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Physiological and Biochemical Responses of *Satureja bachtiarica* Bunge to Cadmium and Lead Toxicity

Ibtihal Ibtihal Khadhim Azra Al-Zobaidi[1], Sajjad Sedaghat[2], Mohammad Jabbari-Oranj[3], Yaghoub Heydarzadeh[4], and Mohamad Norani[2*]

Abstract

Heavy metal contamination in agricultural soils threatens crop productivity and medicinal plant safety. This study evaluated the physiological performance, osmoprotective adjustments, antioxidant machinery, and metal partitioning in *Satureja bachtiarica* Bunge exposed to varying cadmium (Cd: 0–10 mg L^{-1}) and lead (Pb: 0–40 mg L^{-1}) concentrations. Elevated metal levels caused dose-dependent growth impairments; maximal exposure (10 mg L^{-1} Cd and 40 mg L^{-1} Pb) reduced stem dry weight by 48.0% and 46.5%, and root dry weight by 59.0% for both metals, respectively. Concurrently, photosynthetic pigments, including chlorophyll a and b, declined significantly under extreme toxicity. To mitigate stress, *S. bachtiarica* activated robust biochemical defenses. Free proline accumulated predominantly in roots, increasing ~4.0-fold under Cd and 4.3-fold under Pb. Soluble/reducing carbohydrate pools expanded, and shoot ascorbic acid nearly doubled (~94% increase) under maximum Cd stress. Enzymatic defense was also stimulated, with foliar catalase (CAT) and peroxidase (POD) activities significantly upregulated. Crucially, over 90% of absorbed Cd and Pb was compartmentalized in root tissues (up to 98% and 97%, respectively). While the bioaccumulation factor (BF) remained below 0.43, the translocation factor (TF) declined markedly with increasing toxicity (Cd: 0.125 to 0.021; Pb: 0.117 to 0.025; $TF \ll 1$), preventing toxic ion transport to shoots. These findings demonstrate that *S. bachtiarica* combines strong antioxidant/osmoprotective competence with root-mediated metal exclusion, highlighting its potential as an effective phytostabilizer in Cd- and Pb-contaminated soils.

Keywords Antioxidant enzymes, Biochemical responses, Growth medium, Heavy metal contamination, *Satureja bachtiarica.*

[1] University of Al Qadisiyah, Diwaniyah, Qadisiyah, Iraq.
[2] Department of Horticultural Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.
[3] Department of Agronomy and Plant Breeding, Faculty of Agricultural Sciences and Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj, Islamic Republic of Iran.
[4] University of Tabriz, Tabriz, Islamic Republic of Iran.
* Corresponding author; e-mail: noranimohamad@modares.ac.ir

Introduction

Heavy metal contamination in agricultural soils, stemming from anthropogenic activities such as rapid industrialization, mining, agrochemical misuse, and wastewater irrigation, poses a critical threat to ecological integrity and agricultural sustainability (Angon et al., 2024). Among toxic non-essential trace elements, cadmium (Cd) and lead (Pb) are of primary concern due to their high persistence, non-biodegradability, and profound phytotoxicity, readily entering the food web and threatening human health (Alengebawy et al., 2021). Improper agronomic practices, particularly the extensive application of low-grade phosphatic fertilizers and sewage sludge, exacerbate the bioavailable fractions of Cd and Pb in arable lands. According to joint FAO/WHO guidelines, permissible thresholds of Cd and Pb in edible plant materials are strictly regulated at 0.2 mg kg^{-1} and 0.3 mg kg^{-1} dry weight, respectively, underscoring the urgent need to understand trace element dynamics in harvested crops (Rahman et al., 2013).

The physiological toxicity of Cd and Pb begins with their absorption across the root-soil interface. Both toxic ions access root tissues via apoplastic and symplastic routes, often hijacking carrier proteins and ion channels designated for essential divalent cations, including Fe^{2+}, Zn^{2+}, and Ca^{2+} (Shaari et al., 2022). While Pb readily binds to negatively charged polysaccharides within the root cell wall, Cd exhibits higher cellular mobility across plasma membranes driven by electrochemical gradients. Once internalized, excess Cd and Pb trigger extensive metabolic dysfunction; they destabilize the photosynthetic machinery, dismantle chloroplast ultrastructure, accelerate chlorophyll breakdown, and displace essential functional metal cofactors from metalloenzymes (Farid et al., 2013; Vijiyakumar and Prince, 2024). A universal hallmark of heavy metal stress is the hyperaccumulation of reactive oxygen species (ROS), which induces membrane lipid peroxidation and functional decay of vital biomacromolecules.

To alleviate metal-induced oxidative distress, plants have evolved coordinated biochemical defense strategies comprising both enzymatic and non-enzymatic antioxidant networks (Zandi and Schnug, 2022). Plants upregulate key antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), to scavenge cytotoxic radicals. Concurrently, cells orchestrate an osmotic and non-enzymatic buffer system by synthesizing compatible osmolytes and radical scavengers notably free proline, soluble sugars, and ascorbic acid (vitamin C) which stabilize membranes, preserve cell turgor, and protect native protein conformations under metal challenges. Moreover, a plant's agronomic utility and remediation capacity largely depend on its metal

allocation strategy; species that tightly sequester toxic ions within root cells while restricting their upward translocation to aerial tissues through effective compartmentalization represent valuable candidates for phytostabilization (Sharafi and Salehi, 2025).

