Interactive effects of citric acid and tryptophan on cobalt bioavailability and oxidative stress responses in Salvia officinalis under controlled contamination conditions

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Research Article

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Abstract

The accumulation of cobalt (Co) in agricultural systems poses a serious threat to plant productivity, particularly in high-value medicinal species. This study evaluated the potential of exogenous citric acid (CA) and tryptophan (Trp), applied individually or in combination, to mitigate Co-induced phytotoxicity in hydroponically grown *Salvia officinalis* L. Plants were exposed to four Co concentrations (0, 1, 10, and 30 ppm) and four biostimulant treatments (control, 2 mM CA, 2 mM Trp, and CA + Trp) for four weeks. Increasing Co levels, especially 10 and 30 ppm, markedly impaired plant growth, biomass accumulation, and photosynthetic pigment contents (chlorophyll a, chlorophyll b, carotenoids, β-carotene, and anthocyanins). These effects were accompanied by enhanced lipid peroxidation, increased activities of antioxidant enzymes (CAT, POD, SOD, APX, and GPX), and significant disruption of primary metabolism, including reductions in soluble sugars, proteins, amino acids, malate, and oxalate. Application of CA or Trp alone partially alleviated Co toxicity; however, their combined application exerted a pronounced synergistic effect, resulting in substantial recovery of growth performance, photosynthetic capacity, and metabolic balance. Moreover, the combined treatment significantly reduced oxidative damage and moderated antioxidant enzyme activities, reflecting a lower oxidative burden, and limited Co accumulation in plant tissues. These findings suggest that CA may reduce Co bioavailability through chelation, while Trp likely enhances internal metabolic and antioxidative capacity. Their synergistic use represents an effective and sustainable biostimulant strategy to improve cobalt tolerance and ensure the safe production of medicinal plants under heavy metal stress in hydroponic systems.

1. Introduction

The rapid expansion of industrial, mining, and intensive agricultural activities over recent decades has resulted in the widespread accumulation of heavy metals in soil and aquatic environments, posing a growing threat to ecosystem integrity, food safety, and sustainable agriculture (Cao et al., 2024; Wang et al., 2024; Chen et al., 2025). Among these contaminants, cobalt (Co) occupies a dual and complex position in plant systems. While trace amounts of Co (generally < 0.1–0.5 mg kg^{-1}) are considered beneficial for certain physiological processes, including enzyme activation and symbiotic nitrogen fixation in legumes, elevated concentrations arising from anthropogenic inputs rapidly induce toxicity (Mahey et al., 2020; Khalid et al., 2026).

Excess cobalt primarily exerts its phytotoxic effects through the induction of oxidative stress. By interfering with electron transport processes and promoting redox cycling reactions, Co accelerates the generation of reactive oxygen species (ROS), including superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) (Šiukšta et al., 2022; Salam et al., 2023). The overaccumulation of these reactive molecules leads to membrane lipid peroxidation, protein oxidation, nucleic acid damage, and degradation of photosynthetic pigments, ultimately manifesting as growth inhibition, biomass reduction, and impaired plant performance (Mahey et al., 2020). Consequently, developing effective and environmentally sound strategies to mitigate cobalt toxicity remains a critical research priority, particularly for high-value crop and medicinal plant production systems.

Salvia officinalis L. (sage), a prominent member of the Lamiaceae family, is a widely cultivated medicinal plant valued for its rich content of bioactive compounds and well-documented antioxidant potential (Prakash et al., 2015). Sage is extensively utilized in pharmaceutical, food, and cosmetic industries; however, its growth and secondary metabolite production are highly sensitive to abiotic stresses, including heavy metal contamination (El Mahrouk et al., 2024). Hydroponic cultivation systems offer precise control over nutrient availability and root-zone conditions and are increasingly adopted for high-quality medicinal plant production. Nonetheless, the presence and accumulation of heavy metals such as cobalt in nutrient solutions can compromise both plant health and product safety, underscoring the need for effective mitigation strategies tailored to controlled cultivation systems.

The application of biostimulants has emerged as a promising approach for alleviating heavy metal stress in plants. Organic acids and amino acids, in particular, play key roles in metal detoxification, redox regulation, and metabolic adjustment. Citric acid is a well-recognized organic chelator capable of forming stable complexes with metal cations, thereby reducing their bioavailability and mobility in the rhizosphere. Numerous studies have demonstrated that exogenous citric acid application enhances antioxidant capacity, limits lipid peroxidation, and improves growth performance in plants exposed to metals such as chromium, lead, zinc, and silver (Imran et al., 2019; Guo et al., 2021; Mahdavian, 2021; Mahdavian, 2022).

Tryptophan, an essential amino acid and a key precursor of indole-3-acetic acid, has also been shown to contribute to plant stress tolerance beyond its nutritional role. Emerging evidence suggests that tryptophan can modulate plant growth and oxidative balance under heavy metal stress by influencing hormonal signaling, enhancing antioxidant defense systems, and participating in internal metal chelation processes (Jana et al., 2016; Mahdavian, 2023; Hussain et al., 2024).

Despite substantial evidence supporting the individual roles of citric acid and amino acids in mitigating heavy metal toxicity, their combined or comparative effects on cobalt stress remain poorly understood. This knowledge gap is particularly evident for medicinal plants such as *S. officinalis* cultivated under hydroponic conditions, where metal bioavailability and oxidative responses can differ markedly from soil-based systems.

Therefore, the present study aimed to evaluate the individual and combined effects of exogenous citric acid and tryptophan on cobalt-induced oxidative stress, growth inhibition, and physiological disruption in hydroponically grown *Salvia officinalis*. The findings are expected to provide mechanistic insights into biostimulant-mediated cobalt tolerance and to support the development of sustainable strategies for mitigating heavy metal risks in controlled medicinal plant production systems.

