Heliyon 10 (2024) e29528

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Heavy metal tolerance and accumulation in the *Brassica* species (*Brassica chinensis* var. *parachinensis* and *Brassica rapa* L.): A pot experiment

Adzrin Asikin Zunaidi [a,*], Lee Hoon Lim [a], Faizah Metali [b]

[a] *Chemical Sciences Programme, Faculty of Sciences, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong, BE1410, Brunei Darussalam*
[b] *Environmental and Life Sciences Programme, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong, BE1410, Brunei Darussalam*

ARTICLE INFO

Keywords:
Ecotoxicology
Hyperaccumulators
Metal stress response
Phytoremediation
Plant-metal interactions
Soil pollution

ABSTRACT

This study delves into the heavy metal tolerance and accumulation capabilities of *Brassica chinensis* var. *parachinensis* (*B. chinensis*) and *Brassica rapa* L. (*B. rapa*) in a pot experiment, specifically focusing on cadmium (Cd), chromium (Cr) and lead (Pb). Agricultural topsoils were spiked with varying concentrations of these heavy metals (0 mg/kg, 75 mg/kg, 150 mg/kg, 225 mg/kg and 300 mg/kg) for each element. The experiment involved cultivating 15 pots each of *B. chinensis* and *B. rapa* over 60 days. Results indicated that both *Brassica* species experienced delayed germination, with *B. chinensis* exhibiting a significant drop in germination percentage to 53 % at the highest concentration (300 mg/kg), while *B. rapa* showed a tendency for an increased germination percentage of up to 80 % at elevated metal concentrations; however, these differences were not statistically significant. Both *B. chinensis* and *B. rapa* demonstrated a stable decline in growth rate from 0.05 cm/day to 0.04 cm/day with increasing heavy metal concentrations, and the he reduction in relative growth rate was significant at the highest concentration compared to the control. The stress tolerance index revealed a significant decrease in plant heights for *B. chinensis*, in contrast to the stable performance of *B. rapa*, showcasing the tolerance of *B. rapa* to toxic conditions. Despite insignificant differences in fresh biomass due to metal treatments, *B. chinensis* consistently yielded higher biomass, yet it had a lower edible index due to its higher root biomass. Leaf areas increased significantly in both species at higher soil treatments, while root lengths remained unchanged, suggesting their resilience to elevated heavy metal concentrations. Analysis of plant tissues (leaves, stems and roots) using ICP-OES revealed that *B. rapa* accumulated the highest Cd concentration (864 mg/kg), whereas *B. chinensis* accumulated the highest Pb concentration (953 mg/kg) in root parts. Both species significantly accumulated Cr in roots, demonstrating a sequestration mechanism. These findings suggest that both species, particularly, *B. rapa* possess strong tolerance and accumulation capabilities for non-essential heavy metals, making them potential hyperaccumulators for green remediation techniques in toxic soil environments. Understanding the molecular mechanisms driving these responses and validating phytoremediation potential in real-world scenarios is essential for developing sustainable soil management practices.

---

* Corresponding author.
E-mail address: adzrin.asikin@gmail.com (A.A. Zunaidi).

https://doi.org/10.1016/j.heliyon.2024.e29528
Received 18 September 2023; Received in revised form 8 April 2024; Accepted 9 April 2024
Available online 16 April 2024

1. Introduction

Heavy metals pose serious risks to plants and humans due to their high toxicity and persistence in natural ecosystems [1–3]. Their presence in plants can disrupt vital biochemical processes such as nutrient homeostasis, antioxidant and enzyme production, gas exchange components, and photosynthesis [4]. Various remediation techniques, including phytoremediation, chemical and physical methods, and microbial remediation, have been employed to mitigate heavy metal contamination in soil [5]. Phytoremediation, recognized as a cost-effective green technique, involves using plants to absorb and accumulate contaminants, such as heavy metals, through their root systems, effectively removing them from the environment [6,7]. Commonly employed phytoremediation procedures include phytodegradation, phytoextraction, phytostabilisation, phytovolatilisation, and rhizofiltration [8].

In phytoremediation, assessing different plant species' properties is crucial for understanding their tolerance and accumulation abilities against specific contaminants [9]. Phytotolerance analyses are essential to gauge metal tolerance levels, investigating the negative impacts of heavy metals on morphological, physiological, and biochemical activities in plants [10]. Monitoring stress tolerance during seed germination and seedling stages is particularly valuable, given the vulnerability of seeds to toxic environments compared to the vegetative stage [11,12]. Evaluating metal stress in seedlings provides insights into their growth performance under toxic conditions. Numerous studies have highlighted the effects of heavy metal-contaminated soils on germination, often resulting in reduced performance or seedling damage in various plant species [13–16]. These effects can, in turn, impact physicochemical processes such as enzyme activities and photosynthesis [4,17].

Some recognized phytoaccumulators, like *Ariplex halimus* L. and *Halimione portulacoides* Aellen, are deemed suitable for accumulating cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg) and lead (Pb), contributing to phytostabilization in saline soils [18–22]. These hyperaccumulator plants release protons from their roots, increasing soil acidity and facilitating metal ion mobility, thereby enhancing metal uptake into roots and shoots [5]. Ideal hyperaccumulating plants exhibit traits such as high heavy metal concentrations, tolerance to heavy metals, adaptability to toxic environments, fast growth, high biomass, and harvestability [23]. Efficient metal translocation from roots to shoots is also crucial. Currently, only a few plant species, including those from Asteraceae, Brassicaceae, Caryophyllaceae, Cunoniaceae, Cyperaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, and Violaceae, meet these criteria [24–27]. Given that different plant species accumulate various contaminants, careful plant selection for phytoextraction, a key phytoremediation method for heavy metals in soils, is essential [28]. While some studies have explored the use of woody plants like *Populus tremula* and *Picea abies* for soil decontamination, their limited extraction and accumulation of heavy metals pose disadvantages, restricting their use for phytoextraction [29–31].

Several non-woody plants, including *Brassica* species like *Alyssum Bertolonii*, *Alyssum murale*, *Noccaeae caerulescens*, *Streptanthus polygaloides*, *Stanleya pinnata* and *Bornmuellera tymphaea*, are known as metal accumulators [32]. The potential application of *Brassica* species for phytoextraction of heavy metals is mainly due to their underlying tolerance to heavy metals [33]. *Brassica* species are

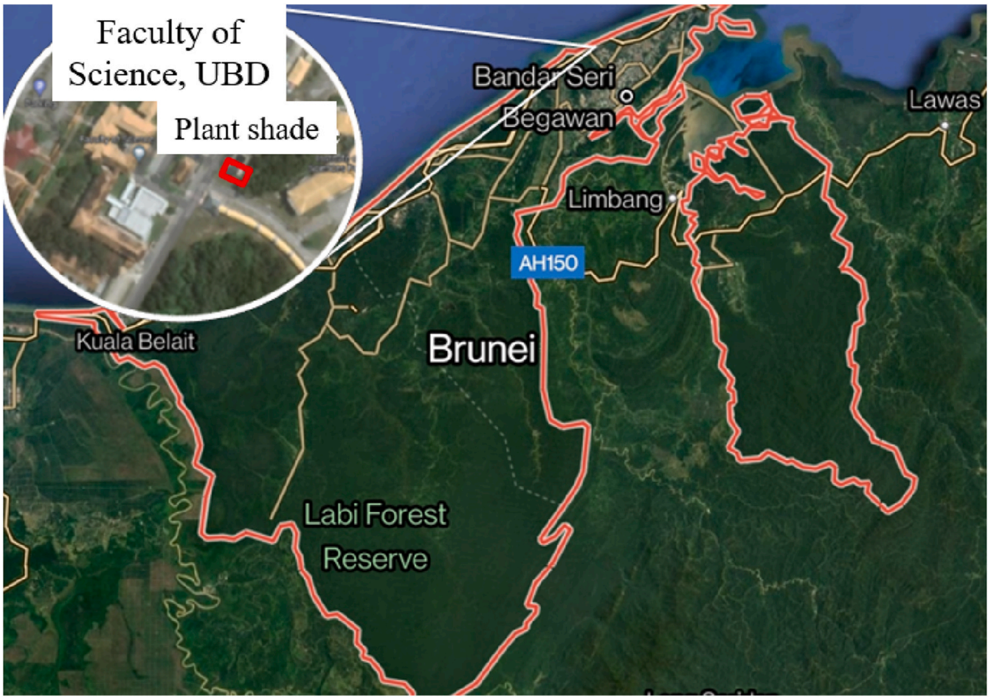

Fig. 1. Location of plant shade at the Faculty of Science, Universiti Brunei Darussalam, Brunei Darussalam (4°58′31.1″ N, 114°53′48.6″ E). Source: https://www.google.com/maps; Accessed 12th November 2023. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

valued for their ability to tolerate heavy metals, particularly *B. juncea*, *B. napus*, *B. oleracea*, *B. carinata* and *B. nigra*, which have shown high tolerance to toxic metal concentrations in soils [34,35]. Studies have demonstrated the accumulation of metals like Cu, nickel (Ni), Pb, and zinc (Zn) in *B. juncea* compared to *B. campestris*, *B. carinata*, *B. napus* and *B. nigra* in a field or pot experiment, indicating its potential for phytoextraction [36]. *B. juncea* accumulated Cd, cobalt (Co), Ni, Pb and Zn in leaves, stems and roots, but only Cu and manganese (Mn), as well as Cr, in the stems and roots based on the metal transfer factor of more than 1 [37]. *B. chinensis* accumulated higher levels of Cd, Cr and Pb in all plant parts than *B. rapa* in a pot study using agricultural soils [38]. Further research is needed to explore metal uptake and tolerance in other *Brassica* species.

This study aims to determine the tolerance and accumulation rates of two *Brassica* species (*Brassica chinensis* var. *parachinensis*, *B. chinensis* hereafter, and *Brassica rapa* L., *B. rapa* hereafter) to Cd, Cr, and Pb, selected due to their prevalence in Brunei Darussalam's agricultural soils [38]. These species were chosen for their common presence in Brunei's farmland and their known high uptake capacities for these metals [38,39]. The study assesses the phytotolerance of *B. chinensis* and *B. rapa* to various concentrations of heavy metal contaminants, alongside evaluating their effects on growth performance. The specific objectives are as follows: (1) to monitor the growth of the two *Brassica* species from seed germination to mature stage at different concentrations of selected heavy metals contaminants (Cd, Cr and Pb), (2) to investigate the heavy metals tolerance levels of *B. chinensis* and *B. rapa*, and (3) to assess the phytoremediation potential of using *B. chinensis* and *B. rapa* to accumulate heavy metals in soils.