Satureja bachtiarica Bunge (Lamiaceae) is an important medicinal and aromatic plant endemic to the arid and semi-arid rangelands of Iran, widely harvested for its valuable pharmacological, culinary, and nutraceutical applications (Yoosefi et al., 2021). Despite extensive knowledge regarding Cd and Pb phytotoxicity in conventional food crops, the physiological plasticity and biochemical tolerance mechanisms of perennial aromatic crops such as *S. bachtiarica* under progressive heavy metal exposure remain poorly characterized. In particular, it remains critical to elucidate whether *S. bachtiarica* can maintain photosynthetic integrity and cellular homeostasis under Cd and Pb exposure, and whether it exhibits root-exclusion behavior suitable for phytostabilization in contaminated soils. Therefore, this study aimed to investigate the comparative physiological and biochemical responses of *S. bachtiarica* to progressive concentrations of Cd and Pb. Specifically, we evaluated: (i) growth performance and photosynthetic pigment stability, (ii) osmotic adjustment mechanisms (proline and soluble/reducing carbohydrate pools), (iii) key non-enzymatic (vitamin C) and enzymatic antioxidant systems (CAT and POD), and (iv) heavy metal uptake, bioaccumulation, and translocation patterns (BF and TF) from roots to aerial tissues. Our findings provide fundamental mechanistic insights into the heavy metal tolerance mechanisms of *S. bachtiarica* and assess its viability as a phytostabilizer in Cd- and Pb-impacted agricultural lands.

2. Materials and methods

Experimental plant material and growth environment

Seeds of *Satureja bachtiarica* were obtained from Pakan Bazr Company (Isfahan, Iran). The seeds were surface-sterilized with 70% (v/v) ethanol for 30 s, followed by three thorough rinses with sterile distilled water. The sterilized seeds were sown in plastic pots (90 mm diameter) filled with perlite and maintained in a growth chamber (phytotron) with a 16/8 h (light/dark) photoperiod at 26/16 °C (day/night) and a light intensity of 12,000 lx. Plants were irrigated with a modified Hoagland nutrient solution (Arnon, 1950) containing: 1.5 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.75 mM $MgSO_4$, 0.28 mM KH_2PO_4, 10 μM Fe-EDDHA, 25 μM H_3BO_3, 5 μM $MnSO_4$, 1 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.1 μM Na_2MoO_4, and 50 μM KCl. The nutrient solution in each pot was renewed twice a week.

Cadmium and lead exposure

Forty-five days old plants were exposed to cadmium at concentrations of 0, 2, 5, 7, and 10 mg/L and to lead at 0, 10, 20, 30, and 40 mg/L form of $CdCl_2H_2O$ for 15 days. The metals were supplied through Hoagland's nutrient medium, with 200 mL of solution provided per pot. Pots receiving only the nutrient mixture without the addition of Cd or Pb served as controls. The pH of all the solutions was checked daily and adjusted to remain between 5.5 and 5.8.

Biomass evaluation

After 50 days of growth, the plants were collected at the 50% flowering stage, a period known for the maximum yield and quality of essential oils. Following harvest, the roots were rinsed with deionized water to eliminate any cadmium or lead residues from their surface. At this stage, stem and root lengths were recorded, and the fresh biomass of both shoots and roots was determined via a digital balance. The plant material was then oven-dried at 60 °C for 72 hours until a constant weight was achieved, after which the dry mass was measured. The data are presented as the means of three independent replicates for each treatment.

Quantification of Heavy Metals

The tissue samples were first oven dried at 60 °C for 72 hours and subsequently ground into a fine powder. Approximately 0.5 g of powdered tissue was digested in 25 mL of 65% nitric acid and heated at 250 °C for 24 hours in open vessels. After partial evaporation of the acid, 5 mL of 30% hydrogen peroxide was added to complete the digestion process. The concentrations of Cd and Pb were determined via flow-injection atomic absorption spectrometry (Perkin Elmer 700) following the protocol described by Sekabira et al. (2011). The Bioaccumulation Factor (BF), Translocation Factor (TF), and Transfer Coefficient (TC) were calculated according to the methodology described by Yousefi et al. (2018).

Measurement of proline content

The proline content was measured according to the methods of (Bates et al., 1973). Fresh tissue (0.5 g) was extracted with 3% sulfosalicylic acid, reacted with acid ninhydrin and glacial acetic acid, and incubated at 95 °C for 1 h, and the absorbance of the toluene phase was recorded at 520 nm via a UV–Vis spectrophotometer (Shimadzu Mini-1240, USA). Proline levels were calculated from an L-proline standard curve and expressed as µmol g^{-1} FW.

Quantification of soluble and reducing carbohydrates

Soluble and reducing sugars were extracted from 0.5 g of fresh tissue with 80% ethanol and quantified via the anthr one method (Houshmandfar and Asli, 2011). One milliliter of extract was reacted with 4 mL of anthrone reagent and incubated in boiling water for 10 min, and the absorbance was read at 620 nm. The sugar content was calculated from a glucose standard curve and expressed as mg g^{-1} FW.

Determination of chlorophyll content

Photosynthetic pigments were extracted from 0.2 g samples with 7 mL of 80% acetone and centrifuged at 3400 × g for 20 min at 4 °C (IEC 215, USA). The absorbance of the supernatant was measured at 664, 647, and 470 nm via a UV–Vis spectrophotometer (Shimadzu Mini-1240, USA), and the chlorophyll and carotenoid concentrations were subsequently calculated Adelusi et al. (2006).

Chlorophyll a= [(13.19 A_{664}-2.57 A_{647}) V/W] (1)

Chlorophyll b = [(22.1 A_{647} -5.26 A_{664}) V/W] (2)

Carotenoids= [(100 (A_{470})-3.27 (mg Chl a)-104 (mg Chl b)/227) V/W] (3)

Vitamin C measurement

The ascorbic acid (Vitamin C) content was determined spectrophotometrically according to the method described by Atanassova et al. (2011), with slight modifications. Briefly, 5 g of fresh tissue was homogenized in 20 mL of methanol. The homogenate was filtered through Whatman No. 1 filter paper to obtain the extract. The absorbance of the extracts was measured at 293 nm using a spectrophotometer. A standard calibration curve was prepared using pure ascorbic acid. The Vitamin C concentration was calculated using the following equation according to Gouveia and Castilho (2011).