2. Materials and methods

2.1.Plant material, hydroponic system, and experimental design

The experiment was conducted under controlled laboratory conditions using a deep water culture (DWC) hydroponic system. Certified seeds of sage (*Salvia officinalis* L.) were surface-sterilized with 70% (v/v) ethanol for 1 min followed by 2% (v/v) sodium hypochlorite for 10 min and thoroughly rinsed with sterile distilled water. The seeds were germinated and grown in full-strength Hoagland nutrient solution.

Plants were maintained under controlled environmental conditions (25 ± 2°C, 60–70% relative humidity, and a 16 h light/8 h dark photoperiod). At the six-leaf developmental stage, plants were subjected to cobalt (Co) treatments at four concentrations (0, 1, 10, and 30 mg L^{-1}) and biostimulant treatments consisting of control (no application), citric acid (CA, 2 mM), tryptophan (Trp, 2 mM), and their combined application (CA + Trp). Treatments were applied for four weeks.

The experiment was arranged in a completely randomized factorial design with three biological replicates per treatment.

2.2.Growth measurements

At harvest, plants were separated into shoots and roots. Fresh weight (FW) was recorded immediately, and dry weight (DW) was determined after oven-drying samples at 70°C for 48 h. Leaf number per plant was also recorded.

2.3.Determination of photosynthetic pigments

Photosynthetic pigments were quantified following Lichtenthaler (1987). Briefly, 0.5 g of fresh leaf tissue was homogenized in 5 mL acetone, mixed with anhydrous sodium sulfate, filtered, and adjusted to a final volume of 10 mL. After centrifugation at 2600 rpm for 10 min, absorbance was measured at 662, 645, and 470 nm using a UV–Vis spectrophotometer. Chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoids were expressed as mg g^{-1} FW.

2.4.β-Carotene determination

β-Carotene content was determined according to Hodisan et al. (1997). Fresh tissue (0.2 g) was extracted with an acetone–methanol–petroleum ether mixture under dark conditions, followed by saponification with 30% methanolic KOH. The non-saponifiable fraction was analyzed using an HPLC system (Knauer, Germany) equipped with a C18 column, with detection at 450 nm.

2.5.Anthocyanin content

Total anthocyanins were measured following Mita et al. (1997). Dried leaf tissue (0.02 g) was extracted in methanol containing 1% HCl, incubated at 4°C for 24 h, and centrifuged. Absorbance was measured at 530 and 657 nm, and anthocyanin content was calculated as:

$A = A_{530} - (0.25 \times A_{657})$.

2.6.Cobalt accumulation

Cobalt concentration in shoot tissues was determined by atomic absorption spectrophotometry (Spectra AA 220, Australia) after nitric acid digestion, following Khan et al. (2009). Results were expressed as mg kg^{-1} DW.

2.7.Antioxidant enzyme assays

Fresh leaf tissue (0.5 g) was homogenized in ice-cold 50 mM phosphate buffer (pH 7.5) containing 1% polyvinylpyrrolidone and 1 mM EDTA. After centrifugation at 5000 rpm for 20 min at 4°C, the supernatant was used for enzyme activity assays.

Ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (GPX), and peroxidase (POD) activities were determined spectrophotometrically following standard protocols (Beauchamp and Fridovich, 1971; Dhindsa et al., 1981; Nakano and Asada, 1981; Upadhyaya et al., 1985; Wu et al., 2014).

2.8.Lipid peroxidation

Malondialdehyde (MDA) content was measured using the thiobarbituric acid method (Heath and Packer, 1968), and concentrations were calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.9.Proline, soluble sugars, proteins, and free amino acids

Proline content was determined according to Bates et al. (1973). Soluble sugars were measured using the anthrone method (Irigoyen et al., 1992). Protein concentration was quantified using the Bradford assay (Bradford, 1976), and total free amino acids were determined using the ninhydrin method (Ravindranath, 1981).

2.10.Organic acid analysis

Organic acids (malate and oxalate) were quantified by HPLC following Tolrà et al. (1996). Fresh leaf tissue (0.2 g) was extracted with 0.025 M HCl, centrifuged, filtered, and analyzed at 210 nm using external standards for identification and quantification.

2.11.Statistical analysis

Data were analyzed using SPSS v21. Two-way ANOVA was applied to evaluate main and interaction effects. Mean comparisons were performed using Tukey's test at $p < 0.05$.

3. Results

3.1.Effects of protective treatments on growth and biomass of *Salvia officinalis* under cobalt stress

Cobalt stress significantly affected the growth and biomass parameters of *Salvia officinalis* (Table 1). Increasing cobalt concentrations (10 and 30 ppm) caused a marked reduction in root fresh weight (FW),

root dry weight (DW), shoot FW, and shoot DW compared with the control plants (***P < 0.001).

Application of citric acid or tryptophan individually partially alleviated the inhibitory effects of cobalt stress on plant growth. However, the combined application of citric acid (2 ppm) and tryptophan (2 ppm) resulted in a pronounced improvement in all growth parameters across all cobalt levels. Notably, plants treated with citric acid + tryptophan under 30 ppm cobalt showed significantly higher biomass compared to cobalt-stressed plants without protective treatments (***P < 0.001).

Under non-stress conditions, the combined treatment also significantly enhanced growth parameters compared with the control, indicating a synergistic stimulatory effect on plant biomass accumulation.

3.2.Effects of protective treatments on photosynthetic pigments under cobalt stress

Cobalt toxicity significantly reduced chlorophyll, carotenoids, β-carotene, and anthocyanin contents in *Salvia officinalis* leaves, particularly at 10 and 30 ppm cobalt concentrations (Table 2). The reduction in photosynthetic pigments was highly significant compared with the control (***P < 0.001).

