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An Insight to Tolerance and Accumulation in Two Species of *Brassica*: *Brassica carinata* and *Brassica rapa* L. against Heavy Metals Stress

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Abstract

The strategy of tolerance and heavy metal accumulation capacity of two *Brassica sp*., *Brassica carinata* and *Brassica rapa* was investigated with a particular focus on Cd, Cr and Zn. Each of the heavy metals was added to agricultural topsoil at five levels (0, 75, 150, 225 and 300 mg kg^{-1}), in a pot experiment. In this experiment, 15 pots of each *Brassica carinata* and *Brassica rapa* plants were grown for 60 days. Both species displayed retarded germination, with *Brassica carinata* having a significant decrease in germination percentage eat the highest metal concentration (300 mg/kg) and *Brassica rapa* seemingly increasing in germination percentage at higher metal concentrations. *Brassica carinata* and *Brassica rapa* showed a consistent decrease in (0.05 to 0.04 cm/day) as the heavy metal concentration increased, and significant decrease in relative growth rate was seen for the highest concentration as compared to control. The stress tolerance index showed a marked decrease in plant size for *Brassica carinata* and no changes for *Brassica rapa*, highlighting its tolerance to heavy metal toxic condition. The root length did not change significantly in both species while the leaf area increased with the soil treatment levels, suggesting that both species are tolerant to higher levels of heavy metals. The results revealed that both species, *Brassica rapa* in particular, showed high tolerance and accumulation potential of non-essential heavy metals, indicating their potential as hyper accumulator and their support to sustainable soil management for ecofriendly and cost-effective remediation of heavy metal polluted soil.

Key words**:** *Brassica carinata, Brassica rapa,* Germination percentage**,** Heavy metals, Phytoremediation

Brassica carinata A. Braun (Ethiopian mustard) and *Brassica rapa* L. are economically important members of the family Brassicaceae, cultivated worldwide for their edible oil, leafy vegetables, fodder, and industrial applications. In addition to their agronomic importance, *Brassica* species possess the ability to tolerate and accumulate certain heavy metals, attracting considerable attention for their potential use in phytoremediation of contaminated soils. Their extensive root system and efficient translocation of metals from roots to shoots further enhance their suitability for remediation of heavy metal-polluted environments.

Heavy metals are persistent environmental contaminants, that specifically due to the interference with nutrient uptake, photosynthetic activity, enzyme activity, or other physiological processes, cause serious health consequences for plants and humans [1]. Of the various remediation options, phytoremediation has proven to be a sustainable, cost effective and environmentally friendly technique that involves the use of plants to extract, concentrate, immobilize or remove heavy metals from soil by using phytoremediation. Phytoremediation potential is related to the tolerance and accumulation of heavy metals in plants. As germination and early seedling development are very sensitive to metal toxicity, seedling growth can be used as a good indicator for the potential of phytotolerance [2]. Assessment of these early growth stages gives information about the impact of heavy metal stress on plant development and potential for removal of contaminated soils [3].

Heavy metal hyper accumulator plants are important in phytoremediation because they tolerate, accumulate and translocate toxic metal from soil to shoots [4]. They passing through root induced soil acidification, change soil chemistry so that they are more efficient at absorbing metals and thus, can be used in the remediation of contaminated soils [5]. One of the major features of the plants with phytoremediation potential is the efficient movement of heavy metals from roots to shoots. Only a few plant species, however, have a combination of traits, the majority of which are in the families Asteraceae, Brassicaceae, Caryophyllaceae and Violaceae [6]. The tolerance and accumulation capacity of the *Brassica carinata* and *Brassica rapa* were evaluated under different Cd, Cr and Zn concentrations. The metals chosen were common pollutants found in agricultural soils and the *Brassica species* were selected due to their ability of producing high biomass and known phytoremediation properties [7]. Therefore, the present study evaluated the germination, seedling growth, and

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phytoremediation potential of *Brassica carinata* and *Brassica rapa* under Cd, Cr, and Zn stress.

MATERIALS AND METHODS

A pot experiment was conducted at Sharda University, Aligarh, Agro University, Greater Noida, Uttar Pradesh, India (28.4744°N, 77.5040°E). The study was conducted during the month of December 2022 through February 2023 under average environmental conditions of temperature (27.3°C, 25.6 - 28.7°C) and rainfall (8.7 mm, 2.1 - 31 mm) per week during 8 weeks of experimental period.