V=C × V/M (4)

V=AA content (mg. AAE/g), C=ascorbic acid concentration extracted from the standard curve (mg/ml), V=volume of sample extract (ml) and M=sample extract weight (g).

Determination of catalase (CAT) and peroxidase (POD) activities

Fresh leaves (0.5 g) were ground in 5 mL of ice-cold 50 mM phosphate buffer (pH 7.0) containing 1% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000 × g for 15 min at 4

°C, and the resulting supernatant was used as the enzyme extract. CAT activity was determined by monitoring the decrease in absorbance at 240 nm due to the decomposition of 10 mM hydrogen peroxide over 3 min. POD activity was measured as the increase in absorbance at 470 nm resulting from guaiacol oxidation (20 mM) in the presence of 40 mM hydrogen peroxide. The enzyme activity for both CAT and POD was expressed as one unit, defined as a 0.01 change in absorbance per minute under assay conditions.

Statistical analysis

All experiments were conducted in a completely randomized design with three replicates. Data was analyzed using analysis of variance (ANOVA) via SAS (version 9.0). The normality of the residuals was verified using the PROC UNIVARIATE procedure in SAS. Significant differences among treatment means were determined using the least significant difference (LSD) test at $P \leq 0.05$. The data are presented as the means of three independent replicates for each treatment.

3. Results

Effects of cadmium stress on plant growth and biomass accumulation

Cadmium (Cd) exposure induced a marked inhibitory effect on both the biomass and longitudinal growth of *S. bachtiarica* in a dose-dependent manner (Table 1). In control plants, the stem and root dry weights were recorded at 43.9 mg and 4.7 mg, respectively, with shoot and root lengths measuring 27.5 cm and 10.2 cm. Increasing Cd concentrations significantly suppressed these growth metrics. Specifically, exposure to the highest Cd concentration (10 mg L^{-1}) resulted in a 48% reduction in stem dry weight and a 59% decline in root dry weight. Similarly, shoot length progressively decreased from 27.5 cm in the control to a minimum of 10.3 cm at 10 mg L^{-1} Cd, representing a 63% reduction. Root length followed a comparable inhibitory pattern, decreasing to 5.2 cm at the highest Cd level, which corresponds to an approximately 49% reduction.

Alterations in proline, carbohydrate, and vitamin C levels under cadmium stress

Cadmium (Cd) stress triggered a significant biochemical response in *S. bachtiarica* tissues (Table 1). Proline levels significantly increased in both roots and shoots across all tested Cd concentrations. In shoots, proline rose from 1.39 µmol g^{-1} FW in the control to 4.6 µmol g^{-1} FW under 10 mg L^{-1} Cd (approximately 3.3-fold), while in roots it increased from 1.21 to 4.8 µmol g^{-1} FW (approximately 4-fold), indicating a stronger osmotic adjustment response in root tissues. In

terms of carbohydrate metabolism, exposure to 10 mg L^{-1} Cd led to the accumulation of soluble and reducing carbohydrates in shoots (109.2 and 44.4 mg g^{-1} FW, respectively; increases of ~75% and ~131% over the control), while root tissues exhibited values of 72.20 and 29.6 mg g^{-1} FW (increases of ~123% and ~77%, respectively).

Vitamin C levels also demonstrated a significant response in aerial parts; the highest vitamin C content (12.2 mg/100 g FW) was recorded under 10 mg L^{-1} Cd, representing an increase of approximately 94% (nearly two-fold) compared to the control (6.3 mg/100 g FW). Conversely, variations in vitamin C levels within the root tissues under Cd stress were statistically non-significant, remaining almost constant (4.0–5.0 mg/100 g FW) across all treatments (Table 1).

Table 1. Changes in dry weight (mg $Plant^{-1}$), length (cm), proline content (ummol $g^{-1}FW$), soluble and reducing carbohydrates (mg $g^{-1}FW$), vitamin C, and heavy metals content (mg kg^{-1}) in root and shoot of *S. bachtiarica* exposed for 15 days to different concentrations of Cd (0, 2, 5, 7, and 10 mg/L) in growing medium.

		Traits						
Organ	**Treatments**	Dry weight (mg/Plant)	Plant length (cm)	Proline content (ummol g^{-1} FW)	Soluble carbohydrates (mg g^{-1} FW)	Reducing carbohydrates (mg g^{-1} FW)	Vitamin C (mg 100 g^{-1} FW)	Heavy metals content (mg kg^{-1})
Shoot	Control	43.9±2.2^{a}	27.5±1.3^{a}	1.39±0.2^{e}	62.3±4.85^{e}	19.20±2.1^{e}	6.3±0.45^{d}	0^{e}
	Cd2	37.1±1.8^{b}	24.2±1.2^{b}	2.79±0.25^{d}	70.2±5.8^{d}	26.2±2.1^{d}	6.2±0.42^{d}	80.2±6.2^{d}
	Cd5	34.2±1.7^{c}	20.5±1.2^{c}	3.2±0.3^{c}	82.35±6.9^{c}	33.6±3.3^{c}	7.9±0.47^{c}	94.94±7.2^{c}
	Cd7	31.5±1.7^{d}	16.4±0.92^{c}	4.12±0.42^{b}	95.7±9.5^{b}	40.2±4.13^{b}	9.7±0.87^{b}	111.12±10.1^{b}
	Cd10	22.8±1.5^{e}	10.3±0.66^{d}	4.6±0.5^{a}	109.2±10.65^{a}	44.4±4.1^{a}	12.2±1.1^{a}	141.21±13.4^{a}
Root	Control	4.7±0.72^{a}	10.2±0.61^{a}	1.21±0.22^{e}	32.40±3.1^{e}	16.74±1.42^{e}	4.0±0.38^{a}	0^{e}
	Cd2	3.9±0.70^{b}	8.0±0.62^{b}	1.9±0.22^{d}	52.12±4.9^{d}	17.3±1.6^{d}	4.0±0.35^{a}	1159.2±44.3^{d}
	Cd5	3.0±0.65^{c}	7.8±0.41^{b}	3.2±0.22^{c}	60.3±6.2^{c}	20.6±2.2^{c}	4.0±0.38^{a}	2359.2±71.3^{c}
	Cd7	2.1±0.44^{d}	6.0±0.40^{c}	4.15±0.22^{b}	65.2±7.1^{b}	24.8±2.3^{b}	4.4±0.33^{a}	4324.2±100.3^{b}
	Cd10	1.9±0.20^{d}	5.2±0.22^{c}	4.8±0.22^{a}	72.20±8.2^{a}	29.6±3.1^{a}	5.0±0.39^{a}	6549.2±154.3^{a}

