



RESEARCH PAPER

Impact of Silicon and Heavy Metals on Hemp (*Cannabis sativa* L.) Bast Fibres Properties: An Industrial and Agricultural Perspective

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Received: 10 February 2022 / Revised: 25 July 2022 / Accepted: 26 July 2022 / Published online: 3 September 2022
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Abstract

Heavy metal (HM) pollution in agricultural fields reduces crop productivity and quality, and poses a risk for human health. On these areas, the cultivation of crops devoted to biomaterial and bioenergy production thus constitutes an attractive alternative to food crop production. To maintain the soil productivity and to obtain a biomass compatible with the requirements of industry, the use of the beneficial metalloid silicon, applied on leaves during plant growth, has been considered. A field study using *Cannabis sativa* L. (cv. Futura 75) was performed during 2 consecutive years on two sites (one soil parcel contaminated with Cd, Zn and Pb, and one taken as uncontaminated control) in the North of France. One half of the plants received weekly foliar spray of Si (2 mM). Photosynthesis-related parameters were analysed in situ, while mineral concentration and fibre properties were determined after 4 months of culture. Our results suggest that Si application improves and preserves the mechanical properties of fibres when plants are exposed to HM stress. Si was also shown to increase by 27% the Cd concentration in leaves and by 30% the Zn concentration in stems. Contaminated fibres and shives containing immobilized HM could be used for specific purposes such as inclusion in cement agglomerates.

Article Highlights

- Hemp is an attractive species for non-food production on heavy-metal polluted soil.
- Exogenous application of silicon was tested for its protective impact on stressed plants.
- Fibers constitute a preferential site for cadmium sequestration in the stem.
- Si treatments improve fibers mechanical properties in the absence of heavy metals.
- Si preserves the mechanical properties of fibres when plants are exposed to heavy metals.

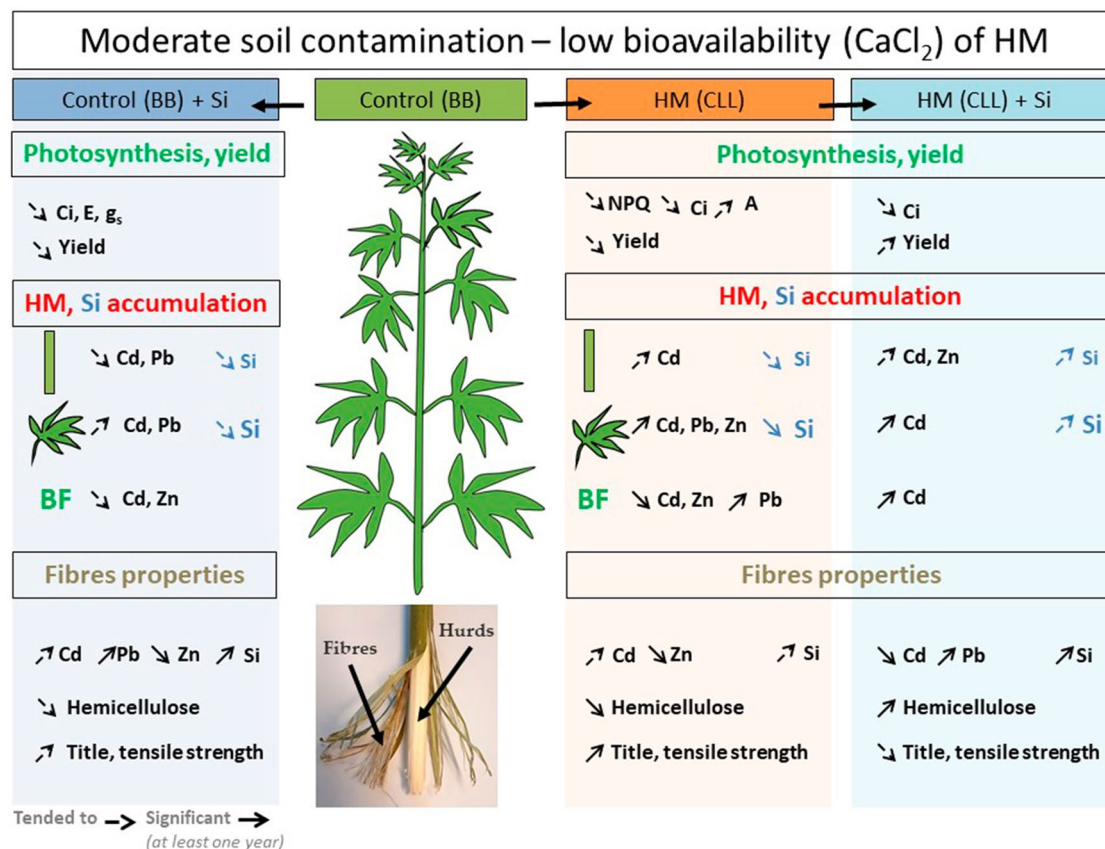
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Graphical Abstract



Keywords Fibers · Heavy metal pollution · Hemp · Phytoremediation · Silicon

Introduction

In numerous areas in the world, activities, such as industrialization, urbanization and agricultural practices have resulted in the release of heavy metals (HM) in the environment. Heavy metals' pollution in agricultural fields frequently results in soil degradation, reduced crop productivity and poor quality of agricultural products. It also induces significant hazards for human and animal health through food chain contamination (Jaskulak et al. 2020; Wang et al. 2016; Peralta-Videa et al. 2009). On these areas, the cultivation of crops devoted to biomaterial and bioenergy production may constitute an attractive alternative to food crops (Fernando et al. 2015). This implies that biomass production should not be compromised by HM from a quantitative point of view, and that the properties of the harvested biomass remain compatible with the requirements of industry from a qualitative point of view (Luyckx et al. 2019).

Fibre crops like textile hemp are important sources of raw materials for paper, textiles and composite production

as they provide in a sustainable manner a great amount of biomass in a relatively short time (Guerriero et al. 2017; Gorshkova et al. 2012). Fibres' commercial applications are largely based on their mechanical properties: as a whole, the structure of the fibre cell wall is similar to a nanocomposite reinforced with cellulose microfibrils and is characterized by high anisotropy, high stiffness and strength in the direction of the fibre axis (reviewed by Gorshkova et al. 2012).

Several silicon (Si) derivatives (silane, siloxane, etc.) are used in industry to improve the mechanical properties and thermal stability of the harvested fibres (Abdelmouleh et al. 2004; Orue et al. 2016; Han et al., 2021). Silicon is known to interact with specific cell wall components, although the mechanisms involved in amorphous silica– SiO_2 precipitation remain elusive (Luyckx et al. 2017a). Besides, the presence of $-\text{SiO}_2$ in hemp woody fibres favour their valorisation in eco-construction. Indeed, the presence of SiO_2 strengthens the bonds between fibres and lime in the manufacture of a lightweight concrete-like material known as “hempcrete” (Luyckx et al. 2017a). Hempcrete is not only very light (six

times lighter than traditional concrete), but also more resistant to degradation. Moreover, the presence of SiO₂ protects it against the attack of chewing insects, termites, fungi and rodents (Luyckx et al. 2017b). SiO₂ is also commonly considered as a fire-retardant (Luyckx et al. 2017b; Piot et al. 2017).

