



Silicon reduces cadmium absorption and increases root-to-shoot translocation without impacting growth in young plants of hemp (*Cannabis sativa* L.) on a short-term basis

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Abstract

Textile hemp (*Cannabis sativa* L.) is a non-edible multipurpose crop suitable for fiber production and/or phytoremediation on moderately heavy metal-contaminated soils. Experiments were conducted in nutrient solution to assess the short-term impact of silicon (Si), a well-known beneficial element, on plants exposed to 20 μ M cadmium (Cd) in nutrient solution. Cd decreased plant growth and affected photosynthesis through non-stomatal effects. Cd translocation factor was higher than 1, confirming the interest of hemp for phytoextraction purposes. Additional Si did not improve plant growth after 1 week of treatment but decreased Cd accumulation in all organs and improved water use efficiency through a decrease in transpiration rate. Si had only marginal impact on Cd distribution among organs. It increased glutathione and phytochelatin synthesis allowing the plants to efficiently cope with oxidative stress through the improvement of Cd sequestration on thiol groups in the roots. Si may thus have a fast impact on the plant behavior before the occurrence of plant growth stimulation.

Keywords Cadmium · *Cannabis sativa* · Hemp · Phytoremediation · Silicon

Introduction

Since the industrial revolution, the phenomenon of heavy metal pollution has been steadily increasing, leading to a rise in atmospheric concentrations and contamination of soil and water in many parts of the world (Barceló and Poschenrieder 2003; Ali et al. 2013; Mahar et al. 2016). Most polluted soils are multi-contaminated, and unlike organic pollutants, heavy metals cannot be degraded but only moved from one place to another (Kumpiene et al. 2008). From an economic point of view, this pollution limits the availability of soils for several uses (residential, commercial, industrial). This is particularly true for agriculture, as environmental legislation prohibits the

production of food on contaminated soils considering obvious risks for human health (Douay et al. 2013; Grčman et al. 2001).

Cd is a widespread pollutant extremely toxic for all living organisms. It may accumulate in farmland soils as a result of the use of fertilizers (especially single superphosphate), sewage sludge, inorganic manures, irrigation wastes, and dry or wet atmospheric deposition (Comnan et al. 2013; Kumarpondit et al. 2017). As a non-essential element, Cd is absorbed by other metal transporters and non-selective cation channels and Cd uptake may consequently compete with the absorption of other divalent cations (Ca, Mg, Fe, Zn, Mn, and Cu). Numerous transporters from the IRT (iron-regulated transporter), YSL (yellow stripe-like), and NRAMP (natural resistance-associated macrophage protein) families were shown to be involved, to various extent, in Cd absorption (Huang et al. 2020). Once it is accumulated within plant tissues, Cd induces a wide range of metabolic disorders, such as oxidative stress; damages to PSII; and decreases in photosynthetic pigments, water stress, leaf chlorosis, and necrosis leading to reduced growth and, ultimately, to plant death (Barylá et al. 2001; Lefèvre et al. 2014; He et al. 2017; Berni et al. 2018). Strategies adopted by the plants to cope with high Cd concentration involve

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reduction of Cd uptake and root-to-shoot translocation, activation of antioxidative systems, and synthesis of sulfur-containing ligands mainly phytochelatins (PC) allowing Cd vacuolar sequestration (Cobbett 2000; Huang et al. 2020).

Plants may be grown on Cd-contaminated substrate for two distinct purposes. The first one is phytoremediation where plants are cultivated to extract, degrade, or immobilize various contaminants from polluted soils (Barceló and Poschenrieder 2003; Ali et al. 2013). Phytoextraction consisting in pollutant accumulation in harvestable parts appears as an interesting cheap alternative to the physicochemical methods traditionally used, since the initial investment is lower although the immobilization of the site is quite longer than for classical engineering approaches (Mahar et al. 2016). The second application of plant culture concerns moderately contaminated substrates and consists in the use of non-edible crop of commercial interest to valorize these marginal farmland soils and provide incomes for the farmer (Tóth et al. 2016; Feng et al. 2020). It is commonly assumed that these two types of plant utilization are somewhat incompatible, since phytoextraction relies on heavy metal absorption by the plant while agricultural application is expected to produce non-contaminated plant products. This, however, partly depends on the nature of the harvested organs in relation to heavy metal distribution at the whole plant level. If the harvested organs are free of pollutant while the remaining part of the shoot contains high amounts of pollutant, it appears possible to combine an agricultural purpose and a phytoremediation process. Hence, determining pollutant distribution among plant organs, as well as bioaccumulation and root-to-shoot translocation factors, is of paramount importance.

Fiber crops are important plant species for bio-economy and are now receiving considerable attention (Guerriero et al. 2017). Hemp (*Cannabis sativa* L.) is considered a rotation head in culture systems. It provides cortical fiber mainly used in paper industries and for the manufacture of various products (composites, insulators, reinforced thermoplastics), and hurds used for the manufacture of animal litter and, in particular, in the composition of building materials (Duqueschumacher et al. 2020; Vandepitte et al. 2020). Seeds may also be harvested and contain valuable essential oil (Ascrizzi et al. 2019). Many authors (Angelova et al. 2004; Shi et al. 2009, 2012; Ahmad et al. 2016; Kumar et al. 2017; Zielonka et al. 2020) have considered using hemp in phytoremediation and believe that the plant is able, to a certain extent, to reduce Cu, Zn, Cd, and Pb contamination in soils. While heavy metals can accumulate within walls in plant tissues (Krzeslowska 2011), the influence of these metals on the growth and development of hemp remains poorly understood, particularly in relation to the kinetics of fiber development. Although seeds may accumulate Cd concentrations above the upper limit (Mihoc et al. 2012), preliminary data demonstrated that bast fibers remain free of heavy metals when plants

were grown in heavy metal-rich solution (Arru et al. 2004) so that fiber production might appear compatible with phytoextraction.

Hemp cultivars differ for their average level of Cd resistance (Zielonka et al. 2020; Kalousek et al. 2020; Huang et al. 2019). Besides a breeding approach aiming to select the most appropriate cultivars, some technical strategies, such as the application of biodegradable chelators (Kos and Leštan 2004) or the inoculation with arbuscular mycorrhiza fungus (Citterio et al. 2005), also contribute to reinforce the capacity of *Cannabis sativa* to accumulate toxic heavy metals. Application of Si fertilizers is a promising option to mitigate the deleterious impact of heavy metal stress on various species (Neumann and Zur Nieden 2001; Doncheva et al. 2009; Wu et al. 2013; Bhat et al. 2019). The young plant stage is often considered the most sensitive one to heavy metals which is mainly due to an incomplete formation of suberized endoderm, thus allowing massive uncontrolled translocation of pollutant through by pass flow (Lefèvre et al. 2009). Si has been reported to deposit in the vicinity of the endodermis, thus contributing to reinforce the barrier against Cd translocation (Shi et al. 2005; Imtiaz et al. 2016) and similar finding were reported for other mineral constraints such as salinity (Yeo et al. 1999). Moreover, Si can accumulate within the parietal structures where it is present in the form of opaline silica (SiO₂) and contributes to reinforce the mechanical properties of the cell wall polymers (Kröger and Poulsen 2004). Adrees et al. (2015) also reported that Si is suspected to influence the root-to-shoot translocation of heavy metals.

The nature and the extent of Si-induced protection depend on the plant species and on the nature of the stressor. As far as hemp is concerned, Guerriero et al. (2019) recently identified aquaporins putatively involved in Si absorption. Berni et al. (2020) confirmed that exogenous Si may mitigate the deleterious effect of salt stress in *C. sativa*. However, to the best of our knowledge, no data are available regarding Si impact on Cd-treated young plants of hemp. The present work was therefore undertaken in order to determine the Si impact on Cd distribution in young hemp plants and the short-term physiological consequences of this impact on photosynthesis-related parameters, water status, and oxidative stress.

