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Synergistic roles of zinc and boron in enhancing growth, stress physiology, and heavy metal tolerance in *Brassica rapa* L.

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Abstract

Heavy metal contamination in soil impairs plant growth and disrupts cellular functions. *Brassica rapa* L. exhibits phytoremediation potential owing to its strong metal tolerance and accumulation capacity. This study evaluated the effects of boron (B) and zinc (Zn), applied alone or together at 10–25 mg kg⁻¹, on growth, biochemical traits, and heavy-metal stress tolerance in *B. rapa* L. The result established B + Zn > B > Zn. Co-application of B and Zn at 15–20 mg kg⁻¹ significantly enhanced growth and tolerance to metal-induced stress compared with individual treatments ($p < 0.05$). At 20 mg kg⁻¹ B + Zn, key biochemical markers, including chlorophyll, sugar, phenolic, carotenoid, protein, and proline levels, were significantly higher compared with individual treatments. Elemental analysis and PCA showed that the optimal 15–20 mg kg⁻¹ B + Zn dose promoted the uptake of essential elements such as Fe and Mn. Simultaneously, reduced toxic metals accumulation (Cd and Pb). Conversely, the 25 mg kg⁻¹ treatment clustered with elevated toxic metals, with Cd showing the highest accumulation, followed by Cr and Pb. The weak linear correlation ($R^2 < 0.1$) observed across parameters indicates that these physiological benefits arise from complex, non-linear regulation. These findings underscore the critical role of precise micronutrient synergy in enhancing heavy-metal tolerance mechanisms in crops, and they are vital for optimizing phytoremediation and ensuring food safety in metal-contaminated agroecosystems.

Keywords Heavy metal stress, Phytoremediation, Soil-plant transfer, Antioxidant defense, Micronutrient

1 Introduction

Accumulated heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), and nickel (Ni) in soil pose serious environmental and biological risks. Essential micronutrients, i.e., iron (Fe), manganese (Mn), magnesium (Mg), and zinc (Zn), become toxic at elevated concentrations [1]. Agricultural intensification also accelerates soil degradation and trace-metal accumulation in ecologically sensitive zones [2, 3]. Excessive accumulation of heavy metals can disrupt physiological homeostasis, leading to toxicity and impaired growth [4]. Heavy metals trigger reactive oxygen species (ROS), causing oxidative



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damage to lipids, proteins, and nucleic acids [5]. To mitigate this, plants activate enzymatic defenses like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), which are commonly upregulated in metal-induced stress conditions [6]. In parallel, metal exposure also induces transcriptional reprogramming of genes involved in metal transporters and detoxification. Notably, the expression of iron-regulated transporter 1 (*IRT1*) and heavy-metal ATPase (*HAM2*) reflects enhanced metal uptake and redistribution, while phytochelatins synthase 1 (*PCS1*) contributes to chelation and sequestration of toxic ions [7]. These coordinated responses highlight the relevance of investigating Zn and B supplementation, as both elements influence redox homeostasis and metal transport pathways [8]. Plants employ crucial defense mechanisms to enhance responses to metal-induced stress. Additionally, plants reduce toxic metal accumulation through avoidance mechanisms such as root exudation and cell wall biosorption [9]. Furthermore, plant tolerance mechanisms are activated to detoxify metal ions. Metal-responsive transcription factors (TFs), such as basic leucine zipper domain (bZIP) [10], and WRKYs (TFs) [11], are upregulated under stress. These TFs regulate the synthesis of detoxifying molecules, i.e., phytochelatins and metallothioneins. These molecules are sequestered into vacuoles via ATP-binding cassette (ABC) transporters [12], cation diffusion facilitator (CDF) proteins, and heavy metal ATPases (HMAs) [13, 14].

Essential micronutrients, including Fe, Mg, Mn, and Zn, are vital for physiological processes like membrane stability and enzyme activation [15]. In contrast, Cr is not essential for plants and is primarily associated with phytotoxic effects, especially under elevated soil contamination [16]. Therefore, it is important to evaluate the inhibitory and stimulating effects of trace metals, i.e., Zn and B, on plant species, especially in soils with variable metal concentrations. Zn is a cofactor for enzymes in antioxidant defense and metabolic regulation [17]. However, excessive Zn can induce toxicity and interfere with enzyme-mediated reactions. Furthermore, excess Zn may disrupt nutrient homeostasis by promoting manganese (Mn) accumulation in plant tissues [18]. Zn-induced toxicity has been shown to inhibit photosystems I and II, thereby impairing ATP synthesis in plants [19]. In vitro and metal-binding studies show that prolonged Zn^{2+} exposure interferes with the function of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), as Zn^{2+} competes with Mg^{2+} for binding at the enzyme's active site. This displacement reduces RuBisCO's carboxylase activity and decreases CO_2 fixation efficiency [20, 21]. Zn toxicity has also been linked to increased formation of calcium oxalate crystals (CaC_2O_4) and elevated free calcium levels in plant tissues [22].

B deficiency impairs key enzymes such as phenylalanine-ammonium lyase (PAL), disrupting phenolic metabolism in vascular plants [23]. Excess B alters the cell wall by binding cis-hydroxyl groups and disrupting cell division [24]. Phytoremediation offers a cost-effective strategy to mitigate soil metal toxicity, relying on plant uptake and translocation [25, 26]. Metal transfer from soil to plant tissues has been previously studied [27]. Soil properties strongly influence metal accumulation in plants [28, 29]. Soil pH, electrical conductivity (EC), organic matter (OM) content, and cation exchange capacity (CEC) critically affect metal uptake in plants [30]. Turnip (*Brassica rapa* var. *rapa* Lin.) is globally cultivated and known for accumulating Cd, Pb, and Cu [31, 32]. Its nutritional value has increased the consumption of *B. rapa* L [33, 34]. Due to micronutrient limitations in soil, farmers apply fertilizers to sustain plant productivity. Imbalanced nutrient supply and soil contamination disrupt Zn and B availability, impairing plant metabolism

and yield [35]. Zn and B deficiencies severely impair *B. rapa* L. growth and development [36]. Although growth regulators have been studied in other *Brassica species*, studies on Zn-B synergy in *B. rapa* L. under heavy metal-induced stress remain limited [37, 38].

We hypothesize that the combined B and Zn application will exert a synergistic effect in *B. rapa* L. This treatment is expected to enhance growth, improve physiological resilience, and improve heavy-metal tolerance. Our study also aimed to evaluate the optimal concentration range for synergistic interaction. Zn and B were selected for their key roles in membrane stability, enzyme activity, and redox homeostasis. Thus, we aimed to optimize B and Zn application to improve yield and stress tolerance under heavy metal-induced stress. Our study advances the understanding of micronutrient synergy in stress tolerance under diverse environmental conditions.

2 Materials and methods

2.1 Experimental workflow

The overall experimental design is illustrated in Fig. 1 and outlines the work described below.

2.2 Preparation of soil to grow *Brassica rapa* L. seeds

Air-dried silt loam soil was collected from an agricultural field with a long-term history of cultivation. The soil was sieved through a 5-mm mesh and divided into separate 1-kg portions for each group: zinc (Zn), boron (B), and a combined Zn + B application. Zn was supplied as zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), and B as boric acid (H_3BO_3).

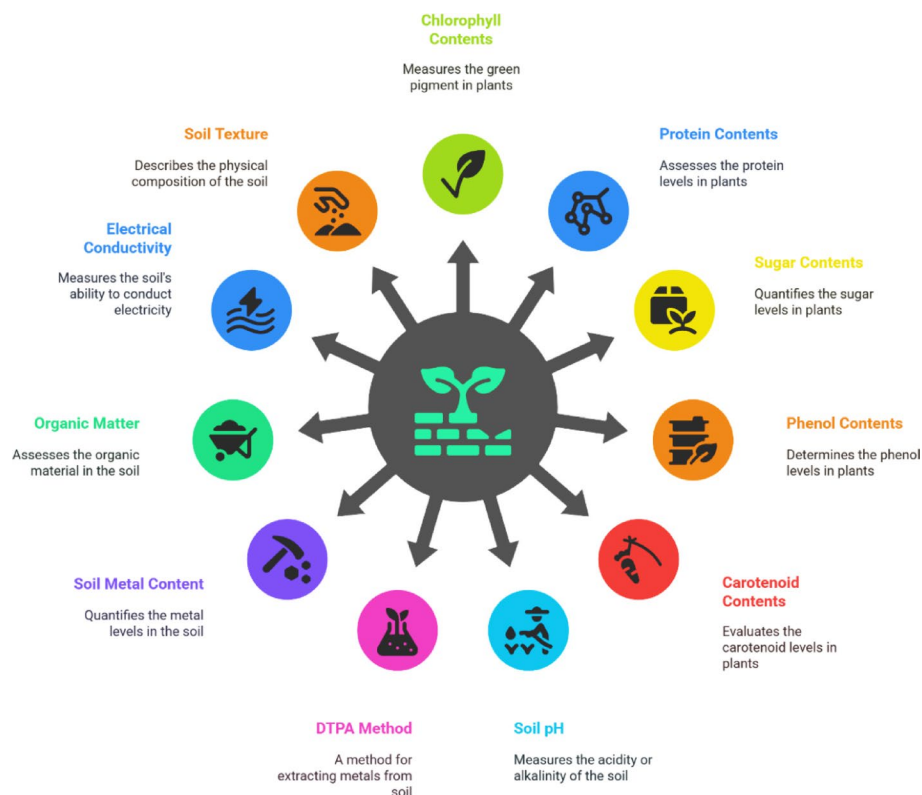


Fig. 1 Schematic representation of the experimental workflow. The process included soil preparation and spiking with varying Boron (B) and Zinc (Zn) concentration, seed germination, plant growth under controlled conditions, and subsequent biochemical and physiological analysis of *B. rapa* L. tissues

Treatments were applied at four concentration levels: 10, 15, 20, and 25 mg kg⁻¹ of soil. In the Zn + B group, each dose consisted of equal concentrations of Zn and B (e.g., 10 mg kg⁻¹ Zn + 10 mg kg⁻¹ B). Untreated soil was analyzed for baseline concentrations of several metals, including Ni, Zn, Cd, Cr, Fe, Pb, Ca, Mg, and Mn, using atomic absorption spectrometry (AAS) (Supplementary Table S1). Although the soil was not sourced from a certified contaminated site, residual metal content was present due to prior field use. After the amendment, soils were thoroughly homogenized and re-sieved before potting. Each treatment, including the non-amended control, was replicated three times ($n = 3$). Plants were cultivated in plastic pots (15-cm diameter × 20-cm height), each filled with 1 kg of treated soil. All pots were maintained under controlled greenhouse conditions with uniform irrigation and temperature.

2.3 Preparation of *B. rapa* L. seeds

Seeds of *B. rapa* L. (var. Maqsood) were purchased from the Institute of Biotechnology and Genetic Engineering (IBGE), University of Agriculture, Peshawar, Pakistan. The cultivation experiments were conducted at the Department of Botany, Abdul Wali Khan University, Mardan (34.2131° N, 72.0680° E). The seeds were surface-sterilized in 30% hydrogen peroxide for 15 min. The samples were carefully washed with deionized water. These disinfected seeds of *B. rapa* L. were then germinated on moistened filter paper placed in Petri dishes. The germination chamber was maintained at 25 ± 1 °C with a 16/8 h light/dark photoperiod and a light intensity of approximately 150 μmol m⁻² s⁻¹. Relative humidity was maintained at approximately 70%, and filter papers were re-moistened daily to ensure consistent hydration. After four days, uniformly germinated healthy seedlings measuring approximately 3–5 cm in height and bearing one to two leaves were selected for transplantation into prepared pots. Plants were cultivated in a climate-controlled greenhouse following the conditions described by [39], where temperature (25 °C Day/19 °C night), relative humidity (52%), and a 12-h photoperiod were actively maintained throughout the experiment. Seedlings were irrigated with distilled water throughout the experiment. Each pot received 100 mL of distilled water every two days, adjusted as needed to maintain consistent soil moisture without waterlogging. Each pot was regularly rotated to ensure uniform exposure to light and temperature. Irrigation volumes were determined based on preliminary trials and monitored visually to ensure uniform hydration across treatments. Two weeks after cultivation, the aboveground parts of *B. rapa* L. plants were harvested and washed with distilled water to remove any adhering soil particles.