Study species, sampling and sample pre-treatment

Brassica carinata and *Brassica rapa* were chosen to determine the phytoremediation potential in heavy metal polluted soils. Top soil from agricultural field was taken, air-dried and sieved (2 mm) in an agricultural experimental farm of Sharda University, Greater Noida, India.

A pot experiment was performed to evaluate the phytotoxic effects of Cd, Cr and Zn applied at six different concentrations (0, 75, 150, 225, 300 mg/kg) in 3 kg of homogenized soil per pot. To test the responses of the plants *Brassica carinata* and *Brassica rapa* under increasing level of heavy metal stress [8], the experiment was designed as a completely randomized design with three replications for each treatment. The seeds were sown in pots containing 10 seeds, watered with distilled water and thinned to 3 seedlings after germination and no fertilizer was used to maintain heavy metals as the only treatment factor. Plants were harvested 60 days after sowing and the growth, germination, heavy metal accumulation, and phytoremediation potential were evaluated to ensure tolerance and dose-dependent response of the species in question [9].

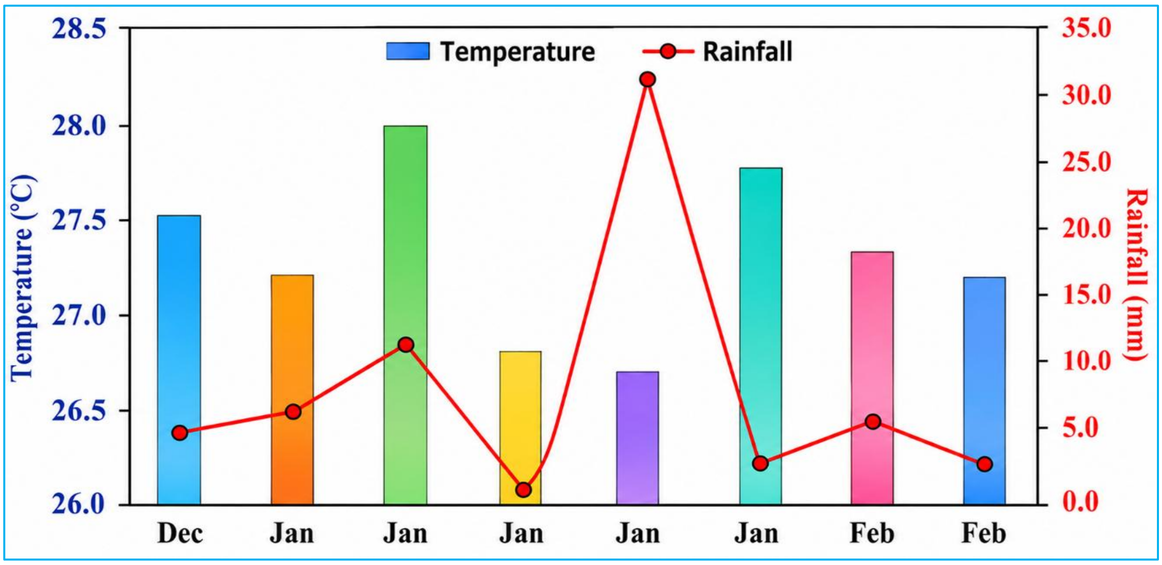

Fig 1 Temperature and Rainfall data recorded at Sharda University, Greater Noida (Uttar Pradesh, India), during the November 2022–January 2023 growth period of *Brassica carinata* and *Brassica rapa* L.

Data analysis

Seven parameters of plant growth were assessed for each of the seven soil treatments and each of the plant species to determine tolerance level and eco-toxicological effects on the germination percentage, relative growth rate, stress tolerance index, leaf area and maximum root length [10].

Germination %: Percentage of the number of seeds sown that successfully germinate. The combined effect of Cd, Cr and Zn stress on seed germination and establishment of early seedlings of *Brassica carinata* and *Brassica rapa* was tested.

$$\text{Germination \%} = \frac{G_1 \times 100\%}{G_2}$$

G_1 and G_2 are germination seeds, G is number of seeds that germinated under each seed germination treatments and N is the number of seeds sown for each treatment.

