Environ Sci Pollut Res
DOI 10.1007/s11356-017-9697-7

RESEARCH ARTICLE

Effects of Cd and Zn on physiological and anatomical properties of hydroponically grown *Brassica napus* plants

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Received: 7 December 2016 / Accepted: 4 July 2017

Abstract Clarifying the connection between metal exposure and anatomical changes represents an important challenge for a better understanding of plant phytoextraction potential. A hydroponic screening experiment was carried out to evaluate the effects of combined interactions of Cd and Zn on mineral uptake (Mg, K, Ca, Na) and on the physiological and anatomical characteristics of *Brassica napus* L cv. Cadeli, Viking, and Navajo. Plants were exposed to 5 μM Cd ($CdCl_2$), 10 μM Zn ($ZnSO_4$), or both Cd + Zn, for 14 days. Cadmium exposure led to a significant reduction in root growth, shoot biomass, and chlorophyll content. After Cd-only and Cd + Zn treatment, primary root tips became thicker and pericycle cells were enlarged compared to the control and Zn-only treatment. No differences between metals were observed under UV excitation, where all treatments showed more intensive autofluorescence connected with lignin/suberin accumulation compared to control conditions. The highest concentrations of Cd and Zn were found in the roots of all tested plants, and translocation factors did not exceed the threshold of 1.0. The root mineral composition was not affected by any treatment. In the shoots, the Mg concentration slightly increased after Cd-only and Cd + Zn treatments, whereas Zn-only treatment caused a sharp decrease in Ca content. Slight increases in K were seen after the addition of Zn. Significantly higher concentrations of Na were induced by Cd- or Zn-only treatment.

Keywords *Brassica napus* · Cadmium uptake · Mineral uptake · Phytoextraction · Root anatomy · Zinc uptake

Responsible editor: Elena Maestri

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Introduction

Metal contamination of agricultural soils represents an important problem in crop production. Nowadays, plants must cope with rapid environmental changes that are mainly due to human activities. Industrialization, traffic, smelting, mining, or excessive use of phosphate fertilizers are the primary causes of increased heavy metal content in soils (Chibuike and Obiora 2014). The uptake of these metals by plants plays a very important role in the entry of these metals into the food chain. Even in low concentrations, heavy metals are harmful for all vertebrates. Cadmium, for example, is a major carcinogen that accumulates in kidneys and bones, where it can pose a serious health threat (Lalor 2008; Clemens et al. 2013).

Zinc (Zn) and cadmium (Cd) are common environmental pollutants that are able to bind to soil particles for extended periods of time (Brown et al. 1998). In small amounts, Zn is an essential micronutrient for plants and represents an important catalytic component of over 300 enzymes (Hänsch and Mendel 2009). It is also necessary for biomass and chlorophyll production and normal pollen function (Wang and Jin 2005; Pandey et al. 2006; Sinclair and Krämer 2012; Emamverdian et al. 2015). In contrast with Zn, Cd is a metal without a physiological function in higher plants. The fourth most toxic trace element for plants (Singh 2005), Cd causes various symptoms of phytotoxicity such as reduced growth, browning of root tips, leaf chlorosis, enhanced production of reactive oxygen species (ROS),

Published online: 16 July 2017

disorders of mineral nutrition, and many other changes in anatomical, morphological, and biochemical properties (Touiserkani and Haddad 2012; Tran and Popova 2013; Choppala et al. 2014).

Although the influence of individual metals on biological properties is well known, the combined effects still need to be investigated. Cadmium and Zn are physically, chemically, and geologically similar elements (Chaney 2010) that are taken in by plants as divalent cations. The effects of these metals, when in combination, have been shown to have very different impacts. In some cases, studies have revealed both antagonistic (Balen et al. 2011; Tammam et al. 2016; Versieren et al. 2017) and synergistic interactions (Nan et al. 2002; Cherif et al. 2011; Cojocaru et al. 2016). It must be emphasized that the combined effects of Cd and Zn depend on the individual concentrations of these two metals. It was found that lower concentrations of Zn (1–50 μM) can decrease Cd-induced oxidative stress via increasing antioxidative enzyme activity in Cd-stressed organisms (Hassan et al. 2005; Taspinar et al. 2011; Balen et al. 2011), while higher levels of Zn (150 μM) combined with Cd induced oxidative stress (Cherif et al. 2011). It was also found that foliar application of Zn (0.3% $ZnSO_4$) can effectively decrease Cd concentrations in wheat grains and reduce the negative effects of Cd exposure (Saifullah et al. 2014).

Oilseed rape (*Brassica napus* L.) is an economically important crop plant for oil production worldwide. Due to its fast growth, high biomass productivity, and heavy metal absorption, oilseed rape has been widely studied for its phytoextraction potential, which largely depends on the efficiency of metal uptake and translocation within different plant parts (Marchiol et al. 2004; Solhi et al. 2005; Turan and Esringu 2007). Since the root system is the part of the plant that first comes into contact with heavy metals, its growth characteristics and anatomical changes should not be neglected, but rather deeply studied and evaluated. As reviewed by Lux et al. (2011), higher Cd concentrations inhibit root growth, reduce root hair production, accelerate root maturation, and cause the formation of asymmetrical epidermal and cortical cells, as well as intercellular air spaces.

The objective of this work was to investigate the effectiveness of *B. napus* relative to phytoextraction of Cd and Zn, possible intraspecific differences between *B. napus* cultivars, and the possible role of Zn in protection from Cd toxicity, and to evaluate anatomical changes correlated with metal uptake and bioaccumulation, all of which are important for our understanding of the overall phytoextraction process. Each cultivar has been well described for specific characteristics, e.g., its higher adaptability to drought in warmer conditions, very high tolerance to lodging before harvest, and very high cold and frost tolerance for Cadeli, Viking, and Navajo, respectively (for more information, see https://grinczech.vurv.cz/gringlobal/search.aspx). Cultivars with various growth characteristics were chosen to determine if any of these qualities were connected with metal accumulation and phytoextraction potential. The experiment was conducted under hydroponic conditions to eliminate mass transfer limitations and to gain access to root material, which allowed investigation of the genetic potential for metal root uptake and root to shoot translocation.

