Silicon Reduces Zinc Absorption and Trigger Oxidative Tolerance Processes Without Impacting Growth in Young Plants of Hemp (Cannabis Sativa L.)

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Abstract

Hemp (*Cannabis sativa* L.) is a promising crop for non-food agricultural production on soils contaminated by moderate doses of heavy metals, while silicon, as a beneficial element, is frequently reported to improve stressed plants behavior. Using a hydroponic system, plants of *Cannabis sativa* (cv. Santhica 27) were exposed for one week to 100 µM Zn in the presence or absence of 2 mM Si. Zinc accumulated in all plants organs but was mainly sequestered in the roots. Additional Si reduced Zn absorption but had no impact on Zn translocation. Zn accumulation had a negative impact on biomass and chlorophyll content but additional Si did not mitigate these symptoms. Exogenous Si reduced the Zn-induced membrane lipid peroxidation (assessed by malondialdehyde quantification) and increased the total antioxidant activities estimated by the FRAP index. In the absence of Si, leaf phytochelatin and total glutathione were the highest in Zn-treated plants and Si significantly decreased their concentrations. Additional approaches using *omics* strategies and histological localization of element will provide interesting information regarding the interaction of Zn and Si in hemp.

Introduction

Increasing numbers of agriculturally used areas are contaminated by anthropogenic-derived heavy metals (HM) (Ali et al. 2013; Linger et al. 2002). Management of these areas constitutes a major environmental challenge since toxic materials absorbed by plants may contaminate the food chain and represents a major risk for human health (Linger et al. 2002; Muthusaravanan et al. 2018). These areas are therefore no longer suitable for food crop production. Other crop species may be used for bioenergy production but considering the phytotoxicity of HM, the plant resistance mechanisms have to set up to mitigate. The repair of toxic-associated damages consumes metabolic energy which cannot be affected for growing processes and this often lead to a decrease in biomass production (Kim et al. 2017; Shahid et al. 2019). Consequently the costs associated with environmental pollution are potentially enormous (Etesami et al. 2018).

Several approaches exist to reduce soil pollution. Phytoremediation, based on the ability of plants to extract, degrade or immobilize various contaminants from polluted soils, appears as an interesting and ecologically-friendly alternative to the traditional remediation approaches (Kurade et al. 2021). However, it still faces some limitations: HM hyper-accumulating plants are able to accumulate high concentration of toxic elements but usually clean up only the soil surface because of their shallow root systems. Moreover, they produce low shoot biomass, so that the amounts of elements extracted from the soil remain extremely low (Khan et al. 2000; Muthusaravanan et al. 2018). The possibility of combining phytoremediation and non-food production, with the view of achieving low price decontamination of soil by the production of a commercially usable resource arouses more and more interest (Vareda et al. 2019; Kanwar et al. 2020; Kurade et al. 2021). The implementation of this strategy requires the selection of fast-growing crops with HM uptake ability, rapid biomass gain and that can tolerate heavy metals. Among plants producing a high above ground biomass and a deep root system, *Cannabis sativa* is a multi-purpose promising crop widely employed in many types of non-food industries (Citterio et al. 2003;

Schluttenhofer and Yuan 2017; Charai et al. 2021; Yang et al. 2020; Zhao et al. 2020). The plant indeed provides cortical fibre mainly used in paper and for manufacture of various products (composites, insulators, reinforced thermoplastics), while hurds present in the central part of the stem are used for the manufacture of animal litter and the composition of building materials (Deleuran and Flengmark 2006). Hemp would also be able, to a certain extent, to reduce Cu, Cd and Pb contamination in soils (Angelova et al. 2004; Bona et al. 2007; Shi et al. 2009 et 2012; Ahmad et al. 2016; Kumar et al. 2017), making it a good candidate for soil phytoremediation, even in the case of organic pollution (Wu et al. 2021).

Zinc is an essential element for all living organisms and it assumes key biological functions during plant growth and development, acting as a cofactor for numerous enzymes and being integrated in the electron transport chain within mitochondria and chloroplasts (Zlobin 2021). Zinc excess in the substrate, occurring as a result of anthropogenic activities, may however constitute a serious threat for ecosystem stability and plant development (Luo et al. 2022). Zinc contamination has a detrimental impact on plant growth and reduces yield in several cultivated plants. It affects the plant water status and compromises photosynthesis through a decrease in pigment concentration and stomatal regulation, as well as respiration and nitrogen metabolism. It was also reported to affect absorption and translocation of other essential elements. Zinc toxicity damages the membranes, proteins but also genetic material through association with phosphate group of DNA (Kaur and Garg 2021; Pilon et al. 2013; Andrejić et al. 2018; Bokor et al. 2014). Although Zn is a non-redox heavy metal, overgeneration of reactive oxygen species (ROS) may be possible due to metabolic disturbances in numerous metabolic pathways. In order to cope with oxidative stress, plants may produce antioxidant molecules among which glutathione plays a key role (Goodarzi et al. 2020). Moreover, glutathione acts as a precursor of phytochelatins which are cysteine rich peptides able to bind heavy metals and sequester those toxic compounds in the vacuole avoiding the toxic effects of free heavy metals in cytosol and organels (Fan et al. 2018; Tennstedt et al. 2009).

To enhance crop growth and help the plant to cope with HM toxicity, the use of silicon (Si)-fertilizer is predicted to become a sustainable strategy and an emerging trend in agriculture (Etesami et al. 2018). Silicon is a non-essential element but it can contribute to improve the behavior of plant exposed to wide range of environmental constraints, including HM (Doncheva et al. 2009; Adrees et al. 2015; Imtiaz et al. 2016; Etesami et al. 2018). As far as hemp is concerned, Si was shown to mitigate the deleterious effect of salt (Berni et al. 2021; Guerriero et al. 2021) and Cd (Luyckx et al. 2021a) toxicities, but Si impact on Zn-exposed plants requires additional experiments. Si can also accumulate within the parietal structures where it is present in the form of orthosilicic acid and contribute to reinforce the mechanical properties of the cell wall polymers (Kröger and Poulsen 2004). As far as hemp fibres are concerned, silica treatments after harvest also provides technical advantages such as moisture buffering properties or acting as a fire retardant (Branda et al. 2016; Jiang et al. 2018).

The present work was therefore undertaken in order to evaluate the impact of a toxic dose of Zn on *Cannabis sativa* cultivated in the presence or absence of exogenous Si. Zn accumulation was quantified in different plant organs and the impact of toxic ions on mineral nutrition, photosynthesis and oxidative stress were recorded.

Material And Methods

Plant material and growing conditions

Seeds of a monoecious hemp fibre cultivar (*Cannabis sativa* cv. Santhica 27) were sown in loam substrate in greenhouse conditions. After one week, the obtained seedlings were transferred to nutrient Hoagland solution (in mM: 2.0 KNO_3, 1.7 $Ca(NO_3)_2$, 1.0 KH_2PO_4, 0.5 NH_4NO_3, 0.5 $MgSO_4$, 17.8 Na_2SO_4, 11.3 H_3BO_3, 1.6 $MnSO_4$, 1 $ZnSO_4$, 0.3 $CuSO_4$, 0.03 $(NH_4)_6Mo_7O_{24}$ 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 solution. Tanks were placed in a phytotron under fully controlled environmental conditions (constant temperature of 24 ± 1°C with a mean light intensity of 230 µmoles $m^{-2}s^{-1}$ provided by Phillips lamps (Philips Lighting S.A., Brussels, Belgium) (HPI-T 400 W), a photoperiod of 16h under a relative humidity of 65%). After a week of acclimatization, half of the tank received Si in the form of H_4SiO_4 to a final concentration of 2 mM Si. Metasilicic acid was obtained from a pentahydrate sodium metasilicate (Na_2SiO_3 x 5 H_2O) 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 nutrient solution was permanently aerated by SuperFish Air Flow 4 pump. A week later, Zn was applied in the form of $ZnCl_2$ (100 µM). The pH of the solution was maintained at 5.5. Solubility of added heavy metal was confirmed by the Visual MINTEQ09 software. Four treatments were thus defined, considering the presence of Zn and the concomitant presence or absence of Si and will be hereafter designed as C (control: no heavy metals and no Si), CSi, Zn and ZnSi (7 tanks per treatment).

Harvests were performed after a week of Zn exposure. Stem length and diameter, number of leaves and main root length were considered. Roots were quickly rinsed in deionized water for 30 s under gentle agitation just before harvest to remove ions from the free spaces: roots, stems and leaves were then separated. Roots and leaves from a same treatment were pooled, quickly frozen in liquid nitrogen and then stored at − 80°C until analysis, except 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.

