Chemosphere 351 (2024) 141235

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Early-stage growth and elemental composition patterns of *Brassica napus* L. in response to Cd–Zn contamination

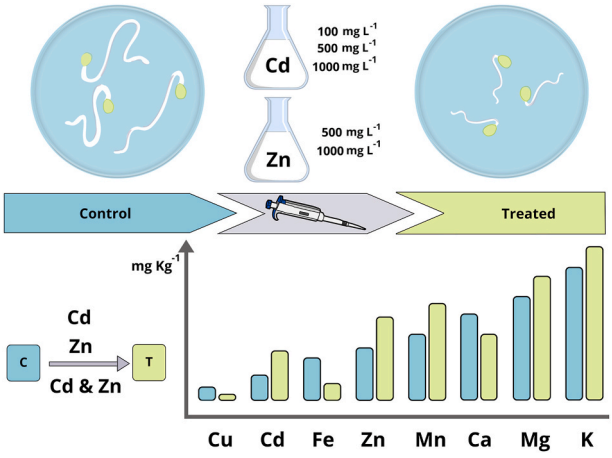
Dávid Tőzsér [a,b], Deborah Osariemen Idehen [a], Jennifer Damilola Osazuwa [a], John Elias Sule [a], Ágota Zsófia Ragyák [c], Zsófi Sajtos [c,*], Tibor Magura [a,d]

[a] Department of Ecology, University of Debrecen, H-4032, Debrecen, Hungary
[b] Circular Economy Analysis Center, Hungarian University of Agriculture and Life Sciences, H-2100, Gödöllő, Hungary
[c] Department of Inorganic and Analytical Chemistry, Agilent Atomic Spectroscopy Partner Laboratory, University of Debrecen, H-4032, Debrecen, Hungary
[d] HUN-REN–UD Anthropocene Ecology Research Group, University of Debrecen, H-4032, Debrecen, Hungary

HIGHLIGHTS

- Initial growth and elemental patterns of rapeseed were studied along Cd–Zn gradients.
- Both Cd and Zn intensified the accumulation of Zn in plantlets.
- Effects of Cd and Zn were significant on Mn and minor on Cu and Fe in tissues.
- The application of Zn substantially decreased the Ca concentration in rapeseed.
- Tissue K and Mg concentrations were determined by Cd and Zn exposure times.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: T Cutright

Keywords:
Rapeseed
Data analysis
Metal interactions
Radicle
Hypocotyl
Phytoremediation

ABSTRACT

Soil contamination caused by the presence of Cd and the excess amount of Zn is a widespread concern in agricultural areas, posing significant risks to the growth and development of crops. In this paper, the early-stage development and metal (Cd and Zn) accumulation potential of rapeseed (*Brassica napus* L.) grown under different metal application schemes were assessed by determining radicle and hypocotyl length and the micro- and macro elemental composition of plantlets after 24, 72, and 120 h. The results indicated that the single and co-application of Cd and Zn significantly reduced the radicle and hypocotyl lengths. Accumulation intensity for Cd and Zn was affected by Cd and the combination of Cd and Zn in the solution, respectively. In addition, both metals significantly influenced the tissue Mn and had a minor effect on Cu and Fe concentrations. Both Cd and Zn significantly affected macro element concentrations by decreasing tissue Ca and influencing K and Mg concentrations in a dose- and exposure time-dependent manner. These findings specify the short-term and support the

* Corresponding author.
E-mail address: sajtos.zsofi@science.unideb.hu (Z. Sajtos).

https://doi.org/10.1016/j.chemosphere.2024.141235
Received 28 November 2023; Received in revised form 11 January 2024; Accepted 15 January 2024
Available online 16 January 2024
0045-6535/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

long-term use of rapeseed in remediation processes. However, interactions of metals are crucial in determining the concentration patterns in tissues, which deserves more attention in future investigations.

1. Introduction

Soil deterioration has been a cause for concern for several centuries, whereby living conditions of organisms in a direct or indirect relationship with the soil – thus, members of the food chain – face severe impairments individually and as part of a community (Sarker et al., 2022). Unfortunately, these unfavourable phenomena get attention only in a small proportion of the actual instances. Nevertheless, increased regard is paid to cases where the area is under agricultural utilization (Wang et al., 2021). In this respect, risks concern not only the scope of stakeholders from the close vicinity of the contamination but – due to the complex processing and supply system – reach quite remote geographical areas, making the adverse effects acutely diversified (Hou et al., 2020).

Among concerned agents, metals constitute a broad range of substances and receive a significant share of the total contamination cases (Hédiji et al., 2015). One of the core issues is that these elements can migrate along the food chain without considerable depletion and excretion from the organisms (Gall et al., 2015; Ali and Khan, 2019; Coimbra and Borges, 2023). This persistence and resistance are also typical for two of the most studied metals in this regard, namely Cd (cadmium) and Zn (zinc). Cd is known to be an unessential, toxic heavy metal with several significant consequences after contact. It was previously indicated that even low concentrations of Cd could trigger the appearance of chlorotic symptoms and hinder plant growth (Haider et al., 2021; Zhao et al., 2021). The metal also decreased chlorophyll content and photosynthesis activity, often complemented by reactive oxygen species (ROS)-based damage to cells and membranes (Huihui et al., 2020). Unlike Cd, Zn is an essential microelement (nutrient) for living organisms. At the same time, it is known to support plant growth and development, and it is a crucial agent in pathogen defense (Cabot et al., 2019). In excess concentrations, however, Zn can also be toxic to organisms. Toxicity symptoms in plants are similar to those of Cd; reduced growth, photosynthetic damage, and ROS-induced harm to tissues are the main issues to be addressed (Kaur and Garg, 2021).

In many cases, when the growing environment contains elements among which interactions (e.g., antagonism, synergism) could be supposed, attributing effects to the actual source becomes more complex (Rietra et al., 2017). Like numerous elements, interactions of various natures were reported for Cd and Zn between the media and the tissues of the organisms affected (Wu et al., 2020; Zhou et al., 2020). Further, due to their physicochemical properties, the two metals are also factors in the dynamics of several micro- and macro elements (Dong et al., 2019).

Along with these characteristics of the metals, it was recognized that plant contact with Cd and Zn does not necessarily conclude in a series of adverse effects; under controlled or monitored conditions, plant accumulation can be managed and used for recovery purposes with the group of approaches called phytoremediation (Sharma et al., 2022; Simon et al., 2022). Among species with proven records of accumulating Cd and Zn in high concentrations from contaminated media, many crops have already been highlighted as good prospects. With simultaneous relevance to these two criteria, rapeseed (*Brassica napus* L.) is a thoroughly studied species (Grispen et al., 2006; Dhaliwal et al., 2020). It is cultivated extensively in many countries of the world primarily for its oil used in the human diet and chemical industry and for its plant parts used as fodder for animals, having an overall global cropland size larger than that of the sunflower and a total yield exceeding 70 million tons a year (Ettl et al., 2020; Raboanatahiry et al., 2021; Romeh, 2021). This widespread and large-scale utilization justifies the necessity of a thorough understanding of the species' functions in metal-contaminated environments.

Regarding the prerequisites of effective metal accumulation, rapeseed is characterized by fast growth, intensive biomass production, and high metal tolerance (Yu et al., 2012). For these reasons, the potential of the species to clean up contaminated environments has also been assessed during its initial stages of development (Chen et al., 2018). Rapeseed is acutely sensitive to stress factors as a plantlet, which raises the need for experiments at this stage involving as many variables as possible (Haj Sghaier et al., 2022). Exposed to metal stress, high concentrations of Cd limit its root and shoot development, which impedes the overall viability of phytoremediation (Zhang et al., 2018). Cultivated in media with the simultaneous presence of Cd and Zn, most relevant studies presented synergism between the accumulations of the two metals in the species (Nan et al., 2002). It was also observed that several rapeseed varieties had very high Zn accumulation potential, even in multi-contaminated soils, without suffering condition-threatening harm (Elizareva et al., 2019). However, variation can be found in the elemental evaluation of the species and the genus *Brassica*, so further studies to provide novel aspects and findings are highly reasonable (Mourato et al., 2015).

The purpose of this paper is to assess the early growth parameters and elemental (Cd, Cu, Fe, Mn, Zn, Ca, K, and Mg) concentration patterns of rapeseed individuals germinating in solutions in single- and co-Cd and Zn treatments, intending to provide novel information on the development and remediation performance of the species in the very short-term (0–120 h). This study hypothesizes that high Cd concentrations hinder radicle and hypocotyl growth, while the adverse effects of Zn are relatively moderate. Further, it is also expected that high single- and co-Cd and Zn doses significantly influence the tissue concentrations of these metals and other interacting elements. Correlations are hypothesized between them and the studied macro elements based on the known interactions of Cd and Zn with other elements.

2. Experimental

2.1. Germination test

During the experiment, the growth of rapeseed individuals was evaluated using solutions with Cd ($3CdSO_4 \times 8H_2O$), Zn ($ZnSO_4 \times 7H_2O$), and their co-application. As for the preparations, filter papers were placed into sterile Petri dishes with a diameter of 55 mm. Filter paper in each dish was moistened using 1 ml of one of the following distilled water-based treatment scheme solutions (the number after the chemical symbol refers to its applied concentration in mg L^{-1}):

Single Cd treatment Cd10 + Zn0, Cd50 + Zn0, Cd100 + Zn0, Cd500 + Zn0, Cd1000 + Zn0; *Single Zn treatment* Cd0+Zn10, Cd0+Zn50, Cd0+Zn100, Cd0+Zn500, Cd0+Zn1000; *Co-treatment* Cd10 + Zn10, Cd10 + Zn500, Cd10 + Zn1000, Cd50 + Zn50, Cd50 + Zn100, Cd50 + Zn500, Cd50 + Zn1000, Cd100 + Zn50, Cd100 + Zn100 Cd100 + Zn500, Cd500 + Zn10, Cd500 + Zn50, Cd500 + Zn100, Cd500 + Zn500, Cd1000 + Zn10, Cd1000 + Zn50, and Cd1000 + Zn1000; *Control* distilled water (Cd0+Zn0).