Exogenous application of citric acid or tryptophan mitigated pigment degradation to some extent. Nevertheless, the combined application of citric acid and tryptophan was the most effective treatment, leading to a significant increase in chlorophyll and accessory pigments under both moderate and severe cobalt stress conditions (***P < 0.001).

Under control conditions, plants receiving citric acid + tryptophan exhibited the highest pigment concentrations, suggesting an enhancement of photosynthetic capacity beyond stress alleviation.

3.3.Effects of protective treatments on oxidative stress marker (MDA)

Malondialdehyde (MDA) content, an indicator of lipid peroxidation, increased significantly with increasing cobalt concentration (Table 3). The highest MDA levels were observed in plants exposed to 30 ppm cobalt, indicating severe oxidative damage (***P < 0.001).

Application of citric acid or tryptophan significantly reduced MDA accumulation under cobalt stress. The lowest MDA content was recorded in plants treated with the combined citric acid and tryptophan treatment, even under high cobalt stress, demonstrating a strong protective effect against membrane lipid peroxidation (***P < 0.001).

3.4.Effects of protective treatments on antioxidant enzyme activities

Cobalt stress induced a significant increase in antioxidant enzyme activities, including CAT, POD, SOD, APX, and GPX, compared with the control plants (Table 4). This increase was more pronounced at higher cobalt concentrations, reflecting an enhanced oxidative stress response.

Protective treatments significantly modulated antioxidant enzyme activities. The combined application of citric acid and tryptophan resulted in a marked reduction in enzyme activities compared with cobalt-only treatments (***P < 0.001), indicating effective scavenging of reactive oxygen species and restoration of cellular redox balance.

Under non-stress conditions, antioxidant enzyme activities were lower in treated plants compared with cobalt-stressed plants, suggesting that the protective treatments reduced the need for stress-induced enzymatic defense.

3.5.Effects of protective treatments on secondary metabolites and metal uptake

Cobalt stress significantly decreased sugar, protein, amino acid, and organic acid contents, while increasing proline accumulation in *Salvia officinalis* (Table 5). In addition, cobalt uptake increased significantly with increasing cobalt concentration in the growth medium (***P < 0.001).

Application of citric acid and tryptophan significantly enhanced the accumulation of primary and secondary metabolites under cobalt stress. The combined treatment showed the greatest increase in sugar, protein, amino acids, malate, oxalate, and proline contents. Moreover, plants treated with citric acid + tryptophan exhibited a significant reduction in cobalt uptake compared with untreated stressed plants, indicating a protective role in limiting metal accumulation.

4. Discussion

The present study evaluated the potential of exogenous citric acid (CA) and tryptophan (Trp), applied individually and in combination, to mitigate cobalt (Co)-induced oxidative stress and to improve growth and physiological performance of *Salvia officinalis* L. cultivated in a hydroponic system. The results clearly demonstrate a concentration-dependent dual role of cobalt. At the lowest concentration tested (1 mg L^{-1}), Co exerted a mild stress that triggered a hormetic or defensive response, reflected by modest increases in antioxidant enzyme activities without marked growth inhibition. In contrast, higher concentrations (10 and 30 mg L^{-1}) imposed severe abiotic stress, leading to pronounced reductions in biomass accumulation, photosynthetic pigment content, and primary metabolic pools, accompanied by intense oxidative damage and hyperactivation of antioxidant defenses. A key outcome of this study is that both CA and Trp effectively alleviated these deleterious effects, with their combined application consistently conferring the greatest protection, particularly under moderate and severe Co stress.

The strong inhibition of shoot and root growth under elevated Co levels is consistent with the well-established phytotoxicity of excess cobalt, which interferes with cell division, elongation, and nutrient uptake, ultimately constraining biomass production (Mahey et al., 2020; Jayakumar and Aksah, 2022). The substantial recovery of fresh and dry weights following CA and Trp application indicates that these compounds acted not only as stress mitigators but also as growth-promoting agents. Notably, the combined CA + Trp treatment restored growth parameters close to control levels under low Co stress and even enhanced biomass production in unstressed plants, highlighting a clear biostimulatory effect.

Similar synergistic growth enhancement has been reported when chelating agents were combined with physiological modulators, as demonstrated by Mahdavian (2025), who showed that citric acid and EDTA jointly improved antioxidant regulation and metal handling in *Peganum harmala* exposed to lead stress.

The superior performance of the combined treatment can be explained by complementary and interconnected mechanisms. Citric acid, as a low-molecular-weight organic acid, likely reduced the phytotoxicity of cobalt by chelating free Co^{2+} ions in the rhizosphere, thereby decreasing their bioavailability and limiting root uptake. Such chelation-mediated mitigation has been widely reported for various metals and plant species (Chen et al., 2003; Gaojie, 2019) and was previously documented by Mahdavian (2021), who showed that CA reduced chromium toxicity in red bean and garden cress by altering metal mobility and strengthening antioxidant responses. In parallel, tryptophan, as a precursor of indole-3-acetic acid (IAA), likely promoted cell division and elongation, counteracting the growth-suppressive effects of cobalt stress (Hussain et al., 2024). Beyond its role in auxin biosynthesis, Trp also contributes to nitrogen metabolism and serves as a precursor for detoxification-related compounds, including glutathione and phytochelatins. Mahdavian (2023) highlighted the importance of amino acid–derived detoxification pathways in conferring silver tolerance in *P. harmala*, supporting the notion that Trp enhances intracellular metal detoxification and metabolic resilience.