Relative growth rate: is the increase in amount of plant biomass or plant size, important measure of plant physiology in response to combined exposure of heavy metals, per unit of existing plant biomass over a specific time interval. Heavy metals like Cd, Cr and Zn have the ability to inhibit the photosynthesis and biomass accumulation, causing growth reduction.

$$\text{RGR} += \frac{\text{In } W_2 - \text{In } W_1}{t_2 - t_1}$$

The plant dry weight at the initial time (W_1) and the final time (W_2) of sampling, and the sampling time in days (t_1, t_2).

The stress tolerance index: was used to compare the ability of the two *Brassica species* to grow under combined Cd, Cr and Zn stress conditions with non-stress (control) conditions.

$$\text{STI \%} = \frac{XS}{XC} \times 100$$

XS = Value of the measured trait in the presence of heavy metals,

XC = value of the measured trait in the absence of heavy metals.

Leaf area

Comparing leaf area of *Brassica carinata* with *Brassica rapa* can be used to determine the species-specific tolerance to combined metal stress.

$$\text{Leaf area (LA)} = \text{leaf length} \times \text{leaf width} \times K$$

The leaf length (cm) is maximum leaf length, the leaf width (cm) is maximum width and the K is correction factors.

Root length: root length is measured from the base of the stem to the tip of the root by using ruler and it is expressed in centimeters (cm). Root length comparison of the two species can be used to establish the species that is more tolerant and can be utilized for the phytoremediation of heavy metal contaminated soils.

Analytical techniques

The USEPA Method 3050B was used to determine heavy metal accumulation in *Brassica carinata* and *Brassica rapa*. In the study, three heavy metals (Cd, Cr and Zn) were selected as they are among the most prevalent heavy metal contaminant in agricultural soils due to various sources including mining, industry, wastewater irrigation, and high usage of fertilizers. Non-essential and highly toxic, cadmium can easily enter into plant tissues at low concentrations. It is recognized that chromium can interfere with plant growth and physiological and biochemical processes, especially in the hexavalent state. Zn is a micronutrient and essential for growth and productivity of crops, but becomes phytotoxic at high concentrations. The leaves, stems and roots were separately oven dried, ground and sieved. A digestion method was used as per [11] using 1 g of dried plant material, and a mixture of HNO_3, H_2O_2 and HCl at 95 ± 5°C for 2 h, before diluting with distilled water. Samples were analysed with Cd, Cr and Zn measured by inductively coupled plasma-optical emission spectroscopy ICP-OES (Thermo Scientific iCAP 7400, Thermo Fisher Scientific, USA) and triplicate and blank samples were analysed to ensure quality assurance.

Statistical methods

The plant growth parameters along with the concentration of heavy metals in the *Brassica carinata* and *Brassica rapa* were analyzed with one way ANOVA and Tukey's HSD test at 5% level of significance in IBM SPSS Statistics 22 (16).

RESULTS AND DISCUSSSION

Germination rate (%)

The seed viability and early establishment of *Brassica carinata* and *Brassica rapa* under heavy metal stress was assessed based on germination percentage. It took approximately 2 days to germinate the metal contaminated seeds than the control, suggesting a decrease in seed vigor and early seedling growth with increasing metal concentrations.