The most appropriate solution quantity was determined in a preliminary experiment. Compared to the addition of 1 ml of the solution, the application of 0.5 and 0.75 ml of the fluid resulted in premature drying and deficient supply for the seeds even after 96 h, while after providing 2, 3, and 5 ml of the solution, a high amount of excess solution remained on the filter paper in each case triggering the development of molds and stunted growth.

Seven seeds were laid onto each filter paper. Petri dishes were packed in aluminium foil to exclude the effects of light and temperature

change. Seeds germinated at permanent room temperature (20.5 ± 1.4 °C). Three replications of each treatment along the concentration gradients were run for 24, 72, and 120 h, respectively. After reaching the end of the indicated exposure intervals, plantlets were thoroughly washed with double distilled water. Then, the radicle and hypocotyl length (with an accuracy of 0.1 mm) were measured on clean laboratory sheets using forcipes and plastic rulers. Tools were thoroughly rinsed among the uses in different treatments. After this, plantlets were moved into clean Petri dish lids and left in an oven (WTC Binder FD53) at 55 °C for 48 h. After cooling down, germinated plantlets were pulverized and homogenized in an agate mortar and then filled into plastic centrifuge tubes before the acid digestion.

2.2. Acid digestion and elemental analyses

During the preparation, 0.1 ± 0.01 g of each sample was measured on an analytical balance (Precisa ES 225SM-DR). Samples were then digested under atmospheric pressure and high temperature (100–120 °C) by adding a mixture of 5.0 ml of 65% (m/m) HNO_3 (reagent grade, Merck) and 1.0 ml of 30% (m/m) H_2O_2 (reagent grade, Merck). Digested samples were diluted to 10.00 ml with ultrapure water (Synergy UV Millipore), filled into centrifuge tubes, and stored at room temperature for further analysis.

The elemental composition (Cd, Cu, Fe, Mn, Zn, Ca, K, and Mg) of the samples was measured using inductively coupled plasma optical emission spectrometry (ICP-OES 5110 Vertical Dual View, Agilent Technologies; Supplementary Materials A1 and A2). An autosampler (Agilent SPS4), Meinhard® type nebulizer, and double pass spray chamber were used, while a 5-point calibration procedure was also applied (ICP VI, Merck). The standard solutions of the microelements (Cd, Cu, Fe, Mn, Zn) were prepared from the multi-element spectroscopic standard solution of 1000 mg L^{-1} (ICP IV, Merck). In contrast, the macro elements (Ca, K, Mg) were prepared from the mono-element spectroscopic standard of 1000 mg L^{-1} (Scharlau). In both cases, a 5-point calibration process was used for which standard solutions were diluted with 0.1 M HNO_3 and prepared in ultrapure water.

2.3. Statistical analyses

The mean values and standard deviations of the three replications were calculated to assess the changes in the radicle and hypocotyl length and elemental concentrations of rapeseed among the treatments and exposure intervals. Single treatments were studied and compared to the relevant results from uncontaminated (control) measurements to reveal the individual effects of Cd and Zn on growth parameters.

To highlight the effects of treatments and exposure intervals on the radicle and hypocotyl length and elemental concentrations, a standardized effect size measure, the unbiased, standardized mean difference (Hedges' *g*), was computed for the contaminated-control comparisons. This calculation was based on the following equations:

$$g = J\frac{\overline{x}_U - \overline{x}_C}{S_{within}} \quad (1)$$

$$S_{within} = \sqrt{\frac{(n_U - 1)S_U^2 + (n_C - 1)S_C^2}{n_U + n_C - 2}} \quad (2)$$

$$J = 1 - \frac{3}{4(n_U + n_C - 2) - 1} \quad (3)$$

where.

$\overline{x}_U$ and $\overline{x}_C$ mean radicle or hypocotyl length (mm), or element concentrations (mg kg^{-1}) in plants grown on uncontaminated (U) and contaminated (C) solution; n_U and n_C sample sizes for plants grown on uncontaminated (U) and contaminated (C) solution; S_U and S_C standard deviation for radicle or hypocotyl length or element concentrations of plants grown on uncontaminated (U) and contaminated (C) solution.

The negative *g* values indicated that element concentration was higher in rapeseed grown in contaminated solutions than those from uncontaminated (control) media. Further, the 95% confidence intervals of the unbiased, standardized mean difference (Hedges' *g*) were computed using the *esc* package (Lüdecke, 2019). The mean effect size was considered statistically significant if the 95% confidence interval did not include zero.

The relationship between elements' accumulation rates was assessed by linear models to study synergism and antagonism between the studied elements. All statistical analyses were performed using the R program environment (version 4.1.2; R Core Team, 2018).

3. Results

3.1. Effects of metal treatments on plant development

The growth parameters of rapeseed were assessed, and further analyses were performed to evaluate germinated seeds. The sample sizes slightly varied across studies due to germination rate values. After 24 h, the germination rate decreased with the increase of Cd dose, primarily by single but also by co-treatment, while after 72 and 120 h, the germination rate varied between 93 and 100% for all treatments (Supplementary Materials B).

In the case of the Cd-contaminated media, significant ($p < 0.05$) radicle and hypocotyl growth was noted for each treatment with the exposure time; the only minor difference occurred in the hypocotyl length of the 72- and 120-h samplings. Further, a considerable variation was observed in the growth of plantlets among different treatments; the radicle and hypocotyl of individuals contaminated by 500 and 1000 mg L^{-1} Cd were significantly shorter after 72 and 120 h than those from the less contaminated media. The growth parameter values in the 100 mg L^{-1} treatment differed from those in the less and more contaminated ones. Radicle lengths from the two least contaminated treatments significantly exceeded that of the control solution, while hypocotyl lengths were similar after 120 h (Fig. 1 and Supplementary Materials C1).

Very intensive radicle and hypocotyl growth was demonstrated with exposure time for the Zn-treated individuals in the case of each treatment, except the 1000 mg L^{-1} treatment for radicle, in which growth was hindered between the 72- and 120-h exposures. Significant differences were also observed among treatments in selected exposure intervals; radicle and hypocotyl had much higher lengths after growing in the three less contaminated media than in the two most contaminated ones. For both organs after 120 h, the most intensive growth was presented for 50 and 100 mg L^{-1} Zn treatments, with values even higher than the control for radicle and significantly higher than the control for hypocotyl (Fig. 2 and Supplementary Materials C2 and C3).

Further, unbiased, standardized mean differences (Hedges' g) based on control values were compared to confirm these findings and broaden the analysis to co-treatment effects. It was found that the single and co-application of the metals significantly decreased the radicle and hypocotyl lengths in most of the treatments. In general, Cd negatively influenced the radicle and hypocotyl elongation applied alone and combined with Zn. However, compared to control individuals, treatments with Zn alone positively affected radicle and hypocotyl length values. Assessing the exposure time-dependent variation in both development factors, a non-significant general difference could be reported between the results of the 24-h sampling and those of the 72- and 120-h samplings with longer radicle and hypocotyl with time (Supplementary Materials C4 and C5).

3.2. Concentration of microelements

The individual assessments of elements indicated significant ($p < 0.05$) differences between the accumulation of rapeseed growing in

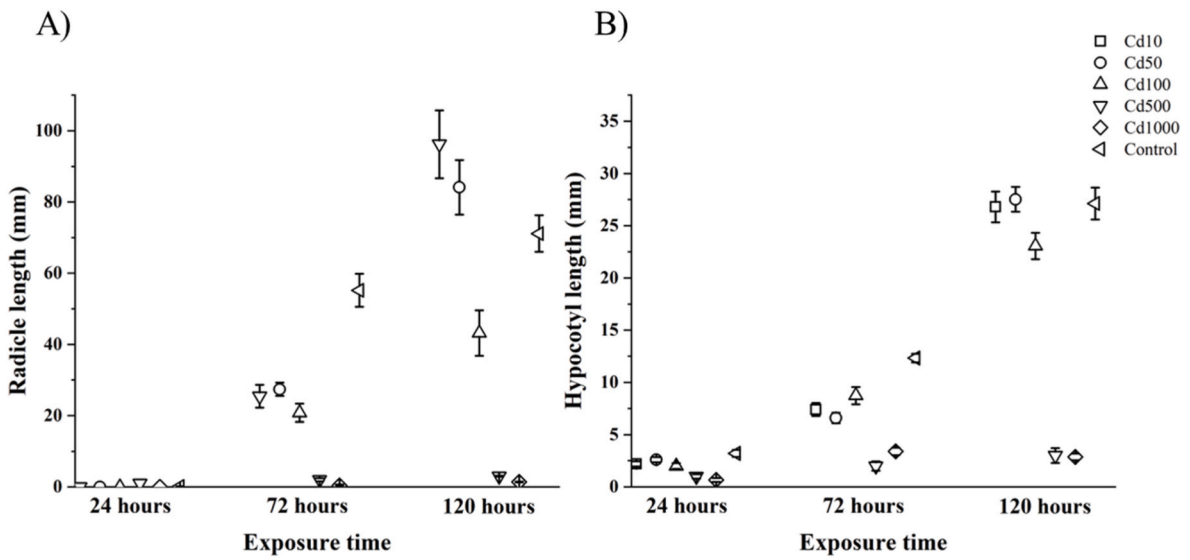


Fig. 1. Radicle (A) and hypocotyl (B) length of rapeseed plantlets in response to different single-Cd concentrations (mg L^{-1}) after 24, 72, and 120 h of growth (mean ± SD). (Control = distilled water – 0 mg L^{-1} Cd.)