2.4 Determination of total chlorophyll contents in fresh *B. rapa* L. leaves

Chlorophyll content in fresh *B. rapa* L. leaves was determined using the [40] method. Briefly, to prepare a 0.1 M ammonium hydroxide solution (NH₄OH), 0.35 g of NH₄OH was dissolved in 100 mL of distilled water. An extraction solution was prepared by mixing NH₄OH solution and 80% acetone in a 1:9 ratio (10 mL:90 mL). An 80% acetone solution was prepared by mixing 20 mL of distilled water with 80 mL of acetone. Fresh *B. rapa* L. leaves were ground; approximately 1.0 g of leaf tissue was homogenized thoroughly using a mortar and pestle. The homogenate was then transferred into an extraction solution containing 10 mL of NH₄OH and 90 mL of acetone. The mixture was centrifuged at 1000 rpm for 10 min, and the supernatant was collected. The volume was

adjusted to 100 mL with 80% acetone and stored at 4 °C. Finally, the mixture was stored in test tubes at 4 °C for 24 h before absorbance measurement. The absorbance of the supernatant was measured at 645 nm and 663 nm for each chlorophyll *a* and *b*, respectively. Chlorophyll contents were expressed in mg g⁻¹ fresh weight (FW), based on a calibration curve prepared using pure chlorophyll standards.

2.5 Determination of total protein content in fresh *B. rapa* L. leaves

Total protein levels in *B. rapa* L. leaves were determined using the method described in [41]. Briefly, 0.1 g of fresh *B. rapa* L. leaves were homogenized in 1 mL of 50 mM phosphate buffer (pH 7.5) using a mortar and pestle. The mixture was then centrifuged at 3000 rpm for 15 min, and 0.1 mL of the supernatant was transferred to test tubes containing distilled water. Subsequently, 1 mL of alkaline copper solution and 0.1 mL of carbonate copper solution were added, and the mixture was shaken for 10 min before being incubated for 30 min. A spectrophotometer (Bio Lambda) was used to measure the absorbance of each sample at 650 nm. Protein concentration was quantified using a bovine serum albumin (BSA) calibration curve and expressed as mg g⁻¹ fresh weight (FW) of the leaves. BSA standards were prepared at various concentrations, including 20, 40, 60, 80, 320, and 640 µg/mL.

2.6 Determination of total sugar contents in fresh *B. rapa* L. leaves

The sugar content of *B. rapa* L. leaves, a protocol [42] was followed. Briefly, 0.5 g of fresh *B. rapa* L. leaves from each pot were homogenized in 10 mL of DW using a mortar and pestle, then centrifuged at 3000 rpm for 5 min. The supernatant was mixed with 0.1 mL of 30% phenol solution and incubated for 4 min at room temperature. Subsequently, 4 mL of concentrated sulfuric acid was added carefully and stored in an incubator for 1 h before the absorbance of each sample was measured using a spectrophotometer (Bio Lambda) at 490 nm. The total sugar content in the leaves was then calculated from the calibration curve using glucose and expressed as mg g⁻¹ fresh weight (FW).

2.7 Determination of total phenol content in fresh *B. rapa* L. leaves

Phenolic levels in *B. rapa* L. leaves were determined using the method described in [43]. Briefly, 50 mg of dried leaves was used for extraction. Leaves were oven-dried at 60 °C for 48 h to constant weight, then ground to a fine powder using a mortar and pestle. The powdered material was extracted in 70% methanol and centrifuged at 3000 rpm for 10 min. After the supernatant volume reached 10 mL, 70% methanol was added. 1 mL of the extract was transferred to test tubes, and 2 mL of 20% Na₂CO₃ was added. The test tubes were then placed in a water bath at 40 °C for 30 min. After incubation, samples were cooled to room temperature before the absorbance was measured. Total phenolic content was determined using a spectrophotometer (Bio Lambda). Absorbance at 650 nm expressed as mg g⁻¹ dry weight (DW), based on a calibration curve prepared with gallic acid (GAE) g⁻¹ dry weight (DW) as the standard.

2.8 Determination of total proline content in fresh *B. rapa* L. leaves

Proline level in *B. rapa* L. leaves was determined using the method described in [44]. Briefly, 100 mg of fresh leaves from each sample was collected and homogenized with 3% sulfosalicylic acid at a ratio of 5 µL/mg fresh weight (FW) using a mortar and pestle. Test

tubes were maintained during sample processing. After homogenization, the mixtures were centrifuged at room temperature for 5 min. Next, a reaction mixture was prepared in separate test tubes containing 100 μL of 3% 5-aminosalicylic acid, 200 μL of glacial acetic acid, and acidic ninhydrin reagent (1.25 mM ninhydrin in glacial acetic acid). Then, 100 μL of the *B. rapa* L. leaf-derived extract supernatant was carefully added to each reaction mixture and thoroughly mixed to prevent pressure during heating. The test tubes were incubated at 96 $^{\circ}\text{C}$ for 1 h using a thermostatically controlled water bath to ensure uniform heating during the extraction. Finally, 1 mL of toluene was added to each test tube for chromophore extraction, vortexed only for 20 s at room temperature, and allowed to separate into phases within 5 min. The toluene (upper layer) was carefully transferred into fresh test tubes. Absorbance was measured at 520 nm using a spectrophotometer (Bio Lambda). Proline concentration was calculated using a standard calibration curve prepared with proline and expressed as mg g^{-1} fresh weight (FW).

2.9 Determination of total carotenoid contents in fresh *B. rapa* L. leaves

Carotenoid content was determined using the method described in [40]. Briefly, 50 mg of fresh *B. rapa* L. leaves were collected from each pot and homogenized in 5 mL of 80% acetone using a mortar and pestle. After the mixture was filtered through Whatman No. 42 filter paper, the pigment-rich filtrate was recollected repeatedly until the residual leaf tissue appeared pale and colorless, indicating complete extraction. Each extraction cycle used 5 mL of 80% acetone, with additional volumes added until no visible pigment remained in the tissue. Finally, the extract volume was increased to 25 mL. Absorbance was measured at 480 and 510 nm using a spectrophotometer (Bio Lambda), and carotenoid content was expressed in mg g^{-1} fresh weight (FW), based on a calibration curve prepared with β -carotene as the standard.

2.10 Determination of soil pH

Soil pH was measured using a 1:1 (soil: water) suspension as described in [45]. In summary, pH was measured using a 1:1 ratio of soil to 0.01 M CaCl_2 solution, which was carefully prepared. A glass beaker was filled with 100 mL of solution and 50 g of 2-mm air-dried soil. Before incubation for 30 min, 50 mL of distilled water was added to the flask using a graduated cylinder, and the mixture was thoroughly stirred with a glass rod. During incubation, the suspension was stirred every 10 min to ensure homogeneity. After 1 h, the suspension was gently mixed again, and a combination glass electrode was immersed approximately 3 cm into the solution to record the pH. After measurement, as listed in Table 1, the electrodes were removed, rinsed thoroughly with distilled water in a separate beaker, and dried by blotting with clean tissue.

2.11 Diethylene triamine Penta acetic acid (DTPA) method

The bioavailable fraction of soil metals was determined using the DTPA extraction method described in [46]. Briefly, 1 g of soil was extracted with 10 mL of a solution containing 0.005 M DTPA, 0.01 M CaCl_2 , and 0.1 M triethanolamine (TEA) at pH 7.3. The mixture was shaken for 2 h at room temperature and then filtered. The concentration of extractable metals—including chromium (Cr), calcium (Ca), magnesium (Mg), nickel (Ni), zinc (Zn), cadmium (Cd), iron (Fe), lead (Pb), and manganese (Mn)

Table 1 Selected physical–chemical properties under Zn and B supplementation of 10, 15, 20, 25 mg kg^{−1} either individual or combined on *B. rapa* L. seed in silt loam soil

S. no	Groups	pH	OM (%)	EC (dS m ^{−1})	Nitrogen (mg kg ^{−1})
1	Control (<i>untreated</i>)	8.410 ^a	0.000G	0.723 ^b	0.000 ⁱ
2	B (10 mg kg ^{−1})	8.160 ^{de}	0.243 ^a	0.625 ^{bcd}	0.023 ^d
3	B (15 mg kg ^{−1})	8.093 ^g	0.241 ^a	0.597 ^{bcd}	0.038 ^a
4	B (20 mg kg ^{−1})	8.150 ^{de}	0.103 ^e	0.716 ^b	0.016 ^f
5	B (25 mg kg ^{−1})	8.166 ^d	0.067 ^f	0.700 ^b	0.012 ^g
6	Zn (10 mg kg ^{−1})	8.263 ^b	0.000 ^g	0.507 ^{de}	0.000 ⁱ
7	Zn (15 mg kg ^{−1})	8.236 ^c	0.067 ^f	0.655 ^{bc}	0.011 ^h
8	Zn (20 mg kg ^{−1})	8.070 ^h	0.137 ^d	1.020 ^a	0.022 ^e
9	Zn (25 mg kg ^{−1})	8.243 ^{bc}	0.103 ^e	1.020 ^a	0.016 ^f
10	B + Zn (10 mg kg ^{−1})	8.140 ^{ef}	0.137 ^c	0.556 ^{cde}	0.022 ^{de}
11	B + Zn (15 mg kg ^{−1})	8.143 ^e	0.173 ^c	0.484 ^{de}	0.027 ^c
12	B + Zn (20 mg kg ^{−1})	8.150 ^{de}	0.241 ^a	0.426 ^e	0.038 ^a
13	B + Zn (25 mg kg ^{−1})	8.120 ^f	0.226 ^b	0.487 ^{de}	0.036 ^b

The experiment involved collecting soil for each group, mean ($n=3$). Different letters indicate a significant difference (LSD, $p<0.05$). EC: Electrical conductivity (dS m^{−1}); OM: Organic matter (%)

- were determined using atomic absorption spectrometry (AAS) with flame atomization (F-AAS), and graphite furnace (GF-AAS) for elements present at trace level.

2.12 Total digestion for soil metal content

Total metal concentrations in soil were determined using the method described in [47]. Briefly, 1 g of air-dried, finely ground soil was placed in a Teflon beaker and treated with a concentrated acid mixture containing 7 mL HNO₃, 3 mL HClO₄, and 5 mL HF. The mixture was heated on a hot plate under a fume hood at 120–130 °C until a clear solution indicated complete digestion. After cooling, the digest was evaporated to near dryness, and the residue was dissolved in 10 mL of 0.5 N HNO₃. The final solution was filtered using deionized water. The concentrations of total Cr, Ca, Mg, Ni, Zn, Cd, Fe, Pb, and Mn were determined using atomic absorption spectrometry (AAS).

2.13 Determination of the organic matter

Organic matter (OM) content was determined using the procedure described in [48]. Briefly, 1 g of soil was transferred to a conical flask, and 10 mL of 1 N potassium dichromate was added to completely moisten it. Furthermore, KMNO₄ was titrated from a burette into a mixture containing 200 mL of distilled water and 25 mL of ferrous ammonium sulfate, and the color change from pink to purple to red was observed to indicate the endpoint. Finally, a blank was run in the same manner to calculate the OM percentage (%), as listed in Table 1.

2.14 Determination of the electrical conductivity of soil

Soil electrical conductivity was determined using the method described in [48]. Briefly, 20 g of air-dried soil was mixed with 100 mL of distilled water (1:5 w/v) in a beaker. The mixture was stirred intermittently for 30 min. After settling, the supernatant was analyzed for electrical conductivity. Electrical conductivity (EC) values were recorded in dS m^{−1}, and the suspension temperature was also noted, as listed in Table 1.

2.15 Statistical analysis

All experiments were conducted using a completely randomized design (CRD) with three replicates per treatment ($n=3$). The treatment structure included 13 distinct levels: four doses of Zn, four doses of B, four doses of the Zn + B combination, and one control without micronutrient supplementation. The Zn + B combination was applied as an independent treatment rather than a factorial combination of Zn and B. A one-way ANOVA was performed to assess differences among all treatment levels, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test ($p \leq 0.05$).