Material and methods

Plant material and growing conditions

Seeds of a monoecious hemp fiber variety (*C. sativa* cv. Santhica 27) were sown in loam substrate in greenhouse conditions. After 1 week, the obtained seedlings were transferred to nutrient Hoagland solution (in mM: 2.0 KNO₃, 1.7 Ca(NO₃)₂, 1.0 KH₂PO₄, 0.5 NH₄NO₃, 0.5 MgSO₄; in μM: 17.8 Na₂SO₄, 11.3 H₃BO₃, 1.6 MnSO₄, 1 ZnSO₄, 0.3 CuSO₄,

0.03 (NH₄)₆Mo₇O₂₄, and 14.5 Fe-EDDHA) in 5-L tanks: for each tank, the seedling was adapted to plugged hole in a polystyrene plate floating at the top of the nutrient solution. Tanks were placed in a phytotron under fully controlled environmental conditions (permanent temperature of 24 ± 1 °C with a mean light intensity of 230 μmol m⁻² s⁻¹ provided by Phillips lamps (Philips Lighting S.A., Brussels, Belgium) (HPI-T 400 W), with a photoperiod of 16 h under a relative humidity of 65%). After a week of acclimatization, half of the tanks received Si in the form of H₂SiO₃ to a final concentration of 2 mM Si. Metasilicic acid was obtained from a pentahydrate sodium metasilicate (Na₂SiO₃ × 5 H₂O) which was passed through an H⁺ ion exchanger resin IR 20 Amberlite type according to Dufey et al. (2014). Tanks were randomly arranged in the phytotron, and the nutrient solution was permanently aerated by SuperFish Air Flow 4 pump. A week later, Cd was applied in the form of CdCl₂ (20 μM). The pH of the solution was maintained at 5.5. Four treatments were thus defined, considering the presence of Cd and the concomitant presence or absence of Si and will be designated as C (control: no heavy metals and no Si), C_{Si}, Cd, and Cd_{Si} (4 tanks per treatment). The solubility of added heavy metal was confirmed by the Visual MINTEQ09 software; speciation of Cd was Cd²⁺ (93.82%), CdHPO₄ (5.96%), CdCl⁺ (0.129%), and CdNO₃⁺ (0.04%) while speciation of Si indicated H₄SiO₄ (94.06%) and H₃SiO₄⁻ (5.93%). Si had no significant impact on Cd solubility and speciation. Cadmium and Si in the nutrient solution were analyzed at the beginning and at the end of treatment to ensure they remain soluble and available to the plants.

Harvests were performed after a week of Cd exposure. Stem length and diameter, number of leaves, and main root length were considered. Roots were quickly rinsed in deionized water for 30 s just before harvest to remove ions from the free spaces. Roots were then separated from shoots, and leaves were separated from the stem. Roots and leaves from the same treatment were pooled, quickly frozen in liquid nitrogen, and then stored at -80 °C until analysis, except for subsamples of 3 plants per treatment incubated in an oven at 70 °C for 72 h to estimate dry weight and water content and to determine ion content.

Mineral concentration

Fifty–100-mg DM (dry matter) of plant samples was dried at 70 °C for 72 h, then digested in 68% HNO₃ and acid-evaporated to dryness on a sand bath at 80 °C. Minerals were incubated with a mix of HCl 37%–HNO₃ 68% (3:1) and the mixture was slightly evaporated and dissolved in distilled water. After filtration on Whatman no. 1 filter paper, cations were quantified by inductively coupled plasma-optical emission spectroscopy (Varian, type MPX). For Si quantification, 1-g DM was placed in an oven and heated to 500 °C for 48 h.

Ashes were then mixed with 0.4 g tetraborate and 1.6 g metaborate and heated to 1000 °C for 5 min. The obtained pellet was dissolved with 34% HNO₃. Cations were quantified by inductively coupled plasma-optical emission spectroscopy (Varian, type MPX).

Translocation factor reflects the capacity of the plant to translocate heavy metal from the root to the shoot and was estimated (1) on the basis of the concentration expressed on a dry weight basis in each plant part (TF_c) and (2) on the basis of total amount of the considered element (TF_a)

TF_c = Cd concentration in the shoot/Cd concentration in the root

TF_a = Total Cd amount accumulated in the shoot/Cd accumulated in the root

The bioaccumulation factor (BF) considers the capacity of the plant to store heavy metals in relation to external concentration. Since a nutrient solution was used in the present work rather than a solid substrate, the internal concentration in the plant was estimated on a water content basis, considering the proportion of the different organs:

BF = Cd concentration in the plant (mg·L⁻¹)/Cd concentration in the solution (mg·L⁻¹)

Photosynthesis-related parameters

Before plant harvest, physiological measurements were performed on 5 plants per treatment, on the middle portion of the blades on the second fully expanded leaf from the top. Chlorophyll fluorescence was measured using a fluorescence monitoring system (FMS2; Hansatech, King's Lynn, UK). Blade portions were acclimated to darkness for 20 min. The minimal fluorescence level (*F*₀) was obtained by measuring the modulated light (0.1 μmol m⁻² s⁻¹). The maximal fluorescence level (*F*_m) with all photosystem II (PSII) reaction centers open was determined by a 0.8-s saturating pulse at 8500 μmol m⁻² s⁻¹ in dark-adapted leaves. The leaves were then continuously illuminated with white actinic light (320 μmol m⁻² s⁻¹), during 3 min; the steady-state value of fluorescence (*F*_s) was recorded. A second saturating pulse at 8500 μmol m⁻² s⁻¹ was imposed to determine maximal fluorescence level in the light-adapted state (*F*_m′). The actinic light was removed and the minimal fluorescence level in the light-adapted state (*F*₀′) was determined by illuminating the leaf with a 3-s pulse of far-red light. The following fluorescence-related parameters were calculated (Maxwell and Johnson 2000): *F*_v/*F*_m (maximal efficiency of PSII photochemistry in the dark-adapted state), *q*_P (photochemical quenching

coefficient), NPQ (non-photochemical quenching), Φ_{PSII} (actual PSII efficiency).

Leaf stomatal conductance (g_s) was measured using an AP4 diffusion porometer (Delta-T Devices Ltd., Cambridge, UK). The instantaneous CO_2 assimilation under ambient conditions (400 ppm CO_2) (A) and instantaneous evapotranspiration (E) were measured using an infrared gas analyzer (LCA4 8.7 ADC, Bioscience, Hertfordshire, UK).

Total chlorophyll (a + b) and carotenoid concentrations were measured according to Lichtenthaler (1987): 100-mg ground fresh samples were homogenized in 10 mL of cold acetone, then centrifuged at 936g for 10 min at 4 °C. Absorbance was measured on supernatant at 663.2 nm, 646.8 nm, and 470 nm.

For osmotic potential determination (Ψ_s), the middle portion of the leaf blades constituting the second fully formed leaves from the top was quickly collected from five plants, cut in small segments placed in Eppendorf tubes perforated with small holes, and immediately frozen in liquid nitrogen. Samples were then thawed 5 min at ambient temperature to rupture the membranes. Freeze-thawing cycles were repeated three times. Each tube was then encased in a second intact Eppendorf tube and centrifuged at 8000g for 15 min at 4 °C. The osmolality of the collected sap was analyzed with a vapor pressure osmometer (VAPRO® Vapor Pressure Osmometer 5520).

Malondialdehyde content and total antioxidant activities

The level of lipid peroxidation in the control and Cd-treated plants was assessed from the concentration of malondialdehyde (MDA) as determined by the thiobarbituric acid (TBA) reaction (Heath and Packer 1968). To estimate the total global antioxidant activity, ferric-reducing ability of plasma (FRAP) was assayed according to Benzie and Strain (1996), considering the ability of plant extract to reduce ferric to ferrous ion at low pH and to produce a colored ferrous-tripyridyltriazine complex which was spectrophotometrically detected at 593 nm. The hydrophilic (AOAM) and hydrophobic fractions (AOAD) were discriminated. The standard curve was established with Trolox (50–800 μM). The concentration is expressed in micromolars of trolox equivalents (TE) g^{-1} of fresh material.

Glutathione and total non-protein thiols

For reduced (GSH) and total (GSht) glutathione quantification, 200 mg of frozen samples was extracted and derivatized by orthophthalaldehyde (OPA) according to Cereser et al. (2001). GSht was quantified after a reduction step of oxidized glutathione (GSSG) by dithiothreitol. Five microliters of sample was injected into a Shimadzu HPLC system (Shimadzu, 's-Hertogenbosch, The Netherlands) equipped with a

Nucleodur C18 Pyramid column (125 × 4.6-mm internal diameter; 5- μm particle size) (Macherey-Nagel, Duren, Germany). Fluorimetric detection was performed with a spectra system Shimadzu RF-20A fluorescence detector at 420 nm after excitation at 340 nm.