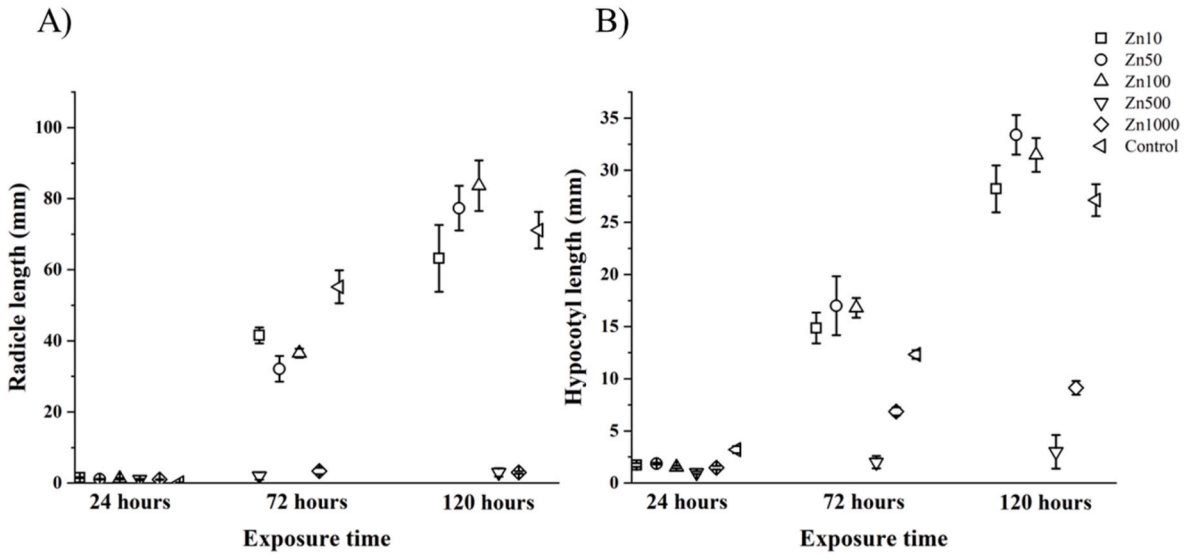


Fig. 2. Radicle (A) and hypocotyl (B) length of rapeseed plantlets in response to different single-Zn concentrations (mg L^{-1}) after 24, 72, and 120 h of growth (mean ± SD). (Control = distilled water – 0 mg L^{-1} Zn.)

contaminated vs. uncontaminated solutions. The variation among the accumulation patterns was also high in several cases.

Compared to control conditions, except for the Cd10 + Zn0 treatment after 72 h, the accumulation of Cd in rapeseed was significantly higher in each single Cd treatment with the most intensive accumulation for the 500 mg L^{-1} Cd dose after 24 h, followed by the same and the 1000 mg L^{-1} doses after 72 h. The single Zn treatments did not influence the accumulation of Cd. As for the co-contaminated treatments, Cd accumulation was very intensive in each treatment in each sampling interval. Further, the degree of tissue metal enrichment was the highest by low Cd doses coupled with low (50 mg L^{-1}) and high (1000 mg L^{-1}) Zn after 24 h and by high Cd doses coupled with low (50 mg L^{-1}) and high (500 mg L^{-1}) Zn after 72 h. Significant accumulation trends were not found within the treatments among exposure intervals (Supplementary Materials D1).

The analysis of tissue Cu concentration in response to single Cd supplies resulted in more intensive accumulation after 72 h, while less intensive after 24 and 120 h compared to the control values. The rapeseed accumulation of Cu was generally lower and, in some cases, significantly lower than in the individuals from uncontaminated solutions for both single Zn-treated and co-contaminated solutions. The highest Cu concentration drop was observed for rapeseed grown in the Cd500 + Zn50 solution, followed by the individuals from the Cd500 + Zn10 and Cd100 + Zn50 treatments, each after 72 h (Supplementary Materials D2).

In general, the concentration of Fe in rapeseed did not differ remarkably in the single contaminated treatments from that of the control tests after any of the sampling intervals. By single Cd doses, a drop in accumulation was found in the Cd10 + Zn0 treatment after 24 h, while by single Zn doses, such variation of any results among the treatments was not indicated. In the co-contaminated treatments, Fe accumulation patterns were also highly similar. Significant accumulation was found only for the Cd50 + Zn1000 treatment after 24 h, while a decrease was indicated in Cd500 + Zn10 after 24 h and Cd500 + Zn500 after 120 h (Supplementary Materials D3).

The accumulation intensity of Mn was significantly increased by high

(500 and 1000 mg L^{-1}) single Cd doses after 72 h, while no consistent patterns were found for the rest of the treatments. As for the single Zn applications, except for three cases, Mn accumulation in rapeseed was more intensive than in individuals from control solutions. However, the relations were insignificant. In the co-contaminated treatments, both increases and decreases in Mn accumulation were observed, while considerable effects were demonstrated with concentration increments only in the minority of the comparisons. In detail, a significant increase in Mn accumulation was found in the Cd10 + Zn1000, Cd50 + Zn500, Cd100 + Zn50, Cd500 + Zn50, and Cd1000 + Zn50 treatments after 24 h, and Cd1000 + Zn50 and Cd1000 + Zn1000 treatments after 72 h (Supplementary Materials D4).

In the case of Zn, its accumulation in rapeseed was only slightly affected by single Cd doses without suggesting any specific correlation. At the same time, in single Zn treatments, apart from growing in 10 mg L^{-1} for 24 and 120 h, accumulation was very high for all the treatments and sampling intervals, with the most intensive enrichment in the Cd0+Zn1000 treatment after 120 h, followed by the application of the same dose after 72 h. In the co-contaminated treatments, the accumulation was more intensive in all the cases, resulting in only six relations with non-significant concentration differences. The most intensive accumulation was observed for the Cd1000 + Zn50 treatment after 72 h, followed by the Cd50 + Zn1000 treatment after 120 h, and the Cd10 + Zn500, Cd1000 + Zn1000, and Cd500 + Zn100 treatments after 72 h, reflecting on the significant role of Cd (Supplementary Materials D5).

3.3. Concentration of macro elements

The concentration of Ca was significantly lowered by applying 500 mg L^{-1} Cd after 24 and 120 h of exposure, with the latter interval having the most significant effect among all the treatments set. Compared to the control solution, a concentration drop of Ca was observed in response to each single Zn treatment. Further, concentration change with time was significant in six cases with 500 mg L^{-1} Zn, causing the most intensive decrease after 120 and 72 h. In the combined treatments, solutions with low levels (10 and 50 mg L^{-1}) of Cd influenced Ca flux substantially after 24 and 72 h when Zn was co-added in 1000 mg L^{-1} concentration. Treatments with moderate levels (100 mg L^{-1}) of Cd had no significant effect on Ca concentration in rapeseed; however, besides a general inhibition in enrichment, the fundamental influence was confirmed by several highly (500 and 1000 mg L^{-1}) Cd-contaminated solutions. In detail, after 120 h, Cd500 + Zn10, Cd1000 + Zn50, and Cd500 + Zn100 treatments lowered the concentration of Ca the most (Supplementary Materials E1).

The concentration change of K in response to single Cd doses was various across the treatments. The only significant relationship was found for 1000 mg L^{-1} Cd after 120 h, triggering a decreased tissue K concentration. As for the single Zn applications, the presence of the metal was positive on the K concentration in rapeseed in most cases. Further, 500 mg L^{-1} Zn after 72 h and 1000 mg L^{-1} Zn after 120 h highly increased K concentration compared to the control treatment. Studying the metal patterns in co-contaminated treatments, the effects were also positive in most comparisons. A significantly positive concentration change was indicated for Cd50 + Zn1000 and Cd500 + Zn500 after 120 h and Cd10 + Zn1000 after 72 h. However, by Cd1000 + Zn50, a significant decrease in K concentration was found after 72 h of growth (Supplementary Materials E2).

The concentration patterns of Mg were various for single Cd treatments. The 10 mg L^{-1} Cd application decreased, while the 100 mg L^{-1} Cd dose increased the tissue concentration of Mg significantly after 24 h. The effects of single Zn doses on the concentration of Mg were also inconsistent, resulting in patterns without any significant relationship. The combined application of Cd and Zn also triggered different Mg concentrations among the treatments. Besides the various relationships compared to the control values, highly positive and negative patterns were also indicated. After 72 h, a substantial increase was found in Mg concentration for the Cd10 + Zn1000, Cd50 + Zn50, Cd50 + Zn500, Cd100 + Zn500, Cd500 + Zn500, and Cd1000 + Zn50 treatments, while a considerable decrease was noted for the Cd1000 + Zn10 treatment (Supplementary Materials E3).

3.4. Interaction of elements

The analysis of element interactions during the single Cd and single Zn accumulations with pooled exposure times resulted in two significant ($p < 0.05$) relationships between studied elements. In the other cases, the nature of the relationship was weak.