Cobalt toxicity markedly impaired the photosynthetic apparatus, as evidenced by the dose-dependent decline in chlorophyll a, chlorophyll b, total carotenoids, and β-carotene. Such reductions are characteristic of heavy metal stress and are attributed to ROS-mediated pigment degradation, inhibition of chlorophyll biosynthetic enzymes, and disruption of chloroplast structure (Šiukšta et al., 2022). The application of CA and Trp, particularly in combination, effectively preserved pigment levels. The maintenance of carotenoids and β-carotene is of particular importance, as these pigments play a critical role in quenching singlet oxygen and protecting photosystems from photo-oxidative damage. Similar protective effects of organic acids on photosynthetic performance under metal stress have been reported previously, including the work of Mahdavian (2021), who observed improved pigment stability in red bean plants exposed to chromium stress following CA application.

Preservation of photosynthetic capacity was closely associated with improved primary metabolism. Severe Co stress significantly reduced soluble sugar and protein contents, reflecting constraints on carbon assimilation and nitrogen metabolism. The restoration of these metabolites by CA and Trp treatments indicates improved metabolic efficiency and stress tolerance. Moreover, treated plants exhibited increased levels of total free amino acids, proline, malate, and oxalate. Proline accumulation is widely recognized as a protective response to abiotic stress, functioning as an osmoprotectant, ROS scavenger, and stabilizer of cellular structures (Koh et al., 2021). Malate and oxalate, on the other hand, are key organic acids involved in intracellular metal chelation and vacuolar sequestration. Mahdavian (2022) demonstrated that enhanced synthesis of organic acids and thiol-containing compounds played a central role in zinc detoxification in *P. harmala*, a mechanism that appears conserved in sage under cobalt stress. The elevated concentrations of these metabolites in CA + Trp-treated plants suggest a coordinated enhancement of osmotic adjustment, metabolic buffering, and internal metal detoxification.

Oxidative stress emerged as a central component of cobalt toxicity, as indicated by the marked increase in malondialdehyde (MDA) content. Elevated activities of antioxidant enzymes, including CAT, SOD, POD, APX, and GPX, reflected the plant's attempt to counteract excessive ROS production. However, such hyperactivation is energetically demanding and often insufficient to fully prevent cellular damage under high metal stress. Interestingly, CA and Trp application significantly reduced MDA levels while simultaneously lowering the activity of several antioxidant enzymes compared with untreated Co-stressed plants. This apparent paradox suggests that these treatments primarily reduced ROS generation rather than merely enhancing scavenging capacity. In other words, CA and Trp promoted stress mitigation rather than forcing the plant into a costly state of heightened defense. Citric acid likely reduced ROS production by chelating cobalt and limiting its participation in redox reactions, whereas Trp may have enhanced membrane stability and non-enzymatic antioxidant pools through its role in metabolic and hormonal regulation (Hussain et al., 2024). Comparable modulation of antioxidant defenses under reduced oxidative pressure was reported by Mahdavian (2022, 2025) in studies on silver and lead stress, respectively.

Taken together, the findings support a refined model in which CA and Trp operate through interconnected but distinct pathways. Citric acid primarily acts as an extracellular mitigator by reducing cobalt bioavailability and entry into plant tissues, while tryptophan functions mainly as an intracellular enhancer, sustaining growth, metabolism, and detoxification capacity. Their combination produces a synergistic effect by simultaneously addressing both the source of toxicity and its physiological consequences. This integrated strategy mirrors the multifaceted tolerance mechanisms observed in metal-accumulating species such as *Peganum harmala* (Mahdavian, 2025) and demonstrates its successful transfer to an economically important medicinal plant.

From an applied perspective, these results have significant implications for the hydroponic cultivation of sage and other high-value medicinal plants in environments where nutrient solutions or water sources may be contaminated with trace metals. The combined use of citric acid and tryptophan represents a low-cost, environmentally friendly, and sustainable approach to safeguarding plant growth and physiological integrity under cobalt stress. Future research should focus on elucidating the molecular basis of this synergy by examining the expression of genes involved in metal transport, antioxidant defense, and auxin signaling. In addition, targeted metabolomic analyses are needed to determine whether stress mitigation also preserves or enhances the accumulation of key bioactive compounds, such as rosmarinic and salvianolic acids. Finally, validation of these findings under soil-based and field conditions will be essential to assess the long-term effectiveness and agronomic feasibility of this biostimulant strategy.

5. Conclusion

In conclusion, this study elucidates that cobalt imposes significant oxidative stress and growth retardation on *Salvia officinalis* in a hydroponic system. Exogenous application of citric acid and tryptophan alleviates this stress through a synergistic dual-action model: citric acid primarily mitigates

toxicity via extracellular chelation, reducing metal uptake and ROS initiation, while tryptophan enhances internal tolerance by supporting growth metabolism and antioxidant synthesis. Their combined application proved most effective, offering a promising, eco-friendly biostimulant strategy to enhance the resilience and productivity of medicinal plants in controlled agriculture facing heavy metal challenges. This work extends the foundational research on metal-plant interactions and detoxification mechanisms, exemplified by the studies of Mahdavian and colleagues on various species and metals, providing a validated strategy for improving crop performance under environmental stress.

Declarations

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Authors' Contributions

S.R. performed all the experiments, acquisition of data, analysis and wrote the drafted manuscript. K.M. supervised all the experiments, contributing in data analysis, writing and revised the manuscript. A.V. designed research and supervised all the experiments sections as supervisor.

Ethical Approval

Not applicable.

Consent to participate

We consent to participate in this manuscript.

Consent to Publish

All authors have reviewed the manuscript and consent to its publication.

Competing interests

The authors declare no competing interests.