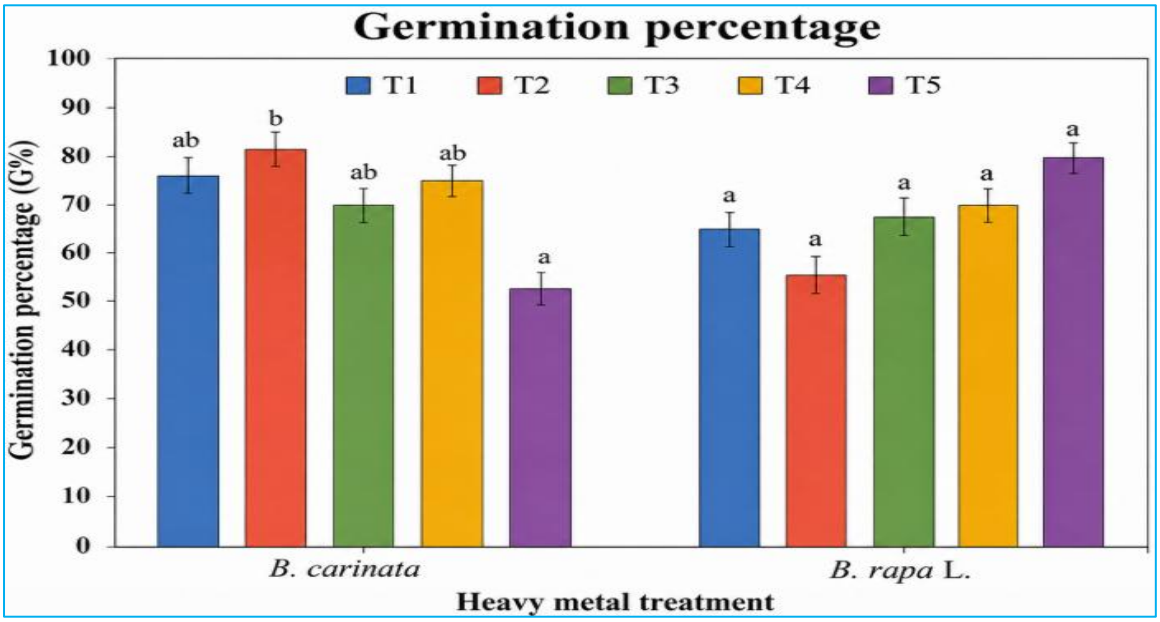

Fig 2 Influence of increasing heavy metal levels on seed germination of *Brassica carinata* and *Brassica rapa* (T_1: Control, T_2: 75mg/kg, T_3: 150mg/kg, T_4: 225mg/kg and T_5: 300mg/kg of Cd, Cr and Zn. Alphabet letters show significantly difference means for the Brassica species using one-way ANOVA at 5% significance level)

The response to heavy metal treatment was dose and species specific for the *Brassica species*. The germination rate of *Brassica rapa* remained constant throughout most treatments. The graph shows in (Fig 2), the germination % decreased considerably, at the highest concentration of heavy metal (300 mg kg^{-1}), with *Brassica carinata*. The germination of the T_1 (control) and T_4 (225 mg/kg) did not significantly differ whereas T_5 (300 mg/kg) significantly reduced germination when compared with T_2 (75 mg kg).

Relative growth rate and stress tolerance assessment

The relative growth rate (RGR) and stress tolerance index (STI) were used to evaluate the growth performance under heavy metal stress and to compare the resistance against the heavy metal stress and control for the two *Brassica species* respectively. RGR was a measure of overall growth performance, and STI was a measure of resistance to metal toxicity, compared with the control (20). The significant effects of heavy metal treatments were observed on RGR and STI for *Brassica carinata*, while for *Brassica rapa*, only RGR showed significant effect. For both species, growth performance decreased with increasing metal concentrations in the soil (0–300 mg/kg Cd, Cr and Zn) with a decrease in RGR for both species from 0.05 cm/day to 0.04 cm/day, which was significantly different from T_1 (75 mg/kg) (Fig 3).

It exhibited contrasting patterns between the two species, in *Brassica carinata*; it decreased gradually with all treatments, while it remained relatively stable in *Brassica rapa* with significant decrease compared with the control (ST1) in 0, 75, 150, 225 and 300 mg kg^{-1} treatments for *Brassica carinata*. Indicating a species and concentration specific impact of heavy metal stress on relative growth rate. This indicates that *Brassica rapa* is highly tolerant to Cd and Zn toxicity in soil, with Cd and Zn accumulation.