As far as plant physiology is concerned, Si is known to have numerous beneficial effects on plants exposed to abiotic and biotic stresses (Frew et al. 2018; Pilon et al. 2013). Various authors reported that the addition of Si improved the growth of plants exposed to HM (Wu et al. 2013; Adrees et al. 2015; Imtiaz et al. 2016; Bhat et al. 2019). Silicon protective mechanisms in alleviating HM toxicity include improved expression of defense genes, increase in antioxidant enzymes' activities, changes in transpiration rates and CO₂ assimilation, osmotic adjustments leading to the maintenance of water content and turgor pressure, alterations in HM uptake and modifications in phytohormone concentrations (Frew et al. 2018). Given the positive effects of Si on plants, Si treatments during fibre development in plants cultivated on HM-contaminated soils could therefore represent an asset for biomass valorization: Si may not only increase fibre production but is also expected to provide improved mechanical properties to the fibres through SiO₂ precipitation in the cell walls (Luyckx et al. 2017a, 2019). Luyckx et al. (2021a and 2021b) recently demonstrated that exogenous Si modified gene expression in Cd-treated *Cannabis sativa* L., including genes involved in secondary cell wall metabolism, which could thus influence fibre development. Khan and Roy (1964) reported increased cell elongation and fineness of the commercial fibre jute as a result of soil application of silicate. However, the availability of Si fertilizer applied into fields is quite low because this silicate slag is hard to dissolve and is easily adsorbed on organic matters and/or minerals in the soils (Wang et al. 2015). In our present study, the foliar application of Si was therefore considered to avoid its immobilization in soil. Si foliar fertilizers with small particle size have already been used and were shown to easily penetrate the leaf but are also able to form a thick SiO₂ layer on the leaf surface (Wang et al. 2015).

The aim of this study performed in situ was to evaluate (i) the influence of Si application on hemp physiological properties when grown on HM-contaminated agricultural soils and (ii) to determine the impact of Si applied during fibres' development on their final properties after harvest.

Material and Methods

Growth Conditions

Two agricultural sites (Courcelles-lès-Lens, CLL (50°38'13" LN; 2°01'01"E), and Bois-Bernard, BB (50°41'62" LN;

3°02'78" E); France) were selected: CLL is located near (about 1 km to the southeast) a former lead and zinc smelter (Metaleurop), while BB is far enough from it (about 9 km to the southwest) not to be influenced by contaminated dust fallout and was taken as a control. Before the beginning of the experiment, three soil samples from ploughed layer (0–25 cm) from each site were characterized as mentioned below. Detailed soil analyses for both sites are provided in Table S1 (supplemental data).

The field study was carried out in 2018 and 2019. Year 2018 was drier and warmer than 2019 (Lille-Lesquin weather station). The accumulated rainfall over the entire vegetation period in 2019 (167.6 mm) slightly exceeded those of 2018 (158.1 mm), while average temperatures were higher in 2018 (23.04 °C) than in 2019 (21.80 °C).

Soil Characterization

Soils samples were collected before plant sowing in 2018 (T0), after plant harvest in 2018 (T1) and 2019 (T2). We analyzed total HM concentrations, HM concentrations after CaCl₂ 0.01 M extraction (HM environmental availability), CEC and pH. Electrical conductivity (EC), total organic C content (OC) and total CaCO₃ were measured at T0 only.

For pseudo-total concentrations, 300-mg DM (dry matter) of soil samples were ground to powder (250 µm), placed in a tube with a mix of HCl 37%–HNO₃ 68% (3:1, v/v), heated to 95 °C for 1 h 30, dissolved in distilled water, and finally filtered through Whatman n°1 filter papers. The environmental available metal concentrations were evaluated using 3 g of ground soil samples, placed in a tube with 30 mL of CaCl₂ (0.01 M), mixed for 2 h before centrifugation (4500g—20 min), and finally filtered on Whatman n°1 filter paper. Total and environmental available heavy metals were quantified by flame atomic absorption spectrometry following the recommendations in Waterlot and Hechelski (2019). Total HM concentrations in CLL soil were homogeneously distributed all over the sites (5.5 ± 0.2 mg Cd, 396.4 ± 31.8 mg Zn, 298 ± 21 mg Pb per kg of soil; MisChar program) and exceeded, for Zn and Pb, the permissible limits (PL) commonly recommended for agricultural use (10 mg Cd kg⁻¹, 250 mg Zn kg⁻¹, and 200 mg Pb kg⁻¹; Tóth et al. 2016). In BB, Cd and Zn concentrations were five times lower than in CLL and below the PL for Cd and Zn (1.1 ± 0.1 mg Cd, 80.9 ± 3.9 mg Zn per kg of soil; MisChar program). The presence of Pb in the topsoil of BB (285 ± 95 mg kg⁻¹) is linked to the presence of some shrapnel issued from World War I. Heavy metals bioavailability was estimated after extraction with CaCl₂ 0.01 M according to Degryse et al. (2003). In both BB and CLL, Cd and Pb presented very low environmental availability since their extractable concentration was below the detection limits of the flame atomic

absorption spectrometer (0.05 mg kg^{-1} for Cd, 0.65 mg kg^{-1} for Pb).

For CEC determination (NF X31-130), 2.5 g DM of soil samples were ground to powder (2 mm), placed in a percolation tube, and rinsed four times with a solution of ammonium acetate (1 M). Residues of ammonium acetate solution were then eliminated with ethanol 95% (v/v), and the tube was dried 24 h at room temperature. Then, samples were transferred in centrifugation tubes with 50 mL NaCl 1 M and agitated during 1 h. The solution was then centrifuged (3000 g, 15 min) and filtered (Minisart filter, $0.45 \mu\text{m}$ cellulose acetate). CEC was determined according to the Berthelot reaction: 400 μL of the solution obtained was mixed with 4 mL of a color reagent (130 g sodium salicylate, 130 g trisodium citrate dihydrate, 970-mg sodium nitroprusside, adjusted to 1 L with distilled water), 4 mL of sodium dichloroisocyanurate (32 g NaOH in 500 mM distilled water, cooled at room temperature and mixed with 2 g of DIC, before volume adjustment to 1 L with distilled water) and adjusted to 40 mL with distilled water. Samples were then agitated, and heated 60 min at 25°C before absorbance measurement (655 nm) (Page et al. 1982).

For pH determination, a volume of 6 mL of dry soil ground to powder (2 mm) was placed in a tube with 30 mL of distilled water and agitated 1 h at 750 rpm. pH was measured 2 h after agitation. Soil electrical conductivity (EC) were determined using glass electrodes (WTW Multi350i) in a 1:5 soil/water past. Total organic carbon was determined by sulfochromic oxidation according to the NFX 31-109 standard. The total carbonate content was measured according to the NFISO 10693 standard considering the measurement of the volume of CO_2 released by reaction with HCl (Douay et al., 2009). In both sites, the CEC was between 17.3 and $19.8 \text{ cmol kg}^{-1}$, and soil pH in CLL (7.9 ± 0.1) was slightly more alkaline than in BB (7.0 ± 0.2). Considered soils had similar EC values (0.87 mS cm^{-1} for CLL and 0.94 mS cm^{-1} for BB) and similar OC (13.6 g Kg^{-1} for CLL and 14.3 g Kg^{-1} for BB). The two sites, however, differ for total CaCO_3 content which was higher in CLL ($6.7 \pm 2.4 \text{ mg Kg}^{-1}$) than in BB ($0.8 \pm 0.3 \text{ mg Kg}^{-1}$).