The total non-protein thiol (NPT) concentration was determined according to De Vos et al. (1992) using Ellman's reagent. The NPT concentration was estimated spectrophotometrically at 412 nm using an extinction coefficient of 13,600 $\text{M}^{-1} \text{cm}^{-1}$. Phytochelatin content was evaluated as the difference between NPT and GSH levels.

Statistical analysis

All analyses were performed on 5 replicates. Normality of the data was verified using Shapiro-Wilk tests, and the data were transformed when required. Homogeneity of the data was verified using Levene's tests. ANOVA 2 were performed at a significant level of P value < 0.05 using R (version 3.3.1) considering the type of heavy metal treatment, and the Si application as main factors. Means were compared using Tukey's HSD all-pairwise comparisons at 5% level as a post hoc test.

Results

Plant growth and morphological parameters

As illustrated in Fig. 1, the presence of Cd in the external nutrient solution strongly hampered plant development and compromised plant growth, inducing a decrease in shoot elongation, stem diameter, and number of leaves while the length of the root remained unaffected by the treatments (Fig. 2). Cd-treated plants exhibited a stunted growth, leaf chlorosis, and leaf rolling behavior. Si had no impact on control plant, except a slight decrease in stem diameter, and it did not attenuate the impact of Cd on morphological parameters of heavy metal-treated plants considering the duration of the treatment (1 week) (Fig. 2).

Cd decreased both fresh and dry weights of roots, stems, and leaves in *C. sativa* (Table 1). It had no impact on the water content of roots and stems but it decreased the leaf WC. Exogenous Si did not interfere with Cd-induced growth inhibition. Cd decreased the leaf osmotic potential, and this effect was even reinforced by the concomitant presence of Si exposure. Si also significantly decreased leaf Ψ_s in the absence of Cd.

Plant mineral content

Cd accumulated to higher concentration in the roots than in the shoot (Table 2). As far as shoot is concerned, Cd accumulation was higher in the stem than in the leaves. The additional

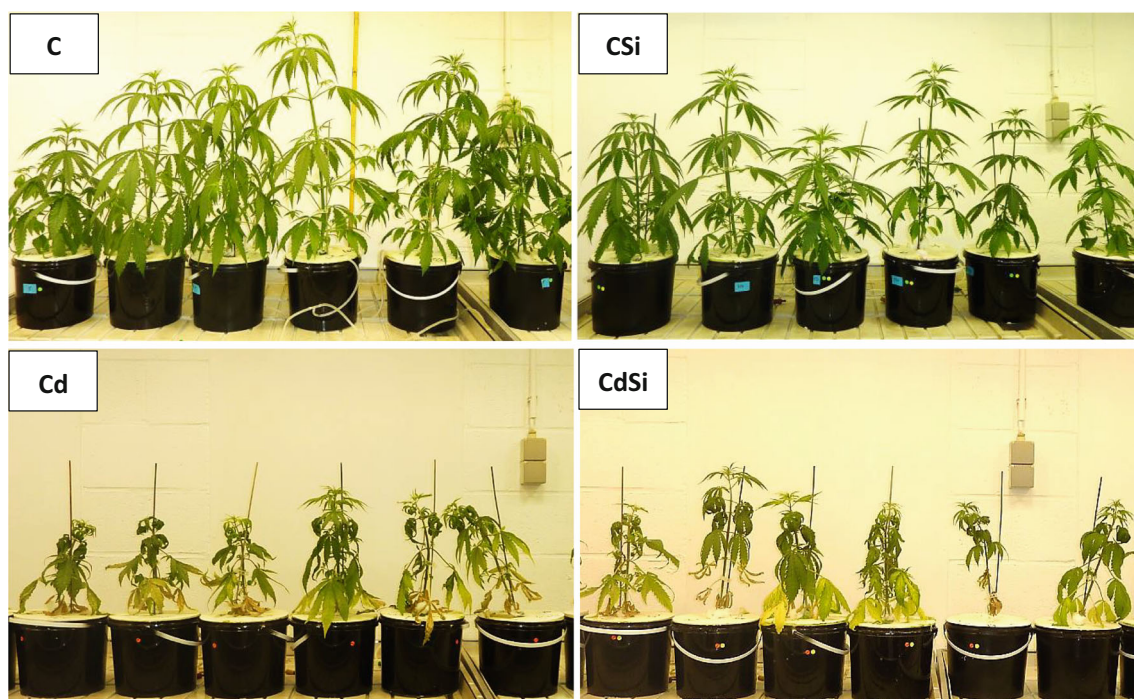


Fig. 1 *Cannabis sativa* (cv. Santhica 27) exposed for 1 week to Cd (20 μ M), in the presence or in the absence of 2 mM H_2SiO_3

presence of Si in the nutrient solution significantly reduced Cd accumulation in all organs, but it increased both TF_c and TF_a values comparatively to plants exposed to Cd in the absence of Si. In all cases, TF_a values were higher than TF_c values. The

Cd bioaccumulation factor was as high as 89.1 in Cd-treated plants and Si only slightly reduced Cd BF.

As expected, Si content increased in all plant parts when unstressed plants were exposed to Si treatment, reaching more

Fig. 2 Total leaf number (a), stem length (b) and diameter (c), and root length (d) of *Cannabis sativa* (cv. Santhica 27) exposed for one week to Cd (20 μ M), in the presence or in the absence of 2 mM H_2SiO_3 . Data are means $\pm$ standard errors ($n = 5$). Values with different letters are significantly different ($P < 0.05$; Tukey's HSD all-pairwise comparisons)

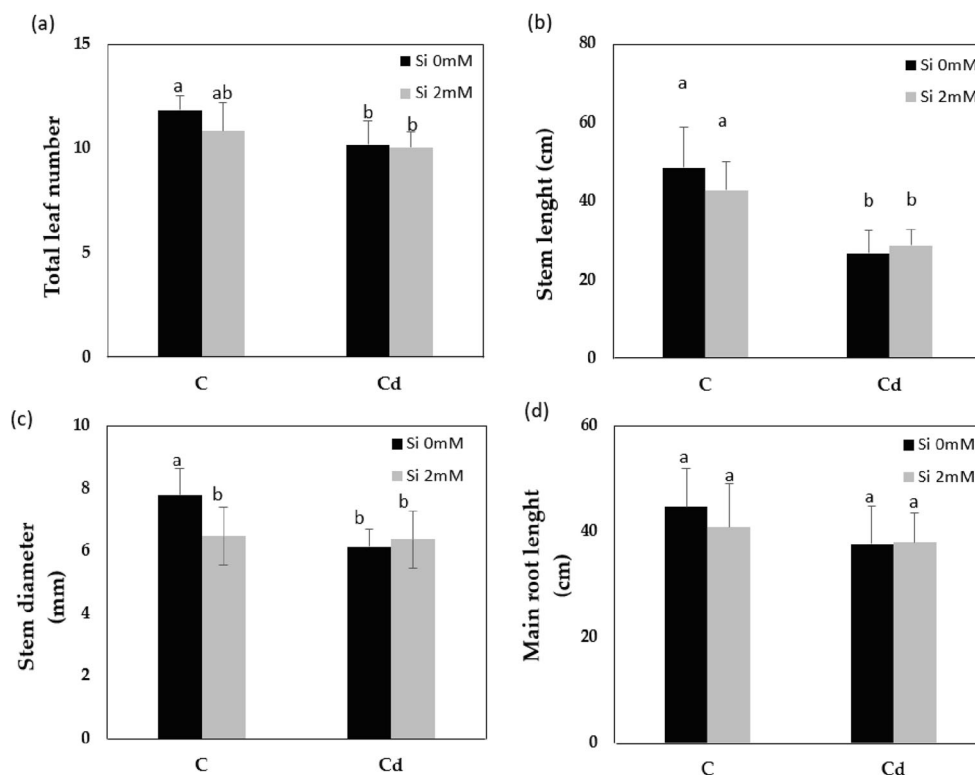


Table 1 Fresh weight (FW), dry weight (DW), water content (WC), and osmotic potential (ψ_s) of *Cannabis sativa* (cv. Santhica 27). Plants were exposed for 1 week to Cd (20 μ M) in the presence or in the absence of 2 mM H_2SiO_3 . For a given organ, different letters indicate significant differences at $P < 0.05$. Each value is the mean of 3 replicates (FW, DW, and WC) or 5 replicates (ψ_s)