Physiological measurements

Before plant harvest, physiological measurements were performed on 5 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 fluorescence monitoring system (Hansatech Instruments). Leaf portions were dark-adapted for at least 30 min. A saturation pulse (18,000 µmol m^{-2} s^{-1}) was then sent to the leaf. The leaf was subsequently exposed to a constant intensity of actinic light (600 µmol m^{-2} s^{-1}) for 3 min, followed by a second saturating pulse of 18,000 µmol m^{-2} s^{-1}. Maximum quantum yield of dark acclimated leaves (F_v/F_m), photosystem II efficiency (Φ_{PSII}), non-photochemical quenching (NPQ) and photochemical quenching (q_P) were estimated according to Maxwell and Johnson (2000).

Leaf stomatal conductance (g_s) was measured using an AP4 diffusion porometer (Delta-TDevices 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 analyser (LCA4 8.7 ADC, Bioscience, Hertfordshire, UK) with a PLC Parkinson leaf cuvette on intact leaves for 1 min (20 records min^{-1}) and an air flow of 3 mL min^{-1}. All measurements were performed between 12 a.m. and 2 p.m. 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 936 *g* for 10 min at 4°C. Absorbance was measured on supernatant at 663.2 nm, 646.8 nm et 470 nm.

For osmotic potential determination (Ψs), the middle portion of the leaf blades constituting the second fully formed leaves from the top was quickly collected from five plants, 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. Then each tube was then encased in a second intact Eppendorf tube and centrifuged at 8,000 *g* for 15 min at 4°C. The osmolarity of the collected sap was analyzed with a vapor pressure osmometer (VAPRO® Vapor Pressure Osmometer 5520).

Mineral concentration

Fresh matter was dried in an oven for at least 48 h until it reaches a constant weight: 50–100 mg dry matter (DM) was 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) and the mixture was slightly evaporated and dissolved in distilled water. After filtration on Whatman n°1 filter papers, cations and sulfur 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% HNO_3. Cations were quantified by Inductively Coupled Plasma-Optical Emission Spectroscopy (Varian, type MPX).

Translocation factor reflects the capacity of the plant to translocate HM from the root to the shoot and was estimated according to Luyckx et al. (2021a) i) on the basis of the concentration expressed on a dry weight basis in each plant part (TF_c) and ii) on the basis of total amount of the considered element (TF_a)

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

TF_a = Total Zn amount accumulated in the shoot / Zn 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 Zn internal concentration in the plant was estimated on a water content basis, considering the proportion of the different organs:

BF = Zn concentration in the plant ($mg \cdot L^{-1}$)/Zn concentration in the solution ($mg \cdot L^{-1}$)

Malondialdehyde (MDA) content and total antioxidant activities

The level of lipid peroxidation in the control and Zn-treated plants was assessed from the concentration of malondialdehyde (MDA) as determined by the thiobarbituric acid (TBA) reaction (Heath and Packer 1968): 0.25 g of ground fresh samples were homogenized in 5 mL of 5% (w/v) trichloroacetic acid (TCA) containing 1.25% glycerol. The homogenate was centrifuged (Sigma 3–30K, Germany) at 12,000 *g* for 10 min at 4°C and filtered on Wathman n°1 filter paper. Two mL of TBA (0.67%) were added to 2 mL of supernatant and the mixture was heated at 100°C for 30 min. The reaction was stopped by placing the reaction tubes in an ice bath. The samples were centrifuged at 12,000 *g* for 1 min and their absorbance was measured at 532 nm (UV-1800 Shimadzu, Belgium). The results were corrected by subtracting the non-specific absorbance component as measured at 600 nm. The concentration of MDA (nmol g^{-1} DW) was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

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 ions at low pH and to produce a colored ferrous-tripyridyltriazine complex which was spectrophotometrically detected at 593 nm: 1 g FM was frozen in liquid nitrogen, ground in the presence of 10 mL methanol, incubated during 12 h at 4°C, and then centrifuged during 20 min at 10,000 *g* at 4°C (Sigma 3–30K, Germany). Supernatant containing the hydrophilic fraction (AOAM) was stored at − 20°C until analysis. Pellets were dissolved in 10 mL dichloromethane, homogenized and incubated at 4°C during 12 h, and centrifuged again at 10,000 *g* during 20 min (Sigma–30K, Germany). The obtained supernatant corresponds to the hydrophobic fraction (AOAD) and was stored at − 20°C. For final analysis, 150 µL of each fraction were separately added to 300 µL of freshly prepared FRAP reagent (25 mL acetate buffer pH 3.6, 2.5 mL of 2,4,6-tripyridyl-s-triazine and 2.5 mL $FeCl_3.6H_2O$ 20 mM). Standard curve was established with Trolox (50–800 µM). The concentration was expressed in µM 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 were extracted and derivatized by orthophthalaldehyde (OPA) according to Cereser et al. (2001). GSHt was quantified after a reduction step of oxidized glutathione (GSSG) by dithiotreitol. Extracts were filtered through 0.45 µm microfilters (Chromafil PES-45/15, Macherey-Nagel) prior to injection and OPA derivatives were separated on a reversed-phase HPLC column with an acetonitrile-sodium acetate gradient system and detected fluorimetrically. Five µL of sample were injected into a Shimadzu HPLC system (Shimadzu, 's-Hertogenbosch, The Netherlands) equipped with a Nucleodur C18 Pyramid column (125×4.6mm internal diameter; 5 µm particle size) (Macherey-Nagel, Duren, Germany). Derivatives were eluted in acetonitril gradient in a 50 mM sodium acetate buffer pH 6.2 at 30°C at a flow rate of 0.7 mL min^{-1}. Fluorimetric detection was performed with a spectra system Shimadzu RF-20A fluorescence detector at 420 nm after

excitation at 340 nm. GSH was quantified using nine-point calibration curves with custom-made external standard solutions ranging from 0.0625 to 50 µM and every ten injections, a check standard solution was used to confirm the calibration of the system. The recovery was determined using GSH as an internal standard. The total non-protein thiol (NPT) concentration was determined according to De Vos et al. (1992): 200 mg FM of tissue were ground in 2 mL of 5% (*w/v*) sulfosalicylic acid plus 6.3 mM diethylenetriaminepentaacetic acid (pH < 1) at 0°C with quartz sand in a mortar. The homogenate was centrifuged at 10,000 *g* for 10 min at 4°C (Sigma 3–30K, Germany). The supernatants were collected and used for the determination of thiols using Ellman's reagent. Three hundred microliters of supernatant were mixed with 630 µL of 0.5M KH_2PO_4 and 25 µL of 10 mM 5,5-dithiobis 2-nitrobenzoic acid (final pH 7.0). The absorbance at 412 nm was recorded after 2 min, and the NPT concentration was estimated using an extinction coefficient of 13,600 M^{-1} cm^{-1}. Phytochelatins content was evaluated as difference between NPT and GSH levels (Schafer et al. 1997*)*.

Statistical analysis

All analysis were performed on 5 replicates. Normality of the data was verified using Shapiro-Wilk tests and the data were transformed when required. Two-way ANOVA were performed at a significant level of *P*-value < 0.05 using R (version 3.3.1) considering the Zn and the Si applications as main factors. Means were compared using Tukey’s HSD all-pairwise comparisons at 5% level as a post-hoc test.

Results

Plant growth

Zinc excess induced leaf chlorosis and early senescence marked by a wilting process, especially in the youngest leaves (Fig. 1). Zinc excess also reduced leaf and stem fresh and dry weights, total leaf number, and stem length compare to control plants (Table 1, Fig. 2), although these trends were not statistically confirmed for roots and leaves dry weight, considering the high level of variability. Zinc excess in the absence of Si did not impact stem diameter or main root length (Fig. 2).

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 one week to Zn (100 µM) in the presence or in the absence of 2 mM H_2SiO_3. For a given organ, different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD all-pairwise comparisons. Each value is mean of 3 replicates (FW, DW and WC) or 5 replicates (Ψ_s).

	C	CSi	Zn	ZnSi
FW (g)				
Roots	11.88 ± 11.31 *a*	8.93 ± 7.54 *ab*	6.04 ± 3.59 a*b*	3.97 ± 3.02 *b*
Stems	10.03 ± 3.49 *a*	9.09 ± 5.00 *a*	4.19 ± 3.04 *b*	4.83 ± 2.94 *b*
Leaves	19.67 ± 9.37 *a*	18.20 ± 10.66 *ab*	10.59 ± 4.96 *bc*	9.45 ± 4.10 *c*
DW (g)				
Roots	0.87 ± 0.19 *ab*	0.95 ± 0.25 *a*	0.57 ± 0.20 *bc*	0.48 ± 0.16 *c*
Stems	1.22 ± 0.28 *a*	1.27 ± 0.23 *a*	0.71 ± 0.41 *a*	1.00 ± 0.47 *a*
Leaves	3.40 ± 1.58 *ab*	3.97 ± 0.83 *a*	2.40 ± 1.03 a*b*	2.09 ± 0.65 *b*
WC (%)				
Roots	88.05 ± 8.00 *a*	94.28 ± 0.58 *a*	92.54 ± 1.26 *a*	91.53 ± 1.68 *a*
Stems	86.17 ± 3.16 *ab*	90.92 ± 0.58 *a*	89.22 ± 2.01 *ab*	86.18 ± 2.42 *b*
Leaves	82.12 ± 2.36 *a*	85.54 ± 1.38 *a*	82.49 ± 2.33 *a*	82.01 ± 3.45 *a*
Ψs (Mpa)				
Leaves	-1.21 ± 0.06 *a*	-1.18 ± 0.04 *a*	-1.34 ± 0.04 *b*	-1.19 ± 0.04 *a*

Silicon addition to control nutrient did not impact plant behaviour in terms of growth or morphological properties. Silicon did not afford obvious protection against Zn toxicity and ZnSi was even the most deleterious treatment for root growth (Table 1).