To specifically evaluate the synergistic potential of the micronutrient. A separate factorial ANOVA was executed on the Zn and B combination database, treating each micronutrient as an independent factor. This approach allowed for the determination of the interaction effect (Zn $\times$ B), which statistically defines synergy. The magnitude of these effects was further quantified using partial eta-squared (η^2) to estimate effect size [49]. Multivariate assessment included Pearson's correlation analysis ($p < 0.05$) to examine trait interdependencies and hierarchical cluster analysis (HCA) to classify treatments based on plant soil element profile [50]. All statistical computations were performed using SPSS version 18.0.

3 Results

3.1 Zn and B supplementation improved *B. rapa* L. growth

The combined application of Zn and B significantly enhanced the morphological development of *B. rapa* L. compared to individual treatment ($p < 0.05$). Specifically, the co-application of 20 mg kg⁻¹ Zn + B treatment maximized shoot length to 20.00 cm. A 13.2% increase over the control (17.66 $\pm$ 0.00 cm). Zn + B treatment significantly enhanced shoot and root elongation relative to individual treatments treatments ($p < 0.05$), as shown in Fig. 2A, B. The most pronounced synergistic effect on root development was observed at the 20 mg kg⁻¹ Zn + B dose, where root length reached 10.00 cm. A 35.4%

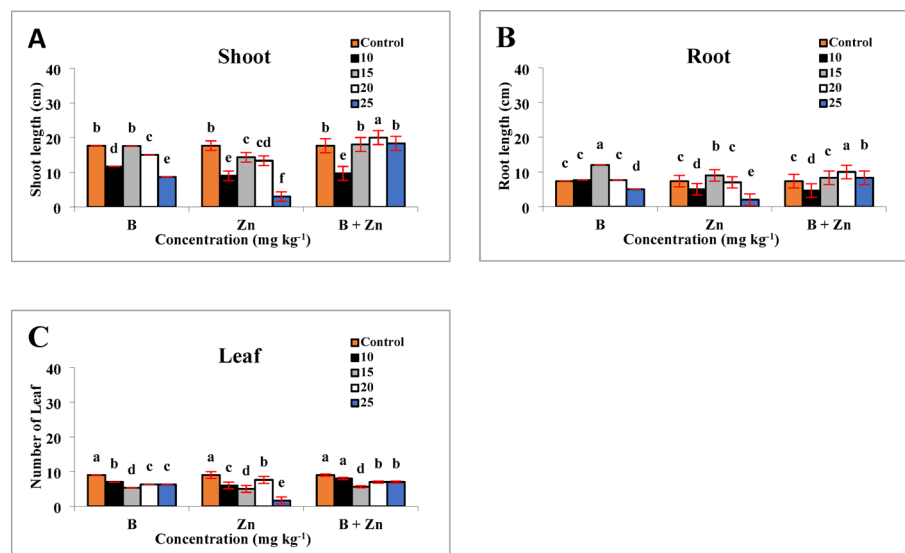


Fig. 2 concentrations Figure 2. Effects of individual and combined application of Boron (B) and Zinc (Zn) on the vegetative growth parameters of *B. rapa* L. (A) Shoot length, (B) Root length, (C) Number of leaves. Following treatment with B, Zn, and B + Zn at concentrations of 10, 15, 20, 25 mg kg⁻¹. Data are presented as mean $\pm$ SD values of at least three independent experiments ($n = 3$). Values with different superscript letter denotes statistically significant differences at $p < 0.05$, as determined by Tukey's HSD test.

improvement over the control (7.33 ± 0.00 cm), and significantly exceeded the individual Zn treatment (7.33 ± 1.58 cm) at the same concentration. Shoot length under Zn + B treatment exceeded all other groups, showing improved nutrient uptake and cell expansion, as shown in Fig. 2A. In contrast, leaf number declined across all treatments relative to the control. However, the 25 mg kg^{-1} Zn + B maintained the highest leaf count among the combined treatments, 7.00 ± 0.65 cm. Showing a small reduction compared to the individual Zn treatments at the same dose (1.66 ± 2.90 cm), which represents a higher retention of foliage (Fig. 2C). Overall, Zn + B co-supplementation was most effective in promoting structural growth parameters.

3.2 Physiology/biochemical investigation

3.2.1 Impact of Zn and B on the chlorophyll contents of *B. rapa* L

A significant interaction between Zn and B was observed, influencing chlorophyll content in *B. rapa* L. leaves. The co-application of Zn and B at 15 mg kg^{-1} yielded the highest total chlorophyll levels ($1.76 \pm 0.31 \text{ mg g}^{-1}$ FW), showing a 10.7% increase over the untreated control ($1.59 \pm 0.45 \text{ mg g}^{-1}$ FW; $p < 0.05$), as shown in Fig. 3B. Conversely, high individual concentrations of these micronutrients proved detrimental. The lowest chlorophyll levels were recorded in the 25 mg kg^{-1} B ($1.38 \pm 0.29 \text{ mg g}^{-1}$ FW) and Zn ($1.51 \pm 0.43 \text{ mg g}^{-1}$ FW), which were 13.2% and 5% lower than the control, respectively. Across all the experimental range ($10\text{--}25 \text{ mg kg}^{-1}$), the Zn + B combination consistently mitigated the inhibitory effect compared with individual applications. Statistically significant increases were observed at 15 and 20 mg kg^{-1} co-application, as shown in Fig. 3B, C. At 10 mg kg^{-1} (Fig. 3A), the combined application showed a modest but significant improvement ($1.46 \pm 0.45 \text{ mg g}^{-1}$ FW) compared to the individual Zn treatment ($1.36 \pm 0.46 \text{ mg g}^{-1}$ FW). While Chlorophyll levels at 25 mg kg^{-1} declined compared to the 15 mg kg^{-1} . The B + Zn group ($1.71 \pm 0.31 \text{ mg g}^{-1}$ FW) still retained 23.9% more pigment compared with 25 mg kg^{-1} B-only treatment, as shown in Fig. 3D, showing a

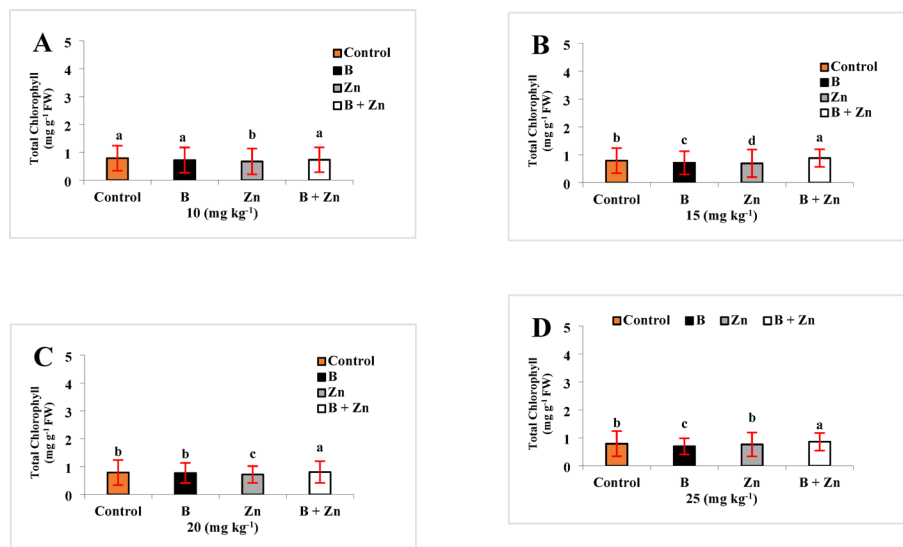


Fig. 3 Total chlorophyll content in the leaves of *B. rapa* L. Following exposure to individual B, Zn and their co-application (B + Zn) at four concentration **A** 10 mg kg^{-1} , **B** 15 mg kg^{-1} , **C** 20 mg kg^{-1} , **D** 25 mg kg^{-1} . Data are presented as mean $\pm$ SD values of at least three independent biological replicates ($n = 3$). Values with different superscript letter denotes statistically significant differences at $p < 0.05$, as determined by Tukey's HSD test. Total chlorophyll represents the sum of chlorophyll *a* and *b*, expressed as mg g^{-1} fresh weight (FW)

concentration-dependent inhibitory effect. In summary, the observed treatment trend is (B + Zn > B > Zn).

3.2.2 Effect of Zn and B on carotenoid contents of *B. rapa* L. leaves

Total carotenoid content was analyzed to evaluate the correlation between accessory pigments and chlorophyll biosynthesis. Combined Zn and B supplementation significantly influenced carotenoid profiles in a dose-dependent manner. Particularly at 15 Zn + B mg kg⁻¹, the accumulation reached 0.29 ± 0.03 mg g⁻¹ FW as shown in Fig. 4B. The maximum synergistic effect at higher concentration was observed at 25 mg kg⁻¹ of Zn + B 0.45 ± 0.13 mg g⁻¹ FW; Fig. 4D). This 25 mg kg⁻¹ combined dose showed a substantial improvement over individual Zn (0.29 ± 0.09 mg g⁻¹ FW) and B (0.34 ± 0.07 mg g⁻¹ FW) treatments ($p < 0.05$). At 10 mg kg⁻¹ (Fig. 4A), co-application also produced a statistically significant increase (0.26 ± 0.01 mg g⁻¹ FW). The individual applications at 20 mg kg⁻¹ Zn and B resulted in levels of 0.29 ± 0.09 and 0.53 ± 0.12 mg g⁻¹ FW, respectively. The Zn + B concentrations maintained a stable level of 0.39 ± 0.13 mg g⁻¹ FW, as shown in Fig. 4C. The results establish a treatment hierarchy of B + Zn > B > Zn regarding pigment stability at supra-optimal doses.

3.2.3 Effect of Zn and B on sugar level in *B. rapa* L. leaves

Sugar content increased significantly at 10 mg kg⁻¹ under both Zn and B treatments, with a declining trend observed at higher concentrations ($p < 0.05$). The co-application of Zn + B at 10 mg kg⁻¹ maximized sugar accumulation to 0.57 ± 0.00 mg g⁻¹ FW. A 2.8% increase over the control (0.55 ± 0.00 mg g⁻¹ FW; Fig. 5A. Among individual treatments at this dose, B (0.55 ± 0.00 mg g⁻¹ FW) showed a stronger stimulatory effect on sugar metabolism compared with Zn (0.53 ± 0.00 mg g⁻¹ FW). Notably, at 15 mg kg⁻¹, the Zn + B treatment maintained an elevated sugar level of 0.56 ± 0.00 mg g⁻¹ FW (Fig. 5C), significantly higher than the individual Zn treatment (0.51 ± 0.00 mg g⁻¹ FW).