Plant Material

For each site, two contiguous blocks (9 m * 10 m) were set up, one for industrial hemp and the other for wheat, to establish a crop rotation. From year to year, hemp and wheat were alternate at the two joint blocks of each site, but only hemp will be considered in this study. Seeds of a monoecious hemp fibre variety (*C. sativa*, cv Futura 75) were sown at the end of April (2018) or at the beginning of May (2019), with a sowing density of 50 kg ha^{-1} . No additional fertilization nor weeding were applied. Foliar application of Si started six weeks after sowing, and was then repeated once

a week until harvest. For Si application, areas of 1 m^2 were delimited within each block: one half without Si application, the other half with Si application. Si was applied as metasilicic acid (2 mM) with a hand sprayer until the solution began to drip off leaves. Metasilicic acid was obtained from a pentahydrate sodium metasilicate ($\text{Na}_2\text{SiO}_3 \cdot 5 \text{ H}_2\text{O}$) which was passed through an H^+ ion exchanger resin IR 20 Amberlite type according to Dufey et al. (2014). Four treatments were thus defined, considering soil contamination (site) and the concomitant presence/absence of Si application, and will hereafter be referred to as BB (control), CLL (contaminated), BBSi (BB with Si application), and CLLSi.

In 2018 and 2019, hemp plants were harvested 4 months after sowing. For each treatment, ten plants were cut 5 cm above the ground, and all the leaves still present on the stem, and stems from a same treatment were then separated. Sub-samples of each treatment and plant part were pooled and dried at 70°C for 72 h before analyses.

Photosynthesis-Related Parameters

Before plant harvest, photosynthesis-related parameters were measured in 2018 and 2019 on five plants per treatment, on the middle portion of the leaf blades constituting the second fully formed leaves from the top. Chlorophyll fluorescence was measured using a fluorimeter (FMS II, Hansatech Instruments, Norfolk, UK) as detailed in Han et al. (2013). Chlorophyll fluorescence parameters (maximum quantum yield of photosystem II (F_v/F_m), photochemical quenching (q_p), non-photochemical quenching (NPQ) and actual efficiency of photosystem II (Φ_{PSII}) were considered (Maxwell and Johnson, 2000).

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 transpiration (E) were measured using an infrared gas analyzer (LCA4 8.7 ADC, Bioscience, Hertfordshire, UK). Gas exchanges were measured using a Parkinson leaf cuvette on intact leaves for 1 min (20 records min^{-1}) with an air flow of 300 mL min^{-1} . All measurements were performed around midday (between 12 a.m. and 2 p.m.).

Fibre Analysis

The stems harvested in 2019 were placed in water-filled containers and incubated during 9 days at 39°C to extract fibres and shives according to Abot et al. (2013). Fibres were separated manually by combing. Fibre bundles title (fibre fineness) and fibre tensile strength (force at break and breaking elongation) were determined according to MO 123 and XP 25-501-3 T standards, respectively. The fibre tensile strength was evaluated using a Zwick tensile bench with 10 N and

500 N load cell, a test speed of 7.5 mm min^{-1} , a pretension of 0.1 N, and a gap between the clamps of 75 mm. Subsequently, we determined lignin, cellulose, hemicellulose through near-infrared spectrometry (NIRS), as well as ion contents in fibres and shives. NIRS was performed using XDS system (Foss Master Lab Rapid Content) with ISIScan software and a spectral range from 400 to 2498 nm. Stem samples were preliminary dried at 60°C for 48 h, cut into small sections with a pair of scissors and then ground with a Grindomix (Retsch) to provide homogenous powder with particle size lower than 1 mm. Analysis was performed with a dual detector system at a data acquisition rate of 2 scan/sec and a spectral resolution of 2 nm.

Mineral Concentration in Stems, Leaves, Fibres and Shives

For Cd, Pb and Zn quantification, 500-mg dry material (DM) were placed in an oven and heated to 500°C for 48 h. The ashes were then digested in 68% HNO_3 and acid evaporated to dryness on a sand bath at 80°C . Minerals were incubated with a mix of HCl 37%– HNO_3 68% (3:1; v/v) and the mixture was slightly evaporated and dissolved in distilled water, and filtered on Whatman n°1 filter papers. 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% v/v HNO_3 . Cations were quantified by Inductively Coupled Plasma-Optical Emission Spectroscopy (Varian, type MPX). The metal bioaccumulation factor (BF) was defined, for each element, as the ratio between the mean value of HM concentration recorded in the whole aerial part divided by the mean value of total concentration in the soil.

Statistical Analysis

Five replicates were analyzed for each treatment. ANOVA analyses were performed separately for each year. Normality of the data was verified using Shapiro–Wilk tests and the data were log-transformed when required. Homogeneity of the data was verified using Levene's tests. Two-way ANOVA were performed at a significant level of P -value < 0.05 using R (version 3.3.1) considering the soil contamination (BB, CLL) and Si application as main factors. Means were compared using Tukey's HSD all-pairwise comparisons at 5% level as a post hoc test.

Table 1 Yield (t ha^{-1}) of hemp grown during 4 months on HM-contaminated (CLL) or on control soil (BB), in the presence or in the absence of 2 mM Si

Year	Site	Si (mM)	Yield (t ha^{-1})
2018	BB	0	$39.6 \pm 4.22 \text{ a}$
		2	$32.25 \pm 4.10 \text{ a}$
	CLL	0	$31.97 \pm 9.26 \text{ a}$
		2	$40.07 \pm 7.32 \text{ a}$
2019	BB	0	$25.47 \pm 3.15 \text{ a}$
		2	$20.73 \pm 3.01 \text{ a}$
	CLL	0	$18.93 \pm 2.63 \text{ a}$
		2	$23.73 \pm 2.52 \text{ a}$

Data are means $\pm$ standard errors ($n=5$). For each column and year, different letters indicate significant difference $P < 0.05$ (Tukey's HSD all-pairwise comparisons)

Table 2 Fibres and shive yield (%) of hemp grown during 4 months on HM contaminated soil (CLL) or on control soil (BB), in the presence or in the absence of 2 mM Si

Year	Site	Si (mM)	Fibres yield (%)	Shive yield (%)
2019	BB	0	$31 \pm 7 \text{ a}$	$68 \pm 7 \text{ a}$
		2	$29 \pm 2 \text{ a}$	$70 \pm 2 \text{ a}$
	CLL	0	$31 \pm 4 \text{ a}$	$68 \pm 7 \text{ a}$
		2	$30 \pm 1 \text{ a}$	$69 \pm 1 \text{ a}$

Data are means $\pm$ standard errors ($n=5$). Different letters indicate significant difference at $P < 0.05$ (Tukey's HSD all-pairwise comparisons)

Results

Yield

The aerial biomass yield of *C. sativa* was higher in 2018 than in 2019 (Table 1). However, for each considered year, neither the presence of pollutants nor the exogenous application of Si had any significant impact on biomass yield; even if Si exposure tended to decrease the yield in BB and to increase it in CLL. For year 2019, the proportions of fibres and shives were not impacted by the treatments (Table 2).