Material and methods

Plant cultivation

Seeds of three cultivars (Cadeli, Viking, and Navajo) of oilseed rape (*B. napus* L.) were obtained from the Crop Research Institute of the Czech Republic. Seeds were germinated in a growth chamber (Percival Scientific) and, after 6 days, seedlings were transferred into a hydroponic medium and cultivated under long-day conditions (16 h light/8 h dark) with a temperature of 21 °C. Plants were grown for 14 days in a modified Hoagland medium [0.1 mM $(NH_4)_2HPO_4$, 0.28 mM $Ca(NO_3)_2$, 0.6 mM KNO_3, 0.2 mM $MgSO_4$, 5 mM of a complex of Fe(III) and *N*,*N*′-di-(2-hydroxybenzyl)-ethylenediamine-*N*,*N*′-diacetate (HBED), 4.63 mM H_3BO_3, 0.032 mM $CuSO_4$, 0.915 mM $MnCl_2$, 0.011 mM MoO_3, and 0.077 mM $ZnSO_4$]. The nutrient solution was changed every 3 days. Cadmium ions (Cd^{2+}) as $CdCl_2$ and Zn ions (Zn^{2+}) as $ZnSO_4$ were added into the Hoagland solution making the desired concentration of 5 μM Cd^{2+} and/or 10 μM Zn^{2+}. These chosen concentrations were based on previous studies in which different concentrations of Cd (1, 5, 10 μM of $CdCl_2$) and Zn (1, 10, 20 μM of $ZnSO_4$) were applied to plants. The pH for all treatment solutions was adjusted to 5.7. In all following experiments, variants were marked as controls (without metal addition), 5 μM Cd, 10 μM Zn, or 5 μM Cd + 10 μM Zn. After 14 days, seedlings were harvested and fresh roots and leaves were weighed and dried.

Elemental analysis

Roots and shoots were harvested separately for elemental analysis. Before drying at 100 °C to constant weight, roots were desorbed for 5 min each in ice-cold Millipore water to remove traces of nutrient medium. Dry plant material (0.1 g) was digested in 3 mL of HNO_3 in a microwave oven (Start 1500, MLS GmbH) using a temperature step gradient (maximum of 210 °C). Digests were, if necessary, diluted with 2% HNO_3. Metal concentrations were measured by ICP-OES on an iCAP 6500 Series spectrometer (Thermo Fisher).

Chlorophyll analysis

Chlorophyll determination was carried out according to Wellburn (1994). Samples were prepared by extraction of

homogenized fresh leaves in 80% acetone (*w*/*v* 1 g FW 20 mL^{-1}) at laboratory temperatures and analysed at wavelengths of 663 and 646 nm (chlorophyll *a* and *b*) on an Agilent Cary 60 spectrophotometer.

Anatomical analysis

Primary roots of treated plants were prepared for embedding according to Roschzttardtz et al. (2009). Roots were vacuum infiltrated with fixation solution, which consisted of 2% (*v*/*w*) paraformaldehyde, 1% (*v*/v) glutaraldehyde, 1% (*w*/*v*) caffeine, and 0.01% TritonX-100 in 0.1 M phosphate buffer (pH 7.0) for 30 min with subsequent incubation overnight at 4 °C. The fixed roots were washed with 0.1 M phosphate buffer (pH 7.4) three times and dehydrated with 50, 70, 90, 95, and 100% ethanol, ethanol/butanol 1:1, and 100% butanol. For embedding in Technovit 7100 resin (Kulzer), we followed the manufacturer's instructions.

For root anatomy analyses, five root tips from each cultivar per treatment were embedded and thin sections (7 μm) were made using a Leica RM2265 microtome. The sections were cut at two locations: (1) 1 cm from the root tip and (2) in the middle part of the root. Sections were put on glass slides and viewed under an Olympus PROVIS AX70 microscope (Olympus Optical Co., Ltd., Japan). Root diameters were calculated using NIS-Elements software (Nikon). Lignin autofluorescence was visualized following UV excitation at a wavelength of 330–385 nm.

Data analysis

All analyses were performed in three replications using two-way ANOVA and Tukey's test (Statistica 10.0, StatSoft Inc., Tulsa, OK, USA) to evaluate the significance ($P < 0.05$) of differences among treatments.

Results and discussion

Dose response of root growth

Treatment concentrations were derived from dose-response studies (Fig. 1) in which several lower and higher concentrations of Cd were applied (i.e., 1, 5, 10 μM of $CdCl_2$) to find suitable metal concentrations that would reduce root growth by approximately 50%. Cadmium at 1 μM caused subtle damage on root growth of Viking, while in Cadeli and Navajo there was a significant reduction in root growth. Concentrations higher than 10 μM were too toxic, particularly for Navajo, showing up as a reduction in plant biomass, root and shoot elongation, leaf chlorosis, and, in the end, plant death (data not shown). After 20 μM treatment, all plants died. The required reduction in root growth for all tested cultivars was seen at 5 μM Cd^{2+}; therefore, we used this concentration for the subsequent experiments. We also tested various Zn concentrations (1, 10, and 20 μM of $ZnSO_4$) to find the dose that would have a positive, or at least not negative, effect on plant growth. The 10 μM Zn was finally chosen. The Cd concentration we used in our experiment was quite low compared to some other hydroponic studies with *B. napus*, where used Cd concentrations were higher than 20 μM (Nouairi et al. 2006; Ali et al. 2013); however, these concentrations resulted in plant death in our study. These differences are most likely because the diluted Hoagland solution used in our study contained lower concentrations of competing ions.

Effects of Cd and Zn on shoot and root growth

Regarding the appearance of visual symptoms, the growth of plants exposed to Cd-only and Cd + Zn treatments was negatively affected by these heavy metals, as shown in Fig. 2d–f, j–l. On the other hand, plants grown in a medium with 10 μM Zn showed no symptoms of toxicity (Fig. 2g–i). A correlation between Cd toxicity and leaf chlorosis was observed, whereas no chlorosis was visible in plants exposed only to excess Zn. Heavy metal-induced leaf chlorosis was previously observed in *Brassicaceae*. This phenomenon is very often connected to inhibition of photochemical activity in chloroplasts (Simonova et al. 2007) together with decreased chlorophyll content (Yan et al. 2016).

Both root lengths and fresh root weights decreased in Cd-only- and Cd + Zn-treated plants compared to controls and Zn-only treatment (Fig. 3a, b). In the presence of Cd and Cd + Zn, the roots of Viking and Navajo were thicker in the middle parts (Table 1). In Cadeli, no significant differences were observed in root diameter (Table 1). Similar results showing thicker roots were also observed in Cd-only-treated roots of *Arabidopsis thaliana* (Suzuki 2005) and *Pisum sativum* (Rodríguez-Serrano et al. 2006). Root growth in plants subjected to Cd + Zn was negatively affected to the same degree as Cd-only-treated plants. As seen in our present study, Zn at lower concentrations did not act negatively on plant growth, based on root length, which did not differ from the control variant (Fig. 3a). However, it must be emphasized that Zn toxicity depends on the concentration used and excess Zn can lead to root growth inhibition (Stoyanova and Doncheva 2002; Sridhar et al. 2007; Li et al. 2012). The negative effects of Cd on root growth have been intensely studied and are well documented in many plant species (for review, see Lux et al. 2011; Tran and Popova 2013).