A significant negative correlation existed between the effect size values calculated for Ca and Zn in rapeseed plantlets (Fig. 3). This interaction pattern suggests that the tissue concentration of Ca decreased in response to the increase in Zn concentration.

Unlike the previous Ca–Zn interaction, a significant positive correlation existed between the effect size values calculated for K and Zn in rapeseed individuals (Fig. 4). This afore interaction indicates that the tissue concentration of K decreased in response to the decrease of tissue Zn concentration. In another aspect, the higher degree of Zn accumulation in plants resulted in a higher K concentration in plant tissues.

4. Discussion

According to the results of this study, a concentration-dependent decrease in germination rate was found for Cd after 24 hours of growth, while after 72 and 120 hours, signs of germination were visible and measurable on almost every seed for all the treatments. It suggests that rapeseed did not suffer irreversible impairments in a 5-day-long period; instead, the development was slowed down by specific treatments after one day, which was successfully compensated with time. The period of this experiment was only five days; however, adverse effects can appear in the longer term. Applying different concentrations of Cd up to 30 mg L^{-1} and Zn up to 100 mg L^{-1}, Montalbán et al. (2012) found non-significant variation in germination rates of rapeseed after two weeks of exposure, explaining the phenomenon with the high degree of

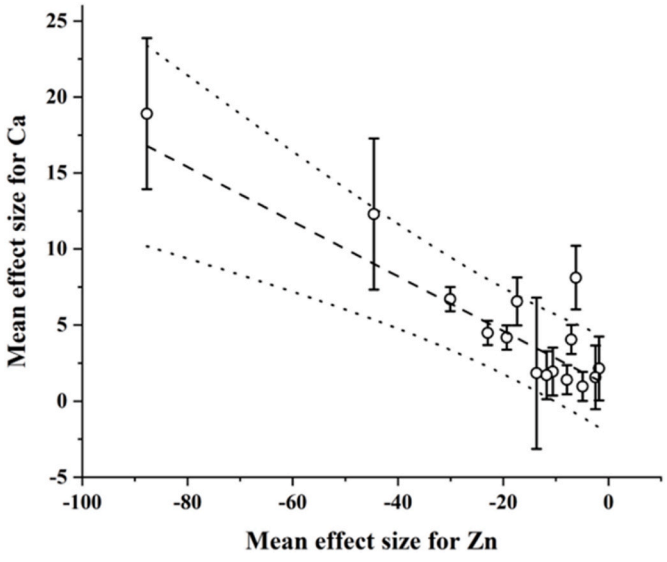


Fig. 3. The relationship between the standardized mean difference (Hedges' *g*) values for Ca and Zn (± SE of the effect size for Ca) from Zn-contaminated vs. uncontaminated comparisons. More negative *g* values denote a higher difference in element concentration of plantlets growing on contaminated vs. uncontaminated solutions (weighted linear regression: F = 21.58, n = 15, $p < 0.001$, r = −0.79). Results are calculated from the mean values of related 24-, 72- and 120-h treatments; the dotted line represents the 95% confidence interval.

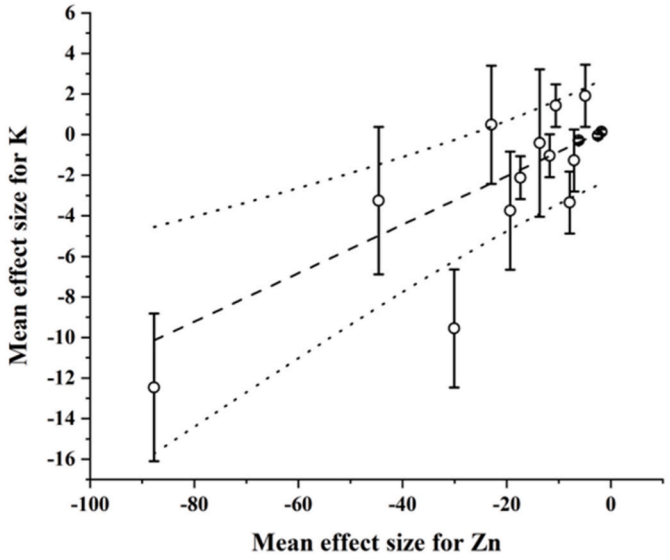


Fig. 4. The relationship between the standardized mean difference (Hedges' *g*) values for K and Zn (± SE of the effect size for K) from Zn-contaminated vs. uncontaminated comparisons. More negative *g* values denote a higher difference in element concentration of plantlets growing on contaminated vs. uncontaminated (weighted linear regression: $F = 19.00$, $n = 15$, $p < 0.001$, $r = 0.77$). Results are calculated from the mean values of related 24-, 72- and 120-h treatments; the dotted line represents the 95% confidence interval.

inherent seed viability under metal stress. Contrasting the findings here, Cojocaru et al. (2016) observed a 92% germination rate for rapeseed with single Cd doses, while that was only 23% grown on a Cd–Zn mixture even after several weeks of exposure. Also, in contrast with the results here, after ten days of growth, Rao et al. (2019) reported a germination rate drop in two *B. napus* cultivars from 97% to 29% when Cd concentration was elevated from 10 to 250 mg L^{-1} in the media. The general negative influence of Cd on development was also noted by Rady and Hemida (2015), underlining the inhibition of cell division and growth as the most significant consequences. At the same time, in line with the result of this paper, the expression of specific genes in response to Cd stress could help proper cell formation and detoxification in rapeseed during development, which thus promotes seed viability and germination in highly Cd-contaminated media (Chen et al., 2018; Zhang et al., 2018). In the case of development under Cd stress, Mwamba et al. (2020) identified various tolerance and adaptation mechanisms among rapeseed genotypes, which account for the different detoxification patterns and germination success observed in individual experiments. Further, Sanjosé et al. (2021) concluded that Zn could foster plant germination and compensate for the adverse effects of other metals even in multi-contaminated environments. Those mentioned above give potential explanations for the observations in this research regarding the 100% germination rate by nearly all the treatments after 72 and 120 h. In another aspect, Kania et al. (2021) indicated that rapeseed exposure to Cd and Zn stress during the first five days of germination promoted extensive molecular responses compared to control conditions; the aminopeptidase activity was altered, resulting in nutrient management deficiencies and hindered germination. The result in this paper also suggests overall delayed germination in response to single- and multi-contaminated treatments; this finding is consistent with the statements made by Kumar Sethy and Ghosh (2013) and Bouchama et al. (2023), underlining that early-stage germination can be retarded by the presence of Cd, showing a moderate variation according to the applied concentration.

Significant differences were found in radicle and hypocotyl length along the exposure interval within the selected treatment and the concentration gradient at the chosen sampling date for Cd and Zn. Unlike in this study, Rosca et al. (2021) measured significantly slower rapeseed growth after 120 h for each Cd supply set between 25 and 300 mg L^{-1} than in the control treatment. It was demonstrated here that development became more intensive after 72 h in the 10, 50, and 100 mg L^{-1} treatments, indicating the positive effect of low concentration Cd on rapeseed growth. Meng et al. (2009) attributed this phenomenon to activating a defense mechanism triggered by low Cd doses. The authors also presented a very restricted development by high and extreme Cd concentrations. This latter is made clear by the fact that the metal is known to disrupt water and nutrient balance, resulting in reduced growth and severe ecophysiological disturbances (Younis et al., 2016; Haider et al., 2021). Peško and Kráľová (2013) noted that rapeseed's metal stress could be indicated by further consequences, such as a decrease in chlorophyll content, leading to multiple developmental disorders. Further, in response to toxic Cd and Zn loads, El Rasafi et al. (2016) emphasized a considerable reduction in growth parameters, identifying crop roots as primary contact organs susceptible to metal stress. At the same time, shoot growth was less limited than by Cd. Ben Ghnaya et al. (2009) noted a variation in the growth parameters among four rapeseed cultivars and also among their plant organs in response to Cd and Zn concentrations, with Cd causing a higher degree of growth inhibition than Zn, based, among others, on the enzyme inhibition induced by the metals. Namely, despite being an essential element, Zn can also have Cd-like toxic effects on plant development, however, only at elevated concentration ranges (Kaur and Garg, 2021). This was confirmed for rapeseed by Wang et al. (2009), who found visible growth reduction in individuals treated with high doses of Zn in a 7-day-long experiment. Accordingly, in this paper, the 500 and 1000 mg L^{-1} treatments hindered radicle and hypocotyl development the most. The radicle elongation was supported by 100 mg L^{-1} Zn the most intensively, while the hypocotyl grew with a permanent intensity throughout the exposure. Other authors confirmed this in a greenhouse and the field (Olama et al., 2014; Ashkiani et al., 2020).

Luo et al. (2019) and Kniuipytė et al. (2023) highlighted that rapeseed germination, metal accumulation, and toxicity symptom occurrence highly depend on environmental factors such as temperature and water availability. For instance, Raza et al. (2022) evidenced that cold stress induces an imbalance in ROS production of rapeseed, thereby indirectly affecting tissue elemental composition patterns. Channaoui et al. (2017) and Batool et al. (2022) named drought stress a primary driver influencing the germination and growth of rapeseed individuals. Further, in response to severe drought stress, Hatzig et al. (2018) identified a transgenerational decline in rapeseed seedling vigor, triggering long-term yield quality deterioration. As another factor, Brunetti et al. (2011) noticed that the metal (e.g., Zn) accumulation of rapeseed can be very different when grown under controlled conditions and in the field, which is in association with the different physiological states and modified role (greenhouse: accumulator, field: tolerant) of individuals between the two setups. Besides these variables, plant development and element accumulation can be affected by further decisive factors, among others: growth stage, condition, type of growing media, pH, weather conditions, exposure time, interactions with other organisms, forms/fractions of elements, and *ex-planta/in-planta* element interactions, which should also be considered when evaluating the potential opportunities and limitations under metal stress (Singh et al., 2016; Tőzsér et al., 2017; Gentili et al., 2018; Zhang and Tielbörger, 2020; Ismukhanova et al., 2022; Xiao et al., 2023).