Photosynthesis-Related Parameters

Light-dependent parameters of photosynthesis (F_v/F_m , Φ_{PSII} , q_p , NPQ, Table 3) were marginally influenced by soil contamination and Si application. In 2018, the F_v/F_m ratio was increased by exogenous Si in CLL. In the absence of Si application, the Φ_{PSII} value recorded in 2018 was similar in BB and CLL treatments, but Si application decreased Φ_{PSII} in BB and significantly increased it in CLL, while an opposite trend was recorded in 2019. Conversely, Si application

Table 3 Light-dependent parameters of photosynthesis

Year	Site	Si (mM)	F_v/F_m	ϕ_{PS2}	q_p	NPQ
2018	BB	0	0.861 ± 0.015 b	0.767 ± 0.030 ab	0.932 ± 0.028 a	0.296 ± 0.205 a
		2	0.792 ± 0.052 ab	0.704 ± 0.059 a	0.990 ± 0.061 a	0.359 ± 0.135 a
	CLL	0	0.815 ± 0.002 a	0.744 ± 0.019 a	0.969 ± 0.014 a	0.252 ± 0.029 a
		2	0.864 ± 0.018 b	0.814 ± 0.033 b	0.944 ± 0.057 a	0.199 ± 0.091 a
2019	BB	0	0.818 ± 0.067 a	0.647 ± 0.174 ab	0.958 ± 0.037 a	0.894 ± 0.944 a
		2	0.878 ± 0.006 a	0.744 ± 0.035 ab	0.933 ± 0.038 a	0.663 ± 0.547 a
	CLL	0	0.834 ± 0.068 a	0.753 ± 0.020 b	0.938 ± 0.056 a	0.322 ± 0.117 a
		2	0.813 ± 0.002 a	0.671 ± 0.032 a	0.964 ± 0.048 a	0.702 ± 0.489 a

Plants were grown during 4 months on HM-contaminated soil (CLL) or on control soil (BB), in the presence or in the absence of 2 mM Si. Data are means $\pm$ standard errors ($n=5$). For each column and year, different letters indicate significant different values at $P < 0.05$ (Tukey's HSD all-pairwise comparisons)

Table 4 Light-independent parameters of photosynthesis

Year	Site	Si (mM)	Ci ($\mu\text{mol mol}^{-1}$)	E ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	A ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)
2018	BB	0	332.67 ± 23.86 b	2.060 ± 0.691 a	3.277 ± 1.229 a	304.33 ± 36.00 ab
		2	285.66 ± 63.32 ab	1.363 ± 0.581 a	3.963 ± 3.547 a	225.00 ± 40.73 a
	CLL	0	258.68 ± 101.36 ab	1.550 ± 0.344 a	4.407 ± 3.640 a	375.33 ± 151.74 ab
		2	160.33 ± 21.94 a	2.707 ± 0.860 a	13.917 ± 3.478 b	442.50 ± 7.50 b
2019	BB	0	392.25 ± 25.99 a	0.878 ± 0.626 a	0.820 ± 0.7332 a	137.70 ± 121.329 a
		2	389.00 ± 32.92 a	0.687 ± 0.291 a	0.610 ± 0.711 a	134.37 ± 89.45 a
	CLL	0	291.50 ± 75.85 a	0.863 ± 0.667 a	2.968 ± 4.249 a	76.10 ± 58.75 a
		2	284.67 ± 42.44 a	0.883 ± 0.212 a	1.637 ± 1.294 a	58.12 ± 30.305 a

Plants were grown during 4 months (in 2018 and 2019) on HM-contaminated soil (CLL) or on control soil (BB), in the presence or in the absence of 2 mM Si. Data are means $\pm$ standard errors ($n=5$). For each column and year, different letters indicate different values at $P < 0.05$ (Tukey's HSD all-pairwise comparisons)

slightly decreased NPQ in CLL treatments in 2018 but increased it in 2019. However, NPQ displayed a high level of variability and the recorded trends remained not significant. Photochemical quenching (q_p) remained similar in all treatments, irrespective of the year considered.

In 2018 and in the absence of Si, CO_2 concentration in intercellular spaces (Ci) and E (Table 4) tended to be higher in BB than in CLL while A and g_s tended to be higher in the plants growing on the contaminated soil CLL comparatively to the control one. Exogenous Si decreased Ci concentration but increased A values. The recorded increase was especially important for plants growing on CLL. Exogenous Si tended to decrease stomatal conductance on BB but to increase it in CLL. Plants cultivated in 2019 exhibited a different trend since E and g_s values were lower than in 2018. Moreover, exogenous Si tended to decrease A values for plants growing on both sites. Nevertheless, net photosynthesis still remained unexpectedly higher in plants cultivated in CLL than in those cultivated in BB, as previously observed in 2018, while g_s values were lower.

Si Accumulation in Hemp

Concentrations of Si in leaves, stems and mean values calculated for the whole aerial parts are reported in Table 5. Silicon content was always higher in the leaves than in the stems. In 2018, Si application unexpectedly decreased Si concentration in both leaves and stems of plants cultivated in the uncontaminated control BB, while it tended to increase Si content in the stem of plants cultivated in CLL. In 2019, Si application significantly increased the leaf Si content in plants cultivated on CLL, resulting in a global significant increase of Si content in the whole aerial parts of plants cultivated on the contaminated site. In the absence of Si application, however, the mean Si concentration in the aerial part was higher in plants growing on BB than on CLL for both years.

HM Accumulation in Hemp

Cadmium, Pb and Zn were detected in plants cultivated in BB and CLL (Table 6). In both sites, foliar concentrations of Cd, Pb and Zn tended to exceed stem concentrations.

Table 5 Silicon concentration (in mg kg⁻¹ DW) in leaves and stems of *Cannabis sativa* and mean value calculated for the whole vegetative aerial part

	Site	Si (mM)	2018	2019
			Si	Si
Leaves	BB	0	7269 ± 1155 b	7013 ± 235 b
		2	4420 ± 20 a	6661 ± 231 b
	CLL	0	6271 ± 428 b	5515 ± 359 a
		2	5646 ± 52 b	6975 ± 121 b
Stems	BB	0	861 ± 76 a	410 ± 193 ab
		2	364 ± 148 a	227 ± 16 b
	CLL	0	319 ± 33 a	157 ± 22 a
		2	988 ± 484 a	259 ± 9 b
Aerial parts	BB	0	2784 ± 293 b	2391 ± 206 b
		2	1581 ± 98 a	2157 ± 81 ab
	CLL	0	2104 ± 151 ab	1764 ± 123 a
		2	2385 ± 354 ab	2274 ± 30 ab

Plants were grown during 4 months on HM-contaminated soil (CLL) or on control soil (BB), in the presence or in the absence of 2 mM Si. Data are means ± standard errors ($n=5$). For each organ and year, different letters indicate significant differences at $P<0.05$ (Tukey's HSD all-pairwise comparisons)

In the absence of Si application, the leaf Cd, Pb and Zn concentrations were always higher in plants growing in CLL than in BB. The difference was however less obvious for stems which even tended to present higher Pb and Zn

concentrations in BB than in CLL in 2018. When mean concentration values were calculated for the whole aerial part, the accumulation was still higher in CLL than in BB.

In 2018, Si application significantly increased Cd, Pb and Zn concentrations in the leaves of plants cultivated in BB while an opposite trend was recorded for the stem. In CLL, Si increased Cd and Pb in the leaves but had no impact on the stem content. In 2019, however, we noticed that Si application almost doubled the concentration of Cd in the stem of plants cultivated on the contaminated site CLL.

To evaluate the impact of Si on hemp HM bioaccumulation factor (BF), the ratio between concentrations of the individual metals in the above-ground biomass and in soil both expressed on a DW basis was calculated (Table 6). In the absence of Si, Cd and Zn BF were higher in BB than in CLL, while Pb BF remained similar for both sites. These results were significant in 2018 and the same trend was recorded in 2019. Exogenous Si had only small impacts on BF values in plants growing on BB. It however significantly increased Cd BF in 2019.