	C	CSi	Cd	CdSi
FW (g)				
Roots	14.67 $\pm$ 3.76 b	13.08 $\pm$ 4.52 b	6.96 $\pm$ 1.62 a	5.84 $\pm$ 1.53 a
Stems	16.37 $\pm$ 3.56 b	12.28 $\pm$ 2.15 b	5.72 $\pm$ 1.36 a	6.01 $\pm$ 1.83 a
Leaves	40.88 $\pm$ 2.09 b	30.24 $\pm$ 8.43 b	8.20 $\pm$ 3.26 a	11.01 $\pm$ 0.12 a
DW (g)				
Roots	1.43 $\pm$ 0.19 b	1.23 $\pm$ 0.03 b	0.56 $\pm$ 0.12 a	0.49 $\pm$ 0.12 a
Stems	1.62 $\pm$ 0.32 b	1.42 $\pm$ 0.28 b	0.72 $\pm$ 0.12 a	0.81 $\pm$ 0.15 a
Leaves	6.34 $\pm$ 0.54 b	5.62 $\pm$ 0.72 b	2.31 $\pm$ 0.50 a	2.18 $\pm$ 0.60 a
WC (%)				
Roots	91 $\pm$ 1.0 a	93 $\pm$ 0.4 b	92 $\pm$ 0.9 ab	91 $\pm$ 0.7 a
Stems	90 $\pm$ 2.3 b	90 $\pm$ 1.0 b	88 $\pm$ 0.8 ab	85 $\pm$ 2.3 a
Leaves	84 $\pm$ 0.2 b	85 $\pm$ 1.0 b	78 $\pm$ 3.3 a	77 $\pm$ 2.9 a
ψ_s (Mpa)				
Leaves	-1.15 $\pm$ 0.03 d	-1.21 $\pm$ 0.04 c	-1.48 $\pm$ 0.05 b	-1.56 $\pm$ 0.04 a

than 2 g·kg⁻¹ in roots and more than 3 g·kg⁻¹ DM in leaves, while the minimal value was recorded in the stem. It is noteworthy that Cd treatment in CdSi-exposed plants significantly reduced Si accumulation in the roots and in the stems but strongly increased it in the leaves, where it reached almost 5 g·kg⁻¹. Cd treatment induced a strong increase of TF_a and TF_c values recorded for Si (Table 2), and it also increased by more than 50% the Si bioaccumulation factor in *C. sativa*.

Table 2 Cadmium (Cd) and silicon (Si) concentrations (in mg kg⁻¹ DW) and translocation factor (TF) in roots, stem, and leaves of *Cannabis sativa* (cv. Santhica 27). Plants were exposed for 1 week to Cd (20 μ M) in the presence or in the absence of 2 mM H_2SiO_3 . TF_c: translocation factor estimated on a concentration basis. TF_a: translocation factor estimated on a total amount basis. BF: bioaccumulation factor. For a given parameter and a given organ, means followed by different letters are significantly different at $P < 0.05$. Each value is the mean of 3 replicates. **Nd**, not detected

	C	CSi	Cd	CdSi
Cd (mg kg ⁻¹ DW)				
Roots	5.59 $\pm$ 1.56 a	nd	2687 $\pm$ 624 c	1819 $\pm$ 532 b
Stems	0.04 $\pm$ 0.08 a	0.25 $\pm$ 0.50 a	1243 $\pm$ 174 c	978 $\pm$ 141 b
Leaves	nd	nd	717 $\pm$ 88 b	623 $\pm$ 76 a
TF _c	0.00 $\pm$ 0.00 a	0.00 $\pm$ 0.00 a	0.31 $\pm$ 0.06 b	0.40 $\pm$ 0.02 c
TF _a	0.01 $\pm$ 0.002 a	0.00 $\pm$ 0.00 a	1.68 $\pm$ 0.27 b	2.40 $\pm$ 0.54 c
BF	0.04 $\pm$ 0.001 a	0.00 $\pm$ 0.00 a	89.10 $\pm$ 5.74 b	81.18 $\pm$ 2.38 b
Si (mg kg ⁻¹ DW)				
Roots	212 $\pm$ 39 a	2002 $\pm$ 212 b	237 $\pm$ 63 a	1626 $\pm$ 156 c
Stems	nd	218 $\pm$ 13 a	nd	89 $\pm$ 35 b
Leaves	70 $\pm$ 13 a	3391 $\pm$ 65 b	nd	4852 $\pm$ 102 c
TF _c	0.26 $\pm$ 0.04 b	1.33 $\pm$ 0.11 c	0.00 $\pm$ 0.00 a	2.19 $\pm$ 0.57 d
TF _a	1.47 $\pm$ 0.20 b	8.64 $\pm$ 1.25 c	0.00 $\pm$ 0.00 a	13.31 $\pm$ 3.25 d
BF	0.22 $\pm$ 0.0a b	7.60 $\pm$ 0.43 c	0.06 $\pm$ 0.01 a	11.76 $\pm$ 0.64 d

In the absence of Cd, Si induced an increase in Ca and Mg concentration in the root, an increase in stem Fe, and a decrease in leaf P (Table 3). Cd increased Ca, P, and S in the stems and in the leaves. It increased Fe concentration in the roots but decreased it in the leaves and also decreased K concentration in the leaves. The addition of Si to Cd-treated plants mitigated the impact of Cd: it strongly increased the leaf Fe content, suppressed the Cd-induced decrease in Ca in the roots and mitigated the Cd-induced P increase in the shoot parts. In contrast, Si had no significant impact on S accumulation in the shoot of Cd-treated plants.

Photosynthesis-related parameters

Si applied in the absence of Cd significantly increased *Chla*, *Chlb*, and carotenoid concentration in the leaves of *C. sativa* (Fig. 3). Conversely, Cd induced a strong decrease in all photosynthetic pigments and Si was unable to reduce the heavy metal impact on chlorophyll and carotenoid concentrations. Neither Si nor Cd had any impact on F_v/F_m , q_P , and Φ_{PSII} which remained constant in all treatments (Table 4). Cd unexpectedly reduced NPQ values: the recorded decrease was similar in Cd- and CdSi-treated plants.

Net photosynthesis (*A*; Table 4) was not affected by Si in the absence of Cd. Cd decreased the *A* values, and Si had no mitigating impact on Cd effects. A high level of variability was observed for stomatal conductance: Si reduced g_s in Cd-treated plants. Similarly, Si had no impact on instantaneous transpiration (*E*) in the absence of Cd and Cd in the absence of Si also had no impact on transpiration. However, Si significantly decreased *E* values in the presence of Cd. Cd-treated plants exhibited higher C_i values than control ones, especially in the absence of Si.

Table 3 Calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), phosphorus (P), and sulfur (S) concentrations and translocation factor (TF) in roots, stem, and leaves of *Cannabis sativa* (cv. Santhica 27). Plants were exposed for 1 week to Cd (20 μ M) in the presence or in the absence of 2 mM H_2SiO_3 . TF_c: translocation factor estimated on a concentration basis. TF_a: translocation factor estimated on a total amount basis. BF: bioaccumulation factor. For a given parameter and a given organ, means followed by different letters are significantly different at $P < 0.05$. Each value is the mean of 3 replicates