Based on the concentration changes of Cd, a moderating effect of Zn was suggested, especially by moderate and high Zn doses. The nature of the interaction between Cd and Zn in plants is evaluated inconsistently across studies. Unlike the findings in this paper, studying rapeseed from various Cd- and Zn-contaminated soils, Cojocaru et al. (2016) observed a very high Cd accumulation rate only by single Cd contamination. In tall fescue individuals, Dong et al. (2019) presented antagonism in roots and various interaction schemes in shoots between the two metals. Wu et al. (2003) highlighted that Zn decreased the concentration of Cd in barley,

thereby moderating the potentially toxic effects exerted by Cd. Zhou et al. (2020) reflected on the same pattern for wheat; the authors evidenced that Zn effectively regulated transport genes of Cd, which also favoured enzyme activity. Moustakas et al. (2011) indicated a dose-level-based inhibitory effect between Cd and Zn fluxes in *Calendula officinalis* L. Moreover, Cherif et al. (2011) found that Zn accumulation decreased the concentrations of Cd in tomato tissues, mitigated Cd-induced toxicity, and restored chlorophyll balance. Such distinct antagonism presented in these studies could not be identified in this paper, which can be attributed to rapeseed's inherent very intensive Cd and Zn accumulation potential. This research also proved that significant Zn accumulation was found by high single and co-Zn-treatment levels and by low and moderate Zn complemented by high Cd doses. Contrasting these results, Yan et al. (2023) did not find a determining effect of Cd on Zn accumulation in *B. napus* individuals. Li et al. (2020) presented that the generally negative correlation between Cd and Zn is caused primarily by the competition between the two metals in the soil and in plant tissues, manifesting itself in low concentration of either metal in the case of their co-presence. Contrarily, the results here show the outstanding initial co-accumulation ability of the metals by rapeseed.

As introduced, both Cd and Zn in the contaminated solutions were found to decrease the concentration of Cu in rapeseed. However, single Cd also had a significant positive effect on its concentration after 72 h. For Zn, these statements are in association with the results by Mousavi et al. (2012), who described a negative interaction between Zn and Cu in plants due to the competition for the same adsorption sites during accumulation. Moreover, Ivanova et al. (2010) established that excess Zn promoted the uptake of Cu in rapeseed, restricting its translocation into aboveground plant organs simultaneously. As a reverse approach, Wang et al. (2022) revealed a negative correlation between Cd and Cu in *Brassica campestris* L. by highlighting that Cu supported plant conditions by compensating for the effects of Cd. Also, in line with the trends introduced here, Wong et al. (1986) reported a species-dependent decrease in the Cu concentration of *Brassica chinensis* (*Brassica rapa* L.) in response to Cd supply. As evidence for the variation mentioned above, Mwamba et al. (2016) outlined a strong synergism between the accumulations of Cd and Cu in rapeseed by low Cd concentration ranges due to their close interaction on the root surfaces. Based on the findings of the relevant literature and the results presented here, the demonstrated divergent patterns justify the need for further research for a more thorough explanation.

It was suggested that both alone and combined, Cd and Zn had a minor role in rapeseed Fe dynamics. In turn, in the cases of both single and co-Cd-treatment, significant effects were found on Mn concentration in rapeseed. In contrast, Wang et al. (2009) proposed that high concentrations of Zn can result in a negative interaction between the metal and other elements, such as Fe and Mn in rapeseed, manifesting itself in a reduced accumulation of afore essential elements due to Zn-induced root injuries. This study did not reveal such interaction between the studied treatments and the Fe- and Mn concentrations. Regarding Mn and Zn in maize, Soltangheisi et al. (2014) confirmed no correlation in their concentration between the media and the shoot, while in roots, Zn lowered the content of Mn. Additionally, for Cd, Mwamba et al. (2016) indicated by two rapeseed cultivars that the metal decreased the accumulation of Fe and Mn as well, presumably due to the toxic effects of Cd during its accumulation. Hussain et al. (2020), using a reverse approach, studied the effects of Fe and Mn on the uptake of Cd in rice. They found that the accumulation of Cd was highly restricted by applying the other two elements due primarily to the differences in the expression of Cd, Fe, and Mn transporters accountant for Cd accumulation. These observations for Cd contradict the results presented for the Cd–Fe relation. As a potential explanation of the results demonstrated here, the concentration of Fe was not dependent on the concentration levels of Cd and Zn, which is considered a species-specific phenomenon. In contrast, the high concentration of solely applied Cd and co-applied Cd or Zn positively affected tissue Mn concentration. This is regarded as a considerable species-specific finding regarding remediation with rapeseed in the short term.

In several treatments, the concentration of Ca was negatively influenced by the single application of both Zn and Cd, while the simultaneous application of the metals also impaired Ca flux. In the literature, the general mediating effects of Ca on Cd uptake are widely described (Huang et al., 2017; Zhang et al., 2020); however, assessments on the role of added Cd in the tissue Ca concentrations are scarce. Accordingly, these relations for *B. napus* and relative species are also sporadic among studies. Zhu et al. (2023) found that nano-Ca supply increased the rate of Cd accumulation by passively and actively enhancing pectin activity and Cd fixation and simultaneously favoring internal translocation of the metal. In *B. campestris*, on the other hand, Chen et al. (2019) recognized a competitive inhibition between Cd and Ca, which triggers a negative correlation by their uptake. Prasad et al. (2016) reported that Zn affects the dynamics of Ca negatively as high concentrations of Zn in the growing media induce the formation of Ca–Zn complexes with low bioavailability, while translocation of Ca from root to shoot is also hindered. These latter corroborate the results of the analyses here, which showed a significant negative correlation in effect size values between tissue Ca and Zn, suggesting that high Zn concentrations substantively decreased Ca concentrations in rapeseed in the long term. Noticing this observation by other species, like in the Zn–Cu relation, Watkins and Ferguson (1982) attributed the Zn-based depletion of Ca in tissues to the competition between the two elements for the same exchange sites. The substitution mechanism between Zn and calcite was depicted as common in tobacco individuals by Sarret et al. (2006), which can also be related to the results presented in this paper.

It was found that single Cd had no significant effect on the K concentration, except for one significant negative relation. In contrast, the influence of single Zn was positive, elevating the concentration of K in two cases applied in high doses. The joint effect remained generally positive with co-application, with significant influences in both directions. As for Mg, concentrations were varied across the treatments. Regarding the relevant literature on *B. napus*, there is a gap in knowledge of well-founded information. Before and after its flowering stage, Anjum et al. (2008) evaluated the elemental composition of *B. campestris* in Cd treatments and experienced different degrees of depletion of tissue K. The authors suggest that Cd can change plasma membrane characteristics; therefore, the nutrient cycle can also be severely impaired. Studying *Pisum sativum*, Abbas et al. (2022) found antagonism between the uptake of Cd and K, explained by the role of K in effectively reducing Cd accumulation intensity. In reverse, Hédiji et al. (2015) concluded that Cd considerably lowered the concentrations of both K and Mg in tomato individuals due to the influence of nutrition balance caused by cell damage. In the literature, the study of interactions between Mg and Cd was mainly dedicated to the protective function of Mg against Cd-induced toxicity (Hermans et al., 2011; Lu et al., 2021). In line with this, Nazar et al. (2012) indicated growth inhibition and Mg depletion under Cd stress in the case of several herbaceous species. Partly supporting the outcomes here, Dong et al. (2019) demonstrated an increase in tissue K and Mg in response to surplus Zn in a Cd-contaminated environment, which was due to the positive effect of Zn on the transpiration dynamics, enabling the build-up of a more balanced nutrient pattern under Cd stress. In this paper, an increase in Zn concentration significantly elevated the concentration of K. Likewise, Molnár et al. (2022) drew a positive relationship between Zn and K in rapeseed, which was the most noticeable in the leaves. Evidence for a positive correlation was also found by Cakmak and Marschner (1988) for three cultivated species; the authors observed that Zn deficiency triggered the exudation of K by roots, causing a significant decrease in tissue concentrations.

5. Conclusions

It was found in this research that the concentration level, treatment, and exposure time of the growing media significantly influenced the development and the micro- and macro element concentrations of rapeseed. Element interactions also determined plant tissue concentrations. The radicle and hypocotyl lengths were significantly reduced by the single and co-application of Cd and Zn. Among microelements, the accumulation intensity of Cd was elevated by applied Cd, the accumulation of Zn was increased by Cd and Zn, and both metals significantly influenced the concentration of Mn. In contrast, the concentrations of Cu and Fe were slightly affected by any of the metals. In the cases of macro elements, both Cd and Zn had significant effects on tissue concentrations; correlations were negative for Ca and variously negative and positive for K and Mg. Based on these findings, rapeseed is recognized as a highly proficient accumulator of both Cd and Zn during its early development stages. However, the interactions involving these two metals are crucial in determining the micro- and macro elemental concentrations in tissues, which deserves more attention in future investigations.