2. Materials and methods

2.1. Study area

The pot experiment with *B. chinensis* and *B. rapa* took place within a plant shade (6 m long x 4 m wide x 2 m high) which is located at the Faculty of Science, Universiti Brunei Darussalam, situated in Brunei Darussalam (4°58′31.1″ N, 114°53′48.6″ E) on the north-west side of the Borneo Island in Southeast Asia (Fig. 1). The plant shade was covered with a black shade netting (50 % light transmission) on the top and sides to shield the growing vegetables from direct sunlight, which could impede their early growth and survival. Additionally, a transparent plastic roof was used to cover the top of the plant shade, ensuring that the experiment remained unaffected by rainfall.

Brunei Darussalam experiences a humid equatorial climate. Throughout our study's growth phases from December 2021 to February 2022, the average monthly temperature ranged from 25.6 °C to 28.7 °C, with an average of 27.3 °C, while the average monthly rainfall varied from 2.1 mm to 31 mm, averaging at 8.7 mm (Brunei Darussalam Meteorological Department, unpublished data). Fig. 2 illustrates the average weekly temperature (°C) and rainfall (mm) over an 8-week period of this study.

2.2. Study species, sampling and sample pre-treatment

The chosen plant species, *B. chinensis* and *B. rapa*, are annual herbaceous plants which are known for their potential in soil heavy metal phytoremediation [40]. These *Brassica* species are versatile, with edible leaves and stems that can be consumed cooked or raw, and they are also utilized for seed oil production and as green manure [35,41]. Seeds of *B. chinensis* and *B. rapa* utilized in this study were sourced from local markets in Brunei Darussalam.

In July 2021, agricultural topsoil (120 kg) was collected from 10 random locations at the Bukit Agok agricultural farmland, located in the Brunei-Muara district of Brunei Darussalam (4°54′58.9″N, 114°47′44.4″E). These soil samples were carefully stored in labeled plastic basins and transported to the laboratory for pre-treatment and analysis. Before any treatment, the soil samples were air-dried and sieved through a 2 mm mesh sieve to remove stone pebbles and plant litter. Following drying, the soil samples ($n = 10$) were analyzed for physicochemical properties with triplicate reading. The soil analyses included texture classification [42], moisture content [43], pH [44], electrical conductivity [45], total organic carbon (OC) and organic matter (OM) content [46], total N, P, K, Ca, Mg and Na [47] and cation exchange capacity (CEC) [48]. The summarized data is presented in Table 1.

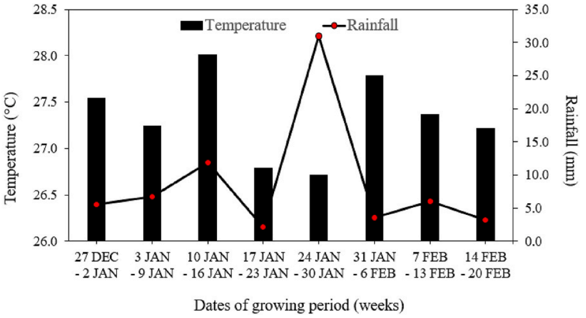

Fig. 2. Average weekly temperature (°C) and rainfall (mm) during the growth period of *Brassica chinensis* var. *parachinensis* and *Brassica rapa* L. from December 2021 to February 2022. Data was sourced from the Brunei Darussalam Meteorological Department (BDMD), Ministry of Transport and Infocommunication, Brunei Darussalam, located approximately 4 km from the study site.

In this study, a total of 30 polyethylene pots (22.5 cm diameter × 20.0 cm height) were arranged inside the plant shade. Following soil analysis, the soils were mixed and bulked. About 3 kg of air-dried soil was then distributed into each pot, spiked with uniform concentrations of Cd, Cr, and Pb at four levels: 75 mg/kg (T1), 150 mg/kg (T2), 225 mg/kg (T3), and 300 mg/kg (T4) of Cd ($CdCl_2$), Cr ($CrCl_3.6H_2O$), and Pb ($Pb(CH_3COO)_2.3H_2O$) as heavy metal pollutants, with triplicates for each treatment. The chosen concentration range is aligned with the maximum allowable limits set by the United States Environmental Protection Agency (USEPA) [50].

Soil spiking in each pot involved preparing individual concentration for each metal (Cd, Cr and Pb), followed by mixing to create the treatment concentrations. This was achieved by adding precise amounts of Cd, Cr and Pb salts dissolved in distilled water into each pot containing 3 kg of soil. Thorough mixing of the soils was conducted using clean plastic trowels, followed by a 2-week period for drying and stabilization at room temperature. This procedure was based on a modified protocol derived from Mkumbo [50] and Amin et al. [51]. Control pots (T0) were also set up with no addition of pollutants. The pot experiment involving varying concentrations of Cd, Cr, and Pb served to systematically explore the responses of *B. chinensis* and *B. rapa* to different levels of heavy metal contamination. This approach established a dose-response relationship, allowing researchers to identify threshold levels impacting germination, growth rates, and metal accumulation. By simulating realistic local soil conditions in Bukit Agok, Brunei Darussalam, the experiment enables the assessment of species-specific responses and evaluates the phytoremediation potential of these *Brassica* species. The controlled environment of the pot experiment enhances the reliability of observations, providing valuable insights into the adaptability and suitability of these plants for sustainable soil management practices.

The pots were arranged in a completely randomized design within the plant shade, resulting in 15 pots (3 pots x 5 treatments) for each species. Each pot was planted with a total of 20 seeds, yielding 60 seeds per treatment for each species. Germination progress for both the *Brassica* species was observed daily until day 10, with plants receiving irrigation of 100 mL distilled water three times weekly. After germination bioassay, seedlings were thinned to maintain three seedlings per pot, resulting in three *B. chinensis* or three *B. rapa* plants per pot. Daily weeding was performed to ensure the unhindered growth of the study species. Both *B. chinensis* and *B. rapa* typically germinated within 5 days and reached maturity in approximately 5 weeks (Fig. 3A0 – A4 and B0 – B4), following the BBCH scale which was used to assess the principal growth stages of the two species under various soil treatments, they can be described using numbers in ascending order [52]. In this experiment, no fertilizer was applied to ensure that the spiked soils remained the sole source of pollutants. After 60 days, both species were harvested in February 2022. Plants from each pot were bulked for chemical analysis in triplicates, resulting in three samples per treatment per species.

2.3. Data analysis

Seven plant growth measurements were conducted for each *Brassica* vegetable to assess their tolerance levels and ecotoxicological effects under varied soil treatments. These measurements included germination percentage, relative growth rate, stress tolerance index, fresh biomass, edible index, leaf area, and maximum root length.

Table 1
Physicochemical properties of agricultural soils at Bukit Agok, Brunei Darussalam, utilized in the pot experiments for assessing heavy metal tolerance in *Brassica* species. Values represent the mean with standard deviation (SD) for $n = 10$.

Soil parameters	Soil samples
Soil classification*	Nudinatric Solonetz
Textural class	Sandy loam
Sand (%) (0.05–2.00 mm)	52.4 (2.40)
Silt (%) (0.002–0.050 mm)	39.8 (2.40)
Clay (%) (<0.002 mm)	7.80 (0.60)
% moisture	15.3 (2.10)
pH	6.50 (0.02)
Electrical conductivity (dS/m)	0.16 (0.00)
Total organic C (%)	5.80 (1.10)
Organic matter (%)	9.90 (1.90)
Total N (mg/kg)	3195 (836)
Total P (mg/kg)	983 (63.1)
Ca (cmol/kg)	85.8 (27.4)
K (cmol/kg)	104 (45.4)
Mg (cmol/kg)	16.0 (3.20)
Na (cmol/kg)	136 (17.4)
CEC (cmol/kg)	342 (50.7)
Cd (mg/kg)	0.85 (0.77)
Cr (mg/kg)	1.64 (0.84)
Pb (mg/kg)	18.7 (1.62)

* Soil classification is based on the World Reference Base for Soil Resources [49].