The effects of Cd- and Zn-only treatments on shoot biomass and chlorophyll attributes are presented in Fig. 3c–f. Reduction of shoot fresh weight under Cd-only or Cd + Zn treatment was between 58 and 64% and between 51 and 60%, respectively, while shoot weight in Zn-only-treated plants was reduced by 15–29% (Fig. 3c). Furthermore, a considerable

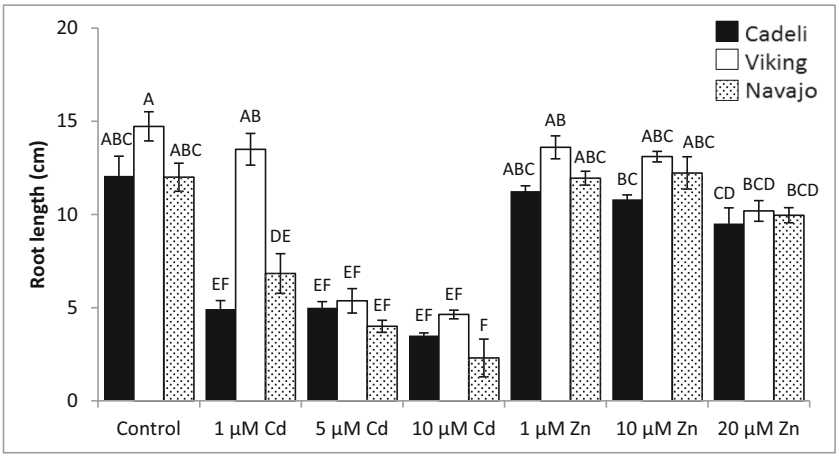

Fig. 1 Root length (cm) in three different cultivars of *B. napus* plants after 14 days of growth in the presence of different concentrations of Cd and Zn. Data are mean ± SD ($n = 3$). Values followed by the *same letter(s)* are not significantly different according to Tukey's test ($P < 0.05$)

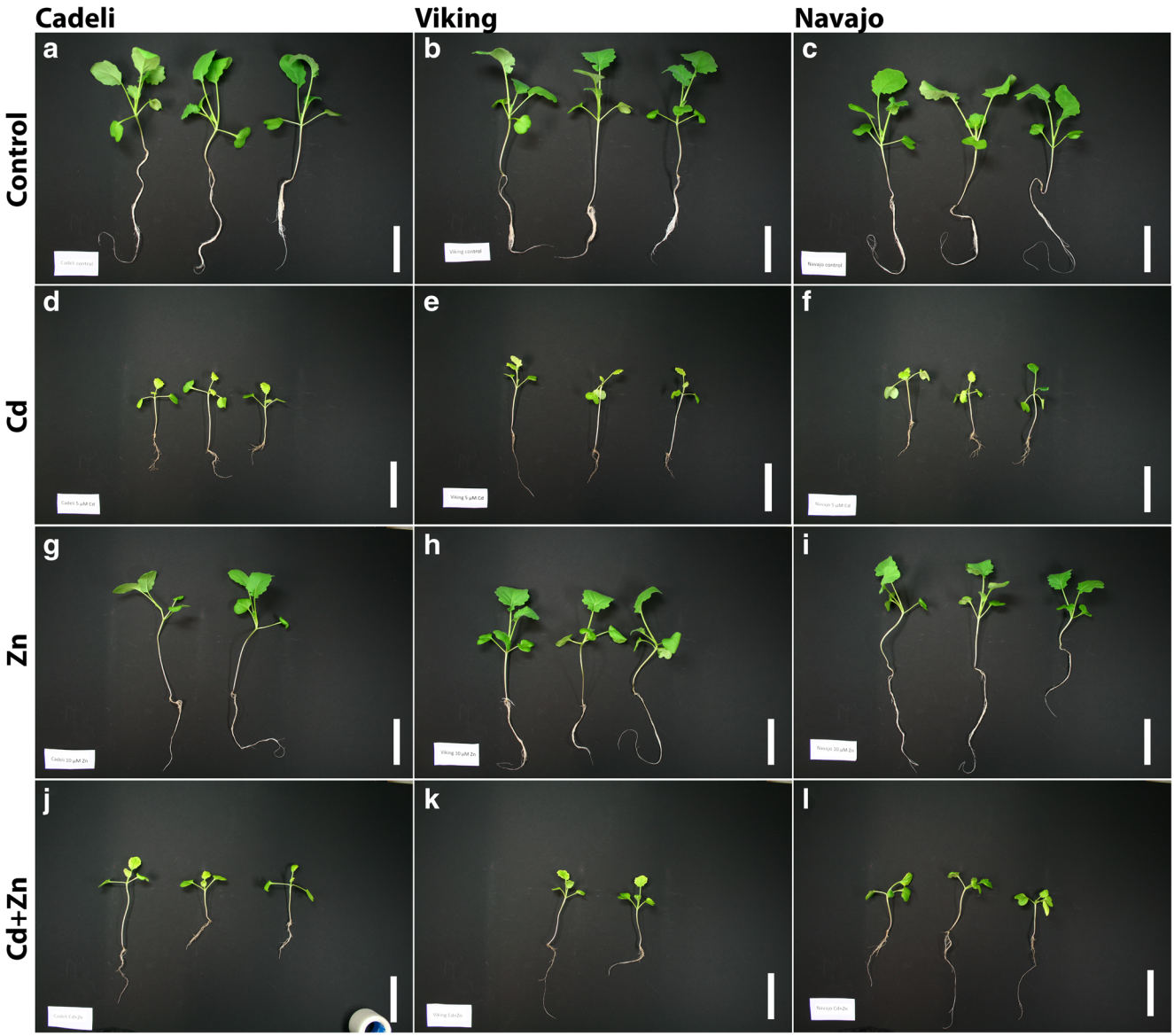

Fig. 2 *B. napus* plants cv. Cadeli, Viking, and Navajo hydroponically grown for 14 days with or without Cd, Zn, or Cd + Zn. **a**–**c** Controls. **d**–**f** Medium with 5 µM Cd. **g**–**i** Medium with 10 µM Zn. **j**–**l** Medium with 5 µM Cd and 10 µM Zn. The *scale bar* (*white*) represents 5 cm