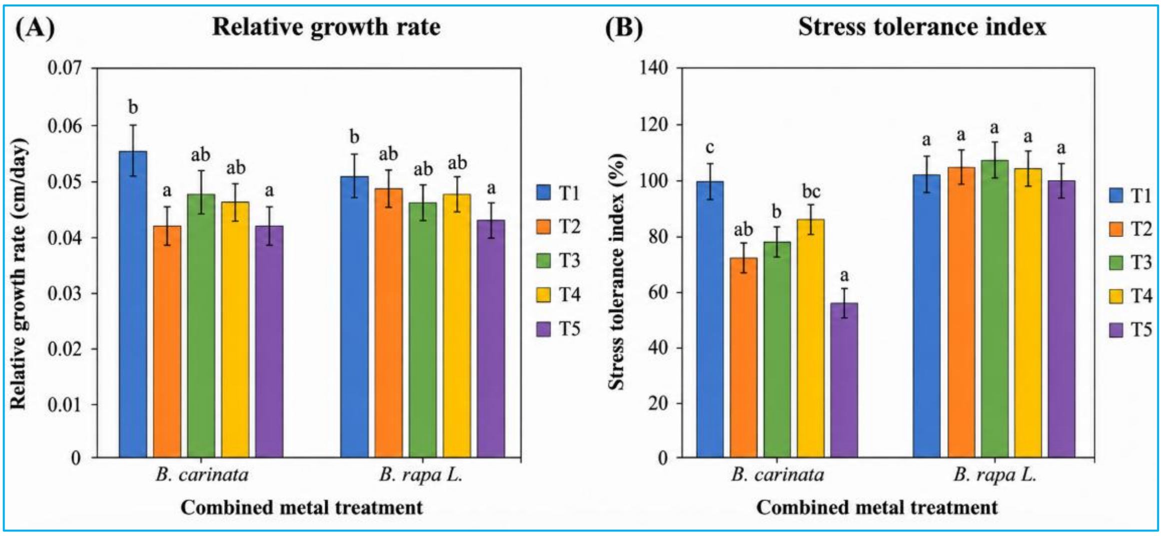

Fig 3 (A) Relative growth rate and (B) stress tolerance index for *Brassica carinata* and *Brassica rapa* for *Brassica carinata* and *Brassica rapa:* T_1: control, T_2:75mg/kg, T_3: 150mg/kg, T_4:225mg/kg and T_5: 300mg/kg of Cd, Cr and Zn. Alphabet letters show significantly difference means for the *Brassica* species using one-way ANOVA at 5% significance level

Assessment of leaf area and root length

The average values of leaf area and root length were calculated to assess the effect of heavy metal stress on *Brassica carinata* and *Brassica rapa*. The leaf area of both species was similar across treatments, with no significant differences, and *Brassica carinata* having a slight increase at the higher metal concentrations suggesting tolerance to and adaptation to the heavy metal contamination treatments (Fig 4).