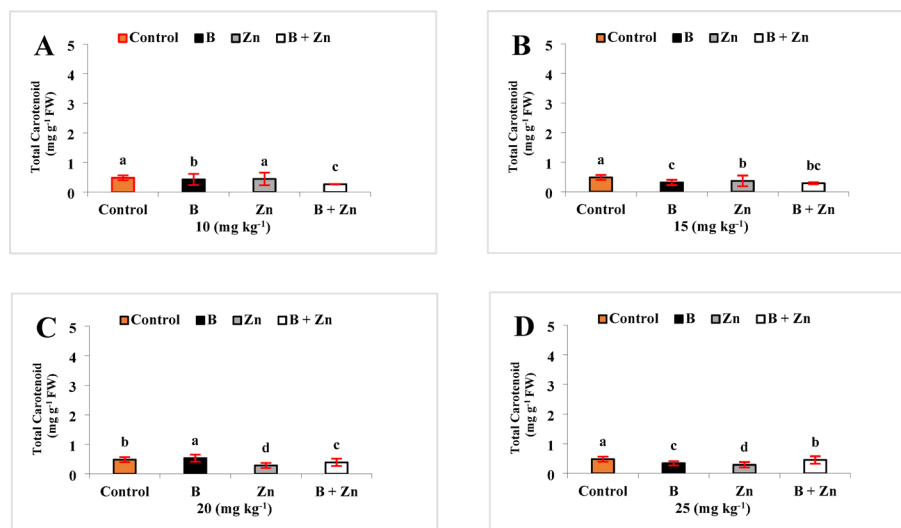


Fig. 4 Total carotenoid content in the leaves of *B. rapa* L. Following exposure to individual B, Zn and their co-application (B + Zn) at four concentration **A** 10 mg kg⁻¹, **B** 15 mg kg⁻¹, **C** 20 mg kg⁻¹, **D** 25 mg kg⁻¹. Data are presented as mean $\pm$ SD values of at least three independent biological replicates ($n = 3$). Values with different superscript letter denotes statistically significant differences at $p < 0.05$, as determined by Tukey's HSD test. Total carotenoids represent the sum of carotenoids (480 nm) and carotenoids (510 nm). Expressed as mg g⁻¹ fresh weight (FW)

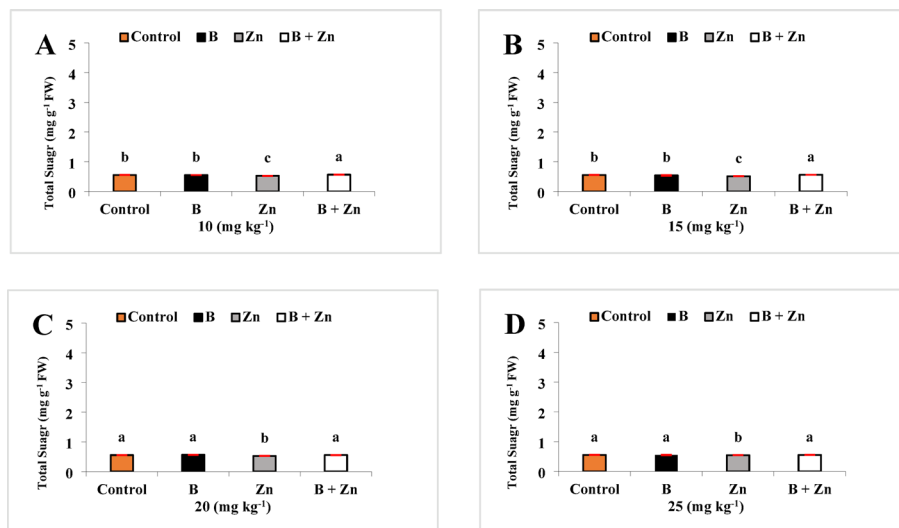


Fig. 5 Total sugar content in the leaves of *B. rapa* L. Following exposure to individual B, Zn and their co-application (B + Zn) at four concentration **A** 10 mg kg⁻¹, **B** 15 mg kg⁻¹, **C** 20 mg kg⁻¹, **D** 25 mg kg⁻¹. Data are presented as mean $\pm$ SD values of at least three independent biological replicates ($n=3$). Values with different superscript letter denotes statistically significant differences at $p < 0.05$, as determined by Tukey's HSD test. Sugar content was expressed as mg g⁻¹ fresh weight (FW)

As concentration increased to 20 and 25 mg kg⁻¹ (Fig. 5C, D), the stimulatory effect diminished, with sugar level either plateauing or declining across all groups. However, even at the 25 mg kg⁻¹, the Zn + B group (0.55 ± 0.00 mg g⁻¹ FW) remained more stable compared with the Zn-only group (0.54 ± 0.00 mg g⁻¹ FW). In summary, the established observed treatment trend is B + Zn > B > Zn.

3.2.4 Impact of Zn and B on the phenolic contents of *B. rapa* L. Leaves

Phenolic content was measured in *B. rapa* L. leaves under heavy metal exposure, as shown in Fig. 6A–D. The synergistic application of Zn and B significantly enhanced phenolic accumulation, at the 15 mg kg⁻¹ dose (1.62 ± 0.00 mg g⁻¹ FW; Fig. 6B). This enhancement is nearly six times higher than the individual B treatment (0.26 ± 0.01 mg g⁻¹ FW) and over twenty times higher than the individual Zn treatment (0.07 ± 0.00 mg g⁻¹ FW; $p < 0.05$). This result is parallel in observed chlorophyll and carotenoid levels at 15 mg kg⁻¹. In contrast, at the 10 mg kg⁻¹ dose (Fig. 6A), phenolic induction remained relatively low across all groups (0.26 ± 0.40 mg g⁻¹ FW) compared to the control (1.91 ± 0.01 mg g⁻¹ FW). As concentration increased to 20 and 25 mg kg⁻¹ (Figs. 6C, D), phenolic levels generally declined across, though individual Zn at 25 mg kg⁻¹ showed a unique increase to 1.29 ± 0.01 mg g⁻¹ FW. The Zn + B group maintained a level of 0.49 ± 0.00 mg g⁻¹ FW. These findings establish a performance hierarchy of Zn + B > Zn > B regarding the induction of secondary metabolite biosynthesis at optimal doses.

3.2.5 Regulation of leaf protein contents by Zn and B in *B. rapa* L. Leaves

Protein contents of *B. rapa* L. leaves were assessed under varying concentrations of Zn and B, as shown in Fig. 7A–D. A concentration-dependent response was observed across treatments ($p < 0.05$). At 10 mg kg⁻¹ treatment (Fig. 7A), individual B (2.34 ± 0.01 mg g⁻¹ FW) and Zn (2.31 ± 0.00 mg g⁻¹ FW) treatments moderately increased protein content

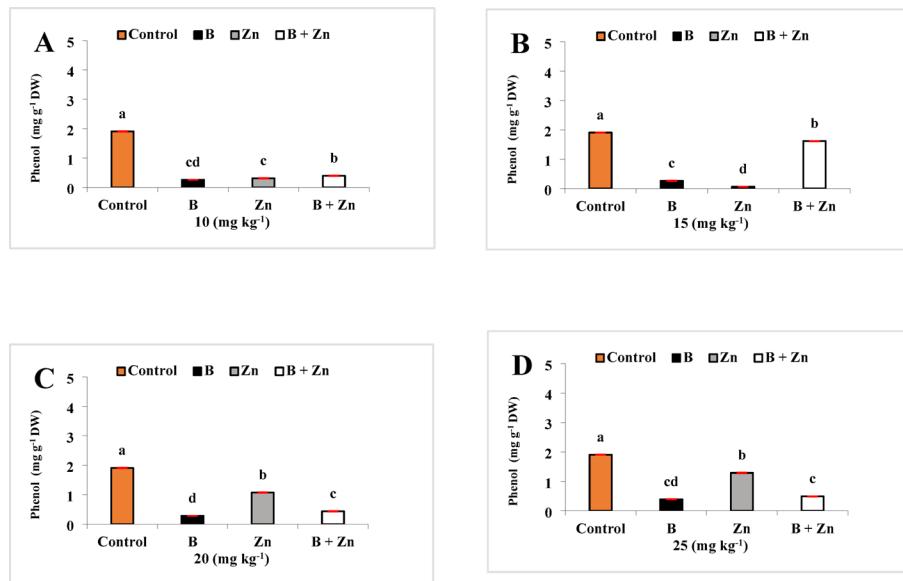


Fig. 6 Total phenolic content in the leaves of *B. rapa* L. Following exposure to individual B, Zn and their co-application (B + Zn) at four concentration **A** 10 mg kg⁻¹, **B** 15 mg kg⁻¹, **C** 20 mg kg⁻¹, **D** 25 mg kg⁻¹. Data are presented as mean $\pm$ SD values of at least three independent biological replicates ($n=3$). Values with different superscript letter denotes statistically significant differences at $p < 0.05$, as determined by Tukey's HSD test. Phenolic content was expressed as mg g⁻¹ dry weight (DW)

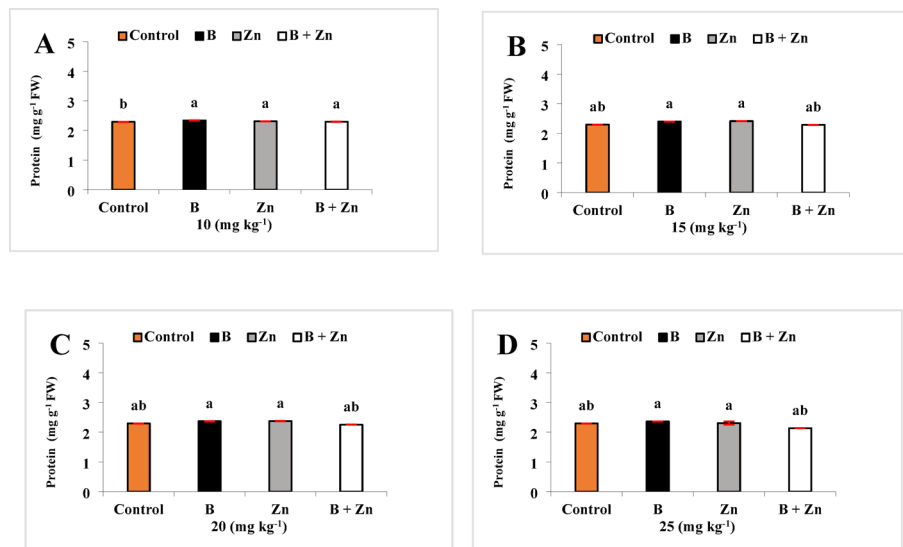


Fig. 7 Total protein content in the leaves of *B. rapa* L. Following exposure to individual B, Zn and their co-application (B + Zn) at four concentration **A** 10 mg kg⁻¹, **B** 15 mg kg⁻¹, **C** 20 mg kg⁻¹, **D** 25 mg kg⁻¹. Data are presented as mean $\pm$ SD values of at least three independent biological replicates ($n=3$). Values with different superscript letter denotes statistically significant differences at $p < 0.05$, as determined by Tukey's HSD test. Protein content was expressed as mg g⁻¹ fresh weight (FW)

compared with the control (2.29 ± 0.00 mg g⁻¹ FW). At 15 mg kg⁻¹ (Fig. 7B), protein levels remained elevated under individual Zn (2.41 ± 0.01 mg g⁻¹ FW) and B (2.40 ± 0.01 mg g⁻¹ FW) application. However, B + Zn co-application resulted in a reduction to (2.28 ± 0.00 mg g⁻¹ FW), showing a shift in stress signaling or metabolic prioritization. As concentration reached (20–25 mg kg⁻¹; Fig. 7C, D), protein levels declined across all treatments. Notably, the 25 mg kg⁻¹ B + Zn group showed the lowest protein content

($2.14 \pm 0.00 \text{ mg g}^{-1} \text{ FW}$), showing a 6.5% decrease relative to the control. The individual B treatment at the same dose remained elevated ($2.36 \pm 0.01 \text{ mg g}^{-1} \text{ FW}$). Collectively, these results show a hierarchy of $B > \text{Zn} > \text{Zn} + \text{B}$ for protein accumulation.

3.2.6 Regulation of proline contents by Zn and B in *B. rapa* L. Leaves

Proline content in *B. rapa* L. was determined to evaluate osmotic adjustment under varying concentrations of Zn and B, as shown in Fig. 8A–D. Individual Zn treatments at 10 mg kg^{-1} ($1.80 \pm 0.00 \text{ mg g}^{-1} \text{ FW}$; Fig. 8A) and B treatment at 25 mg kg^{-1} ($2.18 \pm 0.00 \text{ mg g}^{-1} \text{ FW}$; Fig. 8D) resulted in the highest proline accumulation among single applications, showing a strong stress-induced osmoprotective response. In contrast, the combined Zn + B treatment at 15 mg kg^{-1} led to a balanced accumulation of $1.56 \pm 0.00 \text{ mg g}^{-1} \text{ FW}$ (Fig. 8B), which was nearly identical to the individual Zn treatment ($1.54 \pm 0.01 \text{ mg g}^{-1} \text{ FW}$). Notably, at 10, 20, and 25 mg kg^{-1} (Fig. 8A, C, D), proline level under co-application declined sharply to 0.18 ± 0.00 , 0.02 ± 0.00 , and $0.06 \pm 0.00 \text{ mg g}^{-1} \text{ FW}$, respectively. Compared to the 25 mg kg^{-1} B-only treatment ($2.18 \pm 0.00 \text{ mg g}^{-1} \text{ FW}$). The co-application at the same dose reduced proline by 97.2%, showing significantly reduced physiological stress signaling ($p < 0.05$). These results build a hierarchy of $B/\text{Zn} > \text{control} > \text{B} + \text{Zn}$, showing that individual micronutrient excess triggers high osmotic stress.