Notes: Values are expressed as means ± SD of three independent replicates. All Cd-related data were analyzed separately. In each column, means followed by the same letter are not significantly different according to the least significant difference (LSD), ($p \leq 0.05$). Abbreviations: Cd, Cadmium.

Effects of lead stress on plant growth and biomass accumulation

Similarly, lead (Pb) stress significantly impaired the growth metrics of *S. bachtiarica* in a dose-dependent manner (Table 2). In control plants, the dry weights of stems and roots were recorded at 43.9 mg and 4.7 mg, respectively. The highest Pb concentration (40 mg L^{-1}) induced a 46.5% decrease in stem dry weight and a 59% decline in root dry weight relative to the control (Table 2). Moreover, exposure to 40 mg L^{-1} Pb resulted in the lowest stem length (8.2 cm) and a significant 50% reduction in root length (4.5 cm) compared with non-treated plants. In contrast, the 10 mg L^{-1} Pb treatment did not significantly affect stem length.

Alterations in proline, carbohydrate, and vitamin C levels under lead stress

Lead (Pb) treatment induced substantial under modifications in *S. bachtiarica*, consistent with the stress responses observed under Cd exposure (Table 2). Proline levels significantly increased in both roots and shoots; notably, the 40 mg L^{-1} Pb treatment induced approximately a 4.3-fold increase in root proline content (from 1.11 to 4.78 µmol g^{-1} FW) relative to the control. Carbohydrate concentrations were also markedly elevated, with the highest levels of soluble and reducing carbohydrates under 40 mg L^{-1} Pb recorded in shoots (89.8 and 40.4 mg g^{-1} FW) and roots (69.3 and 34.75 mg g^{-1} FW), respectively. Similar to Cd, Pb stress influenced antioxidant synthesis in shoots, whereas variations in root vitamin C content remained statistically non-significant (4.0–4.9 mg/100 g FW; Table 2).

Table 2. Changes in dry weight (mg $Plant^{-1}$), length (cm), proline content (ummol g^{-1}FW), soluble and reducing carbohydrates (mg g^{-1}FW), vitamin C, and heavy metals content (mg kg^{-1}) in root and shoot of *S. bachtiarica* exposed for 15 days to different concentrations of Pb (0, 10, 20, 30, and 40 mg/L) in growing medium.

		Traits						
Organ	**Treatments**	Dry weight (mg/Plant)	Plant length (cm)	Proline content (ummol g^{-1} FW)	Soluble carbohydrates (mg g^{-1} FW)	Reducing carbohydrates (mg g^{-1} FW)	Vitamin C (mg 100 g^{-1} FW)	Heavy metals content (mg kg^{-1})
Shoot	Control	43.8±2.3^{a}	27.5±1.3^{a}	1.45±0.34^{e}	60.7±6.9^{e}	19.7±2.44^{e}	6.2±0.5^{d}	0^{e}
	Pb10	37.2±1.7^{b}	25.3±1.3^{ab}	2.38±0.49^{d}	68.2±7.35^{d}	22.3±2.5^{d}	6.4±0.54^{d}	80.2±5.13^{d}
	Pb20	33.0±1.6^{c}	21.5±0.9^{b}	3.3±0.75^{c}	74.6±8.1^{c}	29.6±3.3^{c}	7.3±0.6^{c}	129.94±9.4^{c}
	Pb30	27.1±1.4^{d}	14.4±0.81^{c}	4.1±0.9^{b}	78.9±8.32^{b}	34.2±3.73^{b}	8.9±0.9^{b}	174.12±10.2^{b}
	Pb40	23.9±1.3^{e}	8.2±0.52^{d}	4.7±1.1^{a}	89.8±9.2^{a}	40.4±4.2^{a}	11.3±1.2^{a}	205.21±16.2^{a}
Root	Control	4.7±0.42^{a}	10.3±0.7^{a}	1.11±0.12^{e}	29.12±2.74^{e}	16.74±1.7^{e}	4.±0.30^{a}	0^{e}
	Pb10	3.5±0.33^{b}	9.0±0.52^{b}	2.7±0.3^{d}	35.1±3.5^{d}	22.3±1.9^{d}	4.2±0.35^{a}	1174.2±61.2^{d}
	Pb20	3.0±0.34^{c}	7.3±0.4^{b}	3.6±0.4^{c}	41.85±4.8^{c}	25.8±2.4^{c}	4.45±0.3^{a}	2466.2±99.7^{c}
	Pb30	2.1±0.22^{d}	6.1±0.3^{c}	4.35±0.52^{b}	55.4±5.75^{b}	30.3±2.9^{b}	4.5±0.4^{a}	4523.2±125.3^{b}
	Pb40	1.9±0.12^{d}	4.5±0.22^{c}	4.78±0.8^{a}	69.3±6.5^{a}	34.75±3.5^{a}	4.9±0.42^{a}	6751.2±180.9^{a}

Notes: Values are expressed as means ± SD of three independent replicates. All Cd-related data were analyzed separately. In each column, means followed by the same letter are not significantly different according to the least significant difference (LSD), ($p \leq 0.05$). Abbreviations: Pb, Lead.