	C	CSi	Cd	CdSi
Ca (mg kg ⁻¹ DW)				
Roots	2709 $\pm$ 36 <i>a</i>	3050 $\pm$ 133 <i>b</i>	2385 $\pm$ 103 <i>c</i>	2685 $\pm$ 72 <i>a</i>
Stems	8712 $\pm$ 141 <i>a</i>	9889 $\pm$ 320 <i>ab</i>	10,978 $\pm$ 479 <i>b</i>	9021 $\pm$ 602 <i>a</i>
Leaves	50,539 $\pm$ 1061 <i>a</i>	48,008 $\pm$ 919 <i>a</i>	55,234 $\pm$ 1342 <i>b</i>	50,344 $\pm$ 1427 <i>a</i>
Fe (mg kg ⁻¹ DW)				
Roots	588 $\pm$ 39 <i>a</i>	660 $\pm$ 46 <i>a</i>	867 $\pm$ 55 <i>b</i>	847 $\pm$ 88 <i>b</i>
Stems	49 $\pm$ 1 <i>ac</i>	64 $\pm$ 5 <i>b</i>	56 $\pm$ 5 <i>bc</i>	45 $\pm$ 2 <i>a</i>
Leaves	133 $\pm$ 10 <i>a</i>	118 $\pm$ 9 <i>abc</i>	91 $\pm$ 3 <i>c</i>	207 $\pm$ 18 <i>d</i>
K (mg kg ⁻¹ DW)				
Roots	40,923 $\pm$ 972 <i>a</i>	44,665 $\pm$ 2698 <i>a</i>	45,837 $\pm$ 2942 <i>a</i>	40,658 $\pm$ 1979 <i>a</i>
Stems	53,487 $\pm$ 783 <i>b</i>	46,209 $\pm$ 29 <i>ac</i>	46,234 $\pm$ 864 <i>a</i>	40,488 $\pm$ 3354 <i>c</i>
Leaves	43,327 $\pm$ 694 <i>a</i>	42,149 $\pm$ 1605 <i>a</i>	35,444 $\pm$ 663 <i>b</i>	35,736 $\pm$ 293 <i>b</i>
Mg (mg kg ⁻¹ DW)				
Roots	2468 $\pm$ 64 <i>a</i>	3044 $\pm$ 169 <i>b</i>	2823 $\pm$ 151 <i>ab</i>	2862 $\pm$ 186 <i>b</i>
Stems	1660 $\pm$ 16 <i>b</i>	1664 $\pm$ 16 <i>b</i>	2100 $\pm$ 75 <i>a</i>	1776 $\pm$ 156 <i>b</i>
Leaves	5877 $\pm$ 87 <i>a</i>	5679 $\pm$ 80 <i>a</i>	5761 $\pm$ 89 <i>a</i>	5851 $\pm$ 240 <i>a</i>
P (mg kg ⁻¹ DW)				
Roots	11,430 $\pm$ 329 <i>a</i>	10,356 $\pm$ 762 <i>a</i>	11,683 $\pm$ 1057 <i>a</i>	10,353 $\pm$ 835 <i>a</i>
Stems	6000 $\pm$ 35 <i>ab</i>	5188 $\pm$ 7 <i>a</i>	9368 $\pm$ 243 <i>c</i>	7064 $\pm$ 632 <i>b</i>
Leaves	7403 $\pm$ 106 <i>a</i>	6984 $\pm$ 117 <i>b</i>	10,140 $\pm$ 104 <i>c</i>	8472 $\pm$ 230 <i>d</i>
S (mg kg ⁻¹ DW)				
Roots	1871 $\pm$ 472 <i>a</i>	2477 $\pm$ 158 <i>a</i>	2255 $\pm$ 918 <i>a</i>	3348 $\pm$ 410 <i>a</i>
Stems	616 $\pm$ 158 <i>a</i>	412 $\pm$ 138 <i>a</i>	2029 $\pm$ 382 <i>b</i>	1793 $\pm$ 439 <i>b</i>
Leaves	1203 $\pm$ 117 <i>a</i>	967 $\pm$ 61 <i>a</i>	2254 $\pm$ 148 <i>b</i>	1908 $\pm$ 740 <i>b</i>

Oxidative stress-related parameters and phytochelatin estimation

Malondialdehyde (MDA) as an indicator of oxidative stress increased in the leaves in response to Si in the absence of Cd (Fig. 4a). The highest leaf MDA concentration was observed in response to Cd treatment: addition of Si slightly decreased

Cd-induced MDA accumulation but the recorded decrease was not significant from a statistical point of view. At the root level (Fig. 4b), the MDA concentration was the highest for the control plants, while Si and Cd applied separately decreased MDA content to similar extent. The impacts of Si and Cd were somewhat additive since the lowest MDA value was recorded for CdSi treatment.

Table 4 Photosynthesis-related parameters of *Cannabis sativa* (cv. Santhica 27). Plants were exposed for 1 week to Cd (20 μ M) in the presence or in the absence of 2 mM H_2SiO_3 . Maximum quantum yield of dark-acclimated leaves (F_v/F_m), the photochemical efficiency of photosystem II (ϕ_{PSII}), photochemical quenching (q_P), non-photochemical

quenching (NPQ), stomatal conductance (g_s), net photosynthesis (A), instantaneous evapotranspiration (E), CO₂ in intercellular spaces (C_i). For a given organ, different letters indicate significant differences at $P < 0.05$. Each value is the mean of 5 replicates

	C	CSi	Cd	CdSi
F_v/F_m	0.91 $\pm$ 0.01 <i>a</i>	0.91 $\pm$ 0.01 <i>a</i>	0.89 $\pm$ 0.03 <i>a</i>	0.91 $\pm$ 0.01 <i>a</i>
ϕ_{PSII}	0.87 $\pm$ 0.01 <i>a</i>	0.87 $\pm$ 0.01 <i>a</i>	0.84 $\pm$ 0.03 <i>a</i>	0.85 $\pm$ 0.05 <i>a</i>
q_P	0.98 $\pm$ 0.01 <i>a</i>	0.97 $\pm$ 0.02 <i>a</i>	0.98 $\pm$ 0.02 <i>a</i>	0.95 $\pm$ 0.04 <i>a</i>
NPQ	0.25 $\pm$ 0.04 <i>b</i>	0.29 $\pm$ 0.03 <i>b</i>	0.19 $\pm$ 0.03 <i>a</i>	0.17 $\pm$ 0.03 <i>a</i>
g_s (mmol m ⁻² s ⁻¹)	478 $\pm$ 252 <i>b</i>	435 $\pm$ 441 <i>ab</i>	411 $\pm$ 163 <i>b</i>	161 $\pm$ 90 <i>a</i>
A (μ mol m ⁻² s ⁻¹)	4.21 $\pm$ 0.37 <i>b</i>	3.43 $\pm$ 0.63 <i>b</i>	1.79 $\pm$ 0.42 <i>a</i>	1.66 $\pm$ 0.84 <i>a</i>
E (mmol m ⁻² s ⁻¹)	2.47 $\pm$ 0.65 <i>b</i>	2.40 $\pm$ 1.00 <i>b</i>	2.71 $\pm$ 0.34 <i>b</i>	1.72 $\pm$ 0.56 <i>a</i>
C_i (μ mol mol ⁻¹)	333 $\pm$ 20 <i>a</i>	342 $\pm$ 20 <i>ab</i>	371 $\pm$ 8 <i>c</i>	355 $\pm$ 8 <i>b</i>

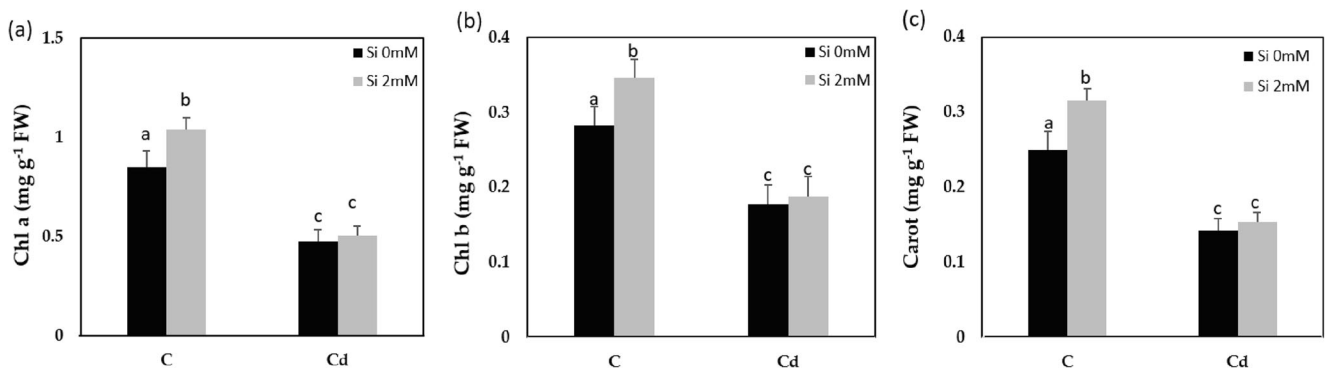


Fig. 3 Chlorophyll a (a), chlorophyll b (b), and carotenoid (c) content of *Cannabis sativa* (cv. Santhica 27) exposed for 1 week to Cd (20 μ M), in the presence or in the absence of 2 mM H_2SiO_3 . Data are means $\pm$

standard errors ($n = 5$). Values with different letters are significantly different ($P < 0.05$; Tukey's HSD all-pairwise comparisons)

The total antioxidant activity was determined for the leaves by using the FRAP index (Fig. 5) and discriminating the AOAD (hydrophobic) and OAOM (hydrophilous) fractions. The FRAP index increased in response to Si for both fractions in control plants. In Cd- and in CdSi-treated plants, the AOAD fraction decreased to lower value than the control while the OAOM fraction was similar in those plants comparative to control.