Funding

This work was financed by the NRDI (National Research, Development and Innovation Office) Fund [grant number 138806].

CRediT authorship contribution statement

Dávid Tőzsér: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Deborah Osariemen Idehen:** Writing – review & editing, Investigation. **Jennifer Damilola Osazuwa:** Writing – review & editing, Investigation. **John Elias Sule:** Writing – review & editing, Investigation. **Ágota Zsófia Ragyák:** Supervision, Investigation, Formal analysis. **Zsófi Sajtos:** Writing – review & editing, Supervision, Resources, Formal analysis. **Tibor Magura:** Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.141235.

References

Abbas, T., Fan, R., Hussain, S., Sattar, A., Khalid, S., Butt, M., Shahzad, U., Atif, H.M., Batool, M., Ullah, S., Li, Y., Al-Hashimi, A., Elshikh, M.S., Al-Yahyai, R., 2022. Protective effect of jasmonic acid and potassium against cadmium stress in peas (*Pisum sativum* L.). Saudi J. Biol. Sci. 29, 2626–2633. https://doi.org/10.1016/j.sjbs.2021.12.051.

Ali, H., Khan, E., 2019. Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs – concepts and implications for wildlife and human health. Hum. Ecol. Risk Assess. 25, 1353–1376. https://doi.org/10.1080/10807039.2018.1469398.

Anjum, N.A., Umar, S., Ahmad, A., Iqbal, M., Khan, N.A., 2008. Ontogenic variation in response of *Brassica campestris* L. to cadmium toxicity. J. Plant Interact. 3, 189–198. https://doi.org/10.1080/17429140701823164.

Ashkiani, A., Sayfzadeh, S., Rad, A.H.S., Valadabadi, A., Masouleh, E.H., 2020. Effects of foliar zinc application on yield and oil quality of rapeseed genotypes under drought stress. J. Plant Nutr. 11, 1594–1603. https://doi.org/10.1080/01904167.2020.1739299.

Batool, M., El-Badri, A.M., Wang, Z., Mohamed, I.A.A., Yang, H., Ai, X., Salah, A., Hassan, M.U., Sami, R., Kuai, J., Wang, B., Zhou, G., 2022. Rapeseed morpho-physio-biochemical responses to drought stress induced by PEG-6000. Agronomy 12, 579. https://doi.org/10.3390/agronomy12030579.

Ben Ghnaya, A., Charles, G., Hourmant, A., Ben Hamida, J., Branchard, M., 2009. Physiological behavior of four rapeseed cultivar (*Brassica napus* L.) submitted to metal stress. C. R. Biol. 332, 363–370. https://doi.org/10.1016/j.crvi.2008.12.001.

Bouchama, K., Rouabhi, R., Reda Djebar, M., 2023. Cadmium phytotoxicity on seed germination, early growth and the differential antioxidant response of guaiacol peroxidase in *Phragmites australis* seedling organs. Pol. J. Environ. Stud. 32, 1531–1544. https://doi.org/10.15244/pjoes/157492.

Brunetti, G., Farrag, K., Rovira, P.S., Nigro, F., Senesi, N., 2011. Greenhouse and field studies on Cr, Cu, Pb and Zn phytoextraction by *Brassica napus* from contaminated soils in the Apulia region. Southern Italy. Geoderma 160, 517–523. https://doi.org/10.1016/j.geoderma.2010.10.023.

Cabot, C., Martos, S., Llugany, M., Gallego, B., Tolrà, R., Poschenrieder, C., 2019. A role for zinc in plant defense against pathogens and herbivores. Front. Plant Sci. 10, 1171. https://doi.org/10.3389/fpls.2019.01171.

Cakmak, I., Marschner, H., 1988. Increase in membrane permeability and exudation in roots of zinc deficient plants. J. Plant Physiol. 132, 356–361. https://doi.org/10.1016/S0176-1617(88)80120-2.

Channaoui, S., El Kahkahi, R., Charafi, J., Mazouz, H., El Fechtali, M., Nabloussi, A., 2017. Germination and seedling growth of a set of rapeseed (*Brassica napus*) varieties under drought stress conditions. Int. J. Environ. Agric. Biotechnol. 2, 487–494. https://doi.org/10.22161/ijeab/2.1.61.

Chen, H., Shu, F., Yang, S., Li, Y., Wang, S., 2019. Competitive inhibitory effect of calcium polypeptides on Cd enrichment of *Brassia campestris* L. Int. J. Environ. Res. Publ. Health 16, 4472. https://doi.org/10.3390/ijerph16224472.

Chen, L., Wan, H., Qian, J., Guo, J., Sun, C., Wen, J., Yi, B., Ma, C., Tu, J., Song, L., Fu, T., Shen, J., 2018. Genome-wide association study of cadmium accumulation at the seedling stage in rapeseed (*Brassica napus* L.). Front. Plant Sci. 9, 375. https://doi.org/10.3389/fpls.2018.00375.

Cherif, J., Mediouni, C., Ben Ammar, W., Jemal, F., 2011. Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative systems in tomato plants (*Solarium lycopersicum*). J. Environ. Sci. 23, 837–844. https://doi.org/10.1016/S1001-0742(10)60415-9.

Coimbra, E.C.L., Borges, A.C., 2023. Removing Mn, Cu and Fe from real wastewaters with macrophytes: reviewing the relationship between environmental factors and plants' uptake capacity. Toxics 11, 158. https://doi.org/10.3390/toxics11020158.

Cojocaru, P., Gusiatin, Z.M., Cretescu, I., 2016. Phytoextraction of Cd and Zn as single or mixed pollutants from soil by rape (*Brassica napus*). Environ. Sci. Pollut. Res. 23, 10693–10701. https://doi.org/10.1007/s11356-016-6176-5.

Dhaliwal, S.S., Taneja, P.K., Singh, J., Bhatti, S.S., Singh, R., 2020. Cadmium accumulation potential of *Brassica* species grown in metal spiked loamy sand soil. Soil Sediment Contam. 29, 638–649. https://doi.org/10.1080/15320383.2020.1758031.

Dong, Q., Hu, S., Fei, L., Liu, L., Wang, Z., 2019. Interaction between Cd and Zn on metal accumulation, translocation and mineral nutrition in tall fescue (*Festuca arundinacea*). Int. J. Mol. Sci. 20, 3332. https://doi.org/10.3390/ijms20133332.

El Rasafi, T., Nouri, M., Bouda, S., Haddioui, A., 2016. The effect of Cd, Zn and Fe on seed germination and early seedling growth of wheat and bean. Ekológia 35, 213–223. https://doi.org/10.1515/eko-2016-0017.

Elizareva, E., Yanbaev, Y., Redkina, N., Kudashkina, N., Elizaryev, A., Khamidullin, I., 2019. Accumulating capacity of different varieties of rapeseed under conditions of anthropogenic pollution of soils by heavy metals. IOP Conf. Ser. Earth Environ. Sci. 403, 012182 https://doi.org/10.1088/1755-1315/403/1/012182.

Ettl, J., Bernhardt, H., Huber, G., Thuneke, K., Remmele, E., Emberger, P., 2020. Evaluation of pure rapeseed oil as a renewable fuel for agricultural machinery based on emission characteristics and long-term operation behaviour of a fleet of 18 tractors. SN Appl. Sci. 2, 1711. https://doi.org/10.1007/s42452-020-03490-8.

Gall, J.E., Boyd, R.S., Rajakaruna, N., 2015. Transfer of heavy metals through terrestrial food webs: a review. Environ. Monit. Assess. 187, 201. https://doi.org/10.1007/s10661-015-4436-3.

Gentili, R., Ambrosini, R., Montagnani, C., Caronni, S., Citterio, S., 2018. Effect of soil pH on the growth, reproductive investment and pollen allergenicity of *Ambrosia artemisiifolia* L. Front. Plant Sci. 9, 1335. https://doi.org/10.3389/fpls.2018.01335.

Grispen, V.M.J., Nelissen, H.J.M., Verkleij, J.A.C., 2006. Phytoextraction with *Brassica napus* L.: a tool for sustainable management of heavy metal contaminated soils. Environ. Pollut. 144, 77–83. https://doi.org/10.1016/j.envpol.2006.01.007.

Haider, F.U., Liqun, C., Coulter, J.A., Cheema, S.A., Wu, J., Zhang, R., Wenjun, M., Farooq, M., 2021. Cadmium toxicity in plants: impacts and remediation strategies. Ecotoxicol. Environ. Saf. 211, 111887 https://doi.org/10.1016/j.ecoenv.2020.111887.

Haj Sghaier, A., Tarnawa, Á., Khaeim, H., Kovács, G.P., Gyuricza, C., Kende, Z., 2022. The effects of temperature and water on the seed germination and seedling development of rapeseed (*Brassica napus* L.). Plants 11, 2819. https://doi.org/10.3390/plants11212819.

Hatzig, S.V., Nuppenau, J.N., Snowdon, R.J., Schießl, S.V., 2018. Drought stress has transgenerational effects on seeds and seedlings in winter oilseed rape (*Brassica napus* L.). BMC Plant Biol. 18, 297. https://doi.org/10.1186/s12870-018-1531-y.

Hermans, C., Chen, J., Coppens, F., Inzé, D., Verbruggen, N., 2011. Low magnesium status in plants enhances tolerance to cadmium exposure. New Phytol. 192, 428–436. https://doi.org/10.1111/j.1469-8137.2011.03814.x.