Mineral concentrations

Zinc significantly accumulated in response to 100 µM $ZnCl_2$ in all plant organs. Zn was mainly accumulated in roots followed by stems and leaves (Table 2). In the absence of Zn stress, H_2SiO_3 application had no impact on Zn content. In the presence of Zn excess, however, exogenous Si significantly reduced Zn accumulation in all organs. Both TF_c and TF_a for Zn increased in response to exogenous Si in the absence of Zn excess, while BF values significantly decreased. Zn excess reduced TF_c and TF_a values and in this case, Si had no impact anymore on translocation factors or BF values.

Table 2

Zinc (Zn), and silicon (Si) concentrations and translocation factor (TF) in roots, stem and leaves of *Cannabis sativa* (cv. Santhica 27). Plants were exposed for one week to Zn (100 µM) in the presence or in the absence of 2 mM H_2SiO_3. TFc: translocation factor estimated on a concentration basis, TF_a: translocation factor estimated on a total amount basis. For a given parameter and a given organ, means followed by different letters are significantly different at $P < 0.05$ according to Tukey's HSD all-pairwise comparisons. Each value is mean of 3 replicates.

	C	CSi	Zn	ZnSi
Zn (mg kg^{-1} DW)				
Roots	464 ± 172 *a*	280 ± 14 *a*	13231 ± 236 *b*	11110 ± 619 *c*
Stems	41 ± 2 *a*	52 ± 9 *a*	1362 ± 95 *c*	1035 ± 36 *b*
Leaves	57 ± 6 *a*	62 ± 4 *a*	1003 ± 31 *c*	904 ± 41 *b*
TF_c	0.11 ± 0.02 *a*	0.21 ± 0.05 *b*	0.08 ± 0.03 *a*	0.09 ± 0.06 *a*
TF_a	0.60 ± 0.12 *a*	1.17 ± 0.18 *b*	0.45 ± 0.15 *a*	0.55 ± 0.31 *a*
BF	294 ± 24.98 c	157 ± 6.59 b	49.95 ± 13.28 *a*	44.45 ± 9.42 *a*
Si (mg kg^{-1} DW)				
Roots	827 ± 38 *a*	3017 ± 101 *c*	945 ± 8 *b*	6948 ± 596 *d*
Stems	103 ± 34 *ab*	174 ± 4 *b*	55 ± 3 *a*	136 ± 21 *b*
Leaves	260 ± 4 *b*	3006 ± 116 *d*	162 ± 5 *a*	1941 ± 100 *c*
TF_c	0.26 ± 0.04 b	0.77 ± 0.19 c	0.15 ± 0.06 *a*	0.20 ± 0.04 *ab*
TF_a	1.41 ± 0.30 *b*	4.22 ± 0.77 *c*	0.79 ± 0.28 *a*	1.24 ± 0.42 *ab*
BF	0	6.20 ± 0.38 *a*	0	6.03 ± 0.61 *a*

Although not intentionally added to the nutrient solution, Si was detected in control plants probably as a consequence of the presence of Si traces in the salt used for nutrient solution preparation. Root Si increased in response to H_2SiO_3 application and the recorded increase was by far higher in Zn-treated plants than in control ones. In contrast, only a slight effect of exogenous Si on Si content was recorded in the stem which remained low comparatively to other organs. In response to H_2SiO_3 application, the leaves displayed a higher concentration than the stem, and the leaf Si concentration was higher in the absence of Zn excess than in the presence of high Zn concentration. In the absence of additional exogenous Si, the Si concentration in the roots and in the leaves were in the same range. TF values for Si increased in response to exogenous application of Si but the recorded increase was significant in the absence of Zn excess only. The addition of Zn excess had no impact on BF values for Si.

Zn excess increased Fe concentration in the roots but decreased it in the leaves (Table 3). Exogenous Si in the presence of Zn excess did not mitigate this trend and even decreased Fe concentration in the stem. Zn excess also increased Mg concentration in the roots and in the stem and slightly decreased it in the leaves. The highest leaf Mg content was recorded for CSi-treated plants and the lowest for ZnSi-exposed ones. Exogenous Si in the absence of Zn excess increased S concentration in all organs. A similar effect was observed when plants were exposed to Zn excess in the absence of Si and was even more marked. In ZnSi-treated plants, S concentration increased in the roots and to a lower extent in the leaves but remained unmodified in the stem comparatively to control plants.

Table 3
Iron (Fe), magnesium (Mg) and sulfur (S) concentrations in roots, stem and leaves of *Cannabis sativa* (cv. Santhica 27). Plants were exposed for one week to Zn (100 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 $P < 0.05$ according to Tukey's HSD all-pairwise comparisons. Each value is mean of 3 replicates.

	C	CSi	Zn	ZnSi
Fe (mg kg^{-1} DW)				
Roots	1526 ± 37 *b*	922 ± 11 *a*	2281 ± 12 *c*	2298 ± 36 *c*
Stems	55 ± 4 *b*	42 ± 14 *ab*	58 ± 1 *b*	22 ± 4 *a*
Leaves	109 ± 4 *b*	115 ± 1 *b*	64 ± 2 *a*	55 ± 3 *a*
Mg (mg kg^{-1} DW)				
Roots	3537 ± 193 *a*	3346 ± 11 *a*	6631 ± 24 *c*	4754 ± 3 *b*
Stems	1295 ± 58 *a*	1639 ± 4 *b*	1599 ± 5 *b*	1419 ± 55 *a*
Leaves	4441 ± 12 *c*	5572 ± 5 *d*	4209 ± 31 *b*	3637 ± 83 *a*
S (mg kg^{-1} DW)				
Roots	2145 ± 205 *a*	2420 ± 107 *ab*	3963 ± 47 *c*	2897 ± 7 *b*
Stems	729 ± 12 *a*	1028 ± 77 *b*	1330 ± 52 *c*	746 ± 11 *a*
Leaves	1016 ± 126 *a*	1721 ± 1 *d*	1597 ± 115 *c*	1242 ± 14 *b*

Photosynthesis-related parameters

Zn exposure had no significant impact on photosynthesis-related parameters but tended to decrease stomatal conductance (g_S), net photosynthesis (A) and instantaneous evapotranspiration (E) (Table 4). Although no significant difference was recorded following H_2SiO_3 application, Si slightly increased gas exchange parameters and stomatal conductance in the absence of Zn excess. In Zn-exposed plants, additional Si tended to decrease the photochemical efficiency of photosystem II (Φ_{PSII}), photochemical

quenching (q_P), stomatal conductance (g_S) and concentration of CO_2 in intercellular spaces (Ci) of Zn exposed plants while it non-significantly increased the NPQ values.

Table 4
Photosynthesis-related parameters of *Cannabis sativa* (cv. Santhica 27). Plants were exposed for one week to Zn (100 µM) in the presence or in the absence of 2 mM H_2SiO_3. Maximum quantum yield of dark acclimated leaves (Fv/Fm), the photochemical efficiency of photosystem II (Φ_{PSII}), photochemical quenching (q_P), non-photochemical quenching (NPQ), stomatal conductance (gs), net photosynthesis (A), instantaneous evapotranspiration (E), CO_2 in intercellular spaces (Ci). For a given organ, different letters indicate significant differences at $P < 0.05$. Each value is mean of 5 replicates.

	C	CSi	Zn	ZnSi
F_v/F_m	0.88 ± 0.01 *ab*	0.88 ± 0.01 *b*	0.85 ± 0.03 *a*	0.86 ± 0.01 *ab*
ϕ_{PSII}	0.83 ± 0.01 *a*	0.83 ± 0.03 *a*	0.80 ± 0.04 *ab*	0.74 ± 0.08 *b*
q_P	0.96 ± 0.02 *a*	0.97 ± 0.01 *a*	0.96 ± 0.02 *ab*	0.89 ± 0.08 *b*
NPQ	0.16 ± 0.04 *a*	0.16 ± 0.03 *a*	0.15 ± 0.03 *a*	0.24 ± 0.11 *a*
g_s (mmol m^{-2} s^{-1})	745 ± 565 *ac*	1012 ± 729 *c*	301 ± 186 *ab*	205 ± 163 *b*
A (µmol m^{-2} s^{-1})	2.44 ± 2.07 *a*	2.67 ± 1.35 *a*	1.57 ± 1.49 *a*	2.23 ± 1.09 *a*
E (mmol m^{-2} s^{-1})	2.95 ± 0.80 *ac*	3.22 ± 0.56 *c*	1.72 ± 1.01 *ab*	1.62 ± 1.16 *b*
C_i (µmol mol^{-1})	402 ± 24 *a*	406 ± 16 *a*	415 ± 15 *a*	385 ± 32 *a*

In the absence of Zn excess, exogenous Si surprisingly decreased Chl a, Chl b and carotenoids content (Fig. 3). In the absence of Si, Zn excess decreased Chl a and carotenoids but had no impact on Chl b. Exogenous Si did not significantly mitigate the deleterious effect of Zn on these parameters.