3.3 Effects of Zn and B supplementation on soil physicochemical properties in *B. rapa* L., cultivation

Zn and B supplementation, individually and in combination, significantly influenced soil physicochemical properties compared with the untreated control ($p < 0.05$). Soil pH decreased slightly across all treatment groups, with the most significant reduction (4.0%) occurring in the 20 mg kg^{-1} . Organic matter (OM) content, which was initially undetectable in the control, increased significantly under supplementation; the B (10 mg kg^{-1}) and Zn + B (20 mg kg^{-1}) groups reached a maximum of 0.243% and 0.241%, respectively.

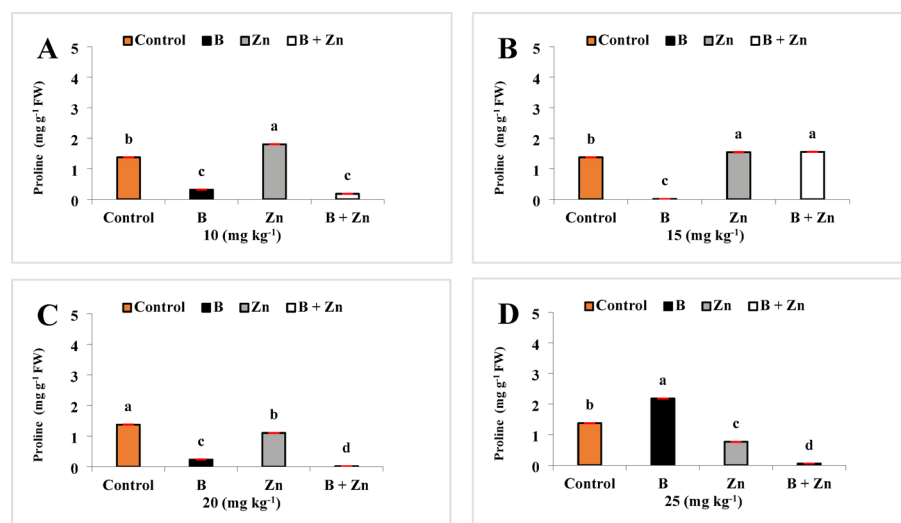


Fig. 8 Total proline content in the leaves of *B. rapa* L. Following exposure to individual B, Zn and their co-application (B + Zn) at four concentration **A** 10 mg kg^{-1} , **B** 15 mg kg^{-1} , **C** 20 mg kg^{-1} , **D** 25 mg kg^{-1} . Data are presented as mean $\pm$ SD values of at least three independent biological replicates ($n = 3$). Values with different superscript letter denotes statistically significant differences at $p < 0.05$, as determined by Tukey's HSD test. Proline content was expressed as mg g^{-1} fresh weight (FW)

Furthermore, Electrical conductivity (EC) increased progressively from 0.723 dS m^{-1} in the control to a peak of 1.020 dS m^{-1} in the 25 mg kg^{-1} Zn treatment, showing a 41.1% increase in ionic activity. Nitrogen level also showed a marked response, rising from 0.00 mg kg^{-1} in the control to a maximum of 0.38 mg kg^{-1} in the 15 mg kg^{-1} B and 20 mg kg^{-1} B + Zn treatments. Collectively, these results establish a trend of $\text{B} + \text{Zn} > \text{B} > \text{Zn}$ for nutrient enrichment, showing that synergistic application optimizes the soil chemical environment, as shown in Table 1.

3.4 Assessment of micro- and macroelements in *B. rapa* L. and soil and their toxic effects

Cd, Pb, and Cr exhibited elevated concentrations compared with Ca, Ni, Fe, Mn, and Mg. Among these, Cd showed the highest levels in both soil (Fig. 9A) and plant tissues (Fig. 9B), indicating strong bioaccumulation and translocation capacity from soil to plant. This indicates high phytoavailability of Cd under applied treatments. Parallel

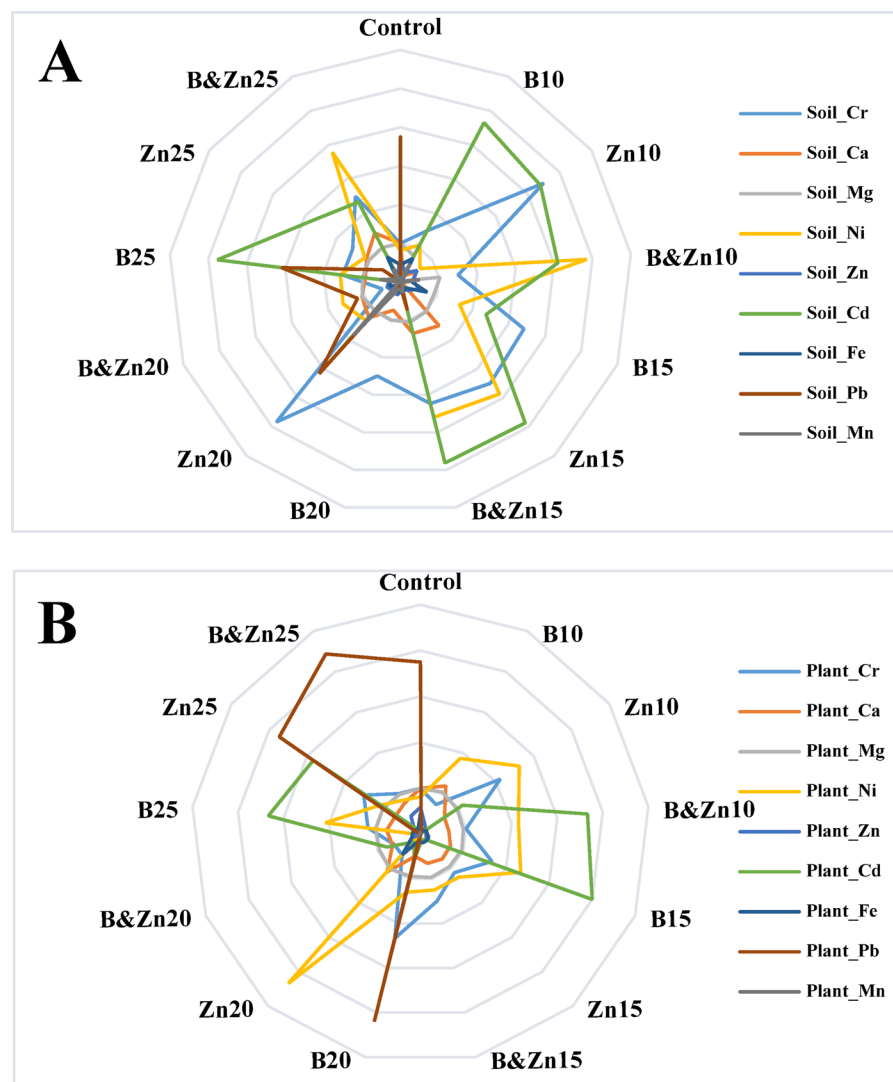


Fig. 9 Radar plots illustrating the concentration profiles of essential heavy metals and micronutrients under different B, Zn, and combined B + Zn treatments in *B. rapa* L. (A) Post-treatment elemental concentrations in soil, and (B) corresponding elemental accumulation in *B. rapa* L. plant tissues grown under B, Zn, and B + Zn at 10, 15, 20, and 25 mg kg^{-1} . Each radar plot represents the mean of three replicates ($n=3$), with elements including Cr, Cd, Ni, Zn, Pb, Fe, Mn, and Cu. Distinct polygonal patterns reflect treatment-specific variations in uptake and translocation

trends of Cd and Pb between soil and plant matrices imply efficient mobilization of these metals from soil into plant tissue. In contrast, Fe, Mn, and Mg remained relatively stable or low, with limited variation across treatments. Cr levels were moderately elevated in both soil and plant samples, indicating moderate mobility and partial uptake by plants. Zn and Ca displayed noticeable treatment-specific variation under Zn-enriched conditions (Zn 20, Zn 25 mg kg⁻¹, and B + Zn combination), reflecting their dual role as essential nutrients and potential competitors with heavy metals during uptake. Overall, Cd exhibited the strongest accumulation trend, followed by Pb and Cr, whereas Ca, Fe, Mn, Ni, and Mg showed comparatively lower concentrations.

3.5 Correlation between soil and plant metal concentration in *B. rapa* L

The transfer of measured metals from soil to plant exhibited weak linear relationships, as shown in Fig. 10A–I. The strongest linear correlation was observed for Cd ($R^2 = 0.0113$), followed by Pb ($R^2 = 0.0876$) and Cr ($R^2 = 0.0067$), as shown in Fig. 10A–C. The weakest linear relationships, indicating poor correlation between soil and plant concentrations, were observed for the essential nutrients Ca ($R^2 = 0.00978$), Mg ($R^2 = 0.0285$), and Mn ($R^2 = 0.044$), as shown in Fig. 10D–F. Regression slopes were highly variable, with positive trends observed only for Cd and Pb. Most other metals, including Fe, Zn, Ni, Cr, Ca, and Mg, exhibited negligible negative slopes. Overall, no metal exhibited a statistically significant linear correlation indicative of concentration-dependent transfer from soil to plant.

3.6 Principal component analysis (PCA): elemental drives and treatment clustering

PCA revealed distinct clustering of samples treated with higher Zn + B concentrations (20–25 mg kg⁻¹), particularly along PC1 and PC2, as shown in Fig. 10. Together, these two components explained 66.33% of the total variance, with PC1 accounting for 49.87% and PC2 for 16.46%. PC1 was primarily driven by toxic trace metals (Cd, Pb, Cr), whose loading vectors pointed strongly in the positive direction, showing their dominant influence on sample separation. PC2 captured variation in essential microelements (Fe, Zn, Mn) and macroelements (Ca, Mg), with Fe and Mn contributing positively along this axis. Loading vectors (Supplementary Tables S2 and S3) showed that Cd, Pb had the highest eigenvalues on PC1, whereas Fe and Mn were most influential on PC2.

Samples treated with Zn + B at 20 mg kg⁻¹ clustered in the positive quadrant of PC2, reflecting enhanced uptake of beneficial elements. In contrast, Zn + B at 25 mg kg⁻¹ aligned more closely with the positive end of PC1, showing elevated accumulation of toxic metals. Zn or B alone exhibited intermediate positioning and partial modulation of elemental dynamics. These separation patterns illustrate the dose-dependent impact of micronutrient synergy: moderate Zn + B supplementation promotes uptake of essential nutrients, whereas higher doses increase translocation of toxic metals, as shown in Fig. 11.