Hédiji, H., Djebali, W., Belkadhi, A., Cabasson, C., Moing, A., Rolin, D., Brouquisse, R., Gallusci, P., Chaïbi, W., 2015. Impact of long-term cadmium exposure on mineral content of *Solanum lycopersicum* plants: consequences on fruit production. South Afr. J. Bot. 97, 176–181. https://doi.org/10.1016/j.sajb.2015.01.010.

Hou, D., O'Connor, D., Igalavithana, A.D., Alessi, D.S., Luo, J., Tsang, D.C.W., Sparks, D. L., Yamauchi, Y., Rinklebe, J., Ok, Y.S., 2020. Metal contamination and bioremediation of agricultural soils for food safety and sustainability. Nat. Rev. Earth Environ. 1, 366–381. https://doi.org/10.1038/s43017-020-0061-y.

Huang, D., Gong, X., Liu, Y., Zeng, G., Lai, C., Bashir, H., Zhou, L., Wang, D., Xu, P., Cheng, M., Wan, J., 2017. Effects of calcium at toxic concentrations of cadmium in plants. Planta 245, 863–873. https://doi.org/10.1007/s00425-017-2664-1.

Huihui, Z., Xin, L., Zisong, X., Yue, W., Zhiyuan, T., Meijun, A., Yuehui, Z., Wenxu, Z., Nan, X., Guangyu, S., 2020. Toxic effects of heavy metals Pb and Cd on mulberry (*Morus alba* L.) seedling leaves: photosynthetic function and reactive oxygen species (ROS) metabolism responses. Ecotoxicol. Environ. Saf. 195, 110469 https://doi.org/10.1016/j.ecoenv.2020.110469.

Hussain, B., Li, J., Ma, Y., Tahir, N., Ullah, A., 2020. Effects of Fe and Mn cations on Cd uptake by rice plant in hydroponic culture experiment. PLoS One 15, e0243174. https://doi.org/10.1371/journal.pone.0243174.

Ismukhanova, L., Choduraev, T., Opp, C., Madibekov, A., 2022. Accumulation of heavy metals in bottom sediment and their migration in the water ecosystem of Kapshagay Reservoir in Kazakhstan. Appl. Sci. 12, 11474 https://doi.org/10.3390/app122211474.

Ivanova, E.M., Kholodova, V.P., Kuznetsov, V.V., 2010. Biological effects of high copper and zinc concentrations and their interaction in rapeseed plants. Russ. J. Plant Physiol. 57, 806–814. https://doi.org/10.1134/S1021443710060099.

Kania, J., Krawczyk, T., Gillner, D.M., 2021. Oilseed rape (*Brassica napus*): the importance of aminopeptidases in germination under normal and heavy metals stress conditions. J. Sci. Food Agric. 101, 6533–6541. https://doi.org/10.1002/jsfa.11325.

Kaur, H., Garg, N., 2021. Zinc toxicity in plants: a review. Planta 253, 129. https://doi.org/10.1007/s00425-021-03642-z.

Kniuipytė, I., Dikšaitytė, A., Praspaliauskas, M., Pedišius, N., Žaltauskaitė, J., 2023. Oilseed rape (*Brassica napus* L.) potential to remediate Cd contaminated soil under different soil water content. J. Environ. Manag. 325, 116627 https://doi.org/10.1016/j.jenvman.2022.116627.

Kumar Sethy, S., Ghosh, S., 2013. Effect of heavy metals on germination of seeds. J. Nat. Sci. Biol. Med. 4, 272–275. https://doi.org/10.4103/0976-9668.116964.

Li, L., Zhang, Y., Ippolito, J.A., Xing, W., Qiu, K., Wang, Y., 2020. Cadmium foliar application affects wheat Cd, Cu, Pb and Zn accumulation. Environ. Pollut. 262, 114329 https://doi.org/10.1016/j.envpol.2020.114329.

Lu, M., Liang, Y., Lakshmanan, P., Guan, X., Liu, D., Chen, X., 2021. Magnesium application reduced heavy metal-associated health risks and improved nutritional quality of field-grown Chinese cabbage. Environ. Pollut. 289, 117881 https://doi.org/10.1016/j.envpol.2021.117881.

Luo, T., Xian, M., Zhang, C., Zhang, C., Hu, L., Xu, Z., 2019. Associating transcriptional regulation for rapid germination of rapeseed (*Brassica napus* L.) under low temperature stress through weighted gene co-expression network analysis. Sci. Rep. 9, 55. https://doi.org/10.1038/s41598-018-37099-0.

Lüdecke, D., 2019. *Esc*: Effect Size Computation for Meta Analysis. https://doi.org/10.5281/zenodo.1249218, Version 0.5.1.

Meng, H., Hua, S., Shamsi, I.H., Jilani, G., Li, Y., Jiang, L., 2009. Cadmium-induced stress on the seed germination and seedling growth of *Brassica napus* L., and its alleviation through exogenous plant growth regulators. Plant Growth Regul. 58, 47–59. https://doi.org/10.1007/s10725-008-9351-y.

Molnár, Á., Kondak, S., Benkő, P., Janovszky, P., Kovács, K., Szőllősi, R., Gondor, O.K., Oláh, D., Gémes, K., Galbács, G., Janda, T., Kolbert, Z., 2022. Limited Zn supply affects nutrient distribution, carbon metabolism and causes nitro-oxidative stress in sensitive *Brassica napus*. Environ. Exp. Bot. 202, 105032 https://doi.org/10.1016/j.envexpbot.2022.105032.

Montalbán, B., Pradas Del Real, A., García, P., Lobo, M., Pérez-Sanz, A., 2012. Germination and early development of *Brassica napus* and *Brachypodium distachyon* growth with Zn, Cr (VI), as (V) or Cd: preliminary results. Acta Phytopathol. Entomol. Hung. 47, 363–371. https://doi.org/10.1556/APhyt.47.2012.2.16.

Mourato, M.P., Moreira, I.N., Leitão, I., Pinto, F.R., Sales, J.R., Martins, L.L., 2015. Effect of heavy metals in plants of the genus *Brassica*. Int. J. Mol. Sci. 16, 17975–17998. https://doi.org/10.3390/ijms160817975.

Mousavi, S.R., Galavi, M., Rezaei, M., 2012. The interaction of zinc with other elements in plants: a review. Intl. J. Agric. Crop Sci. 4, 1881–1884.

Moustakas, N.K., Akoumianaki-Ioannidou, A., Barouchas, P.E., 2011. The effects of cadmium and zinc interactions on the concentration of cadmium and zinc in pot marigold (*Calendula officinalis* L.). Aust. J. Crop. Sci. 5, 277–282.

Mwamba, T.M., Ali, S., Ali, B., Lwalaba, J.L., Liu, H., Farooq, M.A., Shou, J., Zhou, W., 2016. Interactive effects of cadmium and copper on metal accumulation, oxidative stress, and mineral composition in *Brassica napus*. Int. J. Environ. Sci. Technol. 13, 2163–2174. https://doi.org/10.1007/s13762-016-1040-1.

Mwamba, T.M., Islam, F., Ali, B., Lwalaba, J.L.W., Gill, R.A., Zhang, F., Farooq, M.A., Ali, S., Ulhassan, Z., Huang, Q., Zhou, W., Wang, J., 2020. Comparative metabolomic responses of low- and high-cadmium accumulating genotypes reveal the cadmium adaptive mechanism in *Brassica napus*. Chemosphere 250, 126308. https://doi.org/10.1016/j.chemosphere.2020.126308.

Nan, Z., Li, J., Zhang, J., Cheng, G., 2002. Cadmium and zinc interactions and their transfer in soil-crop system under actual field conditions. Sci. Total Environ. 285, 187–195. https://doi.org/10.1016/s0048-9697(01)00919-6.

Nazar, R., Iqbal, N., Masood, A., Khan, M.I.R., Syeed, S., Khan, N.A., 2012. Cadmium toxicity in plants and role of mineral nutrients in its alleviation. Am. J. Plant Sci. 3, 1476–1489. https://doi.org/10.4236/ajps.2012.310178.

Olama, V., Ronaghi, A., Karimian, N., Yasrebi, J., Hamidi, R., Tavajjoh, M., Kazemi, M. R., 2014. Seed quality and micronutrient contents and translocations in rapeseed (*Brassica napus* L.) as affected by nitrogen and zinc fertilizers. Arch. Agron Soil Sci. 60, 423–435. https://doi.org/10.1080/03650340.2013.796588.

Peško, M., Kráľová, K., 2013. Physiological response of *Brassica napus* L. plants to Cu (II) treatment. Proceedings of ECOpole 7, 155–161. https://doi.org/10.2429/proc.2013.7(1)020.

Prasad, R., Shivay, Y.S., Kumar, D., 2016. Interactions of zinc with other nutrients in soils and plants – a review. Int. J. Fertil. 12, 16–26.

Raboanatahiry, N., Li, H., Yu, L., Li, M., 2021. Rapeseed (*Brassica napus*): processing, utilization, and genetic improvement. Agronomy 11, 1776. https://doi.org/10.3390/agronomy11091776.

Rady, M.M., Hemida, K.A., 2015. Modulation of cadmium toxicity and enhancing cadmium-tolerance in wheat seedlings by exogenous application of polyamines. Ecotoxicol. Environ. Safe. 119, 178–185. https://doi.org/10.1016/j.ecoenv.2015.05.008.