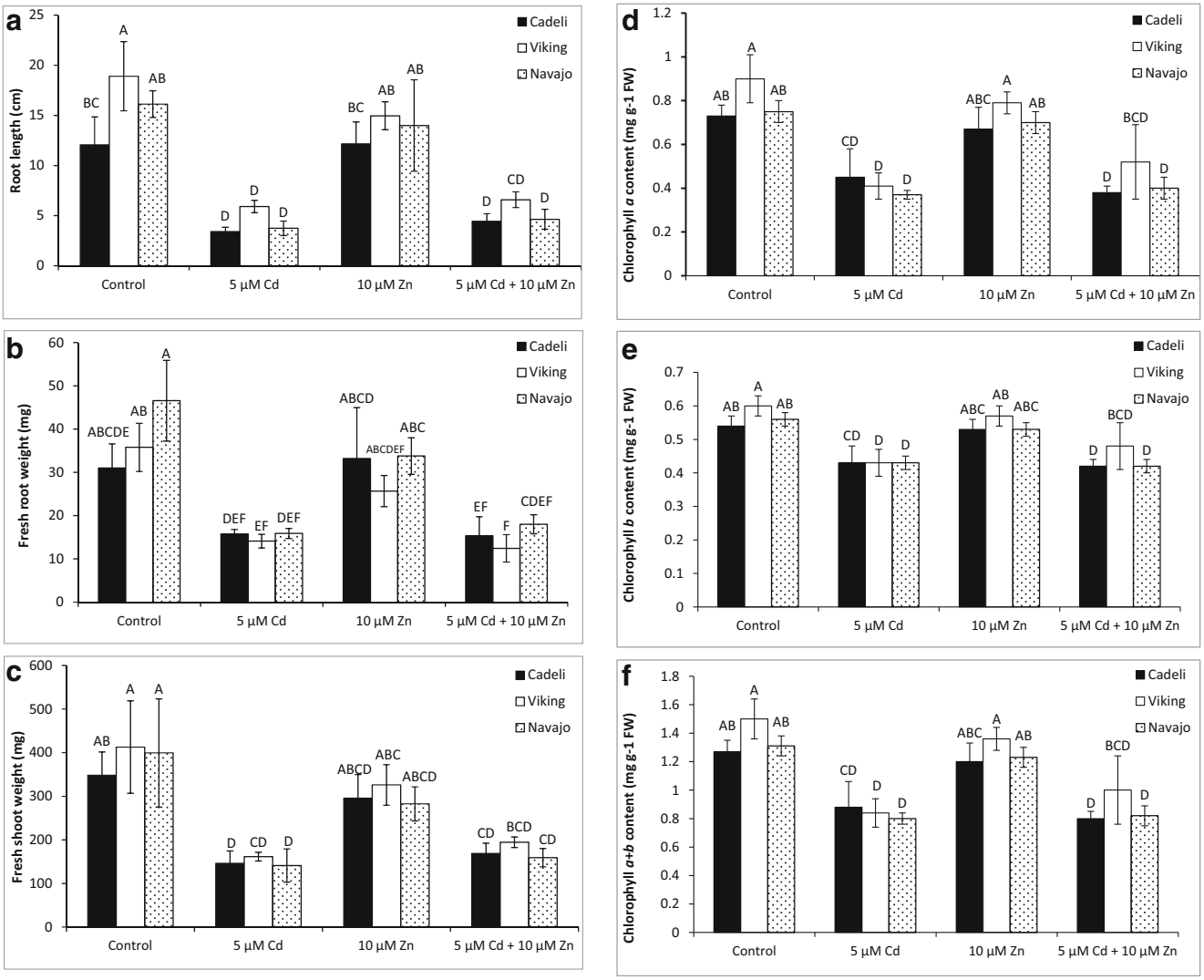

Fig. 3 Changes in root length, root fresh weight, shoot fresh weight ($n = 9$) and chlorophyll content ($n = 3$) in *B. napus* cv. Cadeli, Viking, and Navajo hydroponically grown for 14 days with or without Cd, Zn, or Cd + Zn. Data are mean ± SD. Results of statistic are as in Fig. 1

decrease in chlorophyll content (compared to controls or Zn-only-treated plants) was found in the presence of Cd and Cd + Zn (Fig. 3d–f), which has also been seen in *Brassicaceae* (Mohamed et al. 2012; Ali et al. 2013; Zong et al. 2017). According to the two-way ANOVA, no significant differences were observed between all tested cultivars relative to root length, root weight, shoot weight, chlorophyll content, and concentrations of Cd, Zn, and Cd + Zn.

Effects of Cd and Zn on root anatomy

Cadmium root uptake is allowed via both symplastic (intracellular transport via plasmodesmata) and apoplastic (transport through extracellular solute and gas spaces) pathways (Shahid et al. 2016; Song et al. 2017), while plant root behaviour is affected in numerous ways including changes in root anatomy (Lux et al. 2011), lignin biosynthesis (Yang et al. 2007), plant oxidative status (Romero-Puertas et al. 2002), synthesis of ligands involved in Cd detoxification (Nouairi et al. 2008), and cell wall impregnation, especially by suberin (Vatehova et al. 2012).

In this study, Cd-only and Cd + Zn treatments clearly affected the anatomical and morphological traits of roots. Upon exposure to Cd and Cd + Zn, primary root tips were thicker (Table 1) in all cultivars compared to controls and Zn-only treatment, with the biggest root diameter in Viking treated with Cd alone. In Cadeli, Zn-only treatment caused an enlarged root diameter compared to controls, which is shown in Fig. 4a, c. However, this increase was not statistically significant for Cadeli (Table 1). Pericycle cells in root tips after Cd-only and Cd + Zn treatments were enlarged and showed irregular divisions (Fig. 4b, d), in comparison to control tissue (Fig. 4a). The pericycle layer is responsible for lateral root initiation starting with rapid transverse and subsequent periclinal cell divisions (Laskowski et al. 1995). In several previous studies, it was found that Cd was located in the

Table 1 Root diameters of *B. napus* cv. Cadeli, Viking, and Navajo hydroponically grown for 14 days with or without added Cd, Zn, and Cd + Zn

Root diameter (mm) (n = 5)	Root tip	Middle part
Cadeli		
Control	0.21^{D}	0.28^{BC}
5 μM Cd	0.47^{B}	0.29^{ABC}
10 μM Zn	0.30^{CD}	0.28^{ABC}
5 μM Cd + 10 μM Zn	0.38^{BC}	0.30^{ABC}
Viking		
Control	0.26^{CD}	0.25^{CD}
5 μM Cd	0.69^{A}	0.33^{AB}
10 μM Zn	0.23^{D}	0.25^{CD}
5 μM Cd + 10 μM Zn	0.33^{BCD}	0.29^{BC}
Navajo		
Control	0.22^{D}	0.22^{D}
5 μM Cd	0.46^{B}	0.31^{A}
10 μM Zn	0.24^{D}	0.26^{CD}
5 μM Cd + 10 μM Zn	0.34^{BCD}	0.31^{ABC}

For measurements, sections approx. 0.1 cm from root apex (root tips) and from the middle parts of roots were used. Data are mean ± SD (n = 5). Superscripts with different letter(s) are significantly different (p<0.05) within the same column

middle lamella of the cell walls in the central stele (Van Belleghem et al. 2007), in the endodermis and pericycle (Yamaguchi et al. 2011), or inside the pericycle only (Wojcik and Tukiendorf 2004). It is possible that the presence of Cd inside the endodermis or pericycle affects the development and division of these root tissues (Table 2).