Plant water and oxidative status

Water status was assessed using the osmotic potential (Ψ_S) and the water content (WC) of the leaves. As shown in Table 1, Zn in the absence of H_2SiO_3 significantly lowered Ψ_S values in leaves, while plants of the ZnSi treatment have similar values than control ones. WC was not affected by the treatments applied.

Malondialdehyde (MDA) is a cytotoxic product resulting from lipid peroxidation and commonly considered as an indicator of oxidative stress. Its concentration increased in roots but remained unaffected in leaves of Zn-exposed plants compared to the controls (Fig. 4). The addition of Si significantly decreased MDA concentration in leaves of all treatments. In roots, Si application increased MDA concentration of plants cultivated in the absence of Zn excess.

Data for total antioxidant capacity for the hydrophobic (AOAD) and hydrophilic (AOAM) fractions are provided in Fig. 5. AOAD and AOAM fractions were reduced in plants exposed to Zn, but increased when exposed to Si in the presence or absence of Zn excess.

Zn exposure strongly increased total glutathione (GSHt, Table 5) content in roots and leaves. The addition of silicon significantly decreased GSHt in roots and leaves of plants exposed to Zn, but also in leaves of plants cultivated in the absence of Zn excess. The ratio between oxidized (GSSG) and reduced glutathione (GSH) is an indicator of the oxidative stress undergone. GSSG/GSH ratio was significantly lower in roots and higher in leaves of HM treated plants compare to controls (Table 5). H_2SiO_3 application decreased GSSG/GSH in roots in the absence of Zn excess, and increased this ratio in leaves of plants exposed to Zn.

Table 5
Total glutathione (GSHt), oxidize (GSSG)/reduced (GSH) glutathione, and phytochelatine (PC) concentrations in roots and leaves of *Cannabis sativa* (cv. Santhica 27). Plants were exposed for one week to Zn (100 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 mean of 5 replicates.

	C	CSi	Zn	ZnSi
Roots				
GSHt (nmol g^{-1} FW)	176 ± 20 *a*	82 ± 34 *b*	1622 ± 171 c	762 ± 37 d
GSSG/GSH	14.38 ± 6.85 *a*	5.89 ± 5.84 *b*	3.61 ± 0.73 *b*	8.43 ± 4.27 *ab*
PC (nmol g^{-1} FW)	241 ± 28 *ab*	209 ± 20 *a*	222 ± 37 *ab*	275 ± 55 *b*
Leaves				
GSHt (nmol g^{-1} FW)	412 ± 19 *a*	294 ± 33 *b*	824 ± 161 *c*	564 ± 48 *d*
GSSG/GSH	6.03 ± 0.27 *a*	7.07 ± 0.76 *a*	11.12 ± 2.28 *b*	14.56 ± 1.70 *c*
PC (nmol g^{-1} FW)	821 ± 84 *a*	658 ± 24 *b*	1243 ± 73 *c*	974 ± 121 *d*

As far as roots are concerned, PC concentration was significantly higher in ZnSi-treated plants than in CSi-exposed ones. Zn in the absence of Si had no effect on root PC concentration. PC was higher in the leaves than in the roots for all treatments: the highest value was recorded for plants exposed to Zn in the absence of Si, since exogenous Si significantly reduced the PC concentration of Zn-treated plants.

Discussion

The possibility of combining non-food production and phytoremediation arouses more and more interest. The implementation of this strategy requires the selection of fast-growing crops that can accumulate and tolerate HM. Hemp (*Cannabis sativa* L.) is a multi-uses plants providing cortical fibres used in the

manufacture of various products, and hurds that enter in the composition of building materials (Deleuran and Flengmark 2006), and is thought to be able to reduce HM contamination in soils thus contributing to the remediation of contaminated sites (Angelova et al. 2004; Bona et al. 2007; Shi et al. 2009 and 2012; Ahmad et al. 2016; Kumar et al. 2017).

The present study confirmed that Zn significantly accumulated in *C. sativa* cultivated in nutrient solution under Zn excess. However, growth properties were only marginally affected by Zn excess: the recorded reduction in fresh and dry weight remained not significant from a statistical point of view, considering the registered variability between plants but was clearly detectable (see Fig. 1). It has however to be mentioned that we used a short-term exposure of one week and it can therefore not be excluded that a longer duration would lead to a significant yield decrease.

In plants exposed to Zn excess, Zn concentration in stem was higher than in the leaves. This could be linked to the fixing properties of the fibres which are exploited for biosorption purposes by numerous authors (Pejic et al. 2011; Vukcevic et al. 2014a, b). Zinc is indeed able to bind to carboxyl and hydroxyl groups of cell wall polymers such as those occurring in bast fibres and Loiacono et al. (2018) recently demonstrated that hemp fibres may be efficiently used to clean up waste water contaminated by numerous HM. Wall retention could also occur *in planta*, during the formation of the fibres, although this implies that HM need to be in close contact with the fibre and bast fibres are located in the phloem rather than in the xylem where transpiration stream occurs. More accurate techniques of histological localization would allow us to precise the location of Zn *in situ*.

Zn translocation from roots to shoots, estimated on a concentration basis (TF_c), was however limited ($TF_c \sim 0.1$) but it could also be interesting to estimate TF on a total amount basis (TF_a, Table 3) based on the quantities actually exported from the substrate (Ali et al. 2013). For all treatments, translocation factor estimated on a total amount basis (TF_a) was higher than translocation factor estimated on a concentration basis (TF_c) but remained always lower than 1. This suggests that, despite a good level of tolerance, hemp adopted an excluding strategy. A similar observation has already been reported by Angelova et al. (2004), Löser et al. (2002) and Shi et al. (2009) in hemp exposed to HM. Root sequestration is a strategy widely developed by non-hyperaccumulating plants to avoid the accumulation of toxic elements in photosynthetic tissues. Hence, the use of *Cannabis sativa* cv Santhica 27 in phytoremediation should be restricted to phytostabilization but not phytoextraction of Zn from contaminated soils. This implies that hemp is also able to display tolerance mechanisms.

We demonstrated that metallic stress had only a slight negative impact on biomass production (FW, DW, total leaf number, stem length) and pigment concentration, although we did not observe any mortality in plants nor a significant impact on photosynthesis-related parameters. According to Piotrowska-Cyplik and Czarnecki (2003) and Linger et al. (2005) a reduction of pigments content, and therefore in the availability of photoassimilats, as well as the energy cost of tolerance mechanisms, may explain the reduced biomass production (Ghnaya et al., 2009). According to Küpper et al. (1998), Zn may substitute to Mg within chlorophyll and this could explain both an alteration in the efficiency of light phase, and a

decrease in unstable chlorophyll prone to degradation by chlorophyllase. This, however, is considered to mainly occur in response to Mg deficiency while we demonstrated in the present study that Zn excess reduced the Mg content in the leaves by only 5.2% (Table 3). Accordingly, data provided by chlorophyll fluorescence indicated that the light phase by itself is only marginally affect by Zn excess.

We also paid attention to plant water and antioxidative status. Water content was not affected by the treatments applied but Zn excess significantly lowered Ψ_S values in leaves, which is a clear indication that adaptations were required for Zn-treated plants to maintain internal water potential and turgor. Such accumulation of osmotic compounds contributes to turgor maintenance and CO₂ diffusion within the mesophyll. This probably explain stable *Ci* values in plants exposed to Zn excess compare to controls while other gas exchange parameters tended to decrease. Beside CO_2 diffusion within the mesophyll, the highest resistance encountered by CO_2 to reach the sites of carboxylation is located at the stomatal level. Zinc tended to decrease the g_s value but this had no impact on mean *Ci* which suggest that the carboxylation efficiency might be hampered by Zn excess. Indeed, Zn-treated plants exhibited the lowest *A* value: Cambrollé et al. (2013) mentioned that Zn may also substitute to Mg in Rubisco itself, leading to under-utilization of ATP and NADPH in CO_2 fixation. Nevertheless, this hypothesis might be invalid in our case since no important Mg decrease was recorded in the leaves. Data are still missing regarding the impact of Zn on activity of other enzymes involved in the Calvin cycle.