4 Discussion

Crop plants, the foundation of human nutrition, are highly vulnerable to heavy metal toxicity, which impairs physiology and reduces yield. In this study, *B. rapa* L. (turnip) was cultivated under varying soil concentrations of B and Zn. We assessed their individual and combined effects on growth and physiology. Treatments were applied at 10,

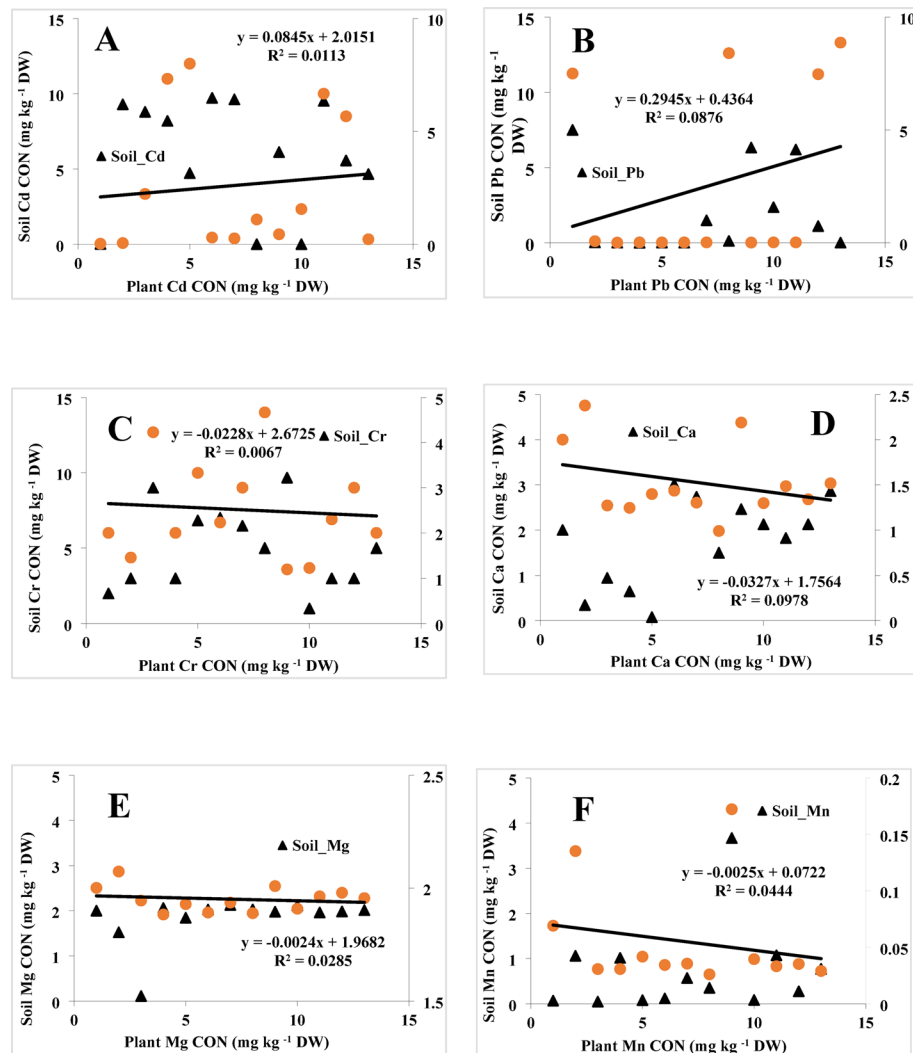
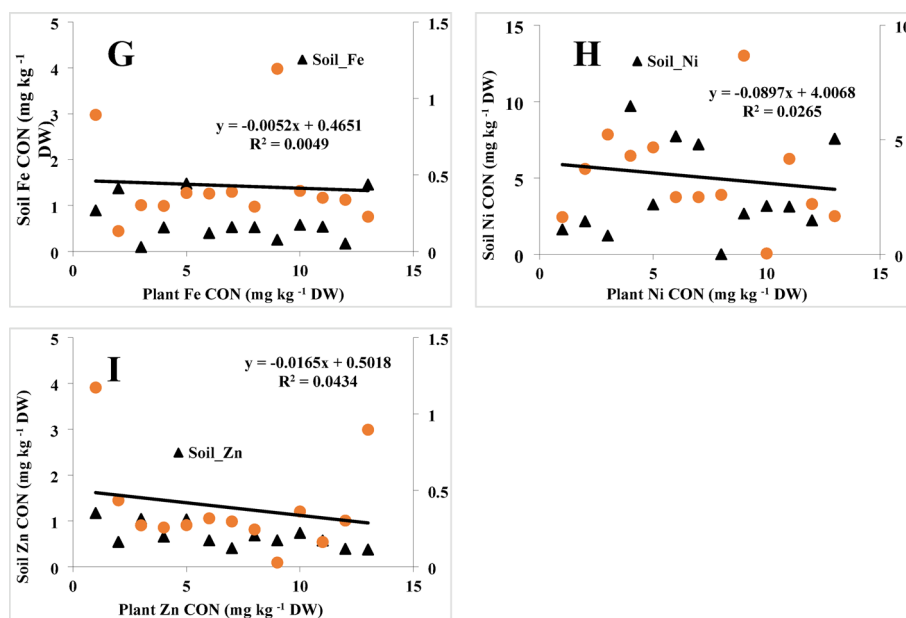


Fig. 10 Relationship between *B. rapa* L. metal and soil metal concentrations from nine different elements: **A** Cd **B**, Pb, **C** Cr, **D** Ca, **E** Mg, **F** Mn, **G** Fe, **H** Ni, **I** Zn. Each subplot displays their linear regression analysis, the triangle (▲) points represent the soil and the circle (●) points represents the plant concentration. The regression line and equation ($y=mx+b$) for the soil versus plant concentration are provided, with the co-efficient of determination (R^2). Each data point representing the mean of three replicates ($n=3$)

15, 20, or 25 mg kg⁻¹ in a concentration-dependent manner. The combination of Zn and B at 15–20 mg kg⁻¹ was associated with increased growth and chlorophyll content, consistent with reports in other *Brassica* species. This synergistic peak likely reflects optimal coordination of Zn-mediated enzymatic activity and B-dependent cell wall stabilization [51]. Such co-application therefore mitigates metal-induced stress and promotes structural development in *B. rapa* L. Carbohydrate metabolism was also sensitive to heavy-metal exposure. Sugars, the most abundant soluble compounds in *B. rapa* L., exhibited dose-dependent changes, with moderate Zn + B supplementation sustaining accumulation. In contrast, higher concentration suppressed it. Prior studies have shown that Zn enhances physiological and biochemical processes, thereby boosting plant productivity [52, 53]. Similarly, continuous availability is essential for reproductive success in *Brassica napus* (canola) [54]. Optimal Zn availability not only supports growth but also improves crop quality in a concentration-dependent manner compared with untreated control

**Fig. 10** (continued)

[55]. In our combined Zn + B supplementation at 15–20 mg kg⁻¹, enhanced growth and chlorophyll were observed more effectively than individual treatments, as shown in Figs. 2 and 3B stabilizes membranes, facilitates sugar transport, and supports lignin biosynthesis, thereby strengthening stress tolerance [56]. However, excess B impairs cell division and elongation, particularly in roots [57], and disrupts cell wall structure by binding cis-hydroxyl groups [58].

Our findings support Zn's enzymatic role in mitigating oxidative stress under metal exposure. Zn contributes to pigment biosynthesis, and low Zn concentrations are associated with increased chlorophyll levels [59]. In our study, chlorophyll levels rose at 15–20 mg kg⁻¹, but declined at 25 mg kg⁻¹. Growth enhancement may also reflect B's role in strengthening the cell wall through rhamnogalacturonan II cross-linking [60]. At 10 mg kg⁻¹ B, carotenoid and chlorophyll levels were lower than at 15–20 mg kg⁻¹ (Fig. 3), showing suboptimal pigment biosynthesis. Prior studies reported that 4 µg g⁻¹ B increased carotenoid accumulation, whereas higher levels caused a decline [59, 61]. Similarly, carotenoid contents were reduced under B-induced stress in *Cucurbita pepo* (pumpkin) compared with *Cucumis sativus* (cucumber) and *B. rapa* L. [62]. B deficiency also reduced phenolic accumulation [63]. Thus, moderate Zn availability appears to stabilize photosynthesis [64, 65]. In our study, low-dose B treatment reduced phenolic content in *B. rapa* L., consistent with reports that abiotic and biotic stresses, such as UV radiation and nutrient deficiency, affect phenolic accumulation [6]. At 4 µg g⁻¹ B, phenolic content decreased by 23.6% compared with the control, confirming that low-dose B reduces phenolic biosynthesis. However, Zn co-application at 15 mg kg⁻¹ significantly increased phenolic levels. This suggests that Zn mitigates B-induced oxidative stress, enabling phenolic biosynthesis. A pattern was also observed under Cu stress in wheat (*Triticum aestivum*) [66, 67]. Reduced oxidative stress under Zn + B synergy reflects improved tolerance mechanism in *B. rapa* L. Furthermore, our results show that low individual doses of Zn and B increased proline levels, supporting stress resilience in *B. rapa* L.

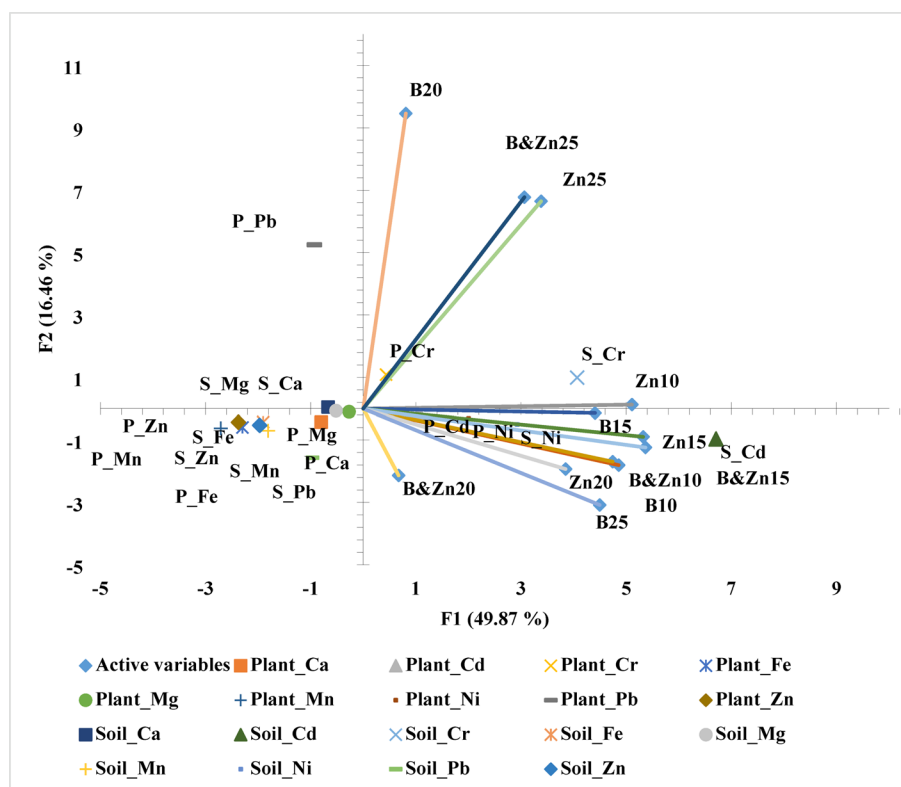


Fig. 11 PCA biplot depicting the spatial distribution of *B. rapa* L. and the correlation of soil and plant variables with the first two principal components. PC1 (49.87%) and PC2 (16.46%), cumulatively 66.33% of the total variance. Treatment involved the application of B and Zn, and B + Zn at concentration of 10, 15, 20, 25 mg kg⁻¹ to soil in which *B. rapa* L. seeds were grown. Vectors represent means of three replicates $\pm$ SD. Abbreviation: P (plant), S (soil), Pb (lead), Cr (chromium), Cd (cadmium), Mn (manganese), Fe (iron), Mg (magnesium), and Ca (calcium), indicating their directional influence to the sample separation of treatments ($n=3$). The proximity and direction treatment points relative to vectors reflects the correlation strength and patterns among variable across treatments. Elemental concentrations were expressed in mg kg⁻¹ dry weight (DW). PCA was performed using Pearson correlation matrix.

Previous studies have shown that amino acid and protein levels increase at low Zn concentrations, particularly at 10 and 25 mg kg⁻¹ [68, 69]. However, excess Zn can bind to protein sulfhydryl groups, disrupting normal protein synthesis and reducing specific proteins [70, 71]. Co-application of B and Zn has been reported to enhance carbohydrate stability, nitrogen assimilation, and protein synthesis under drought conditions in wheat [72]. Proline accumulation is a key stress-resistance mechanism, supporting osmoregulation, protecting enzymes, and stabilizing protein synthesis under abiotic stress [73]. In our study, proline accumulation increased with rising Zn concentration, especially in B-treated plants. Similar trends have been observed in wheat, where B supplementation increased proline and phenolic accumulation more than Zn alone [74]. Heavy metal-induced stress is also known to elevate proline and abscisic acid levels in plants [75]. Zn's function as a cofactor influences key enzymatic reactions. In cluster beans, Zn supplementation reduced sugar and starch levels [69]. Nonetheless, Zn-dependent enzymes, such as tryptophan synthase, carbonic anhydrase, and RNA polymerase, play critical roles in supporting photosynthesis, root growth, and transcription under stress conditions [76, 77].

