Annals of Arid Zone 64(4): 635-642, 2025

Assisted Bioremediation by *Bacillus* sp.: Impact on the Growth of *Vicia faba L.* under Copper Contamination Conditions

Rima Belattar[1,2], Abdelhakim Sellal[1,2*], Amor Bencheikh[4], Mondher Fradjia[3] and Nora Laouicha[4]

[1]*Health and Environment Laboratory, Faculty of Nature and Life Sciences and Earth and Universe Sciences, Mohamed El Bachir El Ibrahimi University, Bordj Bou Arreridj 34000, Algeria.*

[2]*Faculty of Nature and Life Sciences, Ferhat Abbas Setif1 University, Setif 19000; Algeria.*

[3]*Laboratory of Phytotherapy Applied to Chronic Diseases, Department of Biochemistry, Faculty of Nature and Life Sciences, University Ferhat Abbas Setif 1, 19000 Setif, Algeria*

[4]*Laboratory of Applied Microbiology, Faculty of Nature and Life Sciences, Ferhat Abbas setif1 University, Setif, Algeria, 19000.*

Received: August 11, 2025   Accepted: October 29, 2025

****Correspondence***
Abdelhakim Sellal
sellalhak@univ-setif.dz

Citation
Belattar,R., Sellal, A., Bencheikh, A., Fradjia, M. and Laouicha, N 2025. Assisted Bioremediation by Bacillus sp.: Impact on the Growth of Vicia faba L. under Copper Contamination Conditions. Annals of Arid Zone 64(4): 635-642

https://doi.org/10.56093/aaz.v64i4.170134

https://epubs.icar.org.in/index.php/AAZ/article/view/170134

Abstract: Under greenhouse conditions stem length, branching, leaf area, and total chlorophyll content of Vicia faba plants grown in copper-contaminated soil were similarly improved by both EDTA (positive control) and *Bacillus* sp. treatments. Stem height at flowering and maturation stages was slightly higher in *Bacillus* sp.–treated plants (35.33 ± 1.52 cm and 46.33 ± 1.52 cm) than in EDTA-treated plants (34.33 ± 3.51 cm and 44.00 ± 5.56 cm), although the difference was not statistically significant. Branching and leaf area followed a comparable trend, with both treatments showing higher values than the negative control, but no significant differences between them. Total chlorophyll content was consistently higher in both treatments compared with copper-stressed plants, with minor fluctuations over time, indicating partial protection of the photosynthetic system. Overall, these results suggest that *Bacillus* sp. inoculation provides a bioremediation effect comparable to EDTA, promoting growth and mitigating copper toxicity through biologically mediated mechanisms.

Key words: Pollution, heavy metals, copper, phytoremediation, bioremediation, *Vicia faba* L., *Bacillus* sp.

Environmental pollution represents one of the most critical challenges facing modern societies, with heavy metal contamination constituting a particularly serious and persistent threat (Ali *et al*., 2013; Hashem *et al*., 2017). Heavy metals are among the most significant inorganic pollutants of soils and are continuously introduced into terrestrial ecosystems through various anthropogenic activities, including agricultural practices such as the application of sewage sludge and industrial processes, notably metallurgical operations. Certain metallic elements, including lead (Pb), mercury (Hg), and cadmium (Cd), are non-essential and exert exclusively toxic effects on living organisms. In contrast, trace elements such as zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn) are essential for plant growth and metabolism at low concentrations but become toxic when their accumulation exceeds critical thresholds (Yruela, 2009; Sellal *et al*., 2024b).

Among the available strategies for mitigating soil contamination, bioremediation has emerged as a promising and environmentally sustainable approach. This method relies on the use of biological agents, particularly microorganisms, to reduce or eliminate pollutants from contaminated environments. Compared with conventional chemical remediation techniques, bioremediation offers several advantages, including lower ecological impact, reduced labor requirements, and avoidance of long-term soil sterilization (Erneste *et al*., 2017; Bencheikh *et al*., 2025; Ahmedi *et al*., 2025 in press). Microbial-assisted remediation, in particular, has gained increasing attention due to its potential to enhance metal immobilization, transformation, or uptake in contaminated soils.

Phytoremediation, which exploits the natural capacity of plants to tolerate, accumulate, or stabilize pollutants, constitutes another complementary and cost-effective remediation strategy. Numerous plant species possess valuable traits for this purpose. Among them, the broad bean (*Vicia faba* L.) is a robust species characterized by low soil requirements and high adaptability (Alobaidi *et al*., 2020). In Algeria, *V. faba* represents a major food crop, cultivated over approximately 58,000 ha with an annual production of about 254,000 tons (Laamari *et al*., 2008). Moreover, its marked sensitivity to soil contamination makes it a widely used model plant in ecotoxicological and environmental stress studies.

In the present study, copper (Cu) was selected as the target metallic pollutant due to its frequent occurrence in contaminated soils, particularly in areas affected by industrial, mining, and intensive agricultural activities. Copper plays a dual role in plant systems: while it is an essential micronutrient involved in numerous physiological processes, its excessive accumulation induces phytotoxic effects, including growth inhibition, metabolic disturbances, and oxidative stress. This duality makes Cu an appropriate model element for investigating plant tolerance mechanisms and the effectiveness of bioremediation strategies.

The objective of this study was to evaluate the bioremediation potential of *Bacillus* sp. on selected morphological and biochemical parameters of *Vicia faba* L. grown in copper-contaminated soil. The effectiveness of the bacterial treatment was compared with that of the chelating agent ethylenediaminetetraacetic acid (EDTA), which is known to enhance the desorption and mobilization of metallic ions bound to soil mineral particles and organic matter through local soil acidification (Belattar and Sellal, 2020). This comparative approach aims to provide insights into the efficiency of biological versus chemical strategies for mitigating copper-induced stress in plants.