In addition to root tips, the middle parts of the roots were also investigated. After Cd-only, Zn-only, and Cd + Zn treatments, more autofluorescence in the endodermis was detectable under UV light compared to controls in Navajo (Fig. 5a–d). This indicated that metal treatment might enhance deposition of autofluorescing material, such as lignin or suberin. Interestingly, although Zn has a role as a micronutrient, in our study its effect on lignification/suberization was the same as for Cd-only and Cd + Zn treatments (Fig. 5c).

In the cortical intercellular spaces, deposition of extracellular material can be observed and connected to higher levels of toxicity. These blockages indicate root damage (Cd interferes with membranes) but may have protective roles against penetration of cortical apoplast by Cd. Such occlusions were observed in *Oryza* or *Phragmites* under sulphide or organic acid toxicity (Armstrong and Armstrong 2001, 2005) where they may inhibit water and mineral uptake.

Uptake and translocation of Cd and Zn

The metal contents and translocation factors (TFs) in roots and shoots of *B. napus* cv. Cadeli, Viking, and Navajo under different Cd- and Zn-only treatments are given in Fig. 6. The translocation factor was defined as the shoot to root metal concentration ratio, which indicates a plant's ability to translocate metals from roots to shoots. It must be emphasized that

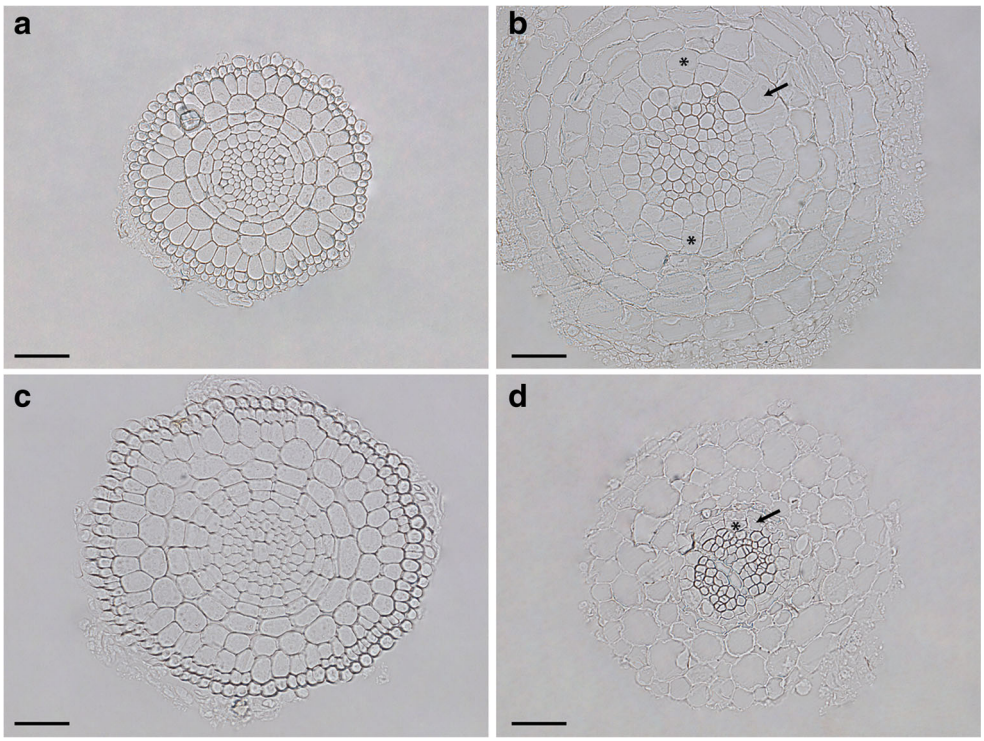

Fig. 4 Effect of Cd and Zn on primary root tip anatomy in *B. napus* cv. Cadeli. In Cd-only and Cd + Zn treatments, enlarged pericycle cells (*arrow*) and irregular cell division (*star*) were observed. **a** Control treatment. **b** Medium with 5 μM Cd. **c** Medium with 10 μM Zn. **d** Medium with 5 μM Cd + 10 μM Zn. Sections were made approx. 0.1 cm from root apex. The *scale bar* (*black*) represents 100 μm

Table 2 Concentration of K, Mg, Ca, and Na (mg g^{-1} DW) in roots and shoots of *B. napus* cv. Cadeli, Viking, and Navajo hydroponically grown for 14 days with or without added Cd, Zn, and Cd + Zn