Heavy metals are also known to disrupt carbohydrate metabolism in *T. aestivum* (wheat) leaves [66]. Most previous studies have focused on individual toxic or essential elements, with limited insight into their comparative distributions in edible plants such as *B. rapa* L. In our study, both toxic and essential elements were assessed within a unified plant-soil system to clarify accumulation and transfer dynamics. Linear regression analysis revealed a weak correlation ($R^2 \leq 0.113$) between individual soil and plant concentrations. This indicates that plant uptake was not strictly proportional to bulk soil levels. It also underscores a complex, non-linear regulator mechanism, where the plant actively modulates ion transport rather than acting as a plant accumulator. Nevertheless, overall elemental dynamics were significantly influenced by micronutrient supplementation. Cd, Pb, and Cr exhibited the highest bioaccumulation factor ($BAF > 1$), reflecting strong root-to-shoot translocation, as shown in Fig. 9A, B. This finding raises concerns about the food safety of *B. rapa* L. cultivated in contaminated soils [78]. In contrast, essential elements, i.e., Fe, Zn, and Mn, were detected at lower concentrations comparatively lower concentrations. Soil microelements levels consistently exceeded plant concentration, indicating the preferential transfer of toxic metals, especially Cd, Cr, and Pb, as shown by PCA loading vectors (Fig. 11).

PCA showed a dose-dependent effect of Zn + B synergy on elemental translocation. Sample treated with Zn + B at 20 mg kg⁻¹ clustered in the positive quadrant of PC2, reflecting enhanced uptake of beneficial elements. In contrast, Zn + B at 25 mg kg⁻¹ aligned more closely with the positive end of PC1, showing elevated accumulation of toxic metals. Treatments with Zn or B alone exhibited intermediate positioning, suggesting partial modulation of elemental dynamics. These separation patterns illustrate that micronutrient synergy and dose level are primary drives of elemental status: moderate Zn + B supplementation promotes uptake of essential nutrients, whereas higher doses increase translocation of toxic metals. Pearson correlation (r) analysis (Table 1) further revealed how soil conditions influence nutrient availability and metal mobility. Soil pH correlated negatively with organic matter (OM; $r = -0.73$) and nitrogen ($r = -0.77$), whereas OM and nitrogen were positively correlated ($r = 0.50$). These relationships suggest that organic-rich soil with low pH may retain nitrogen and modulate B and Zn bioavailability. Differences in micronutrient uptake and metal translocation under varied supplementation (Supplementary Figure S1) support this interpretation. A recent rice study showed that Cd toxicity impairs physiology, while Zn, especially in nanoparticle form, ameliorates antioxidant activity and reduces Cd uptake [79]. The elevated Cd level reflected in our study reflects its high mobility and strong affinity for root uptake. Conversely, lower Zn, Fe, and Mn levels may reflect antagonistic interactions or competitive inhibition by toxic metals. Cd and Pb concentrations consistently exceeded those of essential elements across supplementations. Notably, B + Zn treatments at 20–25 mg kg⁻¹ increased Mn accumulation. These patterns suggest a complex interaction between nutrient supplementation and heavy-metal uptake.

Co-application of Zn and B at 15–20 mg kg⁻¹ improved root, shoot, and leaf growth (Fig. 2), consistent with PCA clustering patterns (Fig. 11). The moderate 20 mg kg⁻¹ dose promoted uptake of beneficial elements, aligning with enhanced growth metrics. At these concentrations, Zn + B doses also enhanced pigments and sugar levels, whereas higher doses suppressed biosynthesis (Fig. 3B, C). Combined treatment enhanced antioxidant activity, thereby improving stress tolerance and detoxification.

Partial eta-squared (η^2) values confirmed strong synergy for protein ($\eta^2 = 0.97$), sugar ($\eta^2 = 0.98$), and chlorophyll ($\eta^2 = 0.70$), with co-application outperforming individual treatments (Supplementary File S3). Comparable detoxification mechanisms have been reported in *Saccharomyces cerevisiae* treated with medicinal plant extract, showing enhanced metal tolerance was achieved through uptake and upregulation of antioxidant and transport genes [80–82]. However, excess Zn or B at 25 mg kg⁻¹ increased toxic elements accumulation and impaired growth. These findings emphasize the importance of precise Zn and B management to optimize productivity while mitigating heavy metal-induced stress in plants.

5 Conclusion

This study demonstrated the dose-dependent synergistic interaction between B and Zn on *B. rapa* L. physiology and elemental dynamics. The results established a hierarchy of B + Zn > B > Zn of 15–20 mg kg⁻¹, significantly enhanced growth, physiological, and biomass ($p < 0.05$) compared with individual treatments. Our results showed that a moderate dose promotes selective nutrient uptake of beneficial elements. It also supports robust *B. rapa* L. root and shoots development. Conversely, the higher dose of 25 mg kg⁻¹ produced a negative shift, correlating with elevated accumulation and translocation of toxic metals, including Cd, Cr, and Pb. Notably, the weak linear correlation observed ($R^2 < 0.1$) between soil and plant metal uptake indicates that the observed benefit arises from a complex, nonlinear regulatory mechanism rather than simple soil availability. These findings highlight the potential of precise B and Zn integration to modulate ion homeostasis and enhance plant resilience against metal-induced stress. Future research is essential to elucidate the molecular mechanism and regulatory gene network underlying B-Zn synergy to support sustainable crop production and ensure food safety in metal-contaminated environments.

6 Limitations of the study

The study elucidated the physiological and biochemical responses of *B. rapa* L. to B and Zn supplementation under heavy-metal stress but did not investigate the underlying molecular pathways. Specifically, gene expression patterns of metal transporters (*IRT1*, *HMA2*), antioxidant enzymes (*SOD*, *CAT*), and detoxification-related genes (*PCS1*) remain unexplored. Future research will incorporate RT-qPCR to quantify these mechanisms and assess their regulation under B-Zn co-treatment. Additionally, transcriptomic and proteomic approaches will be employed to uncover broader regulatory networks and signaling pathways influenced by B-Zn synergy.

Abbreviations

AAS	Atomic absorption spectrometer
ABC	ATP-binding cassette
ATP	Adenosine triphosphate
B	Boron
BAF	Bioaccumulation factor
BSA	Bovine serum albino
bZIP	Basic Leucine zipper domain
<i>B. rapa</i> L.	<i>Brassica rapa</i> L. rapa (turnip)
Ca	Calcium
CAT	Catalase
Cd	Cadmium
CDF	Cation diffusion facilitator
CEC	Cation exchange capacity
Cr	Chromium

dS/m	Deci siemens per meter
DW	Distilled water
DW and FW	Dry weight and fresh weight
DTPA	Diethylene triamine penta acetic acid
TEA	Tri ethanol amine
EC	Electrical conductivity
Fe	Iron
GA	Gallic acid
GAE	Gallic acid equivalent
GPX	Glutathione peroxidase
HCA	Hierarchical cluster analysis
HMA _s	Heavy metal ATPases
HMs	Heavy metals
IAA	Indole acetic acid
IBGE	Institute of Biotechnology and Genetic Engineering
LSD	Least significant difference
Mn	Manganese
Mg	Magnesium
mg kg ⁻¹	Milligram per kilogram
mL	Milli meter
Ni	Nickel
NH ₄ OH	Ammonium hydroxide
nm	Nano meter
OM	Organic matter
PCA	Principal component analysis
PAL	Phenylalanine-ammonium lyase
Pb	Lead
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RuBisCO	Ribulose-1,5-bisphosphate-carboxylase/oxygenase
Rh	Relative humidity
SOD	Superoxide dismutase
TFs	Transcription factors
UV	Ultraviolet radiations
WRKY (TFs)	WRKY transcription factor
Zn	Zinc

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44372-026-00486-3>.

Supplementary Material 1

Author contributions

Hafsa Arif (H.A). Investigation; Conceptualization; Formal analysis; Methodology; Writing the original draft of the manuscript. Unays Siraj (U.S). Investigation; Conceptualization; Resources; Funding acquisition; Project administration; Supervision; Data Curation; Writing—review & editing. Ana (A). Investigation; Writing—review & editing; Formal analysis; Visualization. Sapna Ali (S.A). Investigation; Writing—review & editing; Formal analysis; Data Curation. Sajjad Ali (S. A) and Ashraf Zia (A.Z). Investigation; Conceptualization; Formal analysis; Writing—review & editing. Patricio R. De Lo Rios-Escalante (P.R.D.R). Conceptualization; Formal analysis, Resources; Funding acquisition; Writing—review & editing.

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Declaration.

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Data availability

The data is provided in a supplementary file.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Zulkernain NH, Uvarajan T, Ng CC. Roles and significance of chelating agents for potentially toxic elements (PTEs) phytoremediation in soil: a review. *J Environ Manag.* 2023. <https://doi.org/10.1016/j.jenvman.2023.117926>
2. Ullah A, et al. Ecological and population dynamics of black and grey Francolin (Phasianidae) in totali game reserve. *Pakistan Birds.* 2025;6:26.
3. Ullah A, et al. Influence of nesting habitat and nest emplacement on the breeding success of the black Francolin (*Francolinus francolinus*, Phasianidae): a case study from Pakistan. *Birds.* 2025;6:16.
4. Blankson D, Atiah K, Arthur E, Frimpong KA. Immediate chemical response and potential phytotoxicity of different tropical soils to empty oil palm fruit bunch Biochar application. *J Soil Sci Plant Nutr.* 2025. <https://doi.org/10.1007/s42729-025-02415-x>.
5. Huang S, Yamaji N, Ma JF. Metal transport systems in plants. *Ann Rev Plant Biol.* 2024. <https://doi.org/10.1146/annurev-arplant-062923-021424>.
6. Huang Y, et al. Roles and mechanisms of Boron in reducing cadmium accumulation in crops. *Ecotoxicol Environ Saf.* 2025;296:118216.
7. Rajput VD, et al. Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. *Biology* 2021. 2021;10(10):267.
8. Ebrahimian S, Pirzad A, Jalilian J, Rahimi A. The effect of micronutrient supplementation (Fe, Zn, B, and Mn) on antioxidant activity of milk Thistle (*Silybum marianum* L.) under rainfed conditions. *J Med Plants By-products.* 2021;1:43–50.
9. Shar AG et al. Unlocking biochar's potential: innovative strategies for sustainable remediation of heavy metal stress in tobacco plants. *Scientifica (Cairo).* 2025.
10. Gai WX et al. Characterization of the bZIP transcription factor family in pepper (*Capsicum annuum* L.): CabZIP25 positively modulates the salt tolerance. *Front Plant Sci.* 2020;11.
11. Bakshi M, Oelmüller R. WRKY transcription factors. *Plant Signal Behav.* 2014;9:e27700.
12. Dean M, Hamon Y, Chimini G. The human ATP-binding cassette (ABC) transporter superfamily. *J Lipid Res.* 2001.<https://doi.org/10.1101/gr.184901>.
13. Batool TS, et al. Genome-wide analysis of heavy metal ATPases (HMAs) in Poaceae species and their potential role against copper stress in triticum aestivum. *Sci Rep.* 2023;13:7551.
14. Hossain MA, Piyatida P, da Silva JAT, Fujita M. Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *J Bot.* 2012.
15. Prusty S, Sahoo RK, Nayak S, Poosapati S, Swain DM. Proteomic and genomic studies of micronutrient deficiency and toxicity in plants. *Plants.* 2022;11.
16. Patra HK, Patra DK, Acharya S. Chromium-induced phytotoxicity and its impact on plant metabolism. *Acta Physiol Plant.* 2024;46:1–23.
17. Aziz MA et al. Influence of farmyard manure on retention and availability of nickel, zinc and lead in metal-contaminated calcareous loam soils. *J Environ Eng Landsc Manag.* 2017;25.
18. Nagajyoti PC, Lee KD, Sreekanth TVM. Heavy metals, occurrence and toxicity for plants: a review. *Environ Chem Lett.* 2010. <https://doi.org/10.1007/s10311-010-0297-8>
19. Shanker AK, et al. Zinc nano and zinc Ethylenediaminetetraacetic acid (EDTA) mediated water deficit stress alleviation in Pearl millet (*Pennisetum glaucum* (L.) R. Br.): photosystem II electron transport and pigment dynamics. *Plant Stress.* 2024;14:100651.
20. Flecken M, et al. Dual functions of a Rubisco activase in metabolic repair and recruitment to carboxysomes. *Cell.* 2020;183:457–e47320.
21. Amaral J, Lobo AKM, Carmo-Silva, E. Regulation of rubisco activity in crops. *New Phytol.* 2024;241:35–51.
22. Todeschini V, et al. Effects of high zinc concentration on poplar leaves: a morphological and biochemical study. *Environ Exp Bot.* 2011;71:50–6.
23. Bolaños L, Abreu I, Bonilla I, Camacho-Cristóbal JJ, Reguera M. What can boron deficiency symptoms tell us about its function and regulation? *Plants.* 203;12:777.
24. Attia MS, et al. Nutrient therapies: boosting plant immunity for a sustainable future. *Physiol Mol Plant Pathol.* 2025;139:102788.
25. Sarwar N et al. Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. *Chemosphere.* 2017. <https://doi.org/10.1016/j.chemosphere.2016.12.116>.
26. Gerhardt KE, Gerwing PD, Greenberg BM. Opinion: Taking phytoremediation from proven technology to accepted practice. *Plant Sci.* 2017. <https://doi.org/10.1016/j.plantsci.2016.11.016>.
27. Khan A et al. Toxic metal interactions affect the bioaccumulation and dietary intake of macro- and micronutrients. *Chemosphere.* 2016;146.