The present study also showed that hemp exposed to Zn excess encountered a moderate oxidative stress: MDA content in leaves remained similar to controls, but increased in roots. Zinc accumulated to higher amounts in the roots than in the leaves; hence, a higher oxidative stress in the below part of the plant was not unexpected. This suggests that, in leaves, oxygen species were quickly scavenged. Reduced glutathione (GSH) is involved in the reduction of an important part of ROS generated due to stress (Shao et al. 2007). In our study, HM exposure led to higher GSHt concentration in leaves and elevated GSSG/GSH ratio, suggesting that GSH helped to withstand oxidative stress in leaves. This may also explain the decrease of total antioxidant capacity, GSH being part of the AOAM fraction. Besides its role as an antioxidant, GSH acts as a precursor of phytochelatin (PC) synthesis. In our study, HM exposure led to higher PC content in leaves. Chelating metals by forming PCs or metallothioneins (MTs) metal complex at the intra- and intercellular level are part of the mechanisms used by plants to counteract HM toxicity (Alloway et al., 2013; Citterio et al., 2003; Tennstedt et al. 2009; Emamverdian et al., 2015). Nevertheless, a higher PC concentration in the leaves than in the roots is rare in plants exposed to HM. Wogkaew et al. (2019) suggested that glutathione may be involved in the translocation of Zn from the root to the shoot: the underlying mechanisms however, remains unknown and this observation did not explain why, in the present case, Zn remained sequestered in the roots while glutathione accumulated in the leaves. Besides, oversynthesis of glutathione requires large quantities of S. Hemp increased the S accumulation in roots, stems and leaves in the presence of Zn excess confirming that hemp is able to trigger this protective mechanism. However, it is important to keep in mind that to combine phytostabilization with non-food production, the properties of the harvested biomass have to remain

compatible with the requirements of industry from a qualitative point of view (Luyckx et al., 2019). Additional work is therefore needed to precise the impact of Zn excess on valuable plant parts.

Although Si is not considered as essential for plants, it intervenes as a beneficial element in their defence and growth. As such, it is used in agriculture as a biostimulant. Hemp is not considered as an Si accumulator under normal conditions. However, in this study, Si significantly increased root and leaf Si concentration in the absence and in the presence of Zn excess. In roots, plants of CSi treatment accumulated 3.6x more Si than plants of C treatment while ZnSi treated plants accumulated 7.4x more Si than plants of Zn treatment. This suggests that plants stimulated Si uptake to cope with Zn stress. In rice exposed to heavy metals and Si, Kim et al. (2017) and Ma et al. (2015) reported an increase in the expression of genes involved in the transport of Si (*OsLSi1* and *OsLSi2*) to improve resistance to metal stress. In the present case, however, high Si accumulation in the root of ZnSi-treated plants did not protect the roots from oxidative stress since MDA concentration was similar to Zn-treated plants.

H_2SiO_3 application under Zn exposure interfered with HM absorption. Zn concentration was clearly lower in roots, stems and leaves of Si-treated plants in the presence of Zn excess than in plants exposed to Zn in the absence of Si. A first hypothesis is that the presence of Si in the nutrient solution reduces Zn availability through the precipitation of Zn silicate. Bokor et al. (2014) indeed mentioned that Zn_2SiO_4 may occur in water experiments but this is not confirmed by the speciation program VISUAL MinTEQ which clearly indicated that, for the range of concentration and pH of the solution used in our study, Zn remained fully soluble in the solution. The decrease of Zn concentration cannot be attributed to a decrease in transpiration rate since no significant difference for *E* values was observed between ZnSi and Zn treatments. It has already been observed in rice plants treated with Cu/Cd a decrease in the expression of HM transporters in the presence of Si (Kim et al. 2017; Ma et al. 2015). Huang and Ma (2020) recently demonstrated in rice that Si supply decreased Zn concentration in both the root and the shoots: according to these authors Si acts on Zn uptake by down-regulating *OsZIP1* implicated in Zn uptake. The comparison should however be established with caution considering that rice is a specific plant species for Si hyperaccumulation and that data obtained with rice are not necessarily valid for other plant species, especially dicots. Beside Zn absorption, some authors reported that Si may reduce Zn translocation from the root to the shoot (Zajaczkowska et al. 2020; Naeem et al. 2015) but this was not observed in our experiment since TF value for Zn were hardly modified by Si. This implies that as far as hemp is concerned, Si may impact transporters involved in Zn uptake (especially those encoded by *ZIP* genes) but had no impact on transporters involved in Zn xylem loading and long-distance transport (HMA2 and HMA4) (Zlobin 2021).

It is also interesting to notice that the recorded Zn-induced increase of Zn accumulation was higher than the Si-induced decrease of Zn accumulation in plants of ZnSi treatment. Hence, if Si contributes to defence and growth, silicon could be compatible with phytostabilization purposes. Zn accumulation in control plants was not significantly affected by Si. Although no significant difference was recorded following H_2SiO_3 application, Si slightly improved gas exchange (g_s, *A*, *E*) in controls and net photosynthesis under Zn exposure. In the same treatment (ZnSi), the fact that NPQ was always higher

than in Zn treatment may indicate that plants quickly dissipate an excess of energy in order to maintain an adequate balance between photosynthetic electron transport and carbon metabolism (Gharbi et al., 2017). Si paradoxically decreased pigments content in control plants which was an unexpected result.

Exogenous application of Si under Zn excess increased total antioxidant capacity (AOAM and AOAD fractions) and decreased MDA content mainly in the leaves, as already observed by Kim et al. (2017). This suggests that Si may trigger oxidative tolerance processes in hemp. The same beneficial effect of Si was noticed in leaves of control plants. Increased antioxidant capacity under Si exposure suggested a higher GSHt content. However, in the present study, under Zn excess GSHt content decreased following Si application. The increase in the antioxidant capacity despite a decrease in GSHt content may be due to activation of other antioxidants by Si: the fact that both AOAD and AOAM antioxidant activities increased in response to Si suggests indeed that other compounds, such as ascorbate and α-tocopherol, which were not quantified in the present study, may increase in response to exogenous Si. Moreover, the lower Zn accumulation induced by Si probably decreased ROS production and the need of GSH as an antioxidant and a precursor of PC synthesis. PC content in leaves was indeed decreased in plants of ZnSi treatment comparatively to Zn–treated plants, while we observed an opposite trend in roots, although Si decreased Zn accumulation in both organs. This suggests that PC more efficiently sequester Zn in the leaves than in the roots: while Zn concentrations in the roots are quite high, PC contents were unexpectedly low. Using K-edge extended X-ray absorption fine structure (EXAFS) spectroscopy measurements, Lefèvre et al. (2016) demonstrated that in the roots of HM resistant species *Zygophyllum fabago*, Zn mainly coordinate to Zn–O/N–C groups suggesting that PC did not play a key role in Zn tolerance in this organ and that other compounds, such as organic acid or polyamines, may be involved.

As a matter of fact, exogenous Si provides some metabolic advantages to hemp exposed to Zn excess, especially in relation to a decrease in Zn accumulation in the different parts of the plant. However, this was not sufficient to significantly increase plant growth on a short-term basis. Bokor et al. (2014) found similar data regarding specific maize cultivars simultaneously exposed to Zn and Si. According to these authors, increasing concentration of Si in combination with Zn treatment even increased physiological stress in comparison to Zn treatment. Similar results were observed by Masarovic et al. (2012) in sorghum who observed no positive effect of Si on high Zn in the medium.

Conclusion

Results obtained in nutrient solution indicated that *Cannabis sativa* cv Santhica 27 is able to significantly accumulate Zn when exposed to Zn excess, Zn being mainly accumulated in roots. Hemp can thus not be considered as an hyperaccumulator. In order to be used in phytostabilization strategies, the plant has to be able to tolerate heavy metals. In our study, hemp activated antioxidant defences and coped with the significant accumulation of Zn by limiting its transfer to the aerial parts. Zn excess negatively affected biomass production and we did not observe any mortality in plants. Our experiment was carried out in nutrient solution where Zn bioavailability was high. It could be assumed than in field conditions Zn impact on biomass production would be more limited. Moreover, the use of Si to improve hemp growth on

HM contaminated soils should be considered: in our experiment, Si decreased the proportion of Zn removed by the plants and improved the antioxidant response of control and Zn-exposed plants. Silicon however did not significantly improve growth of hemp exposed to Zn excess. In order to combine phytostabilization with non-food production, the properties of the harvested biomass have to remain compatible with the requirements of industry from a qualitative point of view (Luyckx et al., 2019). It would be therefore appropriate to link morphophysiological observations with the distribution of HM in hemp tissues.