The concentration of total glutathione in the roots (Table 5) was the highest for control plants and decreased in response to Cd, Si, and Cd + Si treatments. The ratio GSSG/GSH was also the highest for control and decreased in all other treatments, being the lowest for CdSi-exposed plants. The leaves displayed a different profile with similar values for total GSH in control and Si-treated plants and a significant drop in plants exposed to Cd or CdSi. A strong increase in the leaf GSSG/GSH ratio was also observed in response to Cd. The non-protein phytochelatin (Table 5) strongly increased in the roots of plants exposed to Cd and reached more than 3000 $\mu\text{mol g}^{-1}$ FW in both Cd- and CdSi-treated plants. Phytochelatin concentration also increased in response to Cd in the leaves, although to a lower extent than in the roots and plants exposed to Cd and to CdSi once again exhibited similar behavior.

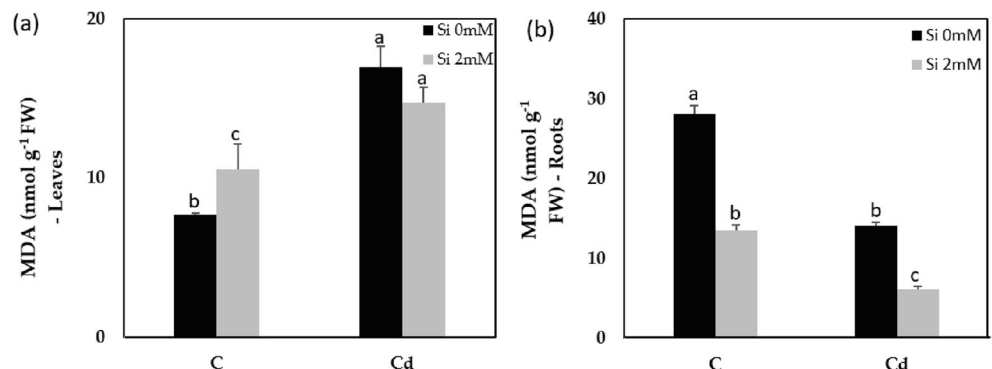
Discussion

Cd impact on hemp behavior

Since a few years, hemp (*C. sativa*) receives considerable attention in relation to fiber production in the stem (Duque-Schumacher et al. 2020; Vandepitte et al. 2020), cannabinoid synthesis in the flowers (Hussain et al. 2019; Yang et al. 2020), and oil in the grains (Mihoc et al. 2012; Ascrizzi et al. 2019) and as an interesting biomass-producing plant (Matassa et al. 2020; Zhao et al. 2020). This promising multipurpose crop was also recently recommended as a possible candidate for phytoremediation of heavy metal-contaminated soils (Ahmad et al. 2016; Kumar et al. 2017; Pietrini et al. 2019; Zielonka et al. 2020).

The use of nutrient solution with high heavy metal bio-availability allows for the quantification of the maximal capacities of the plant for bioaccumulation of pollutants independently of the adsorption process occurring in the soils and which restricts heavy metal transfer from soil to the root system (Kos and Leštan 2004). The present work demonstrates that Cd mainly accumulated in the roots, as recently shown by Praspaliauskas et al. (2020). As far as the shoot is concerned, Cd accumulation was higher in the stem than in the leaves. Although Arru et al. (2004) demonstrated that accumulated

Fig. 4 Malondialdehyde (MDA) content in leaves (a) and roots (b) of *Cannabis sativa* (cv. Santhica 27) exposed for 1 week to Cd (20 μ M), in the presence or in the absence of 2 mM H_2SiO_3 . Data are means $\pm$ standard errors ($n = 5$). Values with different letters are significantly different ($P < 0.05$; Tukey's HSD all-pairwise comparisons)



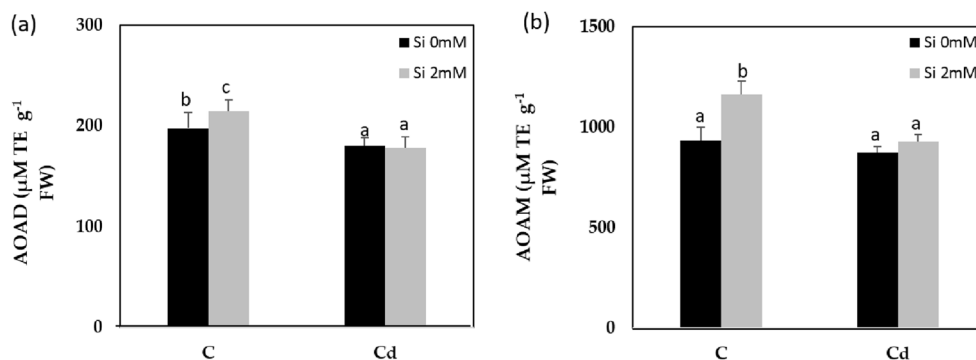


Fig. 5 Total antioxidant activity in the hydrophobic fraction (AOAD; **a**) and the hydrophilic fraction (AOAM; **b**) of leaves of *Cannabis sativa* (cv. Santhica 27). Plants were exposed for 1 week to Cd (20 μM), in the

presence or in the absence of 2 mM H_2SiO_3 . Data are means $\pm$ standard errors ($n = 5$). Values with different letters are significantly different ($P < 0.05$; Tukey's HSD all-pairwise comparisons)

Cu did not fix on hemp bast fibers, a different behavior might be expected for Cd which is able to interact with hemp bast fibers during biosorption processes (Pejic et al. 2009; Vukčević et al. 2014). One may therefore expect that Cd *in planta* could also strongly interact with cell wall polymers as recently demonstrated for *Medicago sativa* (Gutsch et al. 2019) or *Kosteletzkya pentacarpos* (Zhou et al. 2018), and more especially during the process of bast fibers or hurds' formation. The resulting risks for users, as well as the consequence of this localization on fibers' quality, need to be determined. Even if Cd did not accumulate in bast fibers or hurds but in adjacent tissues, a risk of dissemination subsists as a consequence of retting process occurring in the field and which involves selective degradation of the parenchyma cells surrounding the fibers (Bleuze et al. 2018). A sound appraisal of the resulting environmental damages requires the precise determination of Cd distribution in the stem tissues using adapted histological imaging techniques (Lefèvre et al. 2014).

As far as the whole shoot is concerned, hemp accumulates Cd to concentrations quite higher than $100\text{-mg}\cdot\text{kg}^{-1}$ DW commonly referenced as the minimal value for Cd-hyperaccumulating plants (Mahar et al. 2016). Despite such

a high level of accumulation, hemp could not be considered as a hyperaccumulating species since Cd strongly impaired growth. Interestingly, however, the translocation factor calculated on the basis of the total amount of Cd was also clearly higher than 1, suggesting that hemp displays, to some extent, physiological attempts allowing the plant to cope with the high Cd content in photosynthetic tissues. Similarly, the recorded bioaccumulation factor was close to 90 in Cd-treated plants, an extremely high value comparative to most plant species used for phytoextraction purposes (Ali et al. 2013; Barceló and Poschenrieder 2003; Feng et al. 2020; Mahar et al. 2016; Yang et al. 2017).

Besides Cd content itself, the present work demonstrates that this heavy metal affected the distribution of other elements in young hemp plants: Ca translocation was increased by Cd treatment, while Fe exhibited an opposite trend since it accumulated in the roots but decreased in the leaves. Even though Cd is a non-essential element, it competes with numerous Fe and Ca transporters. Some of these transporters are mainly present in the roots (IRT1, IRT2, NRAMP5), while others are more specifically present in vascular tissues (NRAMP3, NRAMP4, HMA2, HMA4) (Huang et al. 2020).

