cd + Si, while da Cunha et al. [43] concluded that Si helped to immobilise Cd and Zn in the endodermis through the thickening of the Casparian strips. Corrales et al. [70] observed that Si decreased Al uptake by maize and suggested that enhanced root exudation or higher pH at the root surface may both lead to Al precipitation at the root surface. A study of the localisation of the respective Cd and Si distributions within the root tissues, as well as a monitoring of Si concentration in the apoplast, would help to conclude on this point.

In our experimental shoots, Si concentrations were similar regardless of the treatment, while Cd concentrations decreased with increasing ASi soil applications. The decrease in Cd concentrations might be related to both, a restricted root–shoot translocation ability, as indicated by a decrease in the shoot/root ratio from 1.1 to 0.7, and a dilution effect, as indicated by a similar Cd output in shoots of all treated plants, while the Si output increased. In shoots, silica precipitation occurs where water evaporates from the plants [42,61], which is in the vicinity of the epidermis and mostly as phytoliths. Zhang et al. [63] have shown that Si decreased Cd accumulation in shoots, and Cd and Si accumulated simultaneously in phytoliths of rice shoots grown hydroponically. This mechanism cannot be excluded in our case, but it would imply that Si has an impact on Cd compartmentalisation in shoots, and thus on its toxicity, rather than on its concentration. This co-precipitation would restrict Cd translocation from shoots to the grain and would help to reduce grain contamination [47]. Neumann et al. [67] reported that zinc was co-precipitated as zinc silicate in the leaf epidermal cell wall of *Minuartia verna* plant species, which served as an explanation for the high zinc tolerance in *M. verna*. More recent studies with cucumber (*Communis sativus* L.) showed that less Mn (10%) was located in the symplast and more Mn (90%) was bound to the cell wall in the Si-treated plants than in the non-Si-treated plants [37], indicating changes in compartmentalisation. In these cases, however, Si concentrations were increased in shoots of the treated plants. Although these mechanisms cannot be ruled out in our case, they do not seem to be the major mechanism involved in the decrease in Cd concentration in shoots.

Silicon concentrations in the soil solution of pots without plants were always larger than those measured in pots with plants. In the pots without plants, Si concentrations in 10 and 15 ton ASi ha^{-1} treated pots were larger between 41 and 64 days after sowing, indicating that application of amorphous silica released Si in the soil solution, whereas the lowest treatment did not exhibit any effect. This was probably because the amount of amorphous silica was too low. After 64 days Si concentrations decreased and returned to concentrations measured in the control pots, indicating that Si was reorganised within the soil either through adsorption of Si on soil surfaces [71], as a result of complexation with organic and inorganic compounds, or was taken up by the microorganisms [42,61,72,73].

The lower Si concentrations measured in solutions from pots with plants, is a good demonstration of plant uptake. Concurrently, similar Si concentrations in plant shoots, regardless of the Si treatment, can be explained both by similar Si concentrations measured in the soil solution and an enhanced Si uptake due to a larger biomass production with increasing Si doses, as already mentioned. This also indicates that, in our case, soil Si release is the limiting factor for Si plant uptake. Except for copper, all metal concentrations in soil solutions were lower in pots with plants than in those without plants. As for Si, these concentration variations can be explained by plant uptake, but the slight increase in pH may also be responsible for a reduction in the metal uptake by the plants through immobilisation in the soil [41,74] and a decreased availability to plants, as also indicated by slightly lower levels of DTPA-TEA-extractable metals in ASi-treated pots (Fig. 5 and Table 5). Gu et al. [75] suggested that, in a contaminated acidic soil amended with FA (fly ash) and SS (steel slag), the reduction in Cd, Zn, Cu and Pb uptake by rice was attributed to two processes, including the in situ immobilisation of heavy metals in soil and the Si-mediated effects on rice. The application of FA and SS decreased the heavy metal DGT pools and the fluxes from the soil solid phase to the solution by transforming the soluble metals to the less soluble and slower exchanging forms, such as metal silicates, phosphates and hydroxides. The mechanism was associated with an increase in pH. Putwattana et al. [76] also showed that silicate fertiliser could effectively immobilise Cd in the soil and decrease its uptake and translocation in sweet basil (*Ocimum basilicum*).

Finally, most of the pot experiments performed with metals and Si used an additional soluble salt to reach high Cd concentrations in soils, and available concentrations were often unrealistic for in situ levels [41,43]. Speciation in the soil may change very quickly in the soil when soil is not equilibrated with the treated solution, which means that the effect of Si addition observed on metal availability might well be a combined effect of aging [77], higher pH levels and silicate phase addition. The soil we used had been contaminated 25 years prior, and no aging effect has been known to occur during the experiment. Although the initial Cd concentration was rather low, we observed a significant alleviating effect of Si addition to soil on Cd transfer to plants, whereas no alleviating effect was observed on Cu, Zn and Pb.

This study suggests that the alleviating effect of ASi on metal-induced stress, as evidenced by an increase of biomass with increasing doses of ASi applied to soil, is mainly due to changes in the Cd balance. As suggested by Liang et al. [41], we observed a combined effect in both the soil and in plants; silica application induced a decrease in available Cd in the soil, while at the plant level, Cd was sequestrated in roots more efficiently when ASi had been added and Cd translocation to shoots was reduced. Biomass production was then increased either because of a Si fertilising effect or because Cd concentrations were reduced and contributed in turn to an additional diluting effect of Cd concentrations in shoots. As suggested by the results, Si release in the soil is the limiting factor for Si plant uptake. This is because Si concentrations in the soil solution of pots with plants were low regardless of the dose application and because no plateau was observed in the Si and Cd concentration trends in plants, although the highest rate applied was already larger than the Japanese recommended optimum rate for rice of 1.5–3.0 t ha^{-1} of silicate slag [78]. Larger ASi doses may thus provide even more significant results than what were established in this study. This would also help to better quantify the respective aspect of the various processes involved in alleviating Cd toxicity in wheat.

5. Conclusion

In conclusion, application of ASi amendment to soil may contribute to decreased Cd concentrations in wheat shoots, even if initial Cd concentrations in soil are limited or if Cd is combined with other metals. Adding ASi to soils may thus be part of a general protocol aiming to control Cd concentrations in wheat shoots and as a consequence in grain, along with other management practices [79,80].

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