	Root mineral concentration				Shoot mineral concentration			
	K	Mg	Ca	Na	K	Mg	Ca	Na
Cadeli								
Control	20.97 ± 2.70^{A}	1.51 ± 0.51^{A}	4.56 ± 0.61^{A}	0.95 ± 0.25^{A}	59.42 ± 1.20^{D}	6.80 ± 0.12^{CD}	32.59 ± 1.30^{CD}	0.31 ± 0.02^{D}
5 μM Cd	25.72 ± 3.92^{A}	1.82 ± 0.26^{A}	6.94 ± 0.78^{A}	4.03 ± 1.67^{A}	45.03 ± 1.64^{F}	9.10 ± 0.08^{A}	34.30 ± 1.32^{ABC}	0.94 ± 0.07^{B}
10 μM Zn	19.52 ± 3.96^{A}	1.65 ± 0.24^{A}	5.78 ± 0.96^{A}	1.37 ± 0.69^{A}	69.56 ± 1.94^{B}	6.43 ± 0.07^{D}	23.74 ± 1.38^{EF}	0.65 ± 0.08^{C}
5 μM Cd + 10 μM Zn	19.78 ± 5.17^{A}	1.82 ± 0.22^{A}	5.70 ± 1.18^{A}	1.41 ± 0.70^{A}	48.07 ± 1.72^{EF}	7.80 ± 0.26^{B}	29.98 ± 1.27^{D}	0.27 ± 0.02^{D}
Viking								
Control	20.00 ± 3.74^{A}	1.44 ± 0.01^{A}	4.42 ± 0.39^{A}	1.27 ± 0.48^{A}	66.76 ± 3.29^{BC}	7.01 ± 0.05^{C}	32.63 ± 0.60^{CD}	0.29 ± 0.01^{D}
5 μM Cd	24.02 ± 4.81^{A}	1.57 ± 0.23^{A}	5.82 ± 0.57^{A}	3.80 ± 3.82^{A}	48.08 ± 3.67^{EF}	8.10 ± 0.09^{B}	32.78 ± 1.05^{BCD}	0.69 ± 0.08^{C}
10 μM Zn	21.99 ± 6.43^{A}	1.80 ± 0.22^{A}	5.63 ± 0.50^{A}	3.93 ± 4.77^{A}	78.86 ± 2.07^{A}	6.53 ± 0.24^{CD}	24.81 ± 1.51^{E}	1.55 ± 0.08^{A}
5 μM Cd + 10 μM Zn	25.20 ± 7.73^{A}	1.79 ± 0.10^{A}	5.89 ± 0.66^{A}	4.44 ± 4.73^{A}	59.01 ± 1.41^{D}	8.02 ± 0.30^{B}	33.01 ± 2.16^{BCD}	0.29 ± 0.03^{D}
Navajo								
Control	19.08 ± 4.31^{A}	1.21 ± 0.17^{A}	4.74 ± 0.24^{A}	1.30 ± 0.58^{A}	63.10 ± 2.23^{CD}	6.97 ± 0.18^{CD}	37.47 ± 0.45^{A}	0.27 ± 0.02^{D}
5 μM Cd	27.95 ± 9.41^{A}	2.08 ± 0.98^{A}	7.26 ± 2.01^{A}	5.42 ± 3.96^{A}	47.32 ± 0.43^{EF}	9.32 ± 0.27^{A}	35.24 ± 1.07^{ABC}	0.69 ± 0.05^{C}
10 μM Zn	25.83 ± 5.07^{A}	1.79 ± 0.54^{A}	5.35 ± 1.22^{A}	3.23 ± 4.04^{A}	75.93 ± 1.22^{A}	5.80 ± 0.37^{E}	20.49 ± 0.53^{F}	0.68 ± 0.01^{C}
5 μM Cd + 10 μM Zn	24.38 ± 5.44^{A}	1.73 ± 0.26^{A}	6.18 ± 1.28^{A}	4.37 ± 5.00^{A}	51.69 ± 0.81^{E}	9.37 ± 0.21^{A}	36.50 ± 1.49^{AB}	0.22 ± 0.03^{D}

Data are mean ± SD ($n = 3$). Superscripts with different letter(s) are significantly different ($p<0.05$) within the same column

the overall process of phytoextraction depends mostly on soil metal phytoavailability as well as soil type, soil pH, soil redox potential, and soil microbial activity (Vig et al. 2003; Alkorta et al. 2006). Still, TFs derived from hydroponic experiments

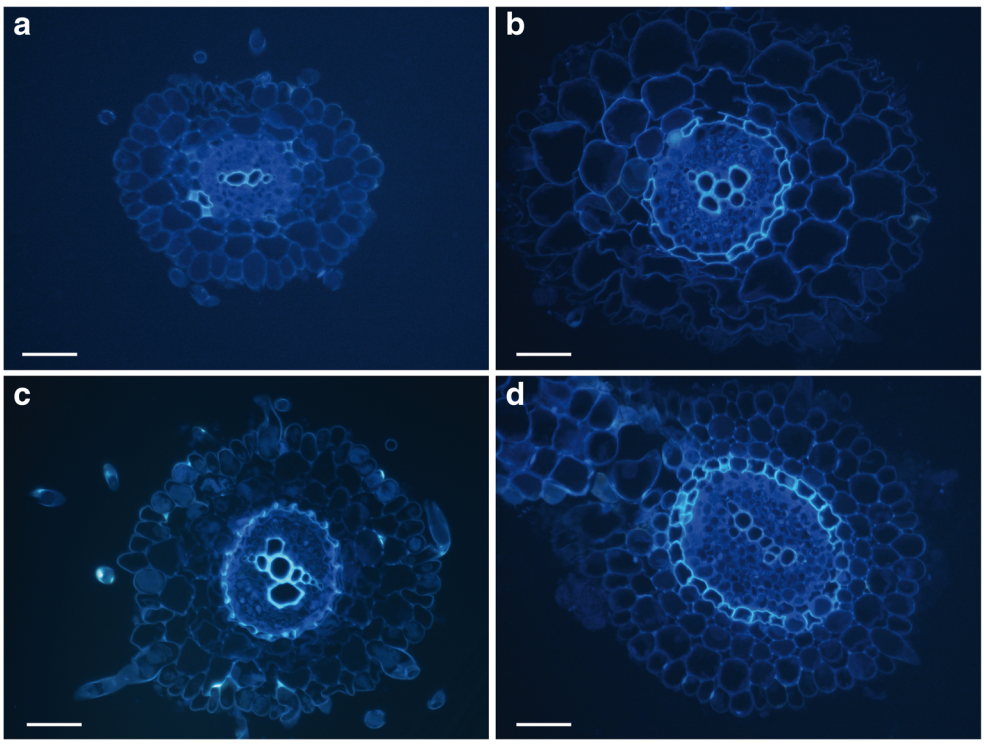

Fig. 5 Effect of Cd and Zn on the accumulation of autofluorescence material (suberin/lignin) in root tissues in *B. napus* cv. Navajo (**a**–**d**); autofluorescence under UV light, middle part of primary root. **a** Control. **b** Medium with 5 μM Cd. **c** Medium with 10 μM Zn. **d** Medium with 5 μM Cd + 10 μM Zn. Sections were made from middle parts of roots. The *scale bar* (*white*) represents 100 μm