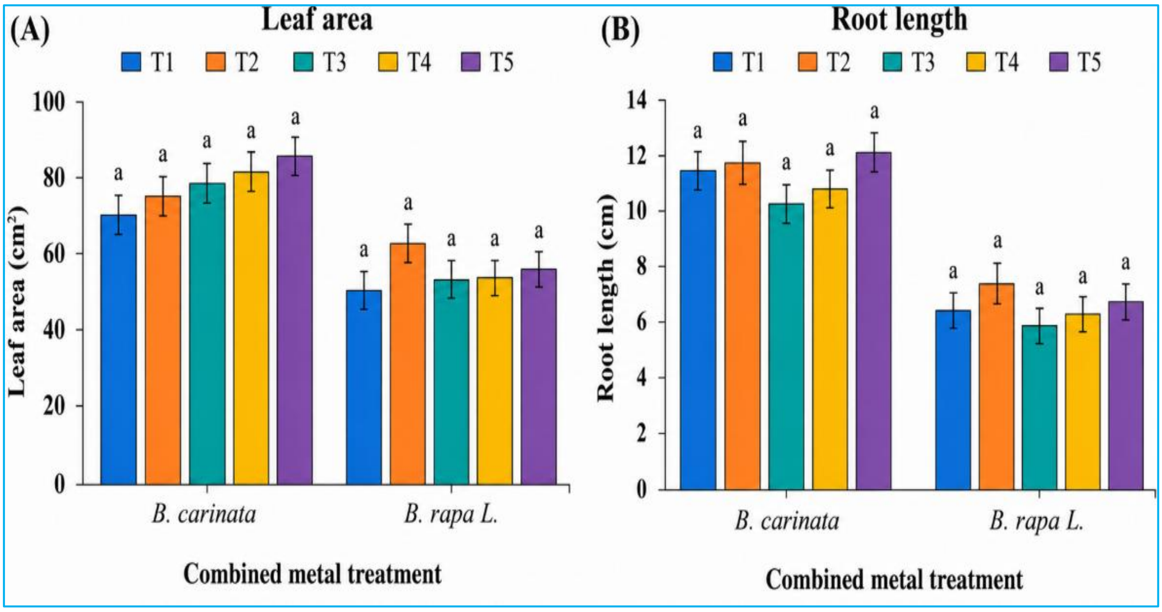

Fig 4 Leaf area (A) and root length (B) for *Brassica carinata* and *Brassica rapa:* T_1: control, T_2:75mg/kg, T_3: 150mg/kg, T_4:225mg/kg and T_5: 300mg/kg of Cd, Cr and Zn. Alphabet letters show significantly difference means for the *Brassica* species using one-way ANOVA at 5% significance level

Heavy metal content in plant species

The concentrations of the heavy metals (Cd, Cr and Zn) obtained in various plant tissues like leaves, stem and roots of *Brassica carinata* and *Brassica rapa* in different soil amendment treatments (0, 25, 50, 100, 200 and 300 mg/kg) are tabulated as follows (Table 1). The heavy metal concentrations in different tissues of the *Brassica species* were quantified to understand the mechanism of uptake, translocation and bioaccumulation of the metals from the rhizospheric soil to evaluate the phytotolerance and phytoremediation potential of the species in the metal polluted environment.

However, the pot experiment was carried out for a relatively short period of 60 days, which may not have been long enough to fully explain the long-term physiological and developmental responses of plant growth to heavy metal stress. The accumulation of Cd in the roots differed among the species in the T_1–T_5 treatments and *Brassica rapa* accumulated more Cd than *Brassica carinata*. The highest concentration of Cd in the roots was found for T_5, with 861 mg/kg in *Brassica rapa* and 737 mg/kg in *Brassica carinata*. In all soils, Cd content was significantly higher in the root tissues of both species compared to control soils (no statistically significant difference detected in control soils). Previous studies have shown that Cd is relatively uniformly distributed in different parts/tissues of plants in several plant species under natural growth conditions [12]. Under high soil Cd levels, Cd observed in the root vacuoles may be evidence of a detoxification system for Cd in these Brassica species, as it is thought that the Cd transporters act in a similar way. The mechanisms involved in Cd uptake and subcellular localization are responsible for reducing the translocation of Cd and its accumulation in shoots [13]. Root vacuoles play a pivotal role in strategies of cellular detoxification and normal developmental processes under toxic stress as they act as safe deposit boxes for ions and metabolites [14]. The results presented here show that *Brassica species*, specifically *Brassica rapa* have a remarkable physiological tolerance to toxic substrates that enables them to show germination competence and continued growth in the presence of heavy metal stress.

Table 1 Mean heavy metal concentrations (standard deviation, SD) for each species following analysis using one-way ANOVA at 5% significance level

Heavy metal	Cd leaf	Cd stem	Cd root	Cr leaf	Cr stem	Cr root	Zn leaf	Zn stem	Zn root
					Brassica carinata				
T_1	30.0b±19.5	6.43a±1.9	14.5ab±4.3	17.10b±6.7	9.92a±1.9	38.3c±3.2	72.0b±22.3	44.0a±4.5	166c±3.0
T_2	47.2a±17.4	31.5a±5.9	136b±35.2	21.1a±6.4	16.1a±2.6	82.5b±13.1	123b±11.5	61.7a±10.6	540c±5.8
T_3	68.0a±11.6	52.2a±8.3	343b±12.7	19.0a±9.2	24.5a±2.8	164b±49.0	202b±22.7	86.2a±10.6	542c±58.5
T_4	92.1a±19.7	69.0a±16.0	452b±16.5	22.1a±8.6	33.6a±2.6	130b±37.4	276b±22.1	114a±8.8	619c±52.9
T_5	104.6a±35.2	78.4±16.1	737b±148	39.5a±12.8	44.1±5.61	160b±37.0	402b±35.0	150a±19.0	950c±83.4
					Brassica rapa				
T_1	62.4a±42.9	12.91±6.62	105a±10.0	12.1a±2.86	4.62a±2.9	32.1b±11.2	80.2b±25.0	54.3±11.2	233b±26.2
T_2	86.3a±48.2	47.1a±25.2	114a±63.0	71.90±17.2	101a±21.0	53.2ab±11.2	104ab±21.0	84.1a±10.0	125b±34.6
T_3	162a±54.4	125a±11.4	406b±70.2	30.6a±12.0	29.6a±2.0	129b±34.0	243b±84.4	99.4a±6.0	269b±71.0
T_4	202a±47.4	215a±61.0	602b±67.2	30.6a±10.3	36.1a±6.3	146b±36.2	288b±32.4	106a±8.5	414a±48.6
T_5	273a±75.1	256a±39.0	861b±38.1	17.0a±9.4	40.2b±2.4	132c±22.0	337b±19.0	121a±9.5	475c±42.0