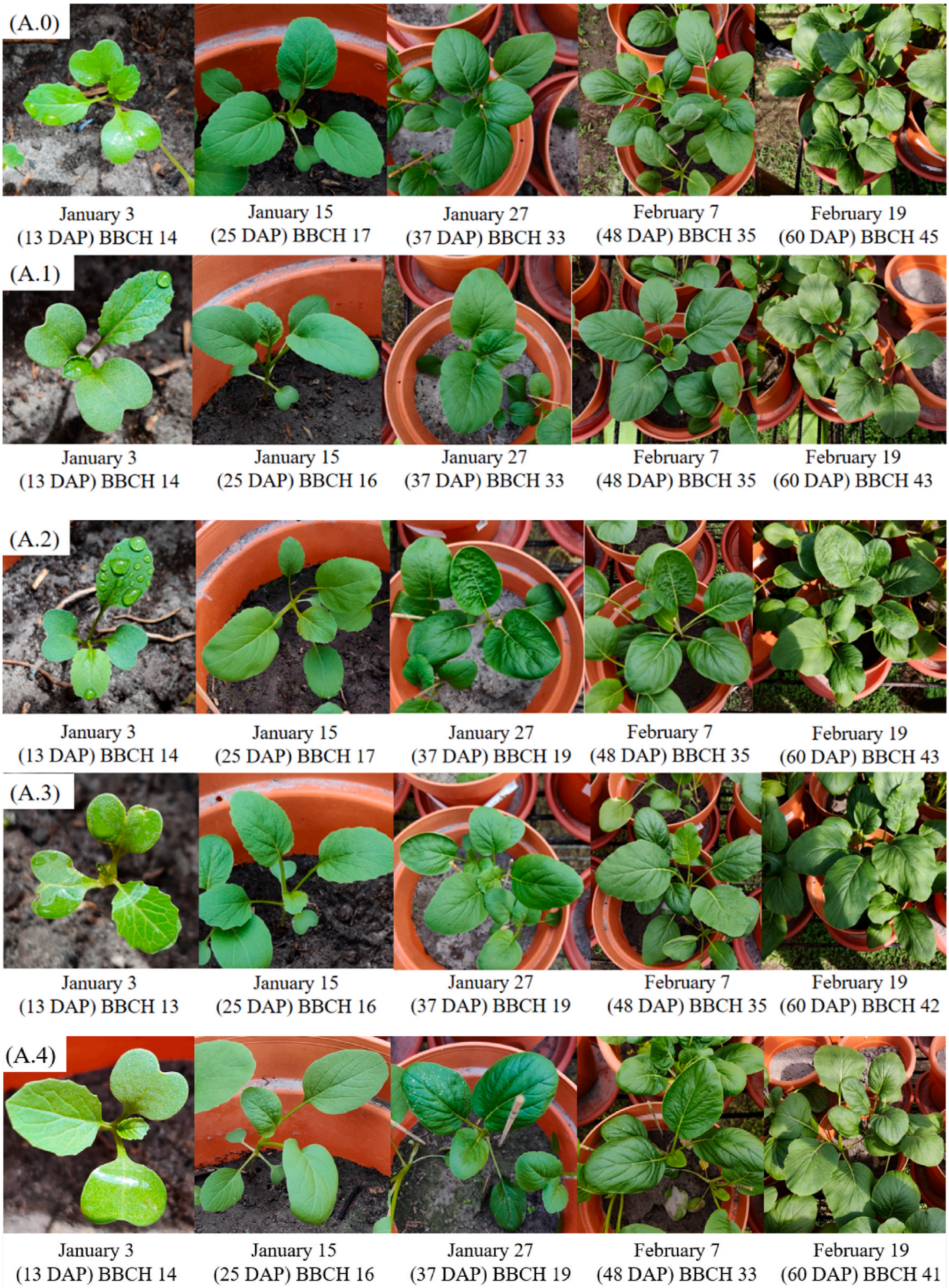

Fig. 3. Phenological stages of (A): *Brassica chinensis* var. *parachinensis* at different heavy metal treatments at different days after planting (DAP) – A.0: Control or Treatment 0, A.1: Treatment 1, A.2: Treatment 2, A.3: Treatment 3 and A.4: Treatment 4, and (B): *Brassica rapa* L. at different treatments – B.0: Control, B.1: Treatment 1, B.2: Treatment 2, B.3: Treatment 3 and B.4: Treatment 4, according to the BBCH scale [52]: 12 (second true leaf unfolded), 13(third true leaf unfolded), 14 (fourth true leaf unfolded), 16 (sixth true leaf unfolded), 17 (seventh true leaf unfolded), 18 (eight true leaf unfolded), 19 (ninth true leaf unfolded), 33 (leaf rosette has reached 30 % of expected diameter), 35 (leaf rosette has reached 50 % of

expected diameter), 41 (10 % of the leaf mass typical of the variety reached), 42 (20 % of the leaf mass typical of the variety reached), 43 (30 % of the leaf mass typical of the variety reached), 44 (40 % of the leaf mass typical of the variety reached, 45 (50 % of the leaf mass typical of the variety reached) and 47 (70 % of the leaf mass typical of the variety reached).

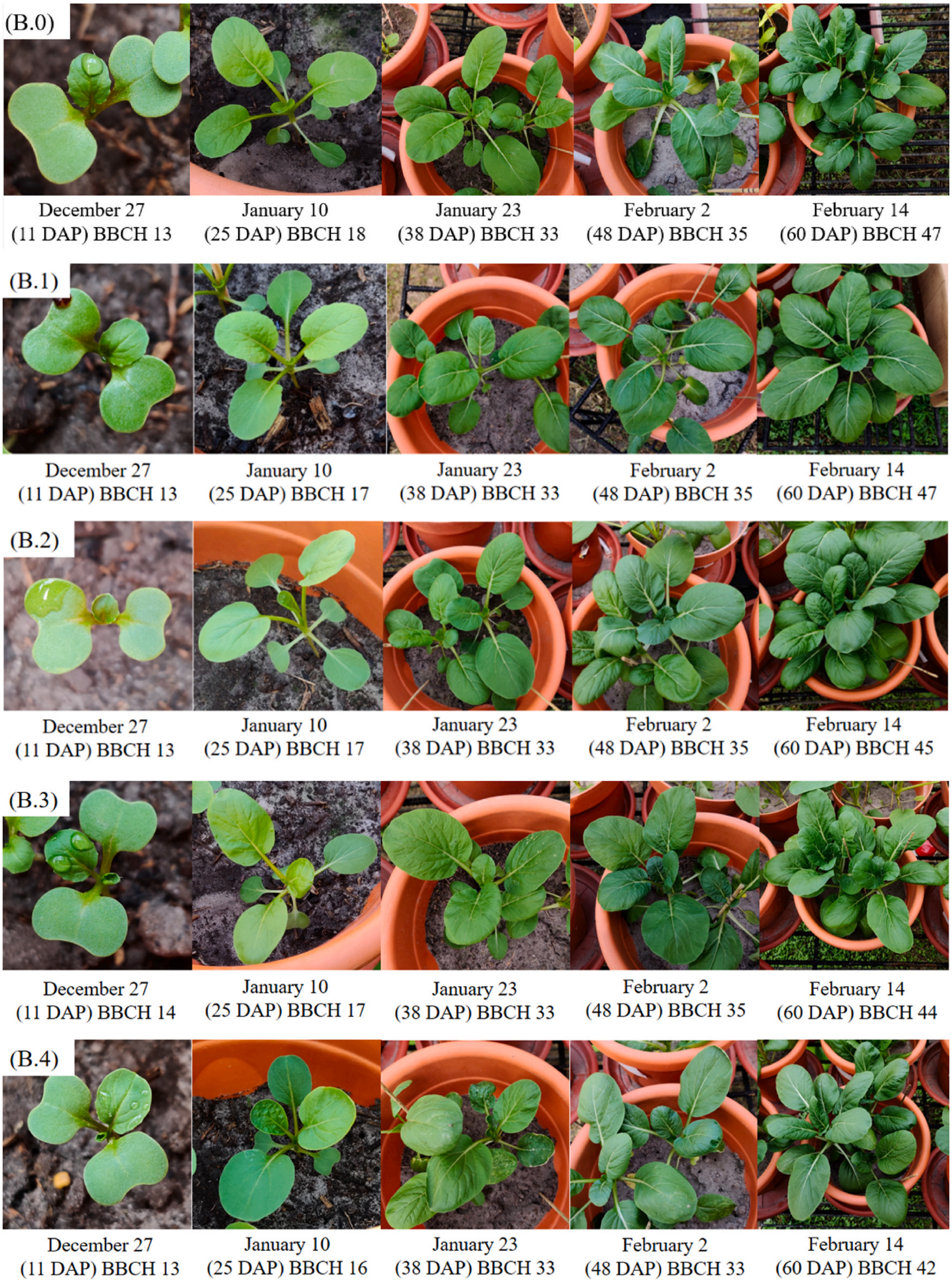

Fig. 3. (*continued*).

2.3.1. Germination percentage

The germination percentage (G%) of *B. chinensis* and *B. rapa* was determined by calculating the ratio of germinated seeds to the total number of viable seeds sown in each metal-spiked treatment, following Eq. (1) [53], observed over a 10-day period:

$$Germination\ percentage\ (G\%) = \frac{\mathrm{G}}{\mathrm{G_o}} x\ 100\% \qquad \text{(Eq. 1)}$$

where G and G_0 represent the number of germinated seeds in each treatment and the total number of seeds sowed in each treatment, respectively. In this study, 20 seeds were sown in each pot, resulting in a total of 300 viable seeds and 60 seeds per treatment for each species.

2.3.2. Relative growth rate of species

The relative growth rate (RGR) or efficiency index (EI) of both *B. chinensis* and *B. rapa* was assessed by measuring plant height under various metal-spiked soil treatments over a 60-day period, as per Eq. (2) [54]:

$$Relative\ growth\ rate\ (RGR) = \frac{\ln(\text{Final height (cm)}) - \ln(\text{Initial height (cm)})}{\text{Day of final measurement} - \text{Day of initial measurement}} \qquad \text{(Eq. 2)}$$

where Final height and Initial height are the heights of treated plant at the day of harvesting and the height of the treated plant during the initial measurement, respectively.

2.3.3. Stress tolerance index

The stress tolerance index (TI_{ph}) was evaluated for plant height by comparing the mean height of treated plants to that of control plants under various heavy metal treatments for both species. This comparison was conducted using Eq. (3) [55]:

$$Stress\ tolerance\ index\ (TIph) = \frac{\text{Height of treated plant (cm)}}{\text{Height of control plant (cm)}} x\ 100\% \qquad \text{(Eq. 3)}$$

2.3.4. Fresh biomass and edible index

Fresh biomass from each plant part (leaves, stems and roots) was determined from both the harvested species. Additionally, the edible index was calculated to assess the edibility of the plants grown in various metal-spiked soil treatments. This calculation involved measuring the total fresh biomass of the species and the fresh biomass of the edible plant parts (leaves and stems), following the method outlined by Mi et al. [56], as per Eq. (4):

$$Edible\ rate\ of\ plant\ (ER) = \frac{\mathrm{FW_e}}{\mathrm{FW}} \qquad \text{(Eq. 4)}$$

where FW and FW_e are the total fresh biomass of plant species and the edible fresh biomass of plants, such as leaves and stems, respectively. The calculation is based on the percentage ratio of FW_e to FW.

2.3.5. Leaf area and root length

Leaf area (LA) and root length measurements were conducted to evaluate the potential impact of heavy metals on plant growth across various metal-spiked soil treatments. LA was determined by multiplying the maximum length (L) and width (W) of a leaf, as outlined in Eq. (5) [57]:

Table 2
Operating conditions, parameters, limit of detection (LOD) and limit of quantification (LOQ) of Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) used for analyzing heavy metal concentrations in *Brassica* species samples collected from pot experiments.

ICP-OES parameter	Elements		
	Cd (214.4)	Cr (283.5),	Pb (220.3)
LOD (ppb)	7.0	2.0	4.0
LOQ (ppb)	23.0	6.0	12.0
Output mode	Intensity		
Plasma viewing mode	Axial		
Spray chamber type	Glass cyclonic spray chamber		
Instrument repeats	3		
RF power	1150 W		
Analysis pump rate	50 rpm		
Auxiliary gas flow rate	0.5 L/min		
Nebulizer gas flow rate	0.7 L/min		
Flush pump rate	100 rpm		
Pump stabilization time	15 s		

$$Leaf\ area\ (LA) = Maximum\ leaf\ length\ (L) \times Maximum\ leaf\ width\ (W) \quad \text{(Eq. 5)}$$

The root length was estimated by measuring the maximum length of the primary or main root, from the base to the root tip using a standard ruler.