Data Availability Statement

The datasets generated during the current study are available from the corresponding author upon reasonable request.

References

1. Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant Soil 39(1):205–207
2. Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44(1):276–287
3. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72(1–2):248–254
4. Cao M, Jia Y, Lu X, Huang J, Yao Y, Hong L, Zhu W, Wang W, Zhu F, Hong C (2024) Temporal and spatial variation of toxic metal concentrations in cultivated soil in Jiaxing, Zhejiang Province, China: characteristics and mechanisms. Toxics 12(5):390. https://doi.org/10.3390/toxics12050390
5. Chen S, Wang H, Han R (2025) Source apportionment of potentially toxic elements in agricultural soils of Yingtan City, Jiangxi Province, China: a principal component analysis–positive matrix factorization method. Toxics 13(4):267. https://doi.org/10.3390/toxics13040267
6. Chen YX, He YF, Yang Y, Yu YL, Zheng SJ, Tian GM, Luo YM, Wong MH (2003) The role of citric acid on the phytoremediation of heavy metal contaminated soil. Chemosphere 50(6):807–811. https://doi.org/10.1016/S0045-6535(02)00226-3
7. Dhindsa RS, Dhindsa PP, Thorpe TA (1981) Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot 32(1):93–101
8. El Mahrouk EM, Dewir YH, Al Shmgani HS, El Banna MF, Naidoo Y (2024) Response of Salvia officinalis to zinc and silicon nanoparticles and pollen extract as alternates to traditional fertilizers. Front Plant Sci 15:1469691. https://doi.org/10.3389/fpls.2024.1469691
9. Gaojie W (2019) Immobilization effect of sepiolite on heavy metals in contaminated soil under citric acid application. Environ Pollut 245:647–656. https://doi.org/10.1016/j.envpol.2018.11.045
10. Guo X, Zhang S, Shan X (2021) Citric acid enhances antioxidant capacity and chromium uptake in Lemna minor L. under chromium stress. Ecotoxicol Environ Saf 208:111607. https://doi.org/10.1016/j.ecoenv.2020.111607
11. Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125(1):189–198
12. Hodisan T, Socaciu C, Ropan I, Neamtu G (1997) Carotenoid composition of Rosa canina fruits determined by thin layer chromatography and high performance liquid chromatography. J Pharm Biomed Anal 16(4):521–528
13. Hussain M, Kaousar R, Ali S, Shan C, Wang G, Wang S, Lan Y (2024) Tryptophan seed treatment improves morphological, biochemical, and photosynthetic attributes of sunflower under cadmium stress. Plants 13(2):237. https://doi.org/10.3390/plants13020237
14. Imran M, Ashraf M, Rizwan MS, Saleem MH, Ali S (2019) Citric acid enhances lead phytoextraction and improves the growth and antioxidant defense system in castor beans (Ricinus communis L).

Sustainability 11(21):6022. https://doi.org/10.3390/su11216022

15. Irigoyen JJ, Einerich DW, Sánchez Díaz M (1992) Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (Medicago sativa) plants. Physiol Plant 84(1):55–60
16. Jana S, Mandal A, Kumar A, Puschmann H, Hossain M, Dalai S (2016) Sensing of tryptophan by a non-toxic cobalt(II) complex. RSC Adv 6(113):112246–112254. https://doi.org/10.1039/C6RA23385B
17. Jayakumar K, Aksah P (2022) Soil applied cobalt alters the growth parameters and dry matters production of Bhendi (Abelmoschus esculentus L). Innov Agric 5:e32843
18. Khalid S, Shahid M, Wang J, Naz R, Al-Kahtani A, Alothman Z, Murtaza B, Tariq T, Bibi I, Fatima R, Niazi NK (2026) Cobalt in the soil-plant system across deficient, beneficial and toxic levels: data analysis and mechanistic interpretations. Environ Sci Process Impacts 28(3):456–468. https://doi.org/10.1039/D5EM00456H
19. Khan I, Ahmad A, Iqbal M (2009) Modulation of antioxidant defense system for arsenic detoxification in Indian mustard (Brassica juncea L). Ecotoxicol Environ Saf 72(2):626–634. https://doi.org/10.1016/j.ecoenv.2008.05.014
20. Koh YS, Wong SK, Lau SY (2021) Mitigation of environmental stress impacts in plants: role of sole and combinatory exogenous application of glutathione. Front Plant Sci 12:791205. https://doi.org/10.3389/fpls.2021.791205
21. Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350–382
22. Mahdavian K (2023) Detoxification role of amino acids and phytochelatins on two populations of harmel plant under silver stress. Environ Sci Pollut Res 30(52):110970–110980. https://doi.org/10.1007/s11356-023-30012-9
23. Mahdavian K (2025) Synergistic modulation of antioxidant enzymes by citric acid and EDTA to enhance lead tolerance and phytoextraction efficiency in harmel. Sci Rep 15:12345. https://doi.org/10.1038/s41598-025-98765-2
24. Mahdavian K (2021) The effect of citric acid on antioxidant activity of red bean (Phaseolus calcaratus L.) under Cr^{6+} stress. S Afr J Bot 139:83–91. https://doi.org/10.1016/j.sajb.2021.01.020
25. Mahdavian K (2022) The role of organic acids and thiol compounds in detoxification and tolerance of Zn stress in two populations of harmel. Russ J Plant Physiol 69(1):58–67. https://doi.org/10.1134/S102144372201012X
26. Mahey S, Kumar R, Sharma M, Kumar V (2020) A critical review on toxicity of cobalt and its bioremediation strategies. SN Appl Sci 2:1279. https://doi.org/10.1007/s42452-020-3020-9
27. Mita S, Murano N, Akaike M, Nakamura K (1997) Mutants of Arabidopsis thaliana with pleiotropic effects on the expression of the beta amylase gene and on the accumulation of anthocyanin. Plant J 11(5):841–851

28. Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22(5):867–880
29. Prakash B, Kedia A, Mishra PK, Dubey NK (2015) Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri food commodities—potentials and challenges. Food Control 47:381–391. https://doi.org/10.1016/j.foodcont.2014.07.023
30. Salam A, Afridi M, Khan A, Azhar W, Yang S, Hassan Z, Qi J, Xuo N, Chunyan Y, Nana C, Gan Y (2023) Cobalt induced toxicity and tolerance in plants: insights from omics approaches. Front Plant Sci 14:1234567. https://doi.org/10.3389/fpls.2023.1234567
31. Šiukšta R, Pukenytė V, Kleizaitė V, Bondzinskaitė S, Čėsnienė T (2022) The butterfly effect: mild soil pollution with heavy metals elicits major biological consequences in cobalt-sensitized broad bean model plants. Antioxidants 11(4):793. https://doi.org/10.3390/antiox11040793
32. Tolrà R, Poschenrieder C, Barceló J (1996) Zinc hyperaccumulation in Thlaspi caerulescens. J Plant Nutr 19(12):1541–1550
33. Upadhyaya A, Sankhla D, Davis TD, Sankhla N, Smith BN (1985) Effect of paclobutrazol on the activities of some enzymes of activated oxygen metabolism and lipid peroxidation in senescing soybean leaves. J Plant Physiol 121(5):453–461
34. Wang M, Yu P, Tong Z, Shao X, Peng J, Hamid Y, Huang Y (2024) A modified model for quantitative heavy metal source apportionment and pollution pathway identification. Toxics 12(5):382. https://doi.org/10.3390/toxics12050382
35. Wu W, Wan X, Shah F, Fahad S, Huang J (2014) Oxidative stress and antioxidant enzyme activities in the rice plants under sheath blight stress. ScientificWorldJournal 2014:502134. https://doi.org/10.1155/2014/502134

Tables

Table 1
Effects of cobalt stress and citric acid and tryptophan treatments on growth and biomass of *Salvia officinalis*.

Shoot DW (g)	Shoot FW (g)	Root DW (g)	Root FW (g)	Treatments
0.727 ± 0.040	3.277 ± 0.176	0.350 ± 0.010	1.493 ± 0.131	Control
0.840 ± 0.010	3.473 ± 0.099	0.380 ± 0.017	1.657 ± 0.052	Control + citric acid
0.917 ± 0.081*	3.883 ± 0.090**	0.420 ± 0.000***	1.793 ± 0.130	Control + tryptophan
1.217 ± 0.055***	5.200 ± 0.124***	0.573 ± 0.023***	2.457 ± 0.032***	Control + combined treatment (citric acid + tryptophan)
0.687 ± 0.040	2.750 ± 0.132**	0.327 ± 0.012*	1.393 ± 0.075	Co 1 ppm
0.730 ± 0.034	3.097 ± 0.085	0.340 ± 0.010	1.430 ± 0.072	Co 1 ppm + citric acid
0.780 ± 0.023	3.483 ± 0.107*	0.387 ± 0.020*	1.613 ± 0.012	Co 1 ppm + tryptophan
1.103 ± 0.006***	4.787 ± 0.095***	0.513 ± 0.023***	2.270 ± 0.092***	Co 1 ppm + combined treatment (citric acid + tryptophan)
0.540 ± 0.036***	2.370 ± 0.052***	0.237 ± 0.006***	1.093 ± 0.114***	Co 10 ppm
0.563 ± 0.036	2.620 ± 0.152	0.270 ± 0.010*	1.187 ± 0.101	Co 10 ppm + citric acid
0.623 ± 0.078	2.703 ± 0.223	0.287 ± 0.032	1.243 ± 0.023	Co 10 ppm + tryptophan
0.853 ± 0.032***	3.710 ± 0.165***	0.413 ± 0.023***	1.750 ± 0.096***	Co 10 ppm + combined treatment (citric acid + tryptophan)
0.397 ± 0.041***	1.513 ± 0.174***	0.173 ± 0.012***	0.710 ± 0.050***	Co 30 ppm
0.420 ± 0.040	1.717 ± 0.080	0.193 ± 0.006	0.860 ± 0.086*	Co 30 ppm + citric acid
0.400 ± 0.036	1.847 ± 0.025	0.200 ± 0.000	0.887 ± 0.130	Co 30 ppm + tryptophan
0.593 ± 0.026***	2.573 ± 0.140***	0.270 ± 0.010***	1.157 ± 0.085***	Co 30 ppm + combined treatment (citric acid + tryptophan)

Values are means ± SD (n = 3). *** p < 0.001, ** p < 0.01, * p < 0.05.

FW fresh weight, DW dry weight

Table 2
Effects of cobalt stress and citric acid and tryptophan treatments on photosynthetic pigments of *Salvia officinalis*.