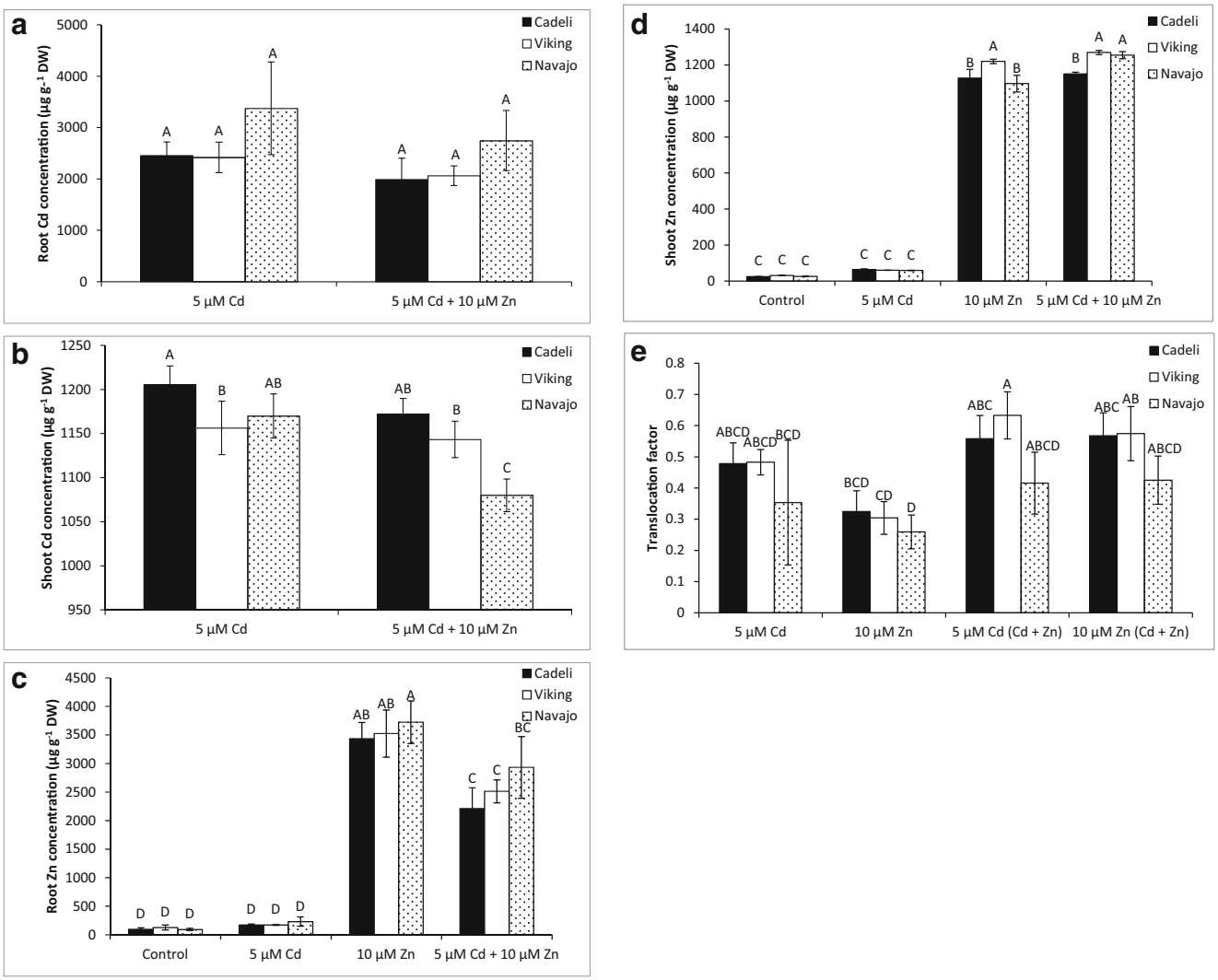

Fig. 6 Concentration of Cd (**a**, **b**) and Zn (**c**, **d**) in roots and shoots and their translocation factor (**e**) [TF = C_{aerial} / C_{root}] in *B. napus* cv. Cadeli, Viking, and Navajo hydroponically grown for 14 days with or without Cd, Zn, and Cd + Zn. Data are mean ± SD (n = 9). Results of statistics are as in Fig. 1

can be indicative of a genetic potential for tested plant genotypes to carry out phytoextraction, as well as their capacity to tolerate higher concentrations of heavy metals.

As expected, the concentration of heavy metals measured in plants grown in medium enriched with Cd, Zn, or both was higher than in the controls. Accumulation of Cd and Zn was higher in roots than in shoots of all genotypes (Fig. 6a–d). Higher Cd shoot translocation was found in Cadeli compared to Viking, whereas, in combined treatment with Zn, greater Cd shoot translocation was observed in Cadeli compared to Navajo (Fig. 6b). Slightly reduced Zn shoot translocation was found in Navajo, which had a TF of 0.26 for Zn (Fig. 6e).

Brassica species are well known as metal accumulators. *Brassica juncea* has been widely studied for its phytoremediation potential with promising results (Qadir et al. 2004; Mohamed et al. 2012). Due to several characteristics such as fast growth, high biomass production, and comparatively high tolerance to heavy metals (Hernández-Allica et al. 2008), *B. napus* represents an excellent candidate plant species for phytoremediation. Some studies have found that, in contaminated soils, the phytoextraction potential of oilseed rape is very limited (Marchiol et al. 2004; Turan and Esringu 2007; Yu et al. 2012). This fact was also evident in our hydroponic study, where the TF values remained below 1 for all tested genotypes (Fig. 6e). However, the findings in the literature are inconsistent and there are similar soil experiments that support the phytoextraction potential of oilseed rape (Szulc et al. 2014; Cojocaru et al. 2016).

As for Cd accumulation, its concentration in root reached similar values in Cd-only and Cd + Zn variants (Fig. 6a). At the shoot level, Cd concentrations remained almost unaltered in both variants, irrespective of Zn (Fig. 6b). Only in Navajo, the cultivar with high frost and cold tolerance, there was a significant reduction in shoot Cd translocation from the medium with Cd + Zn (Fig. 6b). We used Zn in our experiments because it may play a protective role against Cd toxicity, which might be due to Zn's role in antioxidant activity (Agar and Taspinar 2003; Monteiro

et al. 2007; Taspinar et al. 2011). However, our findings did not find any evidence that Zn can reduce Cd toxicity. Moreover, TF values in Cadeli and Viking were significantly higher for Cd translocation in the medium with Cd + Zn (Fig. 6e).

Regarding the root Zn concentration, it was significantly lower in the medium with Cd + Zn compared to the medium with Zn alone (Fig. 6c). For shoots, however, the concentrations of Zn were not significantly different among plants cultivated in the medium with Zn alone or with Zn + Cd (Fig. 6d). These results are partly consistent with the findings of Tkalec et al. (2014), who described that Zn had a positive effect on Cd accumulation in roots, while Cd had an antagonistic effect on Zn accumulation in leaves and roots. In our study, Zn had no negative effect on Cd uptake by roots and translocation of Cd to shoots was also unaffected (Fig. 6a, b). Zn uptake by roots was similar to previous studies in that it was negatively affected by the presence of Cd. On the other hand, Zn transport into stems was unchanged. Contrary to our finding, Wu et al. (2003) observed that Cd decreased Zn concentrations not only in roots but in all plant tissues, and that Zn translocation was significantly inhibited in Cd-treated plants.