2.4. Analytical procedures

The extraction procedure used for analyzing heavy metals in *B. chinensis* and *B. rapa* samples was based on the USEPA method 3050B [58]. Each plant sample was divided into leaves, stems and roots and analyzed separately to investigate the accumulation of spiked heavy metals. Plant samples were oven-dried at 105 °C for 2 h to eliminate moisture [39]. Subsequently, the dried samples were homogenized and ground using a pestle and mortar before passing through a 2 mm sieve.

Oven-dried plant samples (1 g) were digested with 15 mL HNO_3 (Sigma Aldrich, ACS reagent, 65 %), 10 mL H_2O_2 (Sigma Aldrich, 30 %) and 10 mL HCl (Sigma Aldrich, ACS reagent, 35.4 %) for 2 h at 95 ± 5 °C. Following digestion, the samples were cooled down to *ca.* 24 °C and then diluted with distilled water. Subsequently, each sample was analyzed for Cd, Cr and Pb concentrations using an inductively coupled plasma-optical emission spectroscopy (ICP-OES Thermo Scientific iCAP 6000 Series, USA). Blank samples were prepared alongside each sample, and the instrument's accuracy was verified by analyzing the samples in triplicates. The operating conditions for ICP-OES used in this study are summarized as shown in Table 2.

2.5. Statistical analysis

Statistical analyses for all heavy metals and plant parameters in *B. chinensis* and *B. rapa* were performed using a one-way analysis of variance (ANOVA) and TukeyHSD tests at 5 % significance level using SPSS (IBM SPSS Statistics 22) [59]. Moreover, the heavy metal concentrations in both *Brassica* species were used to derive the principal component analysis (PCA) of metal elements across the three different plant parts using SPSS.

3. Results and discussion

3.1. Germination percentage

The study examined the germination percentage to gauge the viability and resilience of seeds in response to adverse environmental conditions induced by heavy metals. This bioassay aimed to offer insights into the early stages of growth and establishment of *B. chinensis* and *B. rapa* seeds under different metal-spiked soils (Fig. 4). In the control treatment, seeds of both species began to germinate around day 5 ± 1, while seeds in soils treated with metal exhibited a delay of at least 2 days (day 7 ± 1). This delay in germination time suggests the possibility of secondary induced dormancy in response to metal concentrations [60]. These current findings are consistent with those of Eze et al. [61], who similarly observed delayed germination in Cr-treated soils, with concentrations of up to 400 mg/kg compared to the control treatment, indicating that germination time increased with higher levels of metal contamination in soils.

The germination percentages of *B. chinensis* and *B. rapa* were significantly affected by the heavy metal treatments (Fig. 4). The results indicated that *B. chinensis* maintained a consistent germination percentage ranging from 70 to 76 % across various treatments, except at 300 mg/kg (Treatment 4), where it significantly decreased to 53 %. Although the difference in germination percentage

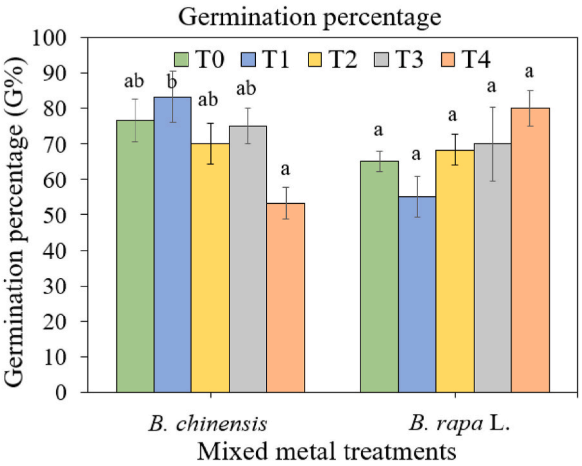

Fig. 4. Germination percentage (%) of *Brassica chinensis* var. *parachinensis* and *Brassica rapa* L. under different soil metal treatments: T0: control soil, T1: 75 mg/kg Cd, Cr and Pb, T2: 150 mg/kg Cd, Cr and Pb, T3: 225 mg/kg Cd, Cr and Pb, and T4: 300 mg/kg Cd, Cr and Pb. Different letters represent significantly different means for each species after analyzing using one-way ANOVA and TukeyHSD tests at 5 % significance level.

between the control and Treatment 4 was not significant, Treatment 4 exhibited a marked decrease compared to Treatment 2 (150 mg/kg Cd, Cr and Pb). Conversely, *B. rapa* displayed trend towards increased germination percentage, reaching levels up to 80 % at higher concentrations of Cd, Cr and Pb in the metal treatments, although these differences were not statistically significant.

The ANOVA results revealed contrasting germination percentages between the two *Brassica* species, highlighting their distinct sensitivity and tolerance to varying heavy metal concentrations in the soil [62]. This indicates that the germination of different plant seeds is influenced by different heavy metal concentrations [60]. The findings imply that elevated metal concentrations could impact the germination process of *Brassica* seeds. This study emphasizes the differential effects of heavy metal concentrations on the germination performance of *Brassica* seeds, consistent with findings from previous research [62].

3.2. Relative growth rate and stress tolerance index

The relative growth rate and stress tolerance index were assessed for both *Brassica* species to evaluate their growth performance under varying heavy metal treatments (Fig. 5). The relative growth rate offers a quantitative assessment of the species' overall growth performance under different soil treatments, reflecting how heavy metals influence their development from seedling to maturity. Similarly, the stress tolerance index offers valuable information regarding the species' ability to withstand adverse conditions caused by heavy metal toxicity. A decline in the stress tolerance index indicates a reduction in plant height relative to control conditions, highlighting the detrimental effects of heavy metals on growth and development. While both relative growth rate and stress tolerance index of *B. chinensis* (Fig. 5A and B) were significantly impacted by metal treatments, only the relative growth rate of *B. rapa* showed a significant effect (Fig. 5A). In the various spiked treatments, the relative growth rate of both species exhibited a decline in growth performance from 0 mg/kg (control) to increasing metal concentrations up to 300 mg/kg of Cd, Cr and Pb in the soil. Both *B. chinensis* and *B. rapa* displayed a consistent decrease in growth rate from 0.05 cm/day to 0.04 cm/day with rising concentrations. However, the reduction in the relative growth rate for 300 mg/kg Cd, Cr and Pb (Treatment 4) was significantly different from the control treatment (T0) for both species.

However, the stress tolerance index exhibited contrasting patterns between *B. chinensis* and *B. rapa* (Fig. 5B). *B. chinensis* displayed a steep decline in the stress tolerance index, plummerting from 100 % to 56.6 % in response to spiked treatments with 300 mg/kg metals, while the stress tolerance index for *B. rapa* remained relatively steady across various metal treatments. Only the stress tolerance index of *B. chinensis* under all metal treatments (T1 to T4) was significantly lower than T0 (control treatment), whereas this difference was not observed for *B. rapa*. This indicates that the relative growth rate of *B. chinensis* was significantly hindered by the presence of heavy metals, suggesting that metal stress was specific to this Brassica species, not *B. rapa*. *B. rapa* accumulated the highest concentrations of Cd and Pb, supporting the notion that it demonstrates high tolerance to elevated soil concentrations of Cd, Pb and Cr [38]. In *B. chinensis*, the transport of heavy metals from the roots to the shoots could disrupt cellular metabolism, contributing to the decline in growth rate and subsequent slowdown in growth performance [60]. Elevated concentrations of heavy metals in the soil have been shown to distress shoot development, affecting plant height, and potentially influencing root growth, thereby reducing nutrient and water uptake by the plants [63,64].

3.3. Fresh biomass and edible index

Fresh biomass of *B. chinensis* and *B. rapa* provided quantitative data on the total plant biomass, including the leaves, stems and roots (Fig. 6A and C), reflecting the overall growth and development of the species under different soil treatments. On the other hand, the edible index determined the proportion of edible biomass (leaves and stems) affected by varying levels of heavy metal contaminations (Fig. 6B and D). *B. chinensis* consistently exhibited higher total fresh biomass (18.2–22.6 g/plant) across all control and treated soils

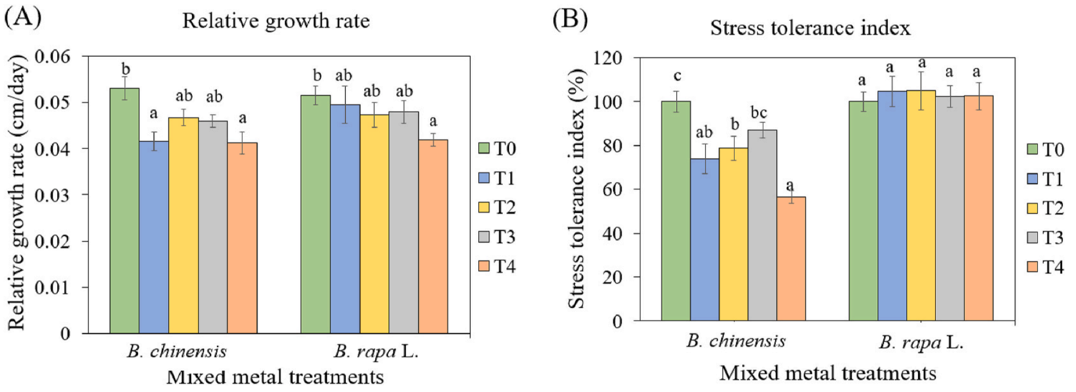

Fig. 5. (A) Relative growth rate (RGR) and (B) stress tolerance index (TI_{ph}) of *Brassica chinensis* var. *parachinensis* and *Brassica rapa* L. under different soil metal treatments: T0: control soil, T1: 75 mg/kg Cd, Cr and Pb, T2: 150 mg/kg Cd, Cr and Pb, T3: 225 mg/kg Cd, Cr and Pb, and T4: 300 mg/kg Cd, Cr and Pb. Different letters represent significantly different means for each species following statistical analysis using one-way ANOVA and TukeyHSD tests at 5 % significance level.