Declarations

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

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References

1. Adrees M, Ali S, Rizwan M, Zia-ur-Rehman M, Ibrahim M, Abbas F, Farid M, Qayyum MF, Irshad MK (2015) Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: a review. Ecotox Environ Saf 119:186–197
2. Ahmad R, Tehsin Z, Malik ST, Asad SA, Shahza M, Bila M et al (2016) Phytoremediation potential of hemp (*Cannabis sativa* L.): identification and characterization of heavy metals responsive genes. CLEAN–Soil Air Water 44:195–201
3. Ali H, Khan E, Anwar Sajad M (2013) Phytoremediation of heavy metals — Concepts and applications. Chemosphere 91:869–881
4. Alloway BJ (2013) Heavy metals in soils: trace metals and metalloids in soils and their bioavailability. Environ Poll 22:11–50
5. Andrejić G, Gajić G, Prica M, Dželetovic Ž, Rakić T (2018) Zinc accumulation, photosynthetic gas exchange, and chlorophyll a fluorescence in Zn-stressed *Miscanthus x giganteus* plants. Photosynthetica 56:1249–1258

6. Angelova V, Ivanova R, Delibaltova V, Ivanov K (2004) Bio-accumulation and distribution of heavy metals in fibre crops (flax, cotton and hemp). Ind Crops Prod 19:197–205
7. Barceló J, Poschenrieder C (2003) Phytoremediation: Principles and perspectives. Contr Sci 2:333–344
8. Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal Biochem 239:70–76
9. Berni R, Mandlik R, Hausman JF, Guerriero G (2021) Silicon-induced mitigatory effects in salt-stressed hemp leaves. Physiol Plant 171:476–482
10. Bokor B, Vaculík M, Slováková L, Masarovič D, Lux A (2014) Silicon does not lways mitigate zinc toxicity in maize. Acta Physiol Plant 36:733–743
11. Bona E, Marsano F, Cavaletto M, Berta G (2007) Proteomic characterization of copper stress response in *Cannabis sativa* roots. Proteomics 7:1121–1130
12. Branda F, Malucelli G, Durante M, Piccolo A, Mazzei P, Costantini A, Silvestri B, Pennetta M, Bifulco A (2016) Silica treatments: a fire retardant strategy or hemp fabric/epoxy composites. Polymers 8:313
13. Cambrollé J, Mancilla-Layton JM, Munoz-Vallès S, Figureuero-Luque E, Luque T, Figureuero-Luque ME (2013) Evaluation of Zn tolerance and accumulation potentiel of the coastal shrub *Limoniastrum monopetalum* (L.) Boiss. Environ Exp Bot 85:50–57
14. Cereser C, Guichard J, Drai J, Bannier E, Garcia I, Boget S, Parvaz P, Revol A (2001) Quantitation of reduced and total glutathione at the femtomole level by high-performance liquid chromatography with fluorescence detection: Application to red blood cells and cultured fibroblasts. J Chro B: Biomedical Sci Appl 752:123–132
15. Charai M, Sghiouri H, Mezrhab A, Karkri M (2021) Therma insulation potential on non-industrial (Moroccan *Cannabis sativa* L.) fibers for green plaster-based building materials. J Clean Prod 292:126064
16. Citterio S, Santagostino A, Fumagalli P, Prato N, Ranalli P, Sgorbati S (2003) Heavy metal tolerance and accumulation of Cd, Cr and Ni by *Cannabis sativa* L. Plant Soil 256:243–252
17. Deleuran LC, Flengmark PK (2006) Yield potential of hemp (*Cannabis sativa* L.) cultivars in Denmark. J Ind Hemp 10:19–31
18. De Vos CR, Vonk MJ, Vooijs R, Schat H (1992) Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*. Plant Physiol 98:853–858
19. Doncheva S, Poschenrieder C, Stoyanova Z, Georgieva K, Velichkova M, Barcelo J (2009) Silicon amelioration of manganese toxicity in Mn-sensitive and Mn-tolerant maize varieties. Environ Exp Bot 65:189–197
20. Dufey I, Gheysens S, Ingabire A, Lutts S, Bertin P (2014) Silicon application in cultivated rice (*Oryza sativa* L. and *O. glaberrima* Steud.) alleviates iron toxicity symptoms through the reduction in iron concentration in the leaf tissue. J Agron Crop Sci 200:132–142

21. Emamverdian A, Ding Y, Mokhberdoran F, Xie Y (2015) Heavy metal stress and some mechanisms of plant defense response. Sci World J 2015: 756120
22. Epstein E (1994) The anomaly of silicon in plant biology. PNAS 91:11–17
23. Etesami H, Jeong BR (2018) Silicon (Si): Review and future prospects on the action mechanisms in alleviating biotic and abiotic stresses in plants. Ecotox Environ Saf 147:881–896
24. Fan W, Guo Q, Liu CY, Liu X, Zhang M, Long D, Xiang Z, Zhao A (2018) Two mulberry phytochelatin synthase genes confer zinc/cadmium tolerance and accumulation in transgenic *Arabidopsis* and tobacco. Gene 645:95–104
25. Gharbi E, Martínez JP, Benahmed H, Hichri I, Dobrev PI, Motyka V, Quinet M, Lutts S (2017) Phytohormone profiling in relation to osmotic adjustment in NaCl-treated plants of the halophyte tomato wild relative species *Solanum chilense* comparatively to the cultivated glycophyte *Solanum lycopersicum*. Plant Sci 258:77–89
26. Ghnaya AB, Charles G, Hourmant A, Hamida JB, Branchard M (2009) Physiological behaviour of four rapeseed cultivar (*Brassica napus* L.) submitted to metal stress. CR Biol 332:363–370
27. Goodarzi A, Namdjoyan S, Soorki AA (2020) Effects of exogenous melatonin and glutathione on zinc toxicity in safflower (*Carthamus tinctorius* L.) seedlings. Ecotox Environ Saf 201:110853
28. Guerriero G, Sutera FM, Torabi-Pour N, Renait J, Hausman JF, Berni R, Pennington HC, Welsh M, Dehorskhi A, Zancan LR, Saffie-Siebert S (2021) Phyto-courrier, a silicon particle-based nano-biostimulant: evidence from *Cannabis sativa* exposed to salinity. ACS Nano 15:3061–3069
29. Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: II. Role of electron transfer. Arch Biochem Biophys 125:850–857
30. Huang S, Ma JF (2020) Silicon suppresses zinc uptake through down-regulating zinc transporter gene in rice. Phyiol Plant 170:580–591
31. Imtiaz M, Rizwan MS, Mushtag MA, Ashraf M, Shahazad SM, Yousaf B, Saeed DA, Rizwan M, Nawaz MA, Mehmood S, Tu S (2016) Silicon occurrence, uptake, transport and mechanisms of heavy metals, minerals and salinity enhanced tolerance in plants with future prospects: a review. J Env Manag 183:521–529
32. Jiang Y, Bourebrab MA, Sid N, Taylor A, Collet F, Pretot S, Hussain A, Ansell M, Lawrence M (2018) Improvement of water resistance of hemp woody substrates through deposition of functionalized silica hydrophobic coating while retaining excellent moisture buffering properties. Sus Chem Eng 6:10151–10161
33. Kanwar VS, Sharma A, Srivastav AL, Ranni L (2020) Phytoremediation of toxic metals present in soil and water environment: a critical review. Environ Sci Poll Res 27:44835–44860
34. Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ (2000) Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197–207
35. Kim YH, Khan AL, Waqas M, Lee IJ (2017) Silicon regulates antioxidant activities of crop plants under abiotic-induced oxidative stress: a review. Front Plant Sci 8:510

36. Kröger N, Poulsen N (2008) Diatoms – from cell wall biogenesis to nanotechnology. Ann Rev Genet 42:83–107
37. Kumar S, Singh R, Kumar V, Rani A, Jain R (2017) *Cannabis sativa*: A Plant Suitable for Phytoremediation and Bioenergy Production. Phytoremediation Potential of Bioenergy Plants. Springer, Singapore, pp 269–285
38. Küpper H, Küpper F, Spiller M (1998) In situ detection of heavy metal substituted chlorophylls in water plants. Photosynth Res 58:123–133
39. Kurade MMB, Ha YH, Xiong JQ, Govindar SP, Jang M, Jeon BH (2021) Phytoremediation as a green biotechnology tool for emerging environmental pollution: a step towards sustainable rehabilitation of the environment. Chem Eng J 415:129040
40. Lefèvre I, Vogel-Mikuš K, Arčon I, Lutts S (2016) How do roots of the metal-resistant perennial bush *Zygophyllum fabago* cope with cadmium and zinc toxicities? Plant Soil 404:193–207
41. Linger P, Müssigg J, Fischer H, Kobert J (2002) Industrial hemp (*Cannabis sativa* L.) growing on heavy metal contaminated soil: fibre quality and phytoremediation potential. Ind Crops Prod 16:33–42
42. Linger P, Ostwald A, Haensler J (2005) *Cannabis sativa* L. growing on heavy metal contaminated soil: growth, cadmium uptake and photosynthesis. Biol Plant 49:567–576
43. Löser C, Zehnsdorf A, Fussy A, Stärk HJ (2002) Conditioning of heavy metal-polluted river sediment by *Cannabis sativa* L. Intern J Phytorem 4:27–45
44. Loiacono S, Morin-Cribni N, Martel B, Chanet G, Bradu C, Torri G, Crini G (2018) Complexation du zinc, du cuivre et du manganese par du chanvre: efficacité chimique et impact écotoxicologique. Environ Risque Santé 17:240–252
45. Luo Y, Zheng Z, Wu P, Wu Y (2022) Effect of different direct revegetation strategies in the mobility of heavy metals in artificial smelting waste slag: implications for phytoremediation. Chemosphere 286:131678
46. Luyckx M, Berni R, Cai G, Lutts S, Guerriero G (2019) Impact of heavy metals on non-food herbaceous crops and prophylactic role of Si. Plant Metallomics and Functional Omics. Springer, Cham, pp 303–321
47. Luyckx M, Hausman JF, Blanquet M, Guerriero G, Lutts S (2021) Silicon reduces cadmium absorption and increases root-to-shoot translocation without impacting growth in young plants o hemp (*Cannabis sativa* L.) on a short- term basis. Environ Sci Poll Res 28:37963–37977
48. Ma J, Cai J, He C, Zhang W, Wang L (2015) A hemicellulose-bound form of silicon inhibits cadmium ion uptake in rice (*Oryza sativa*) cells. New Phytol 206:1063–1074
49. Masarovic D, Slováková L, Bokor B, Budjoš M, Lux A (2012) Effect of silicon application in *Sorghum bicolor* exposed to toxic concentration of zinc. Biologia 67:706–717
50. Maxwell K, Johnson GN (2000) Chlorophyll fluorescence – A practical guide. J Exp Bot 51:659–668