Fibres and Shives Characterization

Fibre bundles title and fibre tensile strength (force at break and breaking elongation) are classical parameters tested in industry to assess the quality of fibres. Soil contamination induced a significant increase of fibres bundles title in the absence of Si treatment (Fig. 1). Exogenous Si increased

Table 6 Cadmium, lead and zinc concentrations (in mg kg⁻¹ DW) in leaves and stems of *Cannabis sativa* and metal bioaccumulation factor (BF)

	Site	Si (mM)	2018			2019		
			Cd	Pb	Zn	Cd	Pb	Zn
Leaves	BB	0	0.018 ± 0.003 a	1.099 ± 0.219 a	25.27 ± 4.10 a	0.075 ± 0.032 a	2.721 ± 1.026 a	41.74 ± 6.35 a
		2	0.032 ± 0.002 b	1.887 ± 0.044 b	34.76 ± 0.22 b	0.081 ± 0.009 ab	2.941 ± 0.205 a	36.27 ± 2.39 a
	CLL	0	0.058 ± 0.004 c	2.171 ± 0.074 c	42.69 ± 1.65 c	0.165 ± 0.040 bc	3.721 ± 0.387 a	65.67 ± 5.06 b
		2	0.076 ± 0.006 d	2.466 ± 0.163 c	37.99 ± 2.34 bc	0.204 ± 0.021 c	2.911 ± 0.225 a	62.61 ± 1.17 b
Stems	BB	0	0.026 ± 0.002 b	0.920 ± 0.005 b	20.47 ± 3.68 d	nd	1.103 ± 0.114 a	10.211 ± 0.879 a
		2	0.014 ± 0.001 a	0.858 ± 0.016 a	8.25 ± 0.02 a	nd	0.997 ± 0.063 a	12.098 ± 2.918 a
	CLL	0	0.039 ± 0.007 bc	0.887 ± 0.083 ab	12.70 ± 0.16 b	0.074 ± 0.015 a	1.193 ± 0.075 ab	12.148 ± 0.961 a
		2	0.040 ± 0.003 c	0.819 ± 0.127 ab	16.07 ± 1.90 c	0.145 ± 0.036 b	1.379 ± 0.068 b	16.333 ± 3.700 a
Aerial parts	BB	0	0.024 ± 0.003 a	0.974 ± 0.069 a	21.915 ± 1.350 a	0.025 ± 0.012 a	1.698 ± 0.272 ab	20.641 ± 1.305 a
		2	0.019 ± 0.004 a	1.167 ± 0.040 b	18.508 ± 3.289 a	0.024 ± 0.002 a	1.594 ± 0.036 a	20.087 ± 1.779 a
	CLL	0	0.045 ± 0.003 b	1.272 ± 0.036 c	21.696 ± 0.382 a	0.106 ± 0.019 b	1.893 ± 0.064 b	28.533 ± 1.379 b
		2	0.051 ± 0.004 b	1.313 ± 0.040 c	22.649 ± 0.629 a	0.164 ± 0.023 c	1.822 ± 0.071 b	31.196 ± 2.415 b
BF	BB	0	0.021 ± 0.002 b	0.003 ± 0.000 a	0.271 ± 0.017 b	0.022 ± 0.011 ab	0.006 ± 0.001 a	0.255 ± 0.016 b
		2	0.018 ± 0.000 b	0.004 ± 0.000 ab	0.229 ± 0.041 b	0.022 ± 0.002 a	0.006 ± 0.000 a	0.248 ± 0.022 b
	CLL	0	0.008 ± 0.001 a	0.004 ± 0.000 b	0.055 ± 0.001 a	0.019 ± 0.003 a	0.006 ± 0.000 a	0.072 ± 0.003 a
		2	0.009 ± 0.001 a	0.004 ± 0.000 b	0.057 ± 0.002 a	0.030 ± 0.004 b	0.006 ± 0.000 a	0.079 ± 0.006 a

Plants were grown during 4 months on HM-contaminated soil (CLL) or on control soil (BB), in the presence or in the absence of 2 mM Si. Data are means ± standard errors ($n=5$). For each element, organ and year, different letters indicate significant differences at $P<0.05$ (Tukey's HSD all-pairwise comparisons)

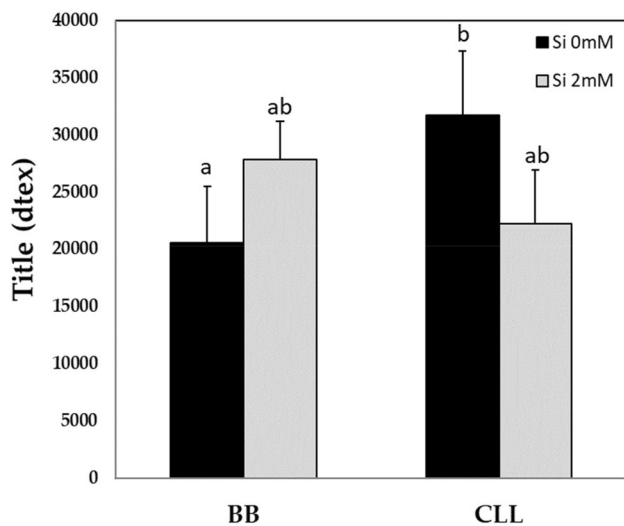


Fig. 1 Fibre bundles title in plants grown on HM-contaminated soil (CLL) or on control soil (BB), in the presence or in the absence of 2 mM Si. Different letters indicate significant difference ($P < 0.05$; Tukey's HSD all-pairwise comparisons)

bundle title in fibres harvested on the BB site but decreased it in CLL, thus suppressing the recorded differences between the two sites (Fig. 1). Fibres derived from plants harvested on the uncontaminated site BB presented significantly lower force break and breaking elongation than those issued from the contaminated site CLL (Fig. 2). Si had no significant impact on these parameters.

Heavy metal accumulation values in shives and fibres of harvested plants are provided in Fig. 3. Cadmium content in shives was higher in plants collected from CLL than in those harvested on BB, and exogenous Si increased Cd accumulation in shives in CLL. In contrast, Pb content in shives was

similar for the two considered sites without Si. Exogenous Si significantly decreased Pb content in shives from BB but had no impact on CLL. Zn could not be accurately quantified in shives. As far as fibres are concerned, Cd accumulation was similar in plants from BB and CLL, but exogenous Si increased Cd content in BB while it strongly reduced it in CLL. Lead content was similar in fibres derived from the two sites and, in both cases, Si significantly increased Pb content in the fibres. Exogenous Si reduced Zn concentration in the fibres issued from BB while the fibres harvested on CLL always exhibited a very low amount of Zn. Silicon concentration in shives remained unaffected by Si treatment. In contrast, it strongly increased in fibres harvested from BB and to a lower extent in those harvested from CLL (Fig. 4).

In the absence of Si treatment, fibres issued from BB had a higher percentage of hemicellulose (Table 7) than those derived from CLL plants. Silicon, however, inverted the trend since it significantly decreased hemicellulose in fibres from BB but increased it in fibres from CLL. No lignin could be accurately detected in fibres, and the cellulose concentration was slightly higher for fibres issued from CLL than those harvested on BB, the difference being significant for Si-treated plants only. Shives contained lower amount of hemicellulose than fibres in the absence of Si on BB site. The hemicellulose amount tended to be higher in plants cultivated on CLL than on BB. Lignin was also higher for shives issued from CLL comparatively to BB. Silicon significantly decreased lignin content in shives derived from BB but had no impact on shives issued from CLL. Neither the sites nor Si treatment had any significant impact on cellulose percentage in shives.