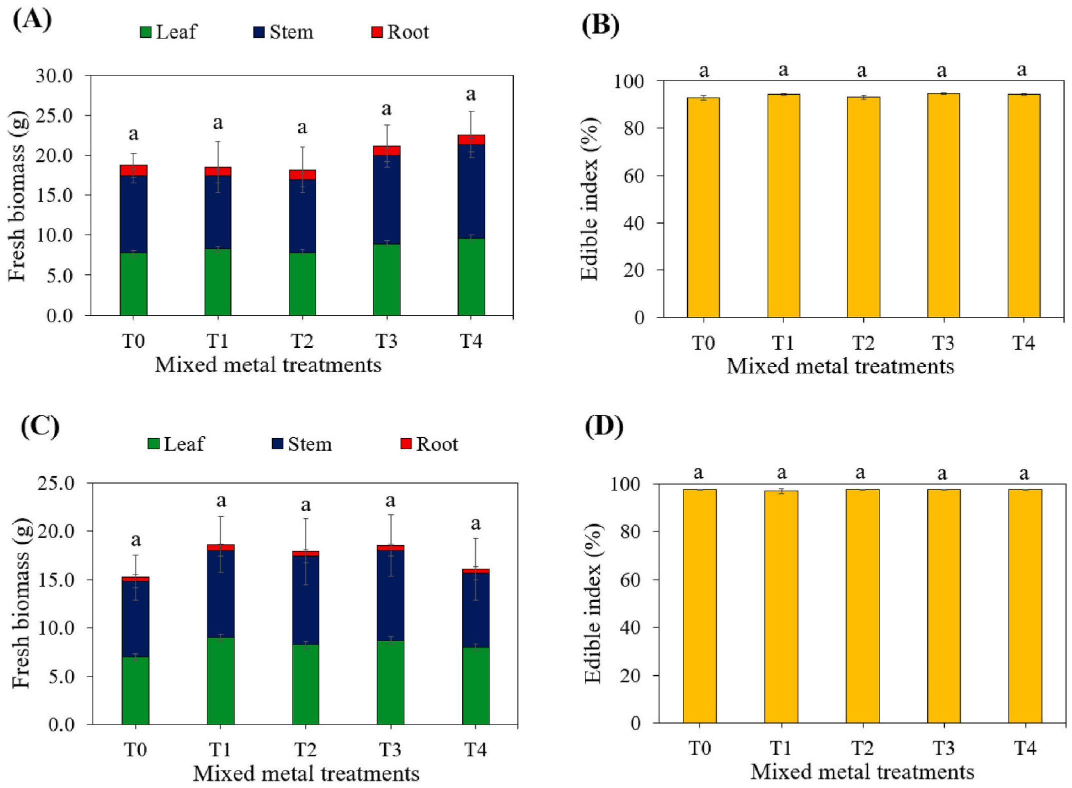

Fig. 6. Fresh biomass and edible indices of (A–B) *B. chinensis* var. *parachinensis*, respectively, and (C–D) *B. rapa*, L., respectively, under different spiked soil treatments: T0: control soil, T1: 75 mg/kg Cd, Cr and Pb, T2: 150 mg/kg Cd, Cr and Pb, T3: 225 mg/kg Cd, Cr and Pb, and T4: 300 mg/kg Cd, Cr and Pb. Different letters represent significantly different means for each species after analyzing using one-way ANOVA and TukeyHSD tests at 5 % significance level.

compared to *B. rapa* (15.2–18.6 g/plant). Furthermore, *B. chinensis* yielded greater root biomass (1.06–1.33 g/plant) across the various spiked treatments compared to *B. rapa* (0.38–0.58 g/plant), leading to a higher edible index of over 97.0 % in *B. rapa* compared to the edible index in *B. chinensis* (92.9–94.8 %). However, this study observed insignificant differences in the fresh biomass of leaf, stem and root parts, as well as edible indices of both species under various soil treatments. The observed decline in relative growth rate in both species (Fig. 5A) and stress tolerance index in *B. chinensis* did not correlate with a decrease in fresh biomass and edible index. It is possible that dry biomass could have been influenced by metal concentrations, as reported by studies by Amin et al. (2013). Unfortunately, this study did not evaluate dry biomass. Overall, these findings suggest that the two *Brassica* species, particularly *B. rapa*,

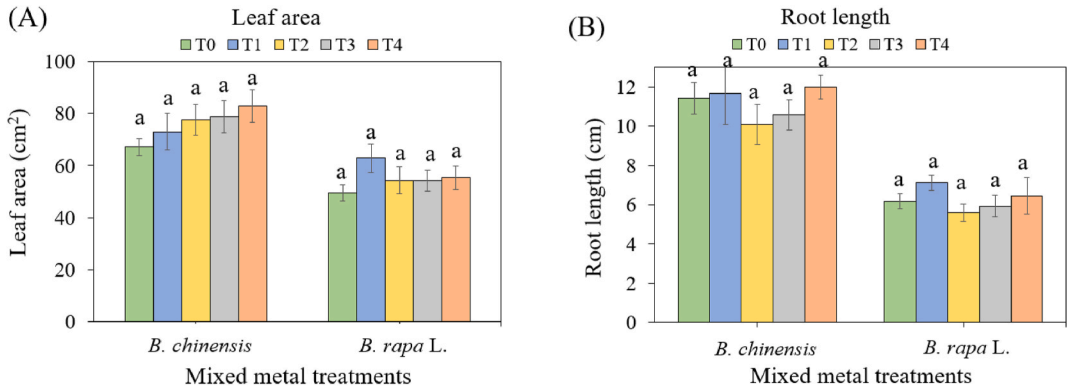

Fig. 7. (A) Leaf areas and (B) root lengths of *Brassica chinensis* var. *parachinensis* and *Brassica rapa* L. under different soil metal treatments: T0: control soil, T1: 75 mg/kg Cd, Cr and Pb, T2: 150 mg/kg Cd, Cr and Pb, T3: 225 mg/kg Cd, Cr and Pb, and T4: 300 mg/kg Cd, Cr and Pb. Different letters represent significantly different means for each species following analysis using one-way ANOVA and TukeyHSD tests at 5 % significance level.

might demonstrate tolerance to Cd, Cr and Pb heavy metals (Figs. 4–6), implying their potential ability to thrive in toxic soil environments [60].

3.4. *Leaf area and root length*

The average leaf areas and root lengths of *B. chinensis* and *B. rapa* were measured to investigate the effects of heavy metals on the physiological growth of *Brassica* plants and the adaptation of species to grow effectively in contaminated environments (Fig. 7A and B). Leaf areas for *B. chinensis* and *B. rapa* showed an increase of 23.5 % and 11.8 %, respectively, in the highest soil treatment compared to the control soils, although these differences were not statistically significant (Fig. 7A). While there were no significant differences in leaf areas of *B. chinensis*, a trend toward larger sizes was observed with increasing soil-metal treatments, up to 300 mg/kg metals. Conversely, consistent leaf areas were observed for *B. rapa* across different soil treatments. No visible signs of toxicity were detected on the leaves of either species, suggesting potential tolerance or adaptive mechanisms to high metal concentrations, particularly in *B. rapa* [65].

The average maximum root lengths for *B. chinensis* and *B. rapa* across all soil treatments were 11.2 cm and 6.26 cm, respectively (Fig. 7B). However, neither species exhibited symptoms of metal contamination, and there were no significant differences in root lengths from the control soil (T0) to the highest soil treatment (T4). Additionally, there were no notable differences between root lengths for both species across different soil treatments, indicating their ability to grow and absorb nutrients normally under stress conditions. Despite a significant reduction in the relative growth rate for both species, this did not translate in significant differences in leaf areas and root lengths, mirroring the findings on fresh biomass and edible index. In theory, a decline in the relative growth rate should negatively impact fresh biomass, edible index, leaf area and root length [51]. Leaf area development and plant growth are typically influenced by the division and enlargement of plant cells, processes that could adversely affected by the presence of Cd and Pb [66]. However, the stimulatory effect of Cd in leaves could be attributed to the plants' defense mechanism, where they synthesize stress proteins and secondary metabolites, alter antioxidant enzyme activity and reduce oxidative stress [67–69]. These results suggest that both *B. chinensis* (to a certain extent, given its low stress tolerance index) and *B. rapa* may exhibit tolerance to metal stress conditions, making them potential candidates for soil remediation techniques.

3.5. *Heavy metals in plant species*

Table 3 presents the concentrations of heavy metals in various plant parts (leaf, stem and root) of *B. chinensis* and *B. rapa* across different soil treatments (0 mg/kg – 300 mg/kg Cd, Cr and Pb). Heavy metal contents in different parts of *Brassica* plants were measured to understand how these plants absorb and accumulate toxins from the soil, assessing their ability to tolerate and potentially remediate contaminated environments. However, the duration of the pot experiment was only 60 days, which is relatively short to fully capture the long-term effects of heavy metal exposure on plant growth and development. Among the soil treatments (T1 – T4), *B. rapa* exhibited the highest accumulation of Cd, with 864 mg/kg Cd in root of T4, compared to *B. chinensis*, which accumulated 739

Table 3
Mean heavy metal concentrations (standard deviation, SD) in the different plant parts (leaf, stem and root) of *Brassica chinensis* var. *parachinensis* and *Brassica rapa* L. Different letters per heavy metal (row) represent significantly different means for the different parts (leaf, stem, and root) for each species following analysis using one-way ANOVA and TukeyHSD tests at 5 % significance level.