Studies describing the interaction between Cd and Zn are inconsistent relative to their combined effects, but the results strongly depend on the metal concentrations used. Cherif et al. (2011) suggested that higher Zn concentrations in combination with Cd increased oxidative stress, while adequate Zn concentrations modulated the effects of Cd toxicity. This was shown in experiments by Sharma et al. (1999), where Zn concentrations of 25 and 50 μM had an antagonistic effect on Cd uptake and vice versa (Sharma et al. 1999). In general, most studies have shown a modulating effect of Zn on oxidative stress in Cd-treated plants (Aravind and Prasad 2003; Balen et al. 2011).

Interaction between Cd and Zn in the growth medium can lead to competition for uptake, transport, and accumulation of these metals by plants, which is due to their chemical similarity (Welch and Norvell 1999). The observed decrease in Zn content in roots of plants grown in the medium with Cd + Zn could have been caused by the Zn-binding domains of certain enzymes or Zn transporters being saturated with Cd, which is a widespread phenomenon in environments where both metals occur (Tang et al. 2014). Moreover, it has been demonstrated that Cd ions can be taken up, sequestrated, and effluxed by the same transporters that are involved in Zn uptake and transport, such as HMA2, 3, 4 (AtHMA2, 3, 4) or NRAMP4 (TcNRAMP4) (Eren and Argüello 2004; Courbot et al. 2007; Oomen et al. 2009; Morel et al. 2009).

Effects of Cd and Zn on uptake and translocation of K, Mg, Ca, and Na

Table 1 shows the concentrations of selected mineral nutrients in roots and shoots after Cd-only and Zn-only treatments. All oilseed rape cultivars showed a similar response to Cd and Zn toxicity in terms of nutrient uptake.

Potassium concentrations in roots were not changed by any of the treatments. On the other hand, some previous studies have shown that K content was decreased in roots after heavy metal treatment (Abu-Muriefah 2008; Siddiqui et al. 2012). In our present study, quite sharp increases in K content were found in the shoots of plants grown in the medium with Zn alone. It has been reported that K addition can have beneficial effects on the uptake of Zn^{2+} ions (Barker and Pilbeam 2015), which might also work the other way around. High K and lower Ca contents in shoots of Zn-treated plants also confirm previously reported antagonistic interactions between K and Ca uptake in plants (Fageria 2008; Barker and Pilbeam 2015).

Higher Mg content was noted in shoots compared to roots. No differences were observed in roots of any of the variants. In shoots, Cd-only and Cd + Zn treatments resulted in a slight increase in Mg content compared to controls or Zn-only treated plants. It is known that Mg can be substituted in chlorophyll by heavy metals (Küpper et al. 1998). Similar results describing an increase in Mg content in shoots after Cd exposure were observed in *B. juncea* (Jiang et al. 2004), *Triticum aestivum*, and *Zea mays* (Nikolic et al. 2014).

Considerably higher concentrations of Ca were found in shoots than in roots; however, no significant changes were observed after the addition of Cd and Cd + Zn. Interestingly, Ca content in shoots was significantly reduced in plants treated with Zn only. On the other hand, no decrease (Viking), or a statistically insignificant slight decrease (Cadeli, Navajo), in Ca content in shoots was observed in Cd + Zn-treated plants. Similar results have been reported by Davis-Carter et al. (1991), who found that Ca content in leaves of peanut plants (*Arachis hypogea*) was reduced by soil application of Zn. However, in leaves of *P. sativum*, Ca content was slightly increased (Stoyanova and Doncheva 2002). It is known that some of the Ca channels and transporters are permeable to many divalent cations such as Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, and Zn^{2+} (White and Broadley 2003). Thus, a Ca decrease in shoots of plants grown in a medium with Zn might occur due to the occupation of Ca^{2+} channels by the highly available Zn^{2+} ions. This effect was not visible in the medium with Cd + Zn, which could have been caused by competition between Cd^{2+} and Zn^{2+} ions, thus leading to a lower occupation of calcium channels.

Sodium concentration in roots was not affected by any treatment. In shoots, a significantly higher concentration of Na was found either after Cd- or Zn-only treatment, but a different behaviour was observed in single cultivars. In Cadeli, a higher Na concentration was found after Cd-only treatment, whereas in Viking, an increase in Na concentration was observed after Zn-only treatment. Similar values of increased Na concentration were obtained after Cd- or Zn-only treatment in Navajo. On the contrary, no effect of Cd-only treatment on Na content was

observed in *P. sativum* (Rodríguez-Serrano et al. 2006). Sodium is a non-essential nutrient, but can be beneficial during K deficiency (Maathuis 2013). Thus, when K content in shoots of plants grown in a Cd medium is reduced, a higher content of Na could play an important metabolic role in the absence of K.

Conclusion

Overall, cadmium treatment had negative effects on plant growth characteristics including root length and root and shoot weight. No protective effects of extra Zn on the growth of Cd-treated plants was observed for any *Brassica* cultivar. Generally, only minor differences between genotypes were observed. After Cd-only-, and to a smaller degree after Cd + Zn-treatment, root anatomy including lignin/suberin accumulation, and enlargement of pericycle cells, was observed. Regarding Cd and Zn translocation, none of the three tested cultivars reached the threshold for having any phytoextraction potential, as indicated by the TFs. However, the TFs from our hydroponic experiments cannot be directly compared to results from soil experiments. Root heavy metal concentrations were consistently higher than shoot heavy metal concentrations. Cadmium accumulation in shoots was affected by Zn-only treatment only in Navajo, where shoot Cd accumulation was significantly reduced in the medium with Cd + Zn. Mineral concentrations did not vary among different cultivars and heavy metal treatment did not influence the mineral composition found in roots. In shoots, K content increased in Zn-only-treated plants, whereas Mg content was higher in the shoots of Cd-only-treated plants. The content of Ca in shoots of Zn-only-treated plants was significantly reduced, but in combined treatment it remained unaltered. Regarding Na, either Cd- or Zn-only treatment induced significantly higher concentrations of Na in shoots compared to controls or Cd + Zn treatment, and the response to these treatments was cultivar dependent.

Acknowledgements This work was financially supported by the Particular Research Program SV 2103/2015, University of Hradec Kralove, and by the Czech Ministry of Education, Youth and Sports, Project LO1417.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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