28. Huang XY et al. A heavy metal P-type ATPase OshMA4 prevents copper accumulation in rice grain. *Nat Commun.* 2016;7.
29. Veličković Z et al. Investigation of soil properties influence on the heavy metals sorption by plants and possibilities for prediction of their bioaccumulation by response surface methodology. *J Serb Chem Soc.* 81;2016.
30. Carvalho MEA et al. Cadmium toxicity degree on tomato development is associated with disbalances in B and Mn status at early stages of plant exposure. *Ecotoxicology.* 2018;27.
31. Li X et al. Cadmium accumulation characteristics in turnip landraces from China and assessment of their phytoremediation potential for contaminated soils. *Front Plant Sci.* 2016;7.
32. Li X, Wang F, Ta N, Huang J. The compositions, characteristics, health benefits and applications of anthocyanins in brassica crops. *Front Plant Sci.* 2025;16:1544099.
33. Cartea ME, Francisco M, Soengas P, Velasco P. Phenolic compounds in Brassica vegetables. *Molecules* vol. 16 Preprint at <https://doi.org/10.3390/molecules16010251> (2011).
34. Singh S, et al. Phyto-nutraceutical promise of brassica vegetables in the post-genomic era: a comprehensive review. *Planta.* 2025;261:1–20.
35. Jayara AS, Pandey S, Kumar R, Micronutrients. Role in plants, their spatial deficiency and management in indian soils: a review. *Agric Rev.* 2021. <https://doi.org/10.18805/AG-R-2162>.
36. Hussain I et al. Choline chloride mediates chromium tolerance in spinach (*Spinacia Oleracea* L.) by restricting its uptake in relation to morpho-physio-biochemical attributes. *J Plant Growth Regul.* 2022;41.
37. Cole JC, Smith MW, Penn CJ, Cheary BS, Conaghan KJ. Nitrogen, phosphorus, calcium, and magnesium applied individually or as a slow release or controlled release fertilizer increase growth and yield and affect macronutrient and micronutrient concentration and content of field-grown tomato plants. *Sci Hortic* 211, (2016).
38. Samreen T et al. Development of organically Complexed-bioaugmented boron-coated DAP and its effect on yield and quality of Canola (*Brassica Napus* L.). *J Plant Growth Regul.* 2022;41.
39. Soussi M, Chaibi MT, Buchholz M, Saghrouti Z. Comprehensive review on climate control and cooling systems in greenhouses under hot and arid conditions. *Agron* 2022. 2022;12(12):626.
40. Esteban R, García-Plazaola JL, Hernández A, Fernández-Marín B. On the recalcitrant use of arnon's method for chlorophyll determination. *New Phytol.* 2018;217:474–6.
41. Lowry OH, Rosebrough, N. J., Farr, A. L., Randall R. J. Protein measurement with the folin phenol reagent. *J Biol Chem.* 1951;193:265–75.
42. Shubhra, Dayal J, Goswami CL, Munjal R. Influence of phosphorus application on water relations, biochemical parameters and gum content in cluster bean under water deficit. *Biol Plant.* 2004;48.
43. Engel E, Baty C, Le Corre D, Souchon I, Martin N. Flavor-active compounds potentially implicated in cooked cauliflower acceptance. *J Agric Food Chem* 50, (2002).
44. Ábrahám E, Hourton-Cabassa C, Erdei L, Szabados L. Methods for determination of proline in plants. 2010. https://doi.org/10.1007/978-1-60761-702-0_20
45. McKeague JA, Stobbe PC. History of soil survey in Canada, 1914–1975. *Soil Res Inst Can Department Agric.* 1978;1.
46. Lindsay WL, Norvell WA. Equilibrium relationships of Zn²⁺, Fe³⁺, Ca²⁺, and H⁺ with EDTA and DTPA in soils. *Soil Sci Soc Am J.* 1969;3.
47. McGrath SP, Cunliffe CH. A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Cd, Pb, Cr, Co and Mn from soils and sewage sludges. *J Sci Food Agric.* 1985;36:794–8.
48. Frank R, Ishida K, Suda P. Metals in agricultural soils of ontario. *Can J Soil Sci.* 1976;56.
49. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Front Psychol.* 2013;4.
50. Addinsoft. {XLSTAT} statistical and data analysis solution. {Paris}, {France}. XLSTAT, Your data analysis solution. 2021.
51. Yang M, Shi L, Xu F, Sen, Lu JW, Wang YH. Effects of B, Mo, Zn, and Their Interactions on seed yield of rapeseed (*Brassica napus* L.) project supported by the program for new century excellent Talents in University, China (No. NCET-05-0666) and the Ministry of Science and Technology of China (No. 2006BAD25B01). *Pedosphere.* 19;2009.
52. Siddiqui SN, Umar S, Iqbal M. Zinc-induced modulation of some biochemical parameters in a high- and a low-zinc-accumulating genotype of *Cicer arietinum* L. grown under Zn-deficient conditions. *Protoplasma.* 2015;252.
53. Zafar S, Ashraf MY, Saleem M. Shift in physiological and biochemical processes in wheat supplied with zinc and potassium under saline condition. *J Plant Nutr.* 2018;41.
54. Samreen T, et al. Co-application of Boron, Sulphur, and Biochar for enhancing growth and yield of *Brassica Napus* under calcareous soil. *Commun Soil Sci Plant Anal.* 2022;53:1050–67.
55. Azam M, Mahmud J, Ahammad K, Gulandaz M, Islam M. Efficiency of different doses of zinc on Growth, productivity and economic returns of mustard in AEZ 11 of Bangladesh. *J Environ Sci Nat Resour.* 2015;6.
56. Hua T, Zhang R, Sun H, Liu C. Alleviation of Boron toxicity in plants: mechanisms and approaches. *Crit Rev Environ Sci Technol.* 2021;51:2975–3015.
57. Reid RJ, Hayes JE, Post A, Stangoulis JCR, Graham R. D. A critical analysis of the causes of Boron toxicity in plants. *Plant Cell Environ.* 2004;27:1405–14.
58. Herrera-Rodríguez MB. N.-G. M. T. Role of Boron in vascular plants and response mechanism to Boron stresses. *Plant Stress.* 2010;4:115–15.
59. Seth K, Aery NC. Effect of Boron on the contents of Chlorophyll, Carotenoid, phenol and soluble leaf protein in mung Bean, *vigna radiata* (L.) Wilczek. *Proc Natl Acad Sci India Sect B: Biol Sci.* 2014;84:713–9.
60. Tanaka M, Fujiwara T. Physiological roles and transport mechanisms of boron: perspectives from plants. *Pflugers Arch.* 2008;456:671–7.
61. Wang JZ et al. Changes in photosynthetic properties and antioxidative system of Pear leaves to Boron toxicity. *Afr J Biotechnol.* 2011;10.
62. Landi M, Remorini D, Pardossi A, Guidi L. Boron excess affects photosynthesis and antioxidant apparatus of greenhouse cucurbita Pepo and cucumis sativus. *J Plant Res.* 2013;126.
63. de Souza RP, et al. Effects of Boron on the emergence and allocation of metabolic compounds in two herbaceous cotton cultivars. *Plants.* 2025;14:576.
64. Smaoui A, et al. Physiological and biochemical responses of *Carthamus tinctorius* L. to zinc at vegetative stage. *J Plant Nutr Soil Sci.* 2023;186:495–506.

65. Sarwar M, et al. Exogenous zinc alleviates post-anthesis heat stress by improving the defense mechanism and leaf physiology of bread wheat. *J Soil Sci Plant Nutr*. 2025. <https://doi.org/10.1007/s42729-025-02516-7>.
66. Ganeva G, Zozikova E. Effect of increasing Cu²⁺ concentrations on growth and content of free phenols in two lines of wheat (*Triticum aestivum*) with different tolerance. *Gen Appl Plant Physiol*. 2008;50:1247–55.
67. Hamid N, Bukhari N, Jawaaid F. Physiological responses of *Phaseolus vulgaris* to different lead concentrations. *Pak J Bot*. 2010;42.
68. Choudhary S, Zehra A, Naeem M, Khan MMA, Aftab T. Effects of Boron toxicity on growth, oxidative damage, antioxidant enzymes and essential oil fingerprinting in *Mentha arvensis* and *Cymbopogon flexuosus*. *Chem Biol Technol Agric*. 2020;7.
69. Manivasagaperumal R, Balamurugan S, Thiyagarajan G, Sekar J. Effect of zinc on Germination, seedling growth and biochemical content of cluster bean (*Cyamopsis Tetragonoloba* (L.) Taub). *Curr Bot* 2, (2011).
70. Chen CT, Chen LM, Lin CC, Kao CH. Regulation of proline accumulation in detached rice leaves exposed to excess copper. *Plant Sci* 160, (2001).
71. Singh Y, Malik CP. Phenols and their antioxidant activity in brassica juncea seedlings growing under HgCl₂ stress. *J Microbiol Biotechnol Res*. 2011;1:124–30.
72. Akhtar N et al. Synergistic effects of plant growth-promoting rhizobacteria and silicon dioxide nanoparticles for amelioration of drought stress in wheat. *Plant Physiol Biochem*. 2021:166.
73. Hemadri Reddy S, et al. Proline and other physiological changes an indicator of abiotic stress caused by heavy metal contamination. *J King Saud Univ Sci*. 2024;36:103313.
74. Hanif S, et al. Proline and Betaine-enriched nano zinc fertilizer for growth and seed quality enhancement in drought-exposed coriander. *Ind Crops Prod*. 2025;230:121136.
75. Zengin FK, Kirbag S. Effects of copper on chlorophyll, proline, protein and abscisic acid level of sunflower (*Helianthus annuus* L.) seedlings. *J Environ Biol*. 2007;28.
76. Pollmann S, DÜchting P, Weiler EW. Tryptophan-dependent indole-3-acetic acid biosynthesis by 'IAA-synthase' proceeds via indole-3-acetamide. *Phytochemistry*. 2009;70:523–31.
77. Kumari V, Thakur R, Kumari J, Kumari A, Khajuria D. Nutritional improvement in soybean (*Glycine max* (L.) Merrill) through plant breeding and biotechnological interventions. *Crop and Pasture Science*. <https://doi.org/10.1071/CP23155>. 2023.
78. Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants: seventy-fourth report of the joint FAO/WHO Expert Committee on Food Additives. 2011.
79. Ghouri F, et al. Polyploidy and zinc oxide nanoparticles alleviated Cd toxicity in rice by modulating oxidative stress and expression levels of sucrose and metal-transporter genes. *J Hazard Mater*. 2023;448:130991.
80. Kerdsoomboon K, Chumsawat W, Auesukaree C. Effects of *Moringa Oleifera* leaf extracts and its bioactive compound Gallic acid on reducing toxicities of heavy metals and metalloid in *Saccharomyces cerevisiae*. *Chemosphere*. 2021;270.
81. Limcharoensuk T, et al. Aqueous extract of *Cissus quadrangularis* L. alleviates heavy metal toxicity in *Saccharomyces cerevisiae* by limiting metal uptake and enhancing detoxification mechanisms. *Ecotoxicol Environ Saf*. 2025;299:1–13.
82. Unays Siraj TLCA. Effect of *pueraria candollei* Grah. Ex Benth. And *Cissus quadrangularis* Linn. extracts on reducing metal toxicities in *Saccharomyces cerevisiae*. 2021;357–61.

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