Heavy metals (mg/kg), dry weight	*B. chinensis*								
	Cd			Cr			Pb		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Control (T0)	29.0^{b} (19.7)	6.47^{a} (1.92)	14.7^{ab} (4.34)	17.3^{b} (6.76)	9.95^{a} (1.96)	38.5^{c} (3.19)	72.2^{b} (22.5)	44.2^{a} (4.50)	169^{c} (30.1)
Treatment 1 (T1)	47.5^{a} (17.6)	31.5^{a} (5.99)	139^{b} (35.6)	21.1^{a} (6.41)	16.3^{a} (2.65)	84.6^{b} (13.3)	123^{b} (16.7)	61.7^{a} (14.1)	314^{c} (44.5)
Treatment 2 (T2)	65.0^{a} (11.8)	52.4^{a} (8.39)	343^{b} (129)	19.1^{a} (10.1)	24.5^{a} (2.78)	164^{b} (49.3)	202^{b} (23.0)	86.2^{a} (10.8)	542^{c} (58.6)
Treatment 3 (T3)	92.3^{a} (19.9)	69.1^{a} (16.3)	456^{b} (169)	22.1^{a} (8.66)	33.6^{a} (2.64)	130^{b} (37.7)	279^{b} (22.4)	116^{a} (8.90)	620^{c} (53.0)
Treatment 4 (T4)	106.5^{a} (35.4)	78.7^{a} (16.4)	739^{b} (150)	39.9^{a} (12.9)	44.5^{a} (5.51)	161^{b} (37.2)	406^{b} (35.2)	152^{a} (19.2)	953^{c} (83.7)
B. rapa									
Control (T0)	62.7^{a} (43.1)	13.1^{a} (6.65)	10.8^{a} (10.4)	12.4^{a} (2.89)	4.66^{a} (2.11)	32.4^{b} (12.2)	80.5^{b} (25.1)	54.9^{ab} (11.5)	23.6^{a} (29.1)
Treatment 1 (T1)	86.5^{a} (48.3)	47.4^{a} (25.5)	115^{a} (63.4)	13.2^{a} (7.15)	19.1^{a} (4.55)	72.0^{b} (17.6)	105^{ab} (21.2)	84.5^{a} (10.3)	125^{b} (35.0)
Treatment 2 (T2)	165^{a} (54.8)	125^{a} (11.9)	409^{b} (70.5)	30.8^{a} (12.4)	29.9^{a} (2.08)	130^{b} (34.1)	243^{b} (84.9)	99.9^{a} (6.09)	270^{b} (71.2)
Treatment 3 (T3)	205^{a} (47.7)	219^{a} (61.1)	603^{b} (67.6)	30.8^{a} (10.5)	36.3^{a} (6.40)	147^{b} (37.6)	290^{b} (32.5)	106^{a} (8.50)	417^{c} (48.9)
Treatment 4 (T4)	273^{a} (75.3)	258^{a} (39.2)	864^{b} (383)	18.0^{a} (9.42)	40.6^{b} (2.50)	132^{c} (23.1)	340^{b} (19.5)	123^{a} (9.52)	478^{c} (42.2)

mg/kg Cd in root of T4. Notably, Cd tended to accumulate significantly in the roots of both species across all treatments, except in the control soils where no significant differences were observed. Previous studies have shown that Cd tends to distribute evenly across all plant parts in various species under natural conditions [38]. The sequestration of Cd in root vacuoles, particularly when exposed to high soil Cd levels, suggests the presence of Cd transporters as detoxification mechanisms in these *Brassica* species. These mechanisms facilitate the uptake and localization of Cd, minimizing its exposure in shoots [70–72]. Under toxic conditions, root vacuoles play a crucial role in storing ions and metabolites, contributing to the detoxification process and normal cell development [73]. These findings underscore the potential and tolerance of *Brassica* species, particularly *B. rapa*, to germinate and thrive in toxic environments.

The patterns of Cr contents in both *B. chinensis* and *B. rapa* reflected the accumulations of Cd in different plant parts (leaf, stem and root) across the various soil metal treatments, with Cr significantly accumulating in the root part of both species. There were no significant differences observed between the leaf and stem parts in both the *Brassica* species, indicating minimal Cr uptake in the aerial parts compared to the roots. Both species accumulated similar amounts of Cr in different plant parts, with *B. chinensis* and *B. rapa* accumulating the highest amounts in the root parts at 161 mg/kg and 132 mg/kg, respectively. Cr, being a non-essential heavy metal, did not have any known biological function in plant physiology, leading to the absence of a specific uptake mechanism for Cr in plants [74,75]. The translocation and distribution of Cr are influenced by factors such as plant species, Cr concentration in the growth medium and its oxidation state and due to its low mobility in plant roots, the concentration of Cr is 100 times higher in roots than in aerial parts of plants [62,76,77]. In this study, Cr concentration was observed to be the highest in the roots, which could be attributed to the sequestration of Cr in the vacuoles of root cells as a protective mechanism [78], thereby naturally enhancing the tolerance of the two species to Cr toxicity [79].

Significant differences were observed in the various plant parts (leaf, stem and root) of *B. chinensis* and *B. rapa* (Table 3), with notable Pb accumulation detected in both the leaf and root parts of both species. This suggests that both species possess the capability to accumulate substantial amounts of Pb in their aerial parts. At spiked Pb concentrations of 300 mg/kg in soil (T4), both *B. chinensis* and *B. rapa* accumulated up to 406 mg/kg and 340 mg/kg Pb, respectively (Table 3), in the leaf part of the plants, indicating the high mobility of Pb in *Brassica* species. *B. chinensis* exhibited the highest Pb content (953 mg/kg Pb) in the root part of the plant compared to *B. rapa*. Some *Brassica* species may regulate the uptake of highly mobile Pb through intracellular detoxification involving sequestration and cell binding in plant cells to reduce the translocation of Pb to the aerial parts of the plants [62,80–82]. Although Pb tends to highly mobile at lower soil pH and is typically found in water-soluble complexes such as $CdSO_4$ compounds, $CdCl^+$, $CdHCO_3^+$ cations and Pb^{2+} [83,84], this study demonstrated that Pb could readily accumulate in the plant parts of *Brassica*. These findings suggest that both *Brassica* species have the potential to accumulate significant amounts of Cd, Cr and Pb and could be cultivated in contaminated soil environments containing up to 300 mg/kg of heavy metals. Future studies could also assess the potential impacts of heavy metal contamination on soil microbial communities, which could play a crucial role in nutrient cycling and plant health, to further understand the limitations of these species.

4. Conclusion

Previous studies have highlighted the phytoremediation potential of these species, but their specific responses to heavy metal treatments based on their morphological, physiological and biochemical traits have been relatively understudied. The findings indicate that *B. rapa* and, to some extent, *B. chinensis* possess the capacity to accumulate significant amounts of non-essential heavy metals (Cd, Cr and Pb) from soils, reflecting their tolerance to toxic soil conditions, which is crucial for soil remediation efforts. Germination percentages for both the *Brassica* species were consistent across various soil treatments, albeit with delays in more toxic environments. The relative growth rate notably decreased at higher soil treatments, highlighting the impact of heavy metals on *Brassica* growth rate, particularly evident at 300 mg/kg Cd, Cr and Pb concentrations. The stress tolerance index suggested a substantial decline in *B. chinensis'* plant heights compared to controls, whereas *B. rapa* exhibited stability, suggesting its resilience to toxic conditions. Despite insignificant differences due to varied metal treatments, *B. chinensis* exhibited higher fresh biomass compared to *B. rapa*, with B. chinensis also displaying greater root biomass. Leaf areas increased with higher soil treatments for both species, while root lengths showed no significant differences, indicating their tolerance to high metal concentrations. *B. rapa* accumulated the highest amount of Cd (864 mg/kg Cd), while *B. chinensis* accumulated the most Pb (953 mg/kg Pb), primarily in the root parts. Both species similarly accumulated Cr in the roots. The hypothesis underlying this research posited that *B. chinensis* and *B. rapa* would exhibit varying degrees of tolerance and accumulation of Cd, Cr, and Pb in response to increasing concentrations of selected heavy metals in soil. Further investigations through long-term effects field studies can provide insights into the effects of heavy metal contamination on both soil and plant health in real-world agricultural settings. The study suggests that both *Brassica* species, particularly *B. rapa*, exhibit high tolerance to non-essential heavy metals and can thrive in toxic soil conditions, potentially serving as hyperaccumulators for green remediation techniques.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Adzrin Asikin Zunaidi: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lee Hoon Lim:** Writing – review

& editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization. **Faizah Metali:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

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.

References

[1] I. Sanjosé, F. Navarro-Roldán, M.D. Infante-Izquierdo, G. Martínez-Sagarra, J.A. Devesa, A. Polo, S. Ramírez-Acosta, E. Sánchez-Gullón, F.J. Jiménez-Nieva, A. F. Muñoz-Rodríguez, Accumulation and effect of heavy metals on the germination and growth of salsola vermiculata l, seedlings, Diversity. 13 (2021) 1–15, https://doi.org/10.3390/d13110539.

[2] X. Shen, M. Dai, J. Yang, L. Sun, X. Tan, C. Peng, I. Ali, I. Naz, A critical review on the phytoremediation of heavy metals from environment: performance and challenges, Chemosphere 291 (2022) 132979, https://doi.org/10.1016/j.chemosphere.2021.132979.

[3] M.A. Hassaan, A. El Nemr, F.F. Madkour, Environmental assessment of heavy metal pollution and human health risk, Am. J. Water Sci. Eng. 2 (2016) 14–19, https://doi.org/10.11648/j.ajwse.20160203.11.

[4] M. Seneviratne, N. Rajakaruna, M. Rizwan, H.M.S.P. Madawala, Y.S. Ok, M. Vithanage, Heavy metal-induced oxidative stress on seed germination and seedling development: a critical review, Environ. Geochem. Health 41 (2019) 1813–1831, https://doi.org/10.1007/s10653-017-0005-8.

[5] G. Wu, H. Kang, X. Zhang, H. Shao, L. Chu, C. Ruan, A critical review on the bio-removal of hazardous heavy metals from contaminated soils: Issues, progress, eco-environmental concerns and opportunities, J. Hazard, Mater 174 (2010) 1–8, https://doi.org/10.1016/j.jhazmat.2009.09.113.

[6] J. Suman, O. Uhlik, J. Viktorova, T. Macek, Phytoextraction of heavy metals: a promising tool for clean-up of polluted environment? Front. Plant Sci. 871 (2018) https://doi.org/10.3389/fpls.2018.01476.

[7] A. Yan, Y. Wang, S.N. Tan, M.L. Mohd Yusof, S. Ghosh, Z. Chen, Phytoremediation: a promising approach for revegetation of heavy metal-polluted land, Front. Plant Sci. 11 (2020) 1–15, https://doi.org/10.3389/fpls.2020.00359.

[8] M. Rigoletto, P. Calza, E. Gaggero, M. Malandrino, D. Fabbri, Bioremediation methods for the recovery of lead-contaminated soils: a review, Appl. Sci. 10 (2020), https://doi.org/10.3390/app10103528.

[9] H. Nouri, S. Chavoshi Borujeni, R. Nirola, A. Hassanli, S. Beecham, S. Alaghmand, C. Saint, D. Mulcahy, Application of green remediation on soil salinity treatment: a review on halophytoremediation, Process Saf. Environ. Protect. 107 (2017) 94–107, https://doi.org/10.1016/j.psep.2017.01.021.