Rao, G., Huang, S., Ashraf, U., Mo, Z., Duan, M., Pan, S., Tang, X., 2019. Ultrasonic seed treatment improved cadmium (Cd) tolerance in *Brassica napus* L. Ecotoxicol. Environ. Saf. 185, 109659 https://doi.org/10.1016/j.ecoenv.2019.109659.

Raza, A., Su, W., Jia, Z., Luo, D., Zhang, Y., Gao, A., Hussain, M.A., Mehmood, S.S., Cheng, Y., Lv, Y., Zou, X., 2022. Mechanistic insights into trehalose-mediated cold stress tolerance in rapeseed (*Brassica napus* L.) seedlings. Front. Plant Sci. 13, 857980 https://doi.org/10.3389/fpls.2022.857980.

Rietra, R.P.J.J., Heinen, M., Dimkpa, C.O., Bindraban, P.S., 2017. Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. Commun. Soil Sci. Plant Anal. 48, 1895–1920. https://doi.org/10.1080/00103624.2017.1407429.

Romeh, A.A., 2021. Potential risks from the accumulation of heavy metals in canola plants. Environ. Sci. Pollut. Res. 28, 52529–52546. https://doi.org/10.1007/s11356-021-14330-6.

Rosca, M., Cozma, P., Minut, M., Hlihor, R.M., Bețianu, C., Diaconu, M., Gavrilescu, M., 2021. New evidence of model crop *Brassica napus* L. in soil clean-up: comparison of tolerance and accumulation of lead and cadmium. Plants 10. https://doi.org/10.3390/plants10102051, 2051.

Sanjosé, I., Navarro-Roldán, F., Infante-Izquierdo, M.D., Martínez-Sagarra, G., Devesa, J. A., Polo, A., Ramírez-Acosta, S., Sánchez-Gullón, E., Jiménez-Nieva, F.J., Muñoz-Rodríguez, A.F., 2021. Accumulation and effect of heavy metals on the germination and growth of *Salsola vermiculata* L. seedlings. Diversity 13, 539. https://doi.org/10.3390/d13110539.

Sarker, A., Kim, J.E., Towfiqul Islam, A.R.M., Bilal, M., Rakib, M.R.J., Rahman, M.M., Islam, T., 2022. Heavy metals contamination and associated health risks in food webs—a review focuses on food safety and environmental sustainability in Bangladesh. Environ. Sci. Pollut. Res. 29, 3230–3245. https://doi.org/10.1007/s11356-021-17153-7.

Sarret, G., Harada, E., Choi, Y.E., Isaure, M.P., Geoffroy, N., Fakra, S., Marcus, M.A., Birschwilks, M., Clemens, S., Manceau, A., 2006. Trichomes of tobacco excrete zinc as zinc-substituted calcium carbonate and other zinc-containing compounds. Plant Physiol. 141, 1021–1034. https://doi.org/10.1104/pp.106.082743.

Sharma, P., Rathee, S., Ahmad, M., Batish, D.R., Singh, H.P., Kohli, R.K., 2022. Biodegradable chelant-metal complexes enhance cadmium phytoextraction efficiency of *Solanum americanum*. Environ. Sci. Pollut. Res. 29, 57102–57111. https://doi.org/10.1007/s11356-022-19622-z.

Simon, L., Uri, Z., Vigh, S., Irinyiné, O.K., Makádi, M., Vincze, G., 2022. Phytoextraction of potentially toxic elements from wastewater solids and willow ash – experiences with energy willow (*Salix triandra* × *S. viminalis* 'inger'). In: Puthur, J.T., Dhankher, O.M. (Eds.), Bioenergy Crops: A Sustainable Means of Phytoremediation. CRC Press, Boca Raton.

Singh, S., Parihar, P., Singh, R., Singh, V.P., Prasad, S.M., 2016. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front. Plant Sci. 6, 1143. https://doi.org/10.3389/fpls.2015.01143.

Soltangheisi, A., Rahman, Z.A., Ishak, C.F., Musa, H.M., Zakikhani, H., 2014. Interaction effects of zinc and manganese on growth, uptake response and chlorophyll content of sweet corn (*Zea mays* var. saccharata). Asian J. Plant Sci. 13, 26–33. https://doi.org/10.3923/ajps.2014.26.33.

Tőzsér, D., Magura, T., Simon, E., 2017. Heavy metal uptake by plant parts of willow species: a meta-analysis. J. Hazard Mater. 336, 101–109. https://doi.org/10.1016/j.jhazmat.2017.03.068.

Wang, C., Zhang, S.H., Wang, P.F., Hou, J., Zhang, W.J., Li, W., Lin, Z.P., 2009. The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. Chemosphere 75, 1468–1476. https://doi.org/10.1016/j.chemosphere.2009.02.033.

Wang, H., Li, W., Zhu, C., Tang, X., 2021. Analysis of heavy metal pollution in cultivated land of different quality grades in the Yangtze River delta of China. Int. J. Environ. Res. Publ. Health 18, 9876. https://doi.org/10.3390/ijerph18189876.

Wang, S., Fu, Y., Zheng, S., Xu, Y., Sun, Y., 2022. Phytotoxicity and accumulation of copper-based nanoparticles in *Brassica* under cadmium stress. Nanomaterials 12, 1497. https://doi.org/10.3390/nano12091497.

Watkins, C.B., Ferguson, I.B., 1982. The interaction of copper and zinc with calcium in apple fruit. Sci. Hortic. 17, 319–325. https://doi.org/10.1016/0304-4238(82)90112-1.

Wong, M.K., Chuah, G.K., Ang, K.P., Koh, L.L., 1986. Interactive effects of lead, cadmium and copper combinations in the uptake of metals and growth of *Brassica chinensis*. Environ. Exp. Bot. 26, 331–339. https://doi.org/10.1016/0098-8472(86)90020-1.

Wu, C., Dun, Y., Zhang, Z., Li, M., Wu, G., 2020. Foliar application of selenium and zinc to alleviate wheat (*Triticum aestivum* L.) cadmium toxicity and uptake from cadmium-contaminated soil. Ecotoxicol. Environ. Saf. 190, 110091 https://doi.org/10.1016/j.ecoenv.2019.110091.

Wu, F., Zhang, G., Yu, J., 2003. Interaction of cadmium and four microelements for uptake and translocation in different barley genotypes. Commun. Soil Sci. Plant Anal. 34, 2003–2020. https://doi.org/10.1081/CSS-120023233.

Xiao, Q., Wang, S., Chi, Y., 2023. Accumulation and chemical forms of cadmium in tissues of different vegetable crops. Agronomy 13, 680. https://doi.org/10.3390/agronomy13030680.

Yan, Q., Fang, H., Wang, D., Xiao, X., Deng, T., Li, X., Wie, F., Liu, J., Lin, C., 2023. Transfer and transformation characteristics of Zn and Cd in soil-rotation plant (*Brassica napus* L and *Oryza sativa* L) system and its influencing factors. Sci. Rep. 13, 7393. https://doi.org/10.1038/s41598-023-34377-4.

Younis, U., Malik, S.A., Rizwan, M., Qayyum, M.F., Ok, Y.S., Shah, M.H.R., Rehman, R. A., Ahmad, N., 2016. Biochar enhances the cadmium tolerance in spinach (*Spinacia oleracea*) through modification of Cd uptake and physiological and biochemical attributes. Environ. Sci. Pollut. Res. 23, 21385–21394. https://doi.org/10.1007/s11356-016-7344-3.

Yu, R., Ji, J., Yuan, X., Song, Y., Wang, C., 2012. Accumulation and translocation of heavy metals in the canola (*Brassica napus* L.)-soil system in Yangtze River Delta, China. Plant Soil 353, 33–45. https://doi.org/10.1007/s11104-011-1006-5.

Zhang, F., Xiao, X., Yan, G., Hu, J., Cheng, X., Li, L., Li, H., Wu, X., 2018. Association mapping of cadmium-tolerant QTLs in *Brassica napus* L. and insight into their contributions to phytoremediation. Environ. Exp. Bot. 155, 420–428. https://doi.org/10.1016/j.envexpbot.2018.07.014.

Zhang, R., Tielbörger, K., 2020. Density-dependence tips the change of plant–plant interactions under environmental stress. Nat. Commun. 11, 2532. https://doi.org/10.1038/s41467-020-16286-6.

Zhang, S., Li, Q., Nazir, M.M., Ali, S., Ouyang, Y., Ye, S., Zeng, F., 2020. Calcium plays a double-edged role in modulating cadmium uptake and translocation in rice. Int. J. Mol. Sci. 21, 8058. https://doi.org/10.3390/ijms21218058.

Zhao, H., Guan, J., Liang, Q., Zhang, X., Hu, H., Zhang, J., 2021. Effects of cadmium stress on growth and physiological characteristics of sassafras seedlings. Sci. Rep. 11, 9913. https://doi.org/10.1038/s41598-021-89322-0.

Zhou, J., Zhang, C., Du, B., Cui, H., Fan, X., Zhou, D., Zhou, J., 2020. Effects of zinc application on cadmium (Cd) accumulation and plant growth through modulation of the antioxidant system and translocation of Cd in low- and high-Cd wheat cultivars. Environ. Pollut. 265, 115045 https://doi.org/10.1016/j.envpol.2020.115045.

Zhu, Z., Tian, H., Tang, X., Li, J., Zhang, Z., Chai, G., Wu, X., 2023. NPs-Ca promotes Cd accumulation and enhances Cd tolerance of rapeseed shoots by affecting Cd transfer and Cd fixation in pectin. Chemosphere 341, 140001. https://doi.org/10.1016/j.chemosphere.2023.140001.