51. Muthusaravanan S, Sivarajasekar N, Vivek JS, Paramasivan T, Naushad M, Prakashmaran J, Al-Duaij OK (2018) Phytoremediation of heavy metals: mechanisms, methods and enhancements. Environ Chem Lett 16:1339–1359
52. Pejic B, Vukcevic M, Kostic M, Skundric P (2009) Biosorption of heavy metal ions from aqueous solutions by short hemp fibers: effect of chemical composition. J Hazard Mat 164:146–153
53. Piotrowska-Cyplik A, Czarnecki Z (2003) Phytoextraction of heavy metals by hemp during anaerobic sewage sludge management in the non-industrial sites. Pol J Environ Stud 12:779–784
54. Schäfer HJ, Greiner S, Rausch T, Haag-Kerwer A (1997) In seedlings of the heavy metal accumulator *Brassica juncea*, Cu^{2+} differentially affects transcript amounts for γ-glutamylcysteine synthetase (γ-ECS) and metallothionein (MT2). FEBS Lett 404:216–220
55. Schluttenhofer C, Yuan L (2017) Challenges towards revitalizing hemp: a multifaced crop. Trends Plant Sci 22:917–929
56. Shahid M, Khalid S, Abbas G, Niazi NK, Murtaza B, Rashid MI, Bibi I (2019) Redox Mechanisms and Plant Tolerance Under Heavy Metal Stress: Genes and Regulatory Networks. Plant Metallomics and Functional Omics. Springer, Cham, pp 71–105
57. Shao HB, Chu LY, Lu ZH, Kang CM (2008) Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. Int J Biol Sci 4:8
58. Shi GR, Cai QS, Liu QQ, Wu L (2009) Salicylic acid-mediated alleviation of cadmium toxicity in hemp plants in relation to cadmium uptake, photosynthesis, and antioxidant enzymes. Acta Physiol Plant 3:969–977
59. Shi G, Liu C, Cui M, Ma Y, Cai Q (2012) Cadmium tolerance and bioaccumulation of 18 hemp accessions. Appl Biochem Biotechnol 168:163–173
60. Tennstedt P, Peisker D, Böttcher C, Trampczynska A, Clemens S (2009) Phytochelatin synthesis is essential for the detoxification of excess zinc and contributes significantly to the accumulation of zinc. Plant Physiol 149:938–948
61. Vareda JP, Valente AJM, Durães L (2019) Assessment of heavy meal pollution from anthropogenic activities and remediation trategies: a review. J Environ Manag 246:101–1018
62. Vukcevic M, Pejic B, Kaliajadis A, Pajic-Lijakovic I, Kostic M, Lausevic Z, Lausevic M (2014a) Carbon material from waste short hemp fibers as a sorbent for heavy metal ions – Mathematical modeling of sorbent structure and ions transport. Chem Eng J 233:284–292
63. Vukcevic M, Pejic B, Lausevic M, Pajic-Lijaovic I, Kostic M (2014b) Influence of chemically modified short hemp fiber structure on biosorption process of Zn^{2+} ions from waste water. Fib Pol 15:687–697
64. Wongkaew A, Nakamura S, Suzui N, Yin YG, Ishii S et al (2019) Elevated glutathione synthesis in leaves contributes to zinc transport from roots to shoots in Arabidopsis. Plant Sci 283:416–423
65. Wu Y, Trejo HX, Chen G, Li S (2021) Phytoremediation of contaminants of emerging concern from soil with industrial hemp (*Cannabis sativa* L.): a review. Environ Dev Sustain 23:14405–14435

66. Yang R, Berthold AC, McCurdy CR, da Silva Benvenue S, Brym ZT, Freeman JH (2020) Development of cannabinoids in flowers of industrial hemp (*Cannabis sativa* L.): A pilot study. J Agr Food Chem 68:6058–6064
67. Zajaczkowska A, Korzeniowska J, Sienkiewicz-Choleva U (2020) Effect of soil and foliar silicon application on the reduction of zinc toxicity in wheat. Agriculture 10:0522
68. Zhao J, Xu Y, Wang W, Griffin J, Roozeboom K, Wang D (2020) Bioconversion of industrial hemp biomass for bioethanol production: A review. Fuel 281:118725
69. Zlobin IE (2021) Current understanding of plant zinc homeostasis regulation mechanisms. Plant Physiol Biochem 162:327–335

Figures

Figure 1

Cannabis sativa plants cultivated in hydroponic conditions; C: control plants; Zn: plants exposed for one week to 100 µM Zn

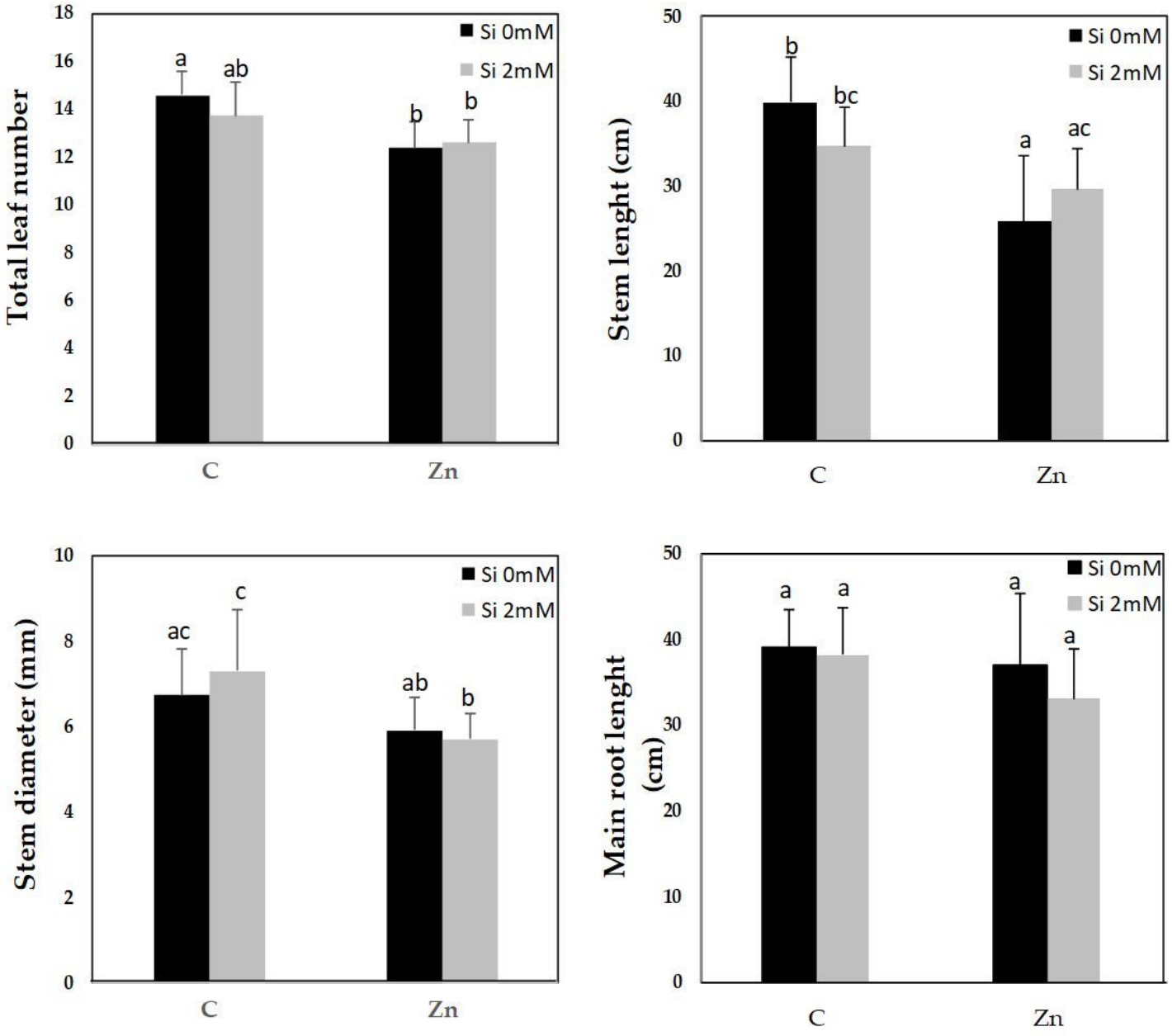

Figure 2

Total leaf number, stem length and diameter, and root length of *Cannabis sativa* (cv. Santhica 27) exposed for one week to Zn (100 µM), in the presence or in the absence of 2 mM H_2SiO_3. Data are means ± standard errors (n = 5). Values with different letters are significantly different ($P < 0.05$; Tukey's HSD all-pairwise comparisons).