[10] M.A. Jayasri, K. Suthindhiran, Effect of zinc and lead on the physiological and biochemical properties of aquatic plant Lemna minor: its potential role in phytoremediation, Appl. Water Sci. 7 (2017) 1247–1253, https://doi.org/10.1007/s13201-015-0376-x.

[11] J. Bae, D.L. Benoit, A.K. Watson, Effect of heavy metals on seed germination and seedling growth of common ragweed and roadside ground cover legumes, Environ. Pollut. 213 (2016) 112–118, https://doi.org/10.1016/j.envpol.2015.11.041.

[12] I. Lefèvre, G. Marchal, E. Corréal, A. Zanuzzi, S. Lutts, Variation in response to heavy metals during vegetative growth in Dorycnium pentaphyllum Scop, Plant Growth Regul. 59 (2009) 1–11, https://doi.org/10.1007/s10725-009-9382-z.

[13] S.A. Moosavi, M.H. Gharineh, R. Tavakkol Afshari, A. Ebrahimi, Effects of some heavy metals on seed germination characteristics of canola (Barassica napus), wheat (Triticum aestivum) and safflower (Carthamus tinctorious) to evaluate phytoremediation potential of these crops, J. Agric. Sci. 4 (2012) 11–19, https://doi.org/10.5539/jas.v4n9p11.

[14] B. Márquez-García, C. Márquez, I. Sanjosé, F.J.J. Nieva, P. Rodríguez-Rubio, A.F. Muñoz-Rodríguez, The effects of heavy metals on germination and seedling characteristics in two halophyte species in Mediterranean marshes, Mar. Pollut. Bull. 70 (2013) 119–124, https://doi.org/10.1016/j.marpolbul.2013.02.019.

[15] J.R. Peralta, J.L. Gardea-Torresdey, K.J. Tiemann, E. Gomez, S. Arteaga, E. Rascon, J.G. Parsons, Uptake and effects of five heavy metals on seed germination and plant growth in Alfalfa (L.), Bull. Environ. Contam. Toxicol. 66 (2001) 727–734, https://doi.org/10.1007/s00128-001-0069-z.

[16] D. Singh, K. Nath, Y.K. Sharma, Response of wheat seed germination and seedling growth under copper stress, J. Environ. Biol. 28 (2007) 409–414.

[17] R.Ø. Aas, Availability of plant nutrients under conventional farming and conservation farming with and without biochar : a field study of maize on acidic, sandy loam in Zambia 52 (2016). https://brage.bibsys.no/xmlui/handle/11250/2435230.

[18] N.A. Anjum, I. Ahmad, M. Válega, I. Mohmood, S.S. Gill, N. Tuteja, A.C. Duarte, E. Pereira, Salt marsh halophyte services to metal-metalloid remediation: assessment of the processes and underlying mechanisms, Crit. Rev. Environ. Sci. Technol. 44 (2014) 2038–2106, https://doi.org/10.1080/10643389.2013.828271.

[19] R. Castro, S. Pereira, A. Lima, S. Corticeiro, M. Válega, E. Pereira, A. Duarte, E. Figueira, Accumulation, distribution and cellular partitioning of mercury in several halophytes of a contaminated salt marsh, Chemosphere 76 (2009) 1348–1355, https://doi.org/10.1016/j.chemosphere.2009.06.033.

[20] R. Reboreda, I. Caçador, Halophyte vegetation influences in salt marsh retention capacity for heavy metals, Environ. Pollut. 146 (2007) 147–154, https://doi.org/10.1016/j.envpol.2006.05.035.

[21] B. Duarte, M. Caetano, P.R. Almeida, C. Vale, I. Caçador, Accumulation and biological cycling of heavy metal in four salt marsh species, from Tagus estuary (Portugal), Environ. Pollut. 158 (2010) 1661–1668, https://doi.org/10.1016/j.envpol.2009.12.004.

[22] M. Válega, A.I. Lillebø, M.E. Pereira, I. Caçador, A.C. Duarte, M.A. Pardal, Mercury in salt marshes ecosystems: Halimione portulacoides as biomonitor, Chemosphere 73 (2008) 1224–1229, https://doi.org/10.1016/j.chemosphere.2008.07.053.

[23] A. Mahar, P. Wang, A. Ali, M.K. Awasthi, A.H. Lahori, Q. Wang, R. Li, Z. Zhang, Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review, Ecotoxicol. Environ. Saf. 126 (2016) 111–121, https://doi.org/10.1016/j.ecoenv.2015.12.023.

[24] I. Alkorta, J. Hernández-Allica, J.M. Becceril, I. Amezaga, I. Albizu, M. Onaindia, C. Garbisu, Chelate-enhanced phytoremediation of soils polluted with heavy metals, Rev. Environ. Sci. Biotechnol. 3 (2004) 55–70, https://doi.org/10.1016/j.envpol.2007.11.015.

[25] A. Bhargava, F.F. Carmona, M. Bhargava, S. Srivastava, Approaches for enhanced phytoextraction of heavy metals, J. Environ. Manag. 105 (2012) 103–120, https://doi.org/10.1016/j.jenvman.2012.04.002.

[26] D. Leštan, C. Luo, X. Li, The use of chelating agents in the remediation of metal-contaminated soils: a review, Environ. Pollut. 153 (2008) 3–13, https://doi.org/10.1016/j.envpol.2007.11.015.

[27] P.K. Padmavathiamma, L.Y. Li, Phytoremediation technology: Hyper-accumulation metals in plants, Water Air Soil Pollut. 184 (2007) 105–126, https://doi.org/10.1007/s11270-007-9401-5.

[28] L. Kacálková, P. Tlustoš, J. Száková, Phytoextraction of risk elements by willow and poplar trees, Int. J. Phytoremediation 17 (2015) 414–421, https://doi.org/10.1080/15226514.2014.910171.

[29] I.D. Pulford, C. Watson, Phytoremediation of heavy metal-contaminated land by trees - a review, Environ. Int. 29 (2003) 529–540, https://doi.org/10.1016/S0160-4120(02)00152-6.

[30] P. Tlustoš, D. Pavlíková, J. Száková, Z. Fischeroá, J. Balík, Exploitation of fast growing trees in metal remediation, Phytoremediation Rhizoremediation (2006) 83–102, https://doi.org/10.1007/978-1-4020-4999-4_7.

[31] I. Brunner, J. Luster, M.S. Günthardt-Goerg, B. Frey, Heavy metal accumulation and phytostabilisation potential of tree fine roots in a contaminated soil, Environ. Pollut. 152 (2008) 559–568, https://doi.org/10.1016/j.envpol.2007.07.006.

[32] N. Rajakaruna, J.E. Gall, The physiology, functional genomics, and applied ecology of heavy metal-tolerant Brassicaceae, in: M. Lang (Ed.), Brassicaceae, Nova Science Publishers, Inc., ME, US, 2016.

[33] S. Neilson, N. Rajakaruna, Roles of rhizospheric processes and plant physiology in applied phytoremediation of contaminated soils using Brassica oilseeds, in: N. A. Anjum, et al. (Eds.), Plant Fam. Brassicaceae Contrib. Towar. Phytoremediation, Environ. Pollut., Springer Science+Business Media B.V., ME, US, 2012, pp. 313–330, https://doi.org/10.1007/978-94-007-3913-0_12.

[34] J. Hernández-Allica, J.M. Becerril, C. Garbisu, Assessment of the phytoextraction potential of high biomass crop plants, Environ. Pollut. 152 (2008) 32–40, https://doi.org/10.1016/j.envpol.2007.06.002.

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

[36] T.J. Purakayastha, T. Viswanath, S. Bhadraray, P.K. Chhonkar, P.P. Adhikari, K. Suribabu, Phytoextraction of zinc, copper, nickel and lead from a contaminated soil by different species of Brassica, Int. J. Phytoremediation 10 (2008) 61–72, https://doi.org/10.1080/15226510701827077.

[37] A.A. Zunaidi, L.H. Lim, F. Metali, Transfer of heavy metals from soils to curly mustard (Brassica juncea (L.) Czern.) grown in an agricultural farm in Brunei Darussalam, Heliyon 7 (2021) e07945, https://doi.org/10.1016/j.heliyon.2021.e07945.

[38] A.A. Zunaidi, L.H. Lim, F. Metali, Comparative assessment of the heavy metal phytoextraction potential of vegetables from agricultural soils : a field experiment, Heliyon 9 (2023) e13547, https://doi.org/10.1016/j.heliyon.2023.e13547.

[39] A.A. Zunaidi, L.H. Lim, F. Metali, Assessments of heavy metals in commercially available fertilizers in Brunei Darussalam, Agric. Res. 10 (2021) 234–242, https://doi.org/10.1007/s40003-020-00500-4.

[40] B. Mongkhonsin, W. Nakbanpote, O. Meesungnoen, M.N.V. Prasad, Adaptive and tolerance mechanisms in herbaceous plants exposed to cadmium, in: M. Hasanuzzaman, M.N.V. Prasad, M. Fujita (Eds.), Cadmium Toxic. Toler. Plants from Physiol. To Remediat., Elsevier Inc., 2019, pp. 73–109, https://doi.org/10.1016/B978-0-12-814864-8.00004-8.

[41] P.H. Williams, C.B. Hill, Rapid-cycling populations of Brassica, Science 13 (80) (1986) 1385–1389, https://doi.org/10.1126/science.232.4756.1385.

[42] D.A. Storer, The Chemistry of Soil Analysis, Terrific Science Press, Ohio, USA, 2005.

[43] S.E. Allen, H.M. Grimshaw, J.A. Parkinson, C. Quarmby, Chemical Analysis of Ecological Materials, Blackwell Scientific Publications, UK: Oxford, 1989.

[44] E.A. Hanlon, Soil pH and electrical conductivity: a county extension soil laboratory manual, Cirl1081. UF/IFAS Ext (2015) 1–10. http://edis.ifas.ufl.edu/ss118.

[45] L. Van Reeuwijk, Procedures for soil analysis, in: International Soil Reference and Information Centre, sixth ed., Food and Agriculture Organization of the United Nations, 2002.

[46] O. Bojko, C. Kabala, Loss-on-ignition as an estimate of total organic carbon in the mountain soils, J. Soil Sci. 47 (2014) 71–79.