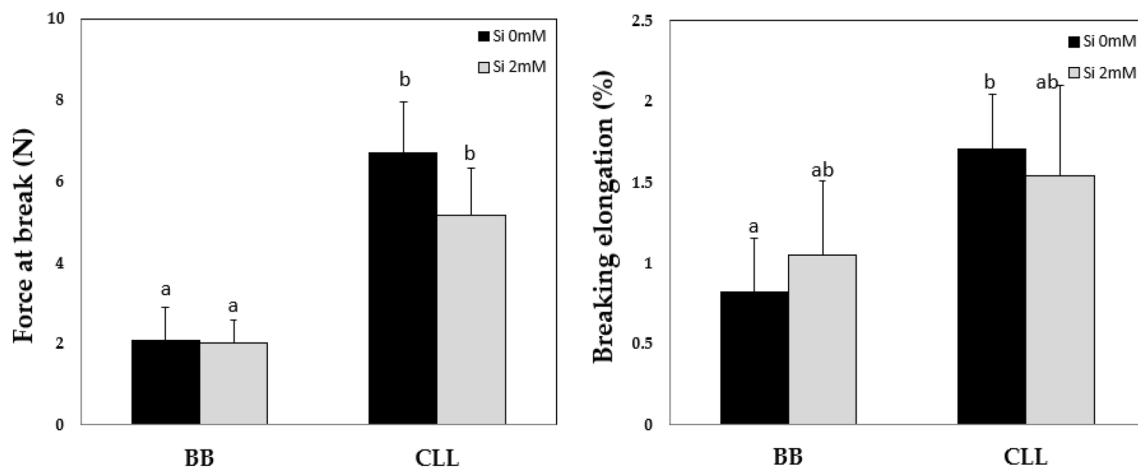


Fig. 2 Force at break (N) and breaking elongation (%) of fibres in plants grown on HM-contaminated soil (CLL) or on control soil (BB), in the presence or in the absence of 2 mM Si. Different letters indicate significant difference ($P < 0.05$; Tukey's HSD all-pairwise comparisons)

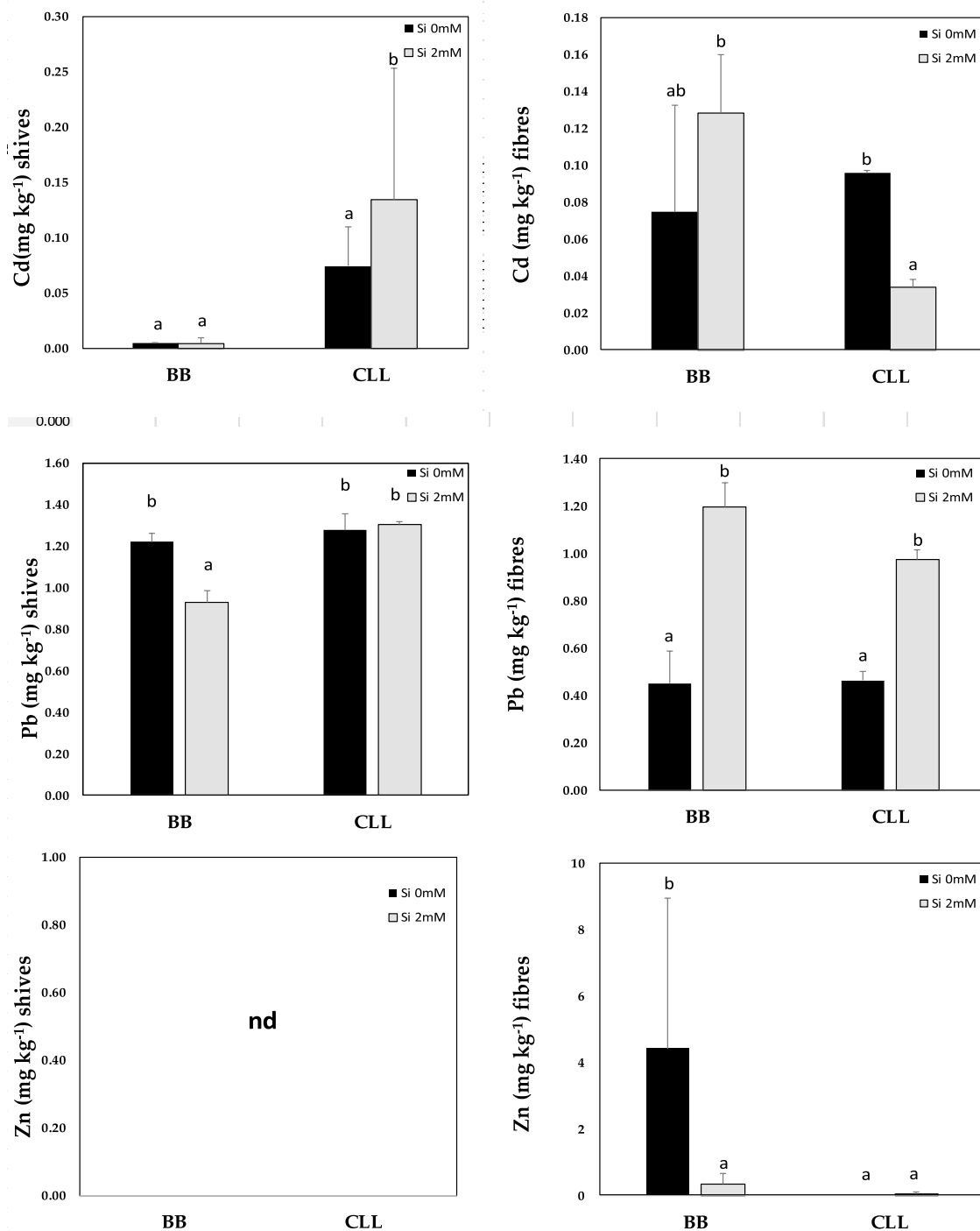


Fig. 3 Cadmium, Pb and Zn concentrations (in mg kg⁻¹ DW) in shives and fibres of *Cannabis sativa* (cv Futura 75). Plants were grown for 4 months (in 2018 and 2019) on HM-contaminated soil (CLL) or on control soil (BB), in the presence or in the absence of

2 mM Si. Data are means $\pm$ standard errors ($n=5$). Nd=not detected. Different letters indicate significant difference ($P<0.05$; Tukey's HSD all-pairwise comparisons)

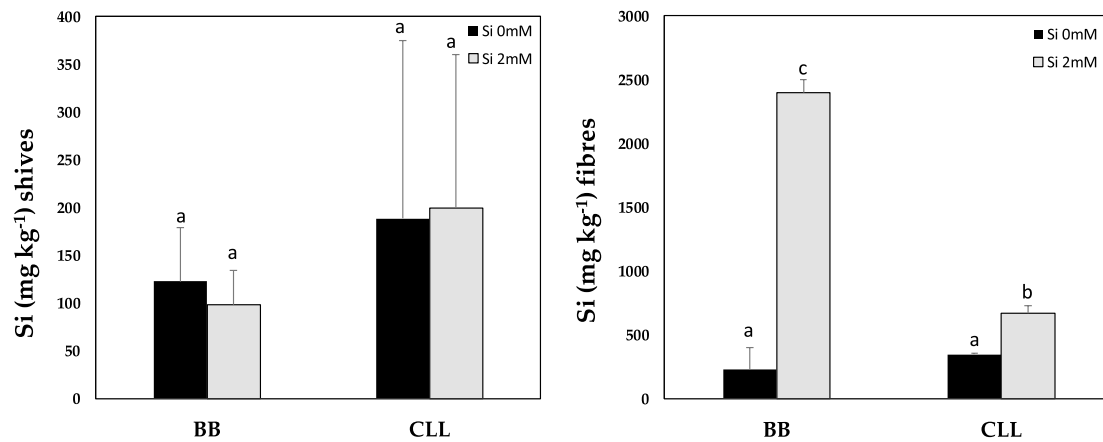


Fig. 4 Silicon concentrations (in mg kg⁻¹ DW) in shives and fibres from plants grown on HM contaminated soil (CLL) or on control soil (BB), in the presence or in the absence of 2 mM Si. Data are

means $\pm$ standard errors ($n=5$). Different letters indicate significant difference ($P < 0.05$; Tukey's HSD all-pairwise comparisons)

Discussion

Several Si derivatives (silane, siloxane, etc.) are used in industry to improve the mechanical properties of plant fibres and their thermal stability (Abdelmouleh et al. 2004; Orue et al. 2016). Moreover, through an enhanced tolerance to stress, Si application could improve hemp biomass production on agricultural soils contaminated with HM and where food production is prohibited. Foliar spraying of Si during hemp cultivation could therefore represent an asset for fibre production and valorization (Luyckx et al. 2017a).