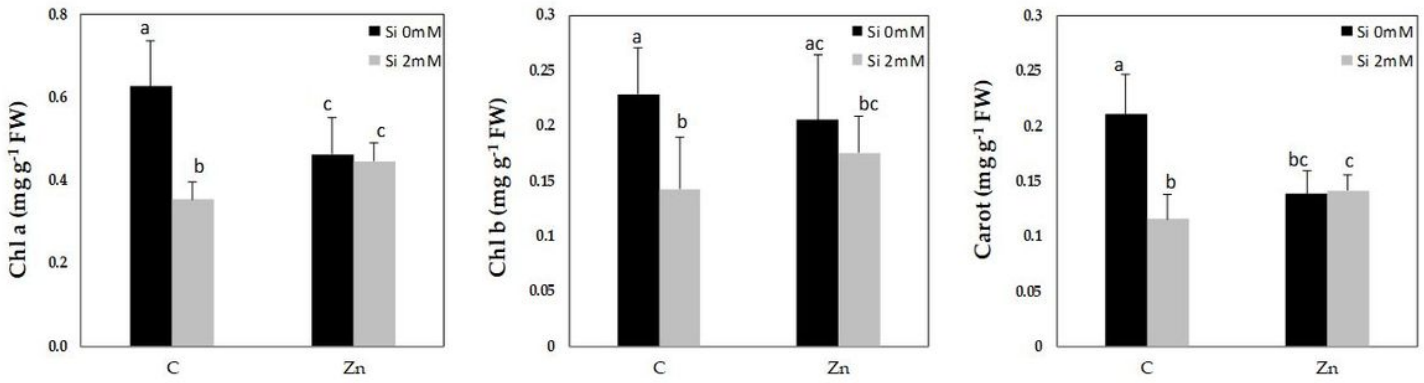

Figure 3

Chlorophyll (a, b) and carotenoid content of *Cannabis sativa* (cv. Santhica 27) exposed for one week to Zn (100 µM), in the presence or in the absence of 2 mM H_2SiO_3. Data are means ± standard errors (n = 5). Values with different letters are significantly different ($P < 0.05$; Tukey's HSD all-pairwise comparisons).

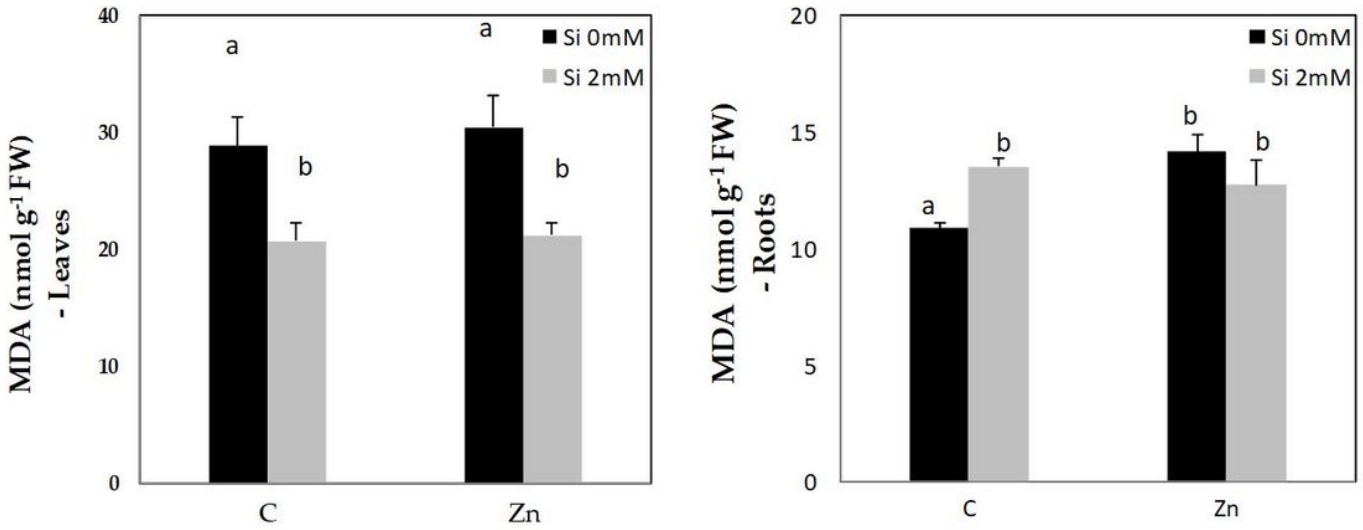

Figure 4

Malondialdehyde (MDA) content in leaves and roots of *Cannabis sativa* (cv. Santhica 27) exposed for one week to Zn (100 µM), in the presence or in the absence of 2 mM H_2SiO_3. Data are means ± standard errors (n = 5). Values with different letters are significantly different ($P < 0.05$; Tukey's HSD all-pairwise comparisons).

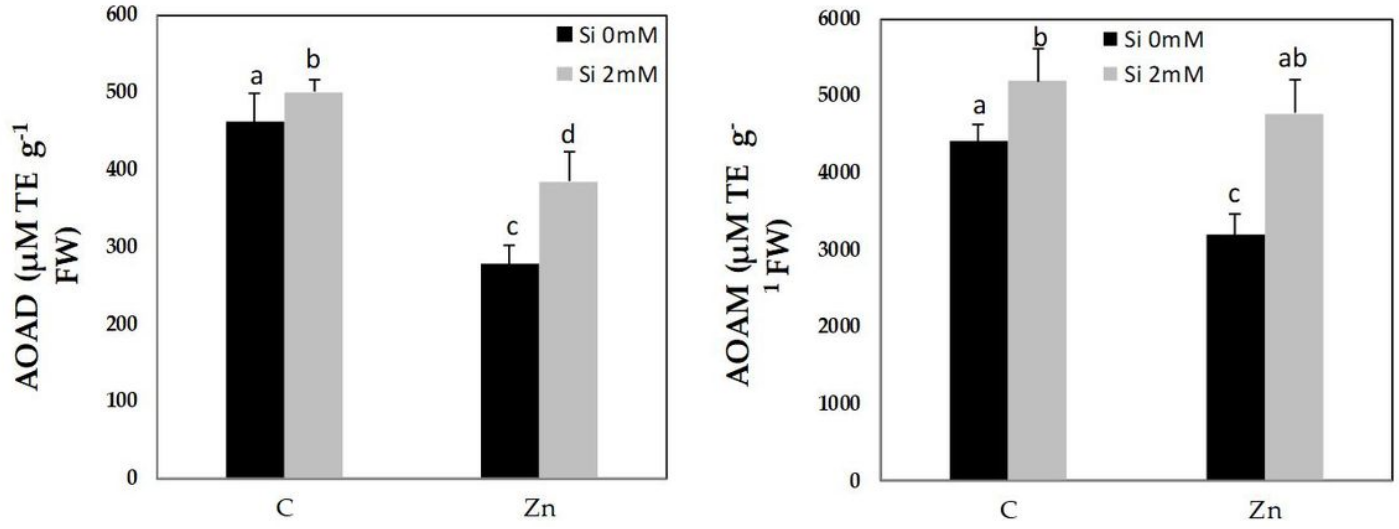

Figure 5

Total antioxidant activity in the hydrophobic fraction (AOAD) and the hydrophilic fraction (AOAM) of leaves of *Cannabis sativa* (cv. Santhica 27). Plants were exposed for one week to Zn (100 µM), in the presence or in the absence of 2 mM H_2SiO_3. Data are means ± standard errors (n = 5). Values with different letters are significantly different ($P < 0.05$; Tukey's HSD all-pairwise comparisons).

Supplementary Files

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- Img.jpg