Table 5 Total glutathione (GSHt), oxidized (GSSG)/reduced (GSH) glutathione, and phytochelatin (PC) concentrations in roots and leaves of *Cannabis sativa* (cv. Santhica 27). Plants were exposed for 1 week to Cd (20 μM) in the presence or in the absence of 2 mM H_2SiO_3 . For a

given parameter and a given organ, means followed by different letters are significantly different at $P < 0.05$. Each value is the mean of 5 replicates

	C	CSi	Cd	CdSi
Roots				
GSHt (nmol g ⁻¹ FW)	116 $\pm$ 9 c	72 $\pm$ 5 a	67 $\pm$ 2 a	92 $\pm$ 31 b
GSSG/GSH	32.70 $\pm$ 5.28 c	7.67 $\pm$ 0.53 b	2.41 $\pm$ 0.15 a	2.11 $\pm$ 0.98 a
PC (nmol g ⁻¹ FW)	296 $\pm$ 19 b	248 $\pm$ 24 a	3473 $\pm$ 181 c	3727 $\pm$ 169 c
Leaves				
GSHt (nmol g ⁻¹ FW)	455 $\pm$ 42 b	455 $\pm$ 24 b	291 $\pm$ 38 a	285 $\pm$ 7 a
GSSG/GSH	2.34 $\pm$ 0.73 a	7.41 $\pm$ 1.21 ac	12.95 $\pm$ 5.96 bc	15.00 $\pm$ 6.00 b
PC (nmol g ⁻¹ FW)	362 $\pm$ 84 a	464 $\pm$ 24 b	1749 $\pm$ 73 c	1613 $\pm$ 120 c

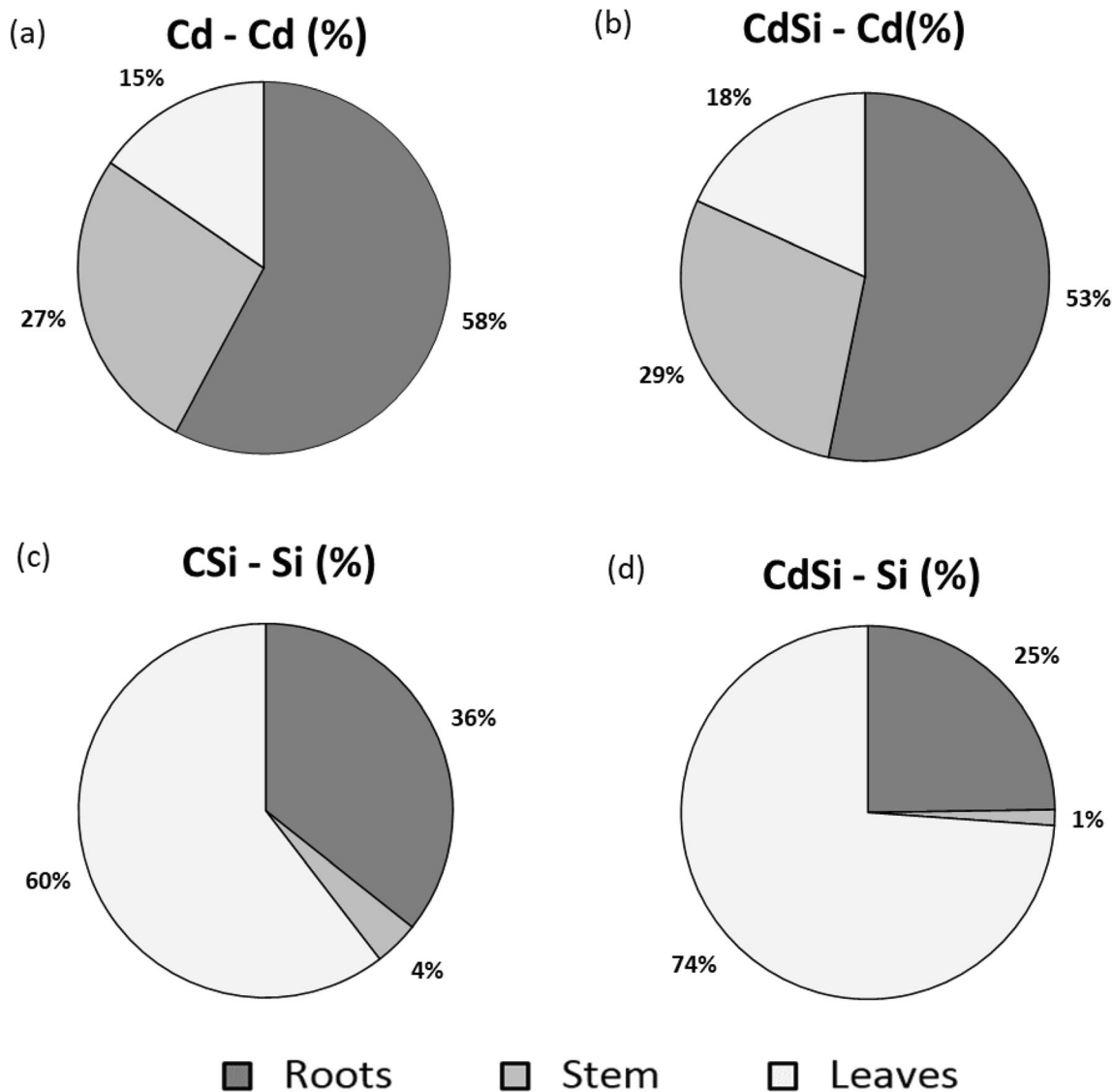


Fig. 6 Distribution (%) of Cd in roots, stem, and leaves of Cd-exposed plants (Cd, CdSi; **a** and **b**), and distribution (%) of Si in roots, stem, and leaves of Si-exposed plants (CSi, CdSi; **c** and **d**). *Cannabis sativa* (cv.

Santhica 27) plants were exposed for 1 week to Cd (20 μ M) in the presence or in the absence of 2 mM H_2SiO_3

According to the relative sensitivities of these transporters to Cd excess, long-distance transport of essential elements such as Ca and/or Fe may be strongly affected. Sulfur strongly increased in all organs, which could be related to the Cd-induced oversynthesis of phytochelatins containing numerous thiol groups (Cobbett 2000). It has however to be mentioned that S mainly increased in the leaves while PC accumulated to a higher extent in the roots than in the shoots.

Photosynthesis is an important physiological process and was reported to be strongly impaired by Cd toxicity (Baryla et al. 2001; Lefèvre et al. 2014). Stress-induced inhibition of photosynthesis may be due to stomatal or to non-stomatal causes. Cadmium did not induce stomatal closure on the second fully expanded leaf in Cd-treated hemp, and a decrease in leaf WC was reported despite osmotic adjustment, reflecting

an alteration in the plant water status. The fact that A strongly decreased while g_s remained unaffected supports the hypothesis that Cd mainly act on this precise leaf through non-stomatal targets.

Carotenoid content also decreased following Cd application. Carotenoids absorb light energy from chlorophyll and have the capability to act as antioxidants by scavenging singlet molecular oxygen ($^1\text{O}_2$) and peroxy radicals (ROO) in chloroplasts (Khalid et al. 2019). It is likely that stress conditions accelerated their degradation and disrupted their protective function (Mlinarić et al. 2016). Neither F_v/F_m , Φ_{PSII} , nor q_P was affected by Cd treatment. This suggests that the plant remained able to trigger protective mechanisms even if very high doses of Cd reduced pigment concentration. Lefèvre et al. (2014) showed that Cd exposure of Cd-resistant plants

lead to the synthesis of chloroplastic proteins contributing to the protection of photosystems and stabilization of the thylakoid membranes. It has however to be mentioned that Cd also induced a decrease in NPQ: this is an unexpected observation since abiotic stresses commonly induce an increase rather than a decrease in NPQ values (Kalaji et al. 2016; Jonar et al. 2020). Non-photochemical quenching would protect against all mechanisms that depend on light absorbed by the PSII antenna. The drop in NPQ recorded in Cd-treated plants suggests that these antennas and/or other parameters influencing the NPQ process started to be affected by Cd toxicity. At the time of photosynthesis measurement, the second fully expanded leaf was photosynthetically active and was therefore of crucial importance for whole plant survival. It did not necessarily reflect all leaf behavior, especially regarding the fact that the oldest leaves are encountering senescence which strongly impact photosynthetic processes at different levels (Mayta et al. 2019); cadmium-induced senescence may depend on Cd distribution among leaves of different ages, and a preferential accumulation of Cd in old leaves could not be ruled out.