In the current study, foliar application of Si had no significant impact on the photosynthetic activity of hemp, although its application tended to decrease C_i , E and g_s values in the absence of soil contamination issued from a former smelter activity. Silicon may have deposited as amorphous silica at the transpiration sites when the liquid fraction of the Si solution has evaporated, and caused partial occlusion of the stomata, resulting in a decrease in E and g_s (Zhu and Gong 2014) as we observed in 2018. Although stomatal conductance conditions the flow of water through the stomata, it also conditions gas exchange, including the entry of CO_2 . The potential obstruction of stomata by silica crystals could thus have limited the CO_2 input and explain the decrease in intercellular CO_2 observed. In the present study, however, such a decrease in g_s did not significantly affect net photosynthesis. In an experiment conducted on wheat cultivars, Maghsoudi et al. (2015) did not observe any significant effect of Si foliar application on photosynthesis, while increased photosynthesis and transpiration rates were reported by Pilon et al. (2013). For the majority of the available studies, the effects of Si under normal conditions are latent (Luyckx et al. 2017a). Under control conditions, Si probably activates the metabolic status of the plant, by making it more efficient in

Table 7 Infra-red prediction of the hemicellulose, cellulose and lignin contents within hemp fibres and shives

	Site	Si (mM)	Hemicellulose (% DW)	Cellulose (% DW)	Lignin (% DW)
Fibres	BB	0	19.13 $\pm$ 0.55 b	61.27 $\pm$ 1.05 a	nd
		2	16.91 $\pm$ 0.97 a	61.17 $\pm$ 0.82 a	nd
	CLL	0	17.12 $\pm$ 0.62 a	62.78 $\pm$ 1.35 ab	nd
		2	20.08 $\pm$ 0.86 b	63.15 $\pm$ 0.67 b	nd
Shives	BB	0	14.21 $\pm$ 0.37 a	60.82 $\pm$ 0.70 a	12.94 $\pm$ 0.49 b
		2	14.15 $\pm$ 1.03 ab	61.38 $\pm$ 0.67 a	11.98 $\pm$ 0.20 a
	CLL	0	15.23 $\pm$ 0.87 ab	60.17 $\pm$ 0.44 a	13.48 $\pm$ 0.20 b
		2	16.21 $\pm$ 0.96 b	59.18 $\pm$ 1.50 a	13.36 $\pm$ 0.67 b

Plants were grown during 4 months on HM-contaminated soil (CLL) or on control soil (BB), in the presence or in the absence of 2 mM Si. Different letters indicate significant difference at $P < 0.05$ (Tukey's HSD all-pairwise comparisons)

responding to exogenous stimuli (Luyckx et al. 2017a): a stimulation of the Calvin cycle might ensure net photosynthesis maintenance and also contributes to C_i decrease.

The present work demonstrates that HM soil contamination did not alter biomass yield and fibres/shives proportion of *C. sativa* and that hemp could therefore be recommended for the valorization of moderately contaminated agricultural soils, as recently suggested by Rheay et al. (2020). Soil contamination slightly decreased NPQ and C_i , and slightly increased net photosynthesis, but Si application had not impact on these parameters. Decreased NPQ values under HM exposure have already been observed in one of

our previous studies (Luyckx et al. 2021a). Non-photochemical-quenching is involved in energy dissipation through heat and is commonly reported to increase in response to abiotic stress (Maxwell and Johnson 2000). The opposite trend observed in the present case suggests that PSII antenna and/or zeaxanthine concentration started to be affected by HM toxicity, leading to a drop in NPQ (Stirbet et al. 2018). Despite this observation, our data confirmed that the light-phase of photosynthesis is only marginally affected by HM, as previously reported by Shi et al. (2012) for numerous hemp accessions.

Plant species strongly differ in their capacity to absorb and accumulate Si (Frew et al. 2018). Monocots usually accumulate more Si than dicots (Luyckx et al. 2017b). The present work demonstrated that hemp is able to accumulate Si in stems and to a higher extent in leaves in the presence as well as in the absence of foliar application of H_2SiO_3 , so that this species could be regarded as capable of absorbing and accumulating Si in its tissues. In the absence of soil contamination, however, application of H_2SiO_3 unexpectedly tended to decrease Si accumulation in leaves and stems (Table 5). Similar results were obtained in an experiment conducted in greenhouse conditions with soil samples from BB (Luyckx et al., unpublished). The highest Si concentrations in plants are found in the major transpiration sites (Zhu and Gong 2014). Guntzer et al. (2012) suggested that Si concentration in a given plant organ reflects its transpiration rate. We may thus hypothesize that the decreased transpiration rate observed in controlled conditions after Si exposure reduced Si accumulation in the above-ground biomass. Mineral nutrition is a highly regulated process. For essential elements such as iron, a shoot-to-root signal downregulates iron absorption when the concentration of this element in the roots is sufficient to cover the requirement for metabolism of photosynthetic leaves (Lucena and Hernandez-Apaolaza 2017). Silicon is not regarded as an essential element and it enters passively in plants with water. The channel-type membrane protein (Lsi1) mediates the passive entry of Si, and its expression level in rice was shown to be downregulated by Si (Ma and Yamaji 2015). Such a downregulation of Lsi1 under Si exposure in hemp could thus not be ruled out.

Plants grown in CLL accumulated higher Si concentrations in leaves and stem when exposed to Si compared to plants grown in CLL without Si application, and this suggests that the response of the plant to Si application is completely different when plants are exposed to pollutants. Pilon et al. (2013) also reported greatest Si concentration in leaves after foliar application of Si. If Si supplementation may be beneficial for the development of plants subjected to HM stress, it is therefore not surprising to observe an increase in Si accumulation in plants of CLL-Si treatment compared to CLL treatment. However, when we compare plants that did not receive Si foliar treatment, we found that Si

accumulation was lower in plants cultivated on contaminated CLL than on less contaminated BB soil and this is valid for both years. It may be hypothesized that Si absorption by roots is somewhat hampered in contaminated soil. Endoderm suberization is frequently increased in HM-treated plants (Pires-Lira et al. 2020). Silicon itself enhances suberization and lignification in root (Fleck et al. 2011). Silicon translocation in accumulators such as rice occurs through the cooperative action of aquaporins and efflux transporters (Ma and Yamaji 2015), and the presence of HM may have an impact on the membrane proteins involved in Si translocation. We may also hypothesize that climatic conditions, and thus plant transpiration rate, rather than soil characteristics are the reason for the difference in the results obtained between plants growing on BB and on CLL. Soil analysis indeed revealed that, except for soil contamination, the two sites were quite similar.