Materials and Methods

The experiment was conducted under greenhouse conditions using a completely randomized design. Twelve plastic pots with a capacity of 1 kg were filled with Terra Brill potting soil, which was sterilized by autoclaving at 120°C for 20 min under a pressure of 1.4 bar. The pots were randomly arranged in the greenhouse. Four treatments were established, each with three replicates: (i) control, consisting of potting soil without any amendment; (ii) negative control, consisting of potting soil irrigated with a copper solution; (iii) positive control, consisting of potting soil supplemented with EDTA and planted with seeds; and (iv) *Bacillus* sp. treatment, consisting of potting soil amended with copper and inoculated with *Bacillus* sp., with seeds planted. Each pot contained four plants.

Copper was applied as copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) to obtain a final concentration of 600 mg Cu kg^{-1} soil. For this purpose, 0.786 g of $CuSO_4 \cdot 5H_2O$ was dissolved in 200 mL of distilled water and thoroughly mixed with 1 kg of soil per pot. The application was repeated thrice as detailed below to reach the final concentration of 600 mg Cu kg^{-1} soil. A solution was prepared by dissolving 200 mg of EDTA in 200 mL of distilled water. Treatments were applied three weeks after planting. EDTA and $CuSO_4 \cdot 5H_2O$ solutions were each administered at a volume of 200 mL per pot, three times within the same week. Pots assigned to the positive control received the EDTA solution, whereas all treatments except the absolute control received the copper sulfate solution (Sellal *et al*., 2019a; Sellal *et al*., 2019b).

A well-isolated colony of *Bacillus* sp. was aseptically collected using a sterile platinum loop and transferred into a test tube containing 10 mL of sterile nutrient broth. After initial incubation, 200 µL of this pre-culture were inoculated into flasks containing 200 mL of sterile nutrient broth. The flasks were tightly sealed and incubated at 37°C for 24 h under gentle agitation to promote bacterial growth. At the end of the incubation period, the optical density of the bacterial suspension was measured at 620 nm using a spectrophotometer and adjusted to an OD between 0.08 and 0.10 to obtain a standardized inoculum (Bencheikh *et al*., 2025). Two days after seedling transplantation, 200 mL of the bacterial suspension were applied directly to the soil at the base of the plants in the corresponding treatment, following the same procedure used for irrigation.

Seeds of *Vicia faba* L. were surface-sterilized with a 2% sodium hypochlorite solution for 5 min, thoroughly rinsed with sterile distilled water, and placed on moistened filter paper for three days to allow germination. After seven days, four uniform seedlings were transplanted into each pot. Irrigation was carried out over a period of ten weeks with a uniform supply of 200 mL of water per pot. Watering was performed every alternate day during moderately humid periods and daily during hot periods to maintain optimal soil moisture and minimize water stress.

Stem length was measured at two growth stages, during flowering (6^{th} week) and at maturity (10^{th} week), from the collar to the terminal bud using a graduated ruler, and values were expressed in centimetres. The total number of branches per plant was recorded three times per week from the 3^{rd} to the 6^{th} week of growth. Leaf area was measured during the same period on fully developed leaves three times per week. Leaves were scanned using a flatbed scanner or a high-resolution camera, and leaf area was calculated using ImageJ software. Total chlorophyll content was measured three times per week from the 3^{rd} to the 6^{th} week using a SPAD chlorophyll meter (CCM-200 Plus). Measurements were taken between 08:00 and 12:00 on the youngest fully expanded leaves near the terminal bud. For each pot, three readings were taken weekly for four consecutive weeks, and the mean value was used for statistical analysis.

All results are expressed as mean ± standard deviation (SD) based on three replicates per treatment. Student's *t*-test was used to compare treatments with the negative control, with differences considered statistically significant at $p \leq 0.05$ and highly significant at $p \leq 0.01$. One-way analysis of variance (ANOVA) was performed using SPSS software, followed by Duncan's multiple range test to compare mean values among treatments, with significance accepted at $p < 0.05$.

Results and Discussion

Stem length (LPF, LPM)

Statistical analysis, performed using Student's *t*-test, revealed no significant difference ($p > 0.05$) in the stem height of *Vicia faba* between the flowering week and the maturation week, under controlled conditions, for samples treated with the chelating agent EDTA (positive control) or with *Bacillus* sp., in the presence of copper. However, the ANOVA results revealed a significant difference between the negative control (C^-) and the other treatments (T, C^+, and *Bacillus* sp.), with an ascending trend as follows: C^{-a} < T^b < C^{+b} < *Bacillus* sp^b. This suggests that *Bacillus* sp. treatment had the most pronounced positive effect compared to the other groups. Nevertheless, the results, illustrated in Figure 4, show a marked increase in stem height during both phases, with a strong similarity between plants treated with *Bacillus* sp. and those of the positive control for both test periods (LPF and LPM). Thus, in the long-period flowering phase (LPF), plants treated with *Bacillus* sp. reached an average height of 35.33 ± 1.52 cm, close to that observed for the positive control (34.33 ± 3.51 cm). In the long-period maturation phase (LPM), these values increased to 46.33 ± 1.52 cm and 44.00 ± 5.56 cm, respectively. In contrast, the negative control plants (untreated) exhibited considerably lower heights, namely 27.00 ± 7.81 cm for LPF and 32.33 ± 8.08 cm for LPM, indicating less favorable vegetative development in the absence of treatment. These observations suggest a potential beneficial effect of *Bacillus* sp., comparable to that of EDTA, in mitigating the impact of copper on the stem growth of *Vicia faba* (Fig. 1).