[47] M.C. Amacher, K.P.O. Neill, R. Dresbach, C. Palmer, Forest Inventory & Analysis: Manual of Soil Analysis Methods, 2003rd ed., 2003.

[48] R.H. GumbaraDarmawan, B. Sumawinata, A comparison of cation exchange capacity of organic soils determined by ammonium acetate solutions buffered at some pHs ranging between around field pH and 7.0, Earth Environ. Sci. (2019), https://doi.org/10.1088/1755-1315/393/1/012015.

[49] IUSS Working Group WRB, World Reference Base for Soil Resources. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps, fourth ed., International Union of Soil Sciences (IUSS), Vienna, Austria, 2022, p. 234.

[50] S. Mkumbo, Development of a low cost remediation method for heavy metal polluted soils, TRITA LWR Licentiate Thesis 2067, Stockholm (2012).

[51] H. Amin, B.A. Arain, F. Amin, M.A. Surhio, Phytotoxicity of chromium on germination, growth and biochemical attributes of Hibiscus esculentus L, Am. J. Plant Sci. 4 (2013) 2431–2439, https://doi.org/10.4236/ajps.2013.412302.

[52] C. Feller, H. Bleiholder, E. Frau, M. Hess, H. Wicke, U. Meier, T. van den Boom, R. Stauss, F.R. Klose, H. Hack, F.L. Buhr, P.D. Lancashire, Growth Stages of Mono-And Dicotyledonous Plants, 2001st ed., BBCH monograph, 1994.

[53] S. Bahira, S. Behera, G. Pattnaik, P. Puhan, Phytoremediation of chromium by chickpea (Ciecer arietinum L.) and mung bean (Vigna radiata L.) A comparative study, Int. J. Curr. Sci. Res. 4 (2018) 1589–1598.

[54] R. Hunt, D.R. Causton, B. Shipley, A.P. Askew, A modern tool for classical plant growth analysis, Ann. Bot. 90 (2002) 485–488, https://doi.org/10.1093/aob/mcf214.

[55] D.A. Wilkins, The measurement of tolerance to edaphic factors by means of root growth, New Phytol. 80 (1978) 623–633, https://doi.org/10.1111/j.1469-8137.1978.tb01595.x.

[56] B. Mi, F. Liu, L. Xie, H. Zhou, F. Wu, X. Dai, Evaluation of the uptake capacities of heavy metals in Chinese cabbage, Ecotoxicol. Environ. Saf. 171 (2019) 511–517, https://doi.org/10.1016/j.ecoenv.2019.01.022.

[57] S.V. Chanda, Y.D. Singh, Estimation of leaf area in wheat using linear measurements, Plant Breed, Seed Sci. 46 (2002).

[58] USEPA, EPA Method 3050B (SW-846): Acid Digestion of Sediments, Sludges, and Soils, 1996, 1996, pp. 1–12, https://doi.org/10.1117/12.528651.

[59] SPSS, IBM Corp, IBM SPSS Statistics for Windows, Version 22 (2018) Amonk, NY, IBM Corp., New York, 2018.

[60] R.O. Ongon'g, J.N. Edokpayi, T.A.M. Msagati, N.T. Tavengwa, G.N. Ijoma, J.O. Odiyo, The potential health risk associated with edible vegetables grown on cr (VI) polluted soils, Int. J. Environ. Res. Publ. Health 17 (2020), https://doi.org/10.3390/ijerph17020470.

[61] C.N. Eze, C.K. Odoh, E.A. Eze, P.I. Orjiakor, S.C. Enemuor, U.J. Okobo, Chromium (III) and its effects on soil microbial activities and phytoremediation potentials of Arachis hypogea and Vigna unguiculata, Afr. J. Biotechnol. 17 (2018) 1207–1214, https://doi.org/10.5897/ajb2018.16566.

[62] M. Shahid, S. Shamshad, M. Rafiq, S. Khalid, I. Bibi, N.K. Niazi, C. Dumat, M.I. Rashid, Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: a review, Chemosphere 178 (2017) 513–533, https://doi.org/10.1016/j.chemosphere.2017.03.074.

[63] S. Bahira, S. Behera, P. Puhan, Phytoremediation of chromium by chickpea (Ciecer arietinum L .) and it's toxic effect, Remarking an Anal 3 (2018) 80–85.

[64] P. Sundarmoorthy, K. Sankarganesh, M. Selvaraj, L. Baskaran, Chromium induced changes in Soybean (Glycine max L.) metabolism, World Sci. News. 16 (2015) 145–178.

[65] B. Piršelová, V. Kubová, P. Boleček, A. Hegedusová, Impact of cadmium toxicity on leaf area and stomatal characteristics in Faba Bean, J. Microbiol. Biotechnol. Food Sci. 11 (2021) 1–4, https://doi.org/10.15414/jmbfs.3718.

[66] M. Hatamian, A. Rezaei Nejad, M. Kafi, M.K. Souri, K. Shahbazi, Interaction of lead and cadmium on growth and leaf morphophysiological characteristics of European hackberry (Celtis australis) seedlings, Chem. Biol. Technol. Agric. 7 (2020) 1–8, https://doi.org/10.1186/s40538-019-0173-0.

[67] H.Y. Lai, Effects of leaf area and transpiration rate on accumulation and compartmentalization of cadmium in Impatiens walleriana, Water Air Soil Pollut. 226 (2015), https://doi.org/10.1007/s11270-014-2246-9.

[68] W.J. Allender, G.C. Cresswell, J. Kaldor, I.R. Kennedy, Effect of lithium and lanthanum on herbicide induced hormesis in hydroponically-grown cotton and corn, J. Plant Nutr. 20 (1997) 81–95, https://doi.org/10.1080/01904169709365235.

[69] E.J. Calabrese, R.B. Blain, Hormesis and plant biology, Environ. Pollut. 157 (2009) 42–48, https://doi.org/10.1016/j.envpol.2008.07.028.

[70] X. Huang, S. Duan, Q. Wu, M. Yu, S. Shabala, Reducing cadmium accumulation in plants: structure – Function relations and tissue-specific operation of transporters in the spotlight, Plants 9 (2020) 18.

[71] D. Ueno, N. Yamaji, I. Kono, C.F. Huang, T. Ando, M. Yano, J.F. Ma, Gene limiting cadmium accumulation in rice, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 16500–16505, https://doi.org/10.1073/pnas.1005396107.

[72] P.M. Kopittke, M.D. de Jonge, P. Wang, B.A. Mckenna, E. Lombi, D.J. Paterson, D.L. Howard, S.A. James, K.M. Spiers, C.G. Ryan, A.A.T. Johnson, N.W. Menzies, Laterally resolved speciation of arsenic in roots of wheat and rice using fluorescence-XANES imaging, New Phytol. 201 (2014) 1251–1262, https://doi.org/10.1111/nph.12595.

[73] E. Martinoia, S. Meyer, A. De Angeli, R. Nagy, Vacuolar transporters in their physiological context, Annu. Rev. Plant Biol. 63 (2012) 183–213, https://doi.org/10.1146/annurev-arplant-042811-105608.

[74] H. Oliveira, Chromium as an environmental pollutant: insights on induced plant toxicity, J. Bot., Le 2012 (2012) 1–8, https://doi.org/10.1155/2012/375843.

[75] A. Sharma, D. Kapoor, J. Wang, B. Shahzad, V. Kumar, A.S. Bali, S. Jasrotia, B. Zheng, H. Yuan, D. Yan, Chromium bioaccumulation and its impacts on plants: an overview, Plants 9 (2020) 1–17, https://doi.org/10.3390/plants9010100.

[76] A.K. Shanker, C. Cervantes, H. Loza-Tavera, S. Avudainayagam, Chromium toxicity in plants, Environ. Int. 31 (2005) 739–753, https://doi.org/10.1016/j.envint.2005.02.003.

[77] A.K. Gupta, S. Sinha, Chemical fractionation and heavy metal accumulation in the plant of Sesamum indicum (L.) var. T55 grown on soil amended with tannery sludge: selection of single extractants, Chemosphere 64 (2006) 161–173, https://doi.org/10.1016/j.chemosphere.2005.10.016.

[78] P.A. Mangabeira, A.S. Ferreira, A.A.F. De Almeida, V.F. Fernandes, E. Lucena, V.L. Souza, A.J. Dos Santos Júnior, A.H. Oliveira, M.F. Grenier-Loustalot, F. Barbier, D.C. Silva, Compartmentalization and ultrastructural alterations induced by chromium in aquatic macrophytes, Biometals 24 (2011) 1017–1026, https://doi.org/10.1007/s10534-011-9459-9.

[79] A.K. Shanker, M. Djanaguiraman, R. Sudhagar, C.N. Chandrashekar, G. Pathmanabhan, Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (Vigna radiata (L.) R.Wilczek. cv CO 4) roots, Plant Sci. 166 (2004) 1035–1043, https://doi.org/10.1016/j.plantsci.2003.12.015.

[80] S. Pasricha, V. Mathur, A. Garg, S. Lenka, K. Verma, S. Agarwal, Molecular mechanisms underlying heavy metal uptake, translocation and tolerance in hyperaccumulators-an analysis: heavy metal tolerance in hyperaccumulators, Environ. Challenges. 4 (2021) 100197, https://doi.org/10.1016/j.envc.2021.100197.

[81] J.H. Park, Contrasting effects of Cr(III) and Cr(VI) on lettuce grown in hydroponics and soil: chromium and manganese speciation, Environ. Pollut. 266 (2020) 115073, https://doi.org/10.1016/j.envpol.2020.115073.

[82] A. Hocaoglu-Ozyigit, B.N. Gene, Cadmium in plants, humans and the environment, Front. Life Sci. Relat. Technol. 1 (2020) 12–21. https://dergipark.org.tr/en/pub/flsrt/issue/56859/781913.

[83] M.M. Sintorini, H. Widyatmoko, E. Sinaga, N. Aliyah, Effect of pH on metal mobility in the soil, IOP Conf. Ser. Earth Environ. Sci. 737 (2021) 6–12, https://doi.org/10.1088/1755-1315/737/1/012071.

[84] A. Kubier, R.T. Wilkin, T. Pichler, Cadmium in soils and groundwater: a review, Appl. Geochem. 108 (2019) 104388, https://doi.org/10.1016/j.apgeochem.2019.104388.