Cadmium and lead uptake and translocation

After two weeks of exposure, the contents of Cd and Pb in the shoots and roots of *S. bachtiarica* increased significantly in response to increasing metal concentrations in the growth media (Table 1, 2). More than 90% of the absorbed Cd was retained in the roots (Figure 1A), reaching 98% at 10 mg L^{-1}, whereas more than 90% of the Pb accumulated in the roots, reaching 97% at 40 mg L^{-1} (Figure 1B). The bioaccumulation factor (BF), reflecting the plant's overall capacity to accumulate metals from the growth medium, remained relatively stable for both Cd and Pb (Figure 1C). Specifically, BF

values for Cd ranged from 0.41 to 0.43, while for Pb, they varied between 0.38 and 0.41, indicating a consistent ability of *S. bachtiarica* to limit metal uptake. In stark contrast, both the translocation factor (TF) and the transfer coefficient (TC) exhibited a significant dose-dependent decrease as metal concentrations increased (Figure 1D, E). For Cd, TF decreased from 0.125 to 0.021, and TC dropped from 0.045 to 0.01. Similarly, under Pb stress, TF values fell from 0.117 to 0.025, and TC values declined from 0.04 to 0.01. These findings demonstrate that while *S. bachtiarica* maintains a stable accumulation pattern, its capacity to translocate and store heavy metals in the aerial tissues is progressively restricted under higher toxicity levels, suggesting an effective phytostabilization strategy.

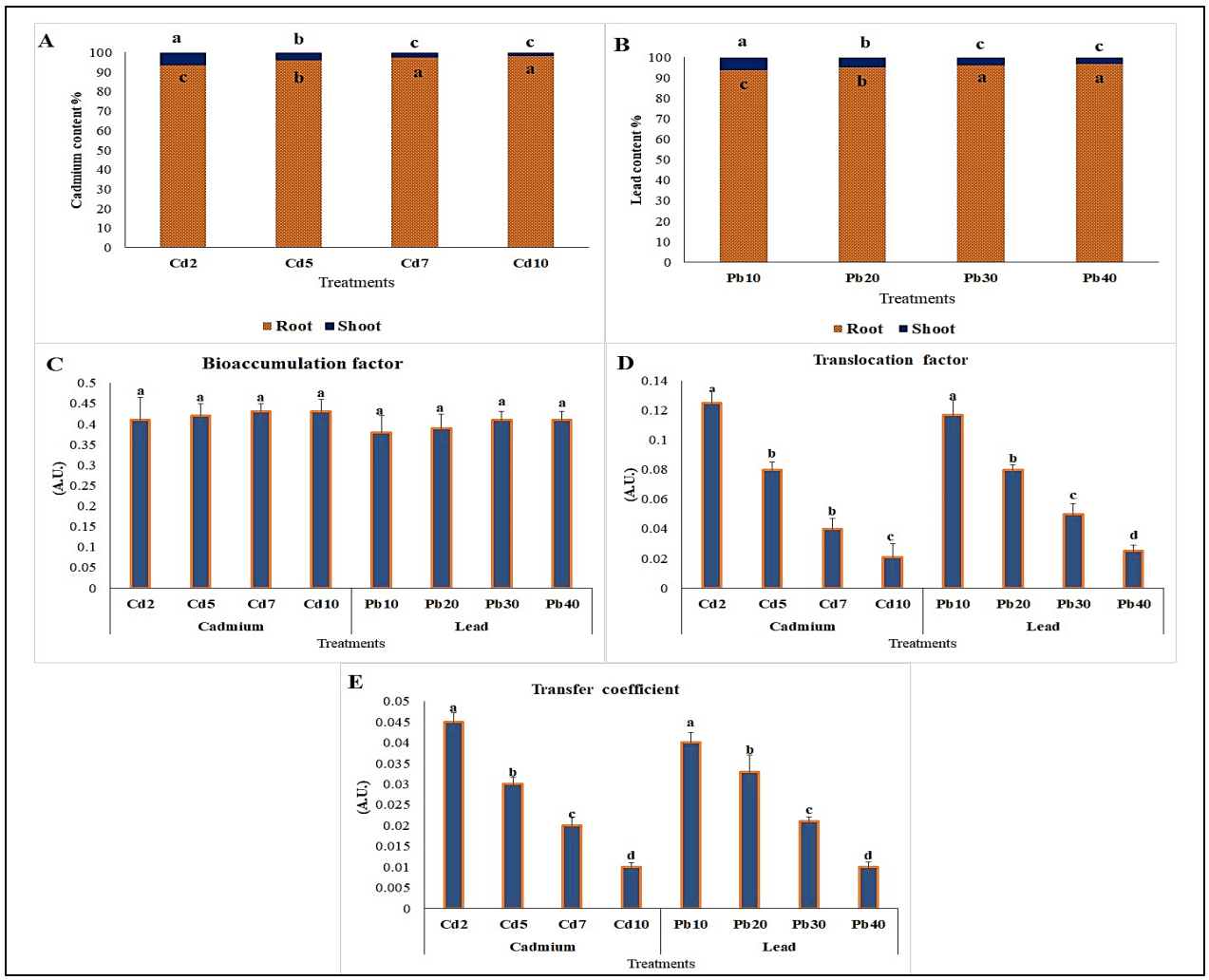

Figure 1. Distribution of absorbed cadmium (A) and lead (B) in roots and shoots, bioaccumulation factor (C), translocation factor (D) and transfer coefficient (E) in S. *bachtiarica* exposed for 15 days to different concentrations of Cd (0, 2, 5, 7, and 10 mg/L) and Pb (0, 10, 20, 30, and 40 mg/L) in growth media. The values are the means ± SDs of three independent replicates. Different letters indicate significant differences ($P \leq 0.05$).

Alterations in chlorophylls, and carotenoids levels under cadmium and lead stress

Exposure to increasing concentrations of Cd and Pb caused significant decreases in the chlorophyll a, chlorophyll b, and carotenoids contents of *S. bachtiarica* (Figure 2A, B, D). In the control plants, the chlorophyll a and b contents were 1.08 and 0.68 mg/g FW, respectively, decreasing to 0.34–0.60 mg/g FW under 10 mg/L Cd and to 0.51 and 0.31 mg/g FW under 40 mg/L Pb (Figure 2A, B). The carotenoids content also decreased from 0.42 mg/g FW in the control under metal stress. Among the Cd treatments, no significant differences were detected between the 2 mg L^{-1} Cd treatment and the control, nor among the 5, 7, and 10 mg L^{-1} Cd treatments (Figure 2D). The 10 mg/L Pb treatment did not differ significantly from the control, whereas the 30 and 40 mg/L treatments showed no significant difference compared to each other.

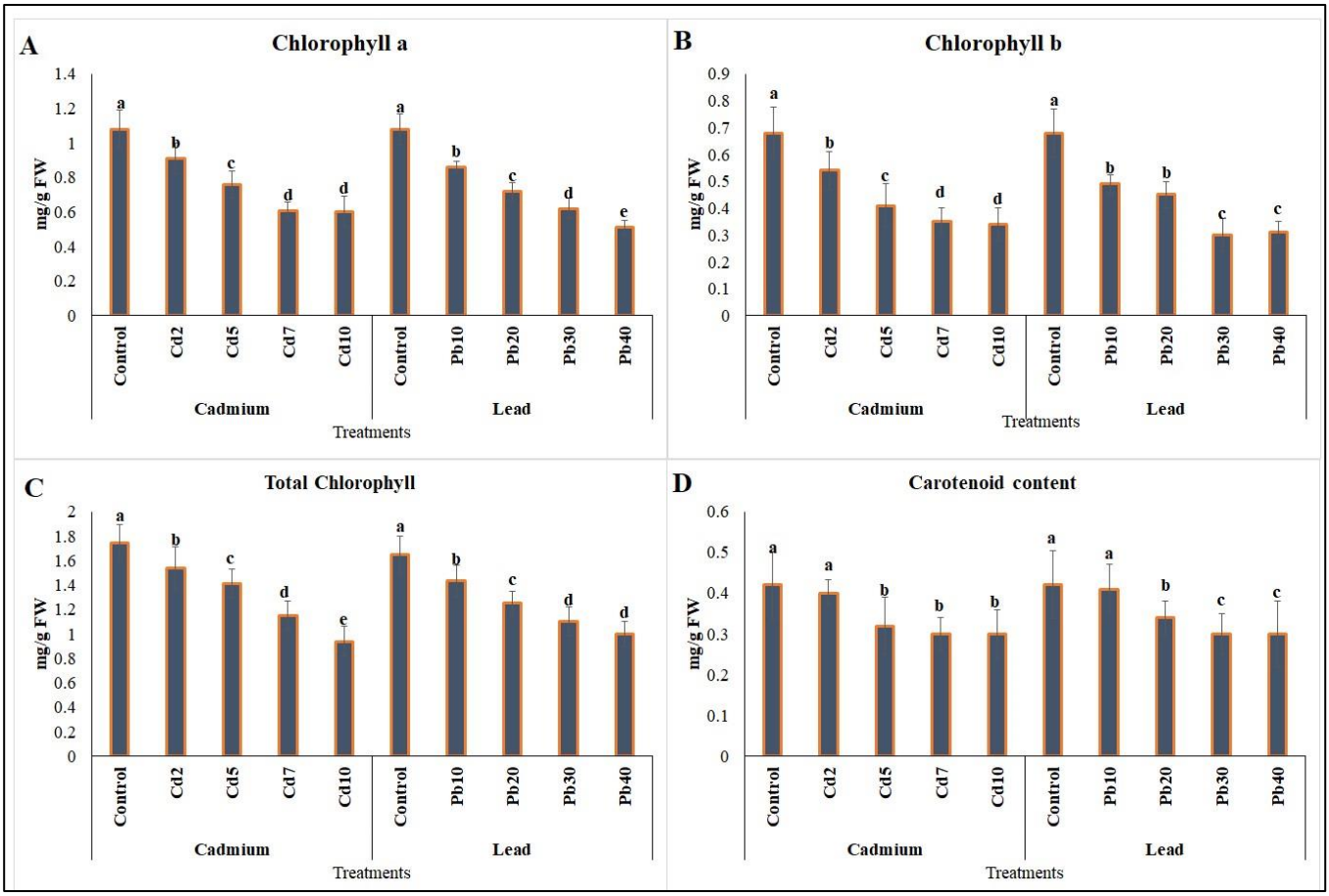

Figure 2. Changes in chlorophyll-a (A), chlorophyll-b (B), total chlorophyll (C), and carotenoid (D) in the leaves of S. *bachtiarica* exposed for 15 days to different concentrations of Cd (0, 2, 5, 7, and 10 mg/L) and Pb (0, 10, 20, 30, and 40 mg/L) in growth media.

Antioxidant enzyme activities under cadmium and lead stress

Figure 3 shows the specific activities of the antioxidant enzymes CAT and POD in the leaves of *S. bachtiarica* under progressive Cd and Pb exposure. CAT activity increased significantly ($p < 0.05$) in response to both metals. The highest CAT activity (80) was recorded in plants exposed to 40 mg/L Cd (Fig. 3A), representing a 2.3-fold increase compared with that of the control and a 1.7-fold increase compared with that of the 10 mg/L Cd treatment. Interestingly, no significant difference was observed between the 5 and 7 mg/L Cd treatments. Similarly, POD activity in response to 2 mg/L Cd did not differ significantly from that in the control, but POD activity increased markedly at higher concentrations, reaching a maximum of 80 at 10 mg/L Cd. Under Pb stress, POD activity also increased significantly from the lowest applied concentration to 40 mg L^{-1}, where it reached 87 (Figure 3B).

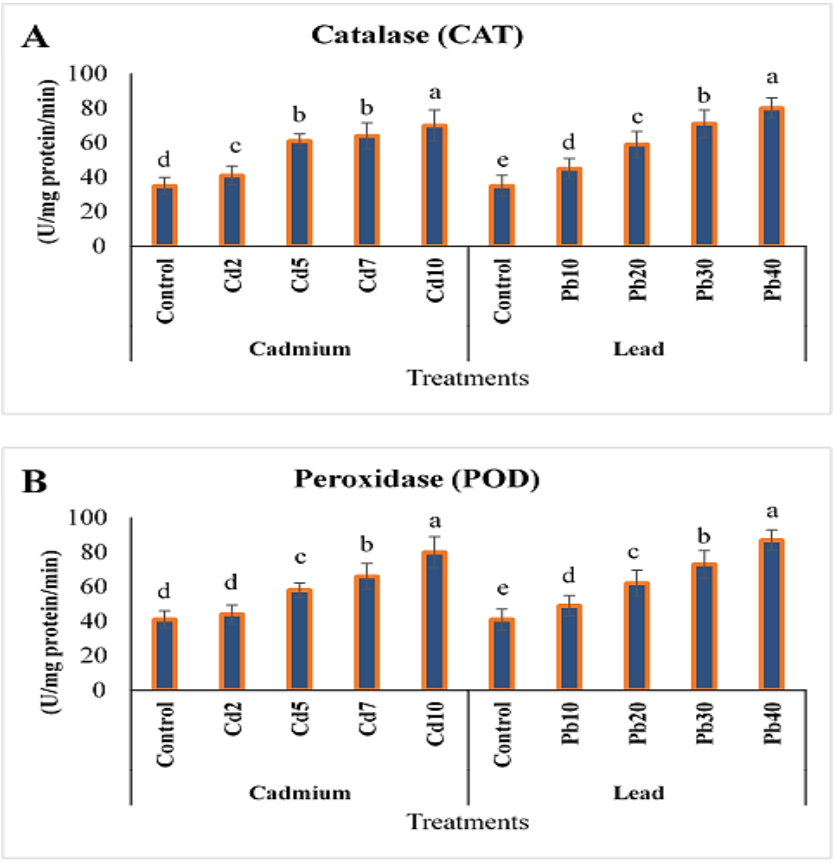

Figure 3. Specific activity of catalase (A) and peroxidase (B) in leaves of S. *bachtiarica* exposed to different concentrations of Cd (0, 2, 5, 7, and 10 mg/L) and Pb (0, 10, 20, 30, and 40 mg/L) in growth media for 15 days.

Discussion

The accumulation of Cd and Pb in the roots and shoots of *S. bachtiarica* increased significantly as the heavy metal concentration in the growth medium increased (Table 1, 2). Variations in metal uptake and internal distribution strongly influence plant tolerance. Indices such as BF, TF, and TC offer practical measures of a plant's ability to accumulate and transport Cd and Pb, providing insights into its potential for phytoremediation (Takarina and Pin, 2017). The bioaccumulation factor (BF) measures the ability of plant roots to absorb Cd and Pb from the soil (Pachura et al., 2016). Typically, a BF > 1 indicates trace element accumulation, whereas a BF < 1 characterizes metal exclusion (Van der Ent et al., 2013). In our study, although the BF values were less than 1, they remained relatively stable with increasing Cd and Pb concentrations in the growth medium, suggesting a consistent root uptake pattern (Figure 1B). These findings indicate the notable capacity of *S. bachtiarica* roots to absorb and sequester Cd and Pb, even under high soil contamination. The translocation factor (TF) reflects a plant's ability to transport metals from roots to shoots, with a TF > 1 indicating efficient translocation (Pachura et al., 2016). The observed reduction in TF and TC values under increasing metal concentrations aligns with recent investigations into the heavy metal tolerance mechanisms of various Satureja species (Tabatabaie et al., 2025). Such findings reinforce the hypothesis that these plants prioritize the sequestration of toxic ions in the root system to safeguard aerial photosynthetic tissues from oxidative damage, a phenomenon increasingly documented in recent botanical studies (Shirkhani et al., 2025). In *S. bachtiarica*, Cd and Pb accumulated predominantly in the roots rather than in the aerial parts, resulting in TF values less than 1. Furthermore, the TF decreased significantly with increasing Cd and Pb concentrations (Figure 1C), indicating a strong restriction of long-distance metal translocation in *S. bachtiarica*, which limits the movement of Cd and Pb from roots to shoots. Consistent with our results, previous studies have shown that in most plants, 70–85% of absorbed Cd is retained in the roots (Pirselova et al., 2016). Moreover, the observed decrease in the transfer coefficient (TC), reflecting the plant's ability to store metals in aerial parts, was due primarily to inhibited root-to-shoot translocation of Cd rather than limited Cd and Pb uptake from the growth medium (Figure 1D). The strong retention of Cd and Pb in roots and limited upward transport indicate that *S. bachtiarica* is well-suited for phytostabilizing heavy metals in contaminated soils. Similar to our findings, several plant species, such as *Satureja montana*, *Satureja hortensis*, and

315 *Brassica nigra*, are able to take up and accumulate high concentrations of heavy metals, suggesting
316 their potential use in phytoremediation (Al-Khayri et al., 2023; Carrubba and Scalenghe, 2012).
317 High Cd and Pb levels can severely impair plant growth and even lead to mortality by disrupting
318 photosynthesis, respiration, water status, and nutrient uptake. Cd and Pb reduce plant dry biomass
319 primarily by impairing photosynthesis through chlorophyll degradation and damage to PSII
320 reaction centers, while their preferential accumulation in roots limits the uptake and translocation
321 of essential micronutrients such as Zn, Fe, and Cu, further disrupting photosynthesis and respiration
322 (Rahman et al., 2024). Cd and Pb affected shoot length more than root length, causing reductions
323 even at low concentrations, while the highest concentrations (10 mg L^{-1} Cd and 40 mg L^{-1} Pb)
324 significantly decreased both shoot and root lengths (Table 1). This effect is partly attributed to Cd
325 and Pb inhibiting cell elongation by disrupting proton pump activity. Additionally, decreased cell
326 water potential, reduced membrane permeability, and lower aquaporin-mediated hydraulic
327 conductivity contribute to the inhibition of root and shoot elongation. Cd and Pb can disrupt the
328 ultrastructure of meristem cells, impair ribosome biogenesis, and thereby inhibit root growth
329 (Hamim et al., 2018). Even low concentrations of Cd and Pb can markedly inhibit shoot growth in
330 medicinal plants such as *Merwilla natalensis* and *Centella asiatica*. This reduction is often
331 accompanied by decreased chlorophyll content, highlighting its role as a sensitive bioindicator of
332 heavy metal toxicity (Biswas et al., 2020; Street et al., 2009).
333 In the present study, Cd and Pb stress significantly reduced the chlorophyll a, chlorophyll b, and
334 total chlorophyll contents in *S. bachtiarica* (Figure 2A–C). The decline in chlorophyll content
335 under Cd and Pb stress may result from the inhibition of chlorophyll biosynthesis enzymes (e.g.,
336 *δ*-aminolevulinic acid and protochlorophyllide reductase) and the disruption of the Mg and Fe
337 availability required for chlorophyll synthesis. The reduced chlorophyll content under Cd exposure
338 is also attributed to the inhibition of carbonic anhydrase due to Cd-induced Zn^{2+} deficiency and the
339 replacement of Mg^{2+} in the tetrapyrrole ring of chlorophylls (Adil et al., 2020). Hu et al. (2023)
340 reported that increasing Cd levels led to decreases in chlorophyll a, chlorophyll b, and carotenoid
341 contents in the leaves of *Phlox divaricata*. Similarly, Fattahi et al. (2021) reported that Pb and Cd
342 stress reduced chlorophyll and carotenoid concentrations in coriander leaves.
343 Enzymatic antioxidants, including catalase and peroxidase, play crucial roles in mitigating Cd- and
344 Pb-induced oxidative stress by decomposing H_2O_2. In this study, Cd and Pb exposure stimulated
345 the activity of antioxidant enzymes (Figure 3). CAT and POX activities were significantly elevated

under Cd and Pb exposure (Figure 3A), which is consistent with previous reports of increased antioxidant enzyme activity in various plant species, including wheat varieties, *Saccharum officinarum*, *Crotalaria juncea*, *Pogonatherum crinitum* seedlings, *Arabidopsis thaliana*, and *Solanum lycopersicum* fruits, under heavy metal stress (Guo et al., 2019).

Proline acts as a key stress-responsive molecule that accumulates in plant tissues to mitigate the adverse effects of Cd and Pb. In addition to its role as an osmolyte, proline stabilizes proteins, scavenges ROS, maintains redox homeostasis, and can increase the activities of antioxidant enzymes such as SOD, CAT, and APX (Singh et al., 2015). It also contributes to metal detoxification by forming proline–metal complexes that protect enzymes from heavy metal inhibition and can directly neutralize ROS, including H_2O_2, hydroxyl radicals, and singlet oxygen. In the present study, the proline content in *S. bachtiarica* increased significantly with increasing Cd and Pb concentrations (Table 1, 2), which is in agreement with reports from Brassica juncea (Wang et al., 2022), Pisum sativum (Zdunek-Zastocka et al., 2021), and other plants (Siddique et al., 2018) under heavy metal stress. Soluble sugars are key osmoprotectants that accumulate under heavy metal stress (Sharma et al., 2019). In the present study, Cd and Pb exposure progressively increased soluble and reducing sugars in both the roots and shoots of *S. bachtiarica* (Table 1, 2). Similar responses have been reported in Spirodela polyrhiza and Festuca arundinacea under Cd and Pb stress (Lou et al., 2017; Qiao et al., 2012). The accumulation of carbohydrates supports stress tolerance by scavenging ROS, maintaining redox balance, and protecting cellular structures such as membranes and macromolecules (Afzal et al., 2021).

Finally, while this study focused on physiological and biochemical defense mechanisms, the impact of heavy metal stress on the secondary metabolic profile of *S. bachtiarica* remains an essential area for future research. Specifically, subsequent studies should investigate how Cd and Pb accumulation influences essential oil yield and the composition of bioactive compounds (such as carvacrol and thymol) to further clarify the pharmacological quality and therapeutic safety of this species when cultivated in metal-impacted environments.

4. Conclusions

Soil contamination by cadmium (Cd) and lead (Pb) severely impairs plant physiological processes and limits medicinal plant productivity. The present findings reveal that *Satureja bachtiarica* copes with heavy metal toxicity primarily via an effective metal-exclusion strategy, characterized by

substantial root sequestration and restricted root-to-shoot translocation (BF, TF<1). Although exposure to high Cd and Pb concentrations reduced dry biomass and chlorophyll pigments, the plant alleviated metal-induced oxidative damage by orchestrating enzymatic antioxidant defenses (CAT, POX) and accumulating key osmoprotectants (proline and soluble carbohydrates). Overall, *S. bachtiarica* demonstrates considerable physiological plasticity under heavy metal stress. The pronounced retention of toxic ions in the subterranean organs not only minimizes metal hazards in shoot tissues but also highlights the high potential of this aromatic shrub for phytostabilization in Cd- and Pb-impacted soils.

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