Although it was considered as not contaminated, BB site presented traces of Cd and Pb in soil and plant samples. Foliar application of H_2SiO_3 tended to decrease stem accumulation of Cd and Pb but it increased the leaf accumulation. This observation demonstrates that foliar application of Si may modify HM distribution within aerial parts of the plants, although the underlying mechanisms still remains elusive. Silicon sprayed onto leaves may have entered leaves and increased Cd and Zn sequestration to the cell wall and trichomes, decreasing the potential for further redistribution (Iwasaki and Matsumura 1999; Wang et al. 2016), but this hypothesis is not supported by the above-mentioned reported decrease in Si for plants cultivated on BB soil (Table 5). The fact that HM were mainly accumulated in leaves highlights the importance to harvest hemp before natural leaf drop to avoid soil recontamination (Di Candilo et al. 2004).

Soil contamination increased leaf and stem HM concentrations compared to plants grown in BB. Plants' exposure to Si increased Cd and Zn accumulation in stems and Cd accumulation in leaves. We suppose that Si increased HM compartmentalization in the stem apoplast to deal with higher toxic ions concentration in plant, in addition to accumulation in leaf cell walls and trichomes. Liu et al. (2013) suggested a mechanism of co-deposition of Si and Cd in the cell walls via a [Si-wall matrix]Cd co-complexation as a plausible explanation for the *in vivo* detoxification of Cd.

Bioaccumulation factor (BF) reflects the ability of plants to take up HM from soil and to accumulate it in the shoots. The results showed that Zn was most easily absorbed from soil, followed by Cd and then Pb, even if BF values remained rather low for these two elements. In the absence of Cd and Zn contamination at BB site, Si tended to reduce Cd and Zn BF, which is in accordance with previous results obtained using foliar application of Si (Wang et al. 2016). Decreased BF may be explained by Si decreased Cd and Zn uptake by roots, and/or by coprecipitation of Cd and Zn with Si in

metabolically less-active tissues, especially in the endodermis cell wall considering that Si reinforces the endoderm structure (Fleck et al. 2011; Wang et al. 2016). However, this implies that Si was able to translocate from the leaves to the roots. We may nevertheless hypothesize that after several weeks of foliar application of Si, part of the solution reached the soil and may have reduced the phytoavailability of HM by changing their speciation in soil solution through the formation of silicate complexes (Bhat et al. 2019): in soil solution, soluble silicate can hydrolyze to generate gelatinous metasilicic acid (H_2SiO_3) which can absorb HM (Gu et al. 2011). However, this hypothesis is not supported by the Si-induced significant increase of Cd BF in the presence of soil contamination on the CLL site. Bioaccumulation was limited by the low level of heavy metal phytoavailability: nevertheless, BF values were lower in plants growing in CLL than in those growing on BB. The two soils displayed similar amounts of organic matter: organic matter may decrease HM solubility by sorbing them on the surface functional groups while the complexation of HM with dissolved organic compounds may increase HM solubility and mobility (Opferlet et al. 2017). The measurement of total organic carbon did not provide the proportion of “stable” and “labile” carbon in the considered soils. Moreover, the fact that CLL contained eight times more CaCO_3 may contribute to low BF value since HM may precipitate with carbonate at the considered pH values (Chen et al. 2022).

Interactions of shives and fibres with HM depend on the chemical composition of the polymers. Although Arru et al. (2004) reported that primary bast fibres seem not to be involved in copper accumulation, the present study showed that both Pb and Cd may accumulate on the fibres and that this was the case for plants growing on BB and on CLL. The two sites contained more or less the same amounts of Pb. It is noteworthy, however, that CLL soil contained five times more Cd than BB soil, but that the Cd accumulated in the fibres was statistically equivalent. Moreover, the mean concentration of Cd in the fibres was higher than the mean stem concentration, suggesting that fibres constitute a preferential site for Cd sequestration. Metal ions are mainly absorbed at carboxylic groups (mainly found in hemicellulose and pectin), and to some extent hydroxyl and carbonyl groups (Pejic et al. 2009). Surprisingly, exogenous Si had an opposite effect on Cd retention by fibres because it increased Cd retention in fibres for plants from BB, but decreased it for fibres issued from CLL. A modification in hemicellulose content was not responsible for this process: indeed, it presented an opposite trend since it decreased in response to Si in BB, while it increased in response to Si for plants growing on CLL (Table 6). Additional information regarding the precise composition of hemicellulose and the presence of pectic compounds could help to answer this question. Silicon was reported to bind to hemicellulose and the type

of binding depends on the nature of the polymers (He et al. 2015). If co-precipitation of silicate and Cd occurs, a different composition in hemicellulose polymers might explain a different impact of Si on Cd fixation on fibres from plants harvested on the two sites. In contrast Si similarly increased the Pb content on the fibre for both sites. Lead may also bind to the cell walls, but Xu et al. (2021) recently reported that it mainly exists in the form of insoluble oxalate precipitate.

As mentioned before, field application of Si could improve fibres' quality (Abdelmouleh et al. 2004; Orue et al. 2016; Han et al. 2021). In controlled conditions, fibres fineness (= bundles title), as well as fibres' breaking elongation increased after Si exposure. Similar results were obtained by Khan and Roy (1964) with the commercial fibre jute after soil application of silicate. Guerriero et al. (2019 and 2020) previously showed, via SIMS nano-analysis, that in hemp bast fibres Si accumulates in a specific region of the G-layer closest to the lumen mainly on the distal side facing the cortex. The presence of Si in the G-layer may account for the mechanical properties modification observed in the present study. Soil contamination significantly increased fibre bundles' title, as well as force at break and breaking elongation. Si application was shown to be beneficial in the presence of soil contamination by counteracting the effects of soil contamination. However, in the present case, the impact of Si during fibre development on final fibre quality requires additional information. Indeed, Si concentration was lower in fibres harvested on CLL comparatively to those harvested on BB (Fig. 4), while force at break and breaking elongation were higher for fibers harvested on CLL than for the fibres harvested on BB (Fig. 2).

To be used in clothes production, heavy metal concentrations in hemp fibres should not exceed the STANDARD 100 established by OEKO-TEX, which is 0.1 mg kg^{-1} for Cd and $0.2\text{--}1.0 \text{ mg kg}^{-1}$ Pb (Linger et al. 2002). In our study, these commercial applications are not recommended, but contaminated fibres and shives could be safely used in combined material where HM could not be set free (insulating material and concrete agglomerates for bioconstruction, as a sound absorbent material in the automotive industry, ...) or to produce energy in thermal power stations with controlled incineration processes (Linger et al. 2002; Di Candilo et al. 2004; Rheay et al. 2020). Since fibres need to be isolated from the stem, retting processes should be preferentially performed ex-situ in liquid medium rather than on the field to avoid HM transfer from the plant to the soil surface or from the soil surface to the valued parts of the harvested biomass.

Conclusion

The present work demonstrated that foliar application of Si had no significant impact on the photosynthetic activity of hemp. Nevertheless, our results suggest that Si treatments during fibre development could represent an asset for biomass valorization by providing improved mechanical properties in the absence of Cd and Zn contamination, and by preserving fibres properties when plants are exposed to HM stress. However, Si increased HM accumulation in above-ground biomass. Contaminated fibres and shives should therefore be used in a way that HM could not be set free, like in cement agglomerates or for energy production with controlled incineration.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s41742-022-00446-1>.

Acknowledgements This work was financed by ADEME (French Agency for the Environment and Energy Management); Convention MisChar n°1672C0044), by FNRS (Fonds National Pour la Recherche Scientifique, convention n°T.0147.21), and by the Luxembourg National Research Fund (INTER/FNRS/20/15045745). The authors are grateful to Aurélien Dubar and Arnaud Lefebvre (LGCgE) for fibre preparation and soil samples analysis, and to Mr. X. Joppin (Celabor—Research and Testing Centre in Food, Packaging, Environmental and Textile Technologies) for his valuable help in fibre analysis.

Declarations

Conflict of Interest The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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