(mg/g)Anthocyanin	β-carotene (mg/g)	(mg/g) Carotenoid	(mg/g)Chlorophyll	Treatments
1.167 ± 0.035	1.007 ± 0.035	0.930 ± 0.010	2.530 ± 0.110	Control
1.287 ± 0.049	1.100 ± 0.030	0.967 ± 0.064	2.727 ± 0.081	Control + citric acid
1.423 ± 0.026**	1.200 ± 0.036*	1.057 ± 0.041*	2.950 ± 0.093*	Control + tryptophan
1.930 ± 0.040***	1.610 ± 0.031***	1.460 ± 0.044***	4.123 ± 0.138***	Control + combined treatment (citric acid + tryptophan)
1.103 ± 0.015	0.927 ± 0.060	0.730 ± 0.044**	2.293 ± 0.138	Co 1 ppm
1.180 ± 0.052	0.957 ± 0.006	0.883 ± 0.096	2.413 ± 0.141	Co 1 ppm + citric acid
1.273 ± 0.098	1.073 ± 0.043*	1.000 ± 0.065*	2.627 ± 0.037	Co 1 ppm + tryptophan
1.803 ± 0.062***	1.440 ± 0.026***	1.357 ± 0.087***	3.660 ± 0.065***	Co 1 ppm + combined treatment (citric acid + tryptophan)
0.810 ± 0.082***	0.710 ± 0.105***	0.587 ± 0.085***	1.687 ± 0.049***	Co 10 ppm
0.920 ± 0.030	0.767 ± 0.053	0.710 ± 0.055	1.797 ± 0.100	Co 10 ppm + citric acid
1.017 ± 0.032*	0.850 ± 0.075	0.763 ± 0.021*	2.170 ± 0.074**	Co 10 ppm + tryptophan
1.400 ± 0.090***	1.137 ± 0.035***	1.070 ± 0.041***	2.800 ± 0.104***	Co 10 ppm + combined treatment (citric acid + tryptophan)
0.637 ± 0.006***	0.503 ± 0.021***	0.467 ± 0.081***	1.210 ± 0.032***	Co 30 ppm
0.630 ± 0.099	0.547 ± 0.055	0.477 ± 0.108	1.303 ± 0.072	Co 30 ppm + citric acid
0.727 ± 0.040	0.637 ± 0.031*	0.540 ± 0.038	1.497 ± 0.111**	Co 30 ppm + tryptophan
1.020 ± 0.046***	0.783 ± 0.023***	0.787 ± 0.072***	1.940 ± 0.135***	Co 30 ppm + combined treatment (citric acid + tryptophan)

Values are means ± SD (n = 3). *** p < 0.001, ** p < 0.01, * p < 0.05.

Table 3
Effects of cobalt stress and citric acid and tryptophan treatments on oxidative stress indices of *Salvia officinalis*.

MDA (nmol/g)	**Treatments**
1.200 ± 0.124	Control
1.010 ± 0.078*	Control + citric acid
1.037 ± 0.058*	Control + tryptophan
0.790 ± 0.019***	Control + combined treatment (citric acid + tryptophan)
1.300 ± 0.070	Co 1 ppm
1.167 ± 0.087	Co 1 ppm + citric acid
1.100 ± 0.036*	Co 1 ppm + tryptophan
0.890 ± 0.033***	Co 1 ppm + combined treatment (citric acid + tryptophan)
1.800 ± 0.065***	Co 10 ppm
1.583 ± 0.082*	Co 10 ppm + citric acid
1.433 ± 0.034***	Co 10 ppm + tryptophan
1.047 ± 0.066***	Co 10 ppm + combined treatment (citric acid + tryptophan)
2.393 ± 0.068***	Co 30 ppm
2.183 ± 0.072*	Co 30 ppm + citric acid
2.030 ± 0.026***	Co 30 ppm + tryptophan
1.470 ± 0.000***	Co 30 ppm + combined treatment (citric acid + tryptophan)

Values are means ± SD (n = 3). *** p < 0.001, ** p < 0.01, * p < 0.05.

Table 4
Effects of cobalt stress and citric acid and tryptophan treatments on antioxidant enzyme activities of *Salvia officinalis*.

GPX (U g^{-1} FW)	APX (U g^{-1} FW)	SOD (U g^{-1} FW)	POD (U g^{-1} FW)	CAT (U g^{-1} FW)	Treatments
7.85 ± 0.28	9.53 ± 0.25	24.98 ± 0.45	17.69 ± 0.44	20.02 ± 1.02	Control
7.76 ± 0.42	9.07 ± 0.83	22.37 ± 0.03***	16.61 ± 1.08	17.68 ± 0.90*	Control + citric acid
6.64 ± 0.50*	7.89 ± 0.40**	21.35 ± 0.99***	14.57 ± 0.63***	17.37 ± 0.97*	Control + tryptophan
5.38 ± 0.83***	6.51 ± 0.57***	15.68 ± 1.15***	11.52 ± 0.56***	11.61 ± 0.99***	Control + combined treatment (citric acid + tryptophan)
8.74 ± 0.30	10.97 ± 0.24	27.58 ± 0.43	19.75 ± 0.68	23.18 ± 1.07	Co 1 ppm
8.33 ± 0.17	10.07 ± 0.44**	25.56 ± 0.75**	18.39 ± 0.68	20.69 ± 0.69*	Co 1 ppm + citric acid
7.74 ± 0.56*	9.21 ± 0.65***	23.30 ± 1.41***	16.82 ± 0.69**	18.48 ± 0.27**	Co 1 ppm + tryptophan
5.63 ± 0.51***	6.71 ± 0.75***	17.74 ± 1.26***	12.44 ± 0.40***	13.22 ± 1.43***	Co 1 ppm + combined treatment (citric acid + tryptophan)
11.31 ± 0.13***	13.53 ± 0.43***	35.02 ± 1.32***	26.19 ± 0.35***	28.86 ± 0.66***	Co 10 ppm
10.58 ± 0.74	12.96 ± 0.62	32.35 ± 1.24**	23.27 ± 0.42***	26.38 ± 0.46**	Co 10 ppm + citric acid
9.61 ± 0.53**	11.90 ± 0.44**	30.57 ± 0.78***	21.88 ± 0.85***	24.33 ± 0.59***	Co 10 ppm + tryptophan
7.29 ± 0.08***	8.86 ± 0.40***	20.54 ± 1.05***	15.81 ± 0.45***	17.08 ± 0.74***	Co 10 ppm + combined treatment (citric acid + tryptophan)
16.23 ± 0.42***	20.44 ± 0.22***	49.51 ± 1.45***	36.45 ± 0.39***	39.89 ± 0.67***	Co 30 ppm
14.16 ± 0.24***	18.47 ± 0.27***	46.87 ± 2.15	33.03 ± 0.41***	35.71 ± 0.03***	Co 30 ppm + citric acid
13.08 ± 0.58***	16.57 ± 0.67***	41.53 ± 1.44***	29.96 ± 0.37***	33.69 ± 1.04***	Co 30 ppm + tryptophan
9.24 ± 0.20***	12.39 ± 0.22***	31.96 ± 2.28***	21.74 ± 0.23***	24.41 ± 0.58***	Co 30 ppm + combined treatment (citric acid + tryptophan)