The distribution of the Cr in the leaf, stem and root tissues of *Brassica carinata* and *Brassica rapa* followed the same pattern as that of the Cd levels in these same plant tissues, with significant preferential accumulation present in the root tissue of both species. The difference between the concentration of Cr in leaf and stem tissues did not show statistical significance in both species of *Brassica*, indicating that the aerial plant organs had low concentration of Cr when compared to the root system. The same concentration of Cr was found in different tissues of plants in both species, and the highest was found in the root tissue, 160 mg/kg *in Brassica carinata* and 132 mg/kg in *Brassica rapa*. Plants have no known mechanism for the acquisition of Cr as a non-essential heavy metal since it is not known to have any metabolic or physiological function [14]. Since the roots have limited mobility, the amount of Cr present in the root tissue is usually about 100 times higher than that of aerial tissues. The present study revealed that the accumulation of Cr was highest in the root tissues, which may be attributed to vacuolar sequestration of Cr in a detoxification mechanism in the cell. Thus, bestowing a natural adaptability to both species to the phytotoxic stress caused by chromium [15].

The distribution of Zn among various plant organs (leaves, stems and roots) of *Brassica carinata* and *Brassica rapa* showed significant differences between the tissues (Table 1). Notably, both species accumulated significant amounts of Zn in the foliar and root tissues and these tissues are likely to be the primary sites of uptake and retention of Zn. This result shows that both species have the ability to store a high amount of Zn in aerial tissues. In soil samples with a spiked Zn concentration of T_5 (300 mg/kg), *Brassica carinata* and *Brassica rapa* showed up to 392 mg/kg and 337 mg/kg of Zn in the foliage, respectively (Table 1), indicating a relatively high translocation efficiency of Zn in *Brassica species*. Zn concentration was found to be significantly higher in the root tissue of *Brassica carinata* (950 mg/kg) than that of *Brassica rapa*. Some *Brassica species* seem to regulate the uptake of highly mobile Zn via intracellular detoxification mechanisms such as sequestration and binding of Zn in the plant's internal organs, which reduces the movement of Zn to the aerial parts of the plant [16]. The present study has shown that Zn was easily taken up by various plant parts of *Brassica species*, although the mobility of Zn in soils is high at low pH and it is mostly present in water soluble forms like $CdSO_4$, $CdCl^+$, $CdHCO_3^+$ cations but not as much as other forms. It is found that both *Brassica species* have high Cd, Cr and Zn accumulation capacity and they can be cultivated in soils contaminated with up to 300mg/kg (T_5) of heavy metals.

CONCLUSION

This study showed that *Brassica rapa* and *Brassica carinata* had the ability to tolerate and accumulate significant levels of Cd, Cr and Zn with *Brassica rapa* having greater tolerance to Cd, and *Brassica carinata* having the highest accumulation of Zn. Whilst growth rates were decreased under heavy metal stress at higher concentrations both species were able to germinate and grow biomass, which means they have a good potential to phytoremediate contaminated soils especially *Brassica rapa,* and can be used for sustainable remediation of polluted soils. The highest Cd accumulation was observed in *Brassica rapa* (861mg/kg) while the highest Zn accumulation was observed in *Brassica carinata* (940mg/kg), with both metals mainly found in the root's tissues. The hypothesis of the present study was that *Brassica carinata* and *Brassica rapa* would exhibit differential tolerance and accumulation of Cd, Cr and Zn under increasing levels of these heavy metals in soil. Notably, there was no difference between the two species with respect to root accumulation of Cr. The understanding of the molecular mechanisms of these responses, as well as validation in the field of their potential for phytoremediation will be important for developing.

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