Among the plethora of injuries induced by Cd toxicity, oxidative stress related to ROS oversynthesis is of paramount importance. In order to prevent oxidative burst, plant cells may increase the endogenous concentration of antioxidant and activities of antioxidative enzymes. Cd induced an obvious increase in leaf MDA, resulting from lipid peroxidation. Surprisingly, Cd induced a decrease in root MDA. Malondialdehyde is often considered a non-toxic end-product reflecting the rate of lipid peroxidation. In fact, it has to be noticed that MDA itself is a toxic molecule able to react with DNA and protein to form several different adducts. It has been referred to as a potentially mutagenic and atherogenic compound and is thought to generate unwanted DNA-protein cross-links (Del Rio et al. 2005). According to Yan et al. (2021), major damages induced by Cd on earthworms result from MDA toxicity rather than from primary ROS. Plants may decompose MDA through activation of aldehyde dehydrogenase or aldo-keto-reductases (AKR) (Sengupta et al. 2015). One hypothesis explaining the low level of root MDA in Cd-exposed plants is that this metabolic pathway was activated in roots to prevent damages induced by MDA.

Among the antioxidants involved in ROS detoxification, GSH plays a dual function since it acts also as a precursor of PC involved in vacuolar sequestration of Cd. Jozefczak et al. (2014) demonstrated that root GSH may be preferentially allocated to PC synthesis for Cd chelation leading to a transient decrease in free GSH. Our results corroborate this observation since we recorded a significant decrease in GSht and a strong increase in root PC with no concomitant increase in MDA concentration suggesting that oxidative stress remained limited thanks to an efficient Cd sequestration. In the leaves, PC concentration was lower than in the roots and a proportion of

free Cd may induce oxidative stress. The total antioxidant of our samples was only marginally affected by Cd, but the high GSSG/GSH ratio observed in Cd-treated leaves suggests that the activity of glutathione reductase may be impaired to some extent by the heavy metal. Besides its role as antioxidant and metal chelator, glutathione also assumes crucial role in transcriptional activation of Cd-induced DNA damages response and an impact in cell cycle regulation so that even a transient depletion of GSht may contribute to growth inhibition (Hendrix et al. 2020).

Si impact on plant response to Cd

It is now well-established that Si is a non-essentially beneficial element contributing to abiotic stress resistance in a wide range of plant species (Epstein 1994; Luyckx et al. 2017; Kim et al. 2017; Berni et al. 2020). As far as heavy metal stress is concerned, Bhat et al. (2019) reported that the beneficial effects of Si are more evident in plants able to accumulate high level of Si. Recently, Guerriero et al. (2019) identified a gene coding for Si channel in hemp. The maximal level of Si accumulation in our material was around 0.5% DW for hemp leaves, suggesting that *C. sativa* is a low Si accumulator when compared with species such as rice or horsetail (Epstein 1994). This explains why Si had only a marginal impact on control plants that were not exposed to Cd stress. In our experimental approach, Si was applied before Cd, allowing the plants to already contain significant amounts of Si when Cd stress was applied. Numerous authors reported that the addition of Si improved growth of plants exposed to heavy metals (Wu et al. 2013; Adrees et al. 2015; Imtiaz et al. 2016; Bhat et al. 2019) but this is a long-term observation and the underlying reasons for this growth stimulation remain poorly documented. In order to unravel the putative first targets of Si, we thus analyzed plants' behavior on a short-term basis before growth stimulation occurred.

In some studies, Si was shown to co-precipitate with Cd in the external medium, leading to a decrease in stress intensity through a reduction in Cd bioavailability (Adrees et al. 2015). Cadmium may be fixed by the walls of the tanks while Cd and Si may be absorbed by microorganisms, thus reducing heavy metal toxicity for plants. At acidic pH, polymerization of the monosilicic acid and capsulation of Cd could not be excluded. If they occurred, these processes remained of limited importance in our work since both VisualMinteq software and nutrient solution analysis clearly indicated that Cd speciation was not affected by added Si and that both Cd and silicic acid remained soluble and fully available during the time course of the experiment. Numerous works also demonstrated that Si reduced root-to-shoot translocation of Cd by blocking the apoplastic bypass flow after Si deposits in the vicinity of the endodermis leading to Cd accumulation in the root (Shi et al. 2005; Adrees et al. 2015; Bhat et al. 2019). In the present

work, Si significantly reduced Cd accumulation in all organs including the roots and this suggests that Si impacted the Cd absorption process and not the translocation step. Indeed, the TF_a and TF_c values increased in CdSi-treated plants comparative to values recorded for Cd-treated ones (Table 2) and root retention was not the strategy adopted by the plant to cope with Cd in the presence of Si. As a consequence, the relative distribution of Cd in the CdSi-treated plants was almost similar to that of Cd-treated ones (Fig. 6). Conversely, Cd reduced Si accumulation in the stem but strongly increased it in the leaves, resulting in an increase in TF_a and TF_c for Si, but also in a strong increase in BF values (Table 2; Fig. 6). In the xylem sap, Si is mostly present in the form of monosilicic acid and aquaporins involved in Si xylem unloading and intervacular transport were identified in some species (Adrees et al. 2015). Our data suggest that Cd might have an impact on these aquaporins which may lead to a higher Si deposit as amorphous silica at the transpiration sites.

Besides distribution between organs of the plants, Si was shown to accumulate within cell walls and this is also the case for Cd (Krzyszowska 2011) so that co-precipitation might occur. Si concentration remained the lowest in the stem comparatively to roots and leaves but Guerriero et al. (2019) clearly demonstrated that Si is not evenly distributed among stem tissues and is specifically present in the wall of bast fibers. This may have a positive impact on the mechanical properties of the fibers since harvested fibers are frequently coated with silica during industrial processing to improve the quality (Branda et al. 2016; Jiang et al. 2018; Koushki et al. 2020). It has to be noticed that Cd reduced by more than 50% Si accumulation in the stem and may thus hamper the Si contact with mature thickened bast fibers.

Si application to Cd-treated plants strongly decreased the g_s and E values in relation to the abovementioned increase in Si content in the leaves, while this phenomenon was not observed in the plants exposed to Si alone. Once again, hemp displayed a specific behavior in this respect, since numerous studies performed on other species reported a Si-induced increase in g_s and transpiration rates of heavy metal-exposed plants (Wu et al. 2013; Adrees et al. 2015; Imtiaz et al. 2016). In our case, however, the fact that A was less impacted than E values by stomatal closure leads to an increase of WUE in CdSi-treated plants comparative to Cd-treated ones. Although a positive impact of Si on chlorophyll content was detected in control plants, this was not observed for plants exposed to Cd and Si was similarly unable to restore the NPQ values of Cd-treated plants. Hence, the photosynthetic machinery did not appear as the first target of Si impact on Cd-treated plants.

Si was also reported to decrease the oxidative stress induced by heavy metals and thus decrease MDA content in various organs (Adrees et al. 2015; Kim et al. 2017). Si obviously decreased MDA content in the roots of hemp, but not in

the leaves. It increased the total antioxidants in the leaves (for both AOAD and AOAM fractions), but only in control plants and not in Cd-exposed ones. At the root level, Si significantly increased the GSHt concentration and slightly increased the PC concentration, allowing the plant to efficiently sequester Cd on thiol groups while maintaining at the same time its capacity to detoxify ROS with free available glutathione. According to Wu et al. (2013), Si is able to stimulate *PCSI* (the major phytochelatin synthase gene) in *Arabidopsis thaliana* while Bhat et al. (2019) reported a Si-induced increase in the transcript levels of *MT2a* and *MT2b* corresponding to metallothionein also involved in Cd sequestration.

Conclusions

The present study indicates that *C. sativa* is able to accumulate high concentration of Cd and is therefore suitable for remediation purposes. The use of produced fibers should however be considered with caution in relation to Cd accumulation in the stem and the resulting risks of Cd dispersion during the retting process. The addition of Si has numerous physiological impacts on the plant behavior occurring rapidly after Cd stress application and before any modification in terms of growth. Si reduced Cd accumulation in all organs but did not impact Cd distribution within the plant. It also improved water use efficiency in relation to stomatal closure and decrease in transpiration rates. Exogenous Si stimulated phytochelatin synthesis at the root level and thus improved Cd sequestration. It also mitigated the deleterious impact of Cd on leaf Fe and root Ca content but was unable to reduce Cd effect on chloroplastic PSII antenna. The Cd-induced increase in Si accumulation may be regarded as an attempt to absorb this beneficial element. Further experimentations are required to analyze the long-term effect of Si impact on plant growth and to precisely map Cd distribution in plant organs.

Author contribution ML, GG, and SL designed the methodology; ML performed the whole experiment, treated, and analyzed the data; MB contributed to mineral analysis; JFH and SL supervised the whole research process; ML and SL wrote the original draft. All authors reviewed the manuscript.

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Data availability All data generated or analyzed during this study are included in this published article

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable

Competing interests The authors declare no competing interests.

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