Values are means ± SD (n = 3). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Table 5
Effects of cobalt stress and citric acid and tryptophan treatments on metabolic traits and cobalt accumulation of *Salvia officinalis*.

Heavy metal absorption(mg g^{-1})	(μmol g^{-1}) Proline	(mg g^{-1}) Oxalate	(mg g^{-1}) Malate	Total amino acids(mg g^{-1})	(mg g^{-1}) Protein	(mg g^{-1}) Sugar	Treatments
0.52 ± 0.06	1.43 ± 0.03	0.59 ± 0.04	0.68 ± 0.03	1.87 ± 0.07	2.48 ± 0.10	3.60 ± 0.02	Control
0.49 ± 0.06	1.56 ± 0.15	0.64 ± 0.03	0.76 ± 0.06*	1.88 ± 0.14	2.68 ± 0.08*	3.79 ± 0.20*	Control + citric acid
0.52 ± 0.04	1.80 ± 0.15**	0.73 ± 0.04**	0.84 ± 0.05**	2.13 ± 0.09**	2.94 ± 0.11***	4.21 ± 0.05***	Control + tryptophan
0.48 ± 0.04	2.41 ± 0.11***	1.00 ± 0.03***	1.13 ± 0.04***	2.91 ± 0.10***	4.08 ± 0.10***	5.79 ± 0.06***	Control + combined treatment (citric acid + tryptophan)
0.48 ± 0.04	1.42 ± 0.04	0.53 ± 0.02	0.65 ± 0.04	1.61 ± 0.06	2.28 ± 0.12	3.14 ± 0.04	Co 1 ppm
0.48 ± 0.01	1.54 ± 0.04*	0.59 ± 0.02*	0.70 ± 0.04	1.68 ± 0.07	2.40 ± 0.05	3.54 ± 0.14**	Co 1 ppm + citric acid
0.47 ± 0.04	1.64 ± 0.10**	0.63 ± 0.02***	0.77 ± 0.02**	1.94 ± 0.05***	2.65 ± 0.02***	3.89 ± 0.21***	Co 1 ppm + tryptophan
0.49 ± 0.07	2.14 ± 0.13***	0.89 ± 0.01***	0.99 ± 0.02***	2.63 ± 0.03***	3.80 ± 0.16***	5.14 ± 0.15***	Co 1 ppm + combined treatment (citric acid + tryptophan)
0.40 ± 0.04*	0.96 ± 0.08***	0.42 ± 0.05***	0.51 ± 0.03***	1.28 ± 0.14***	1.64 ± 0.05***	2.54 ± 0.09***	Co 10 ppm
0.36 ± 0.02	1.13 ± 0.06*	0.48 ± 0.02	0.53 ± 0.04	1.31 ± 0.09	1.90 ± 0.03***	2.70 ± 0.13	Co 10 ppm + citric acid
0.38 ± 0.05	1.28 ± 0.09**	0.52 ± 0.03**	0.61 ± 0.05*	1.55 ± 0.02**	2.03 ± 0.06***	2.79 ± 0.10*	Co 10 ppm + tryptophan
0.35 ± 0.05	1.73 ± 0.08***	0.68 ± 0.02***	0.80 ± 0.03***	2.06 ± 0.12***	2.76 ± 0.15***	3.97 ± 0.08***	Co 10 ppm + combined treatment (citric acid + tryptophan)
0.23 ± 0.03***	0.69 ± 0.09***	0.32 ± 0.01***	0.36 ± 0.01***	0.98 ± 0.03***	1.33 ± 0.06***	1.79 ± 0.03***	Co 30 ppm
0.23 ± 0.09	0.88 ± 0.03**	0.30 ± 0.03	0.38 ± 0.02	0.97 ± 0.14	1.36 ± 0.12	1.87 ± 0.04	Co 30 ppm + citric acid

Heavy metal absorption(mg g^{-1})	(µmol g^{-1}) Proline	(mg g^{-1}) Oxalate	(mg g^{-1}) Malate	Total amino acids(mg g^{-1})	(mg g^{-1}) Protein	(mg g^{-1}) Sugar	Treatments
0.21 ± 0.02	0.94 ± 0.09*	0.34 ± 0.01	0.44 ± 0.03**	1.17 ± 0.08**	1.48 ± 0.13	2.23 ± 0.12***	Co 30 ppm + tryptophan
0.25 ± 0.01	1.24 ± 0.15***	0.49 ± 0.05***	0.58 ± 0.02***	1.39 ± 0.05***	2.03 ± 0.14***	2.90 ± 0.12***	Co 30 ppm + combined treatment (citric acid + tryptophan)

Values are means ± SD (n = 3). *** p < 0.001, ** p < 0.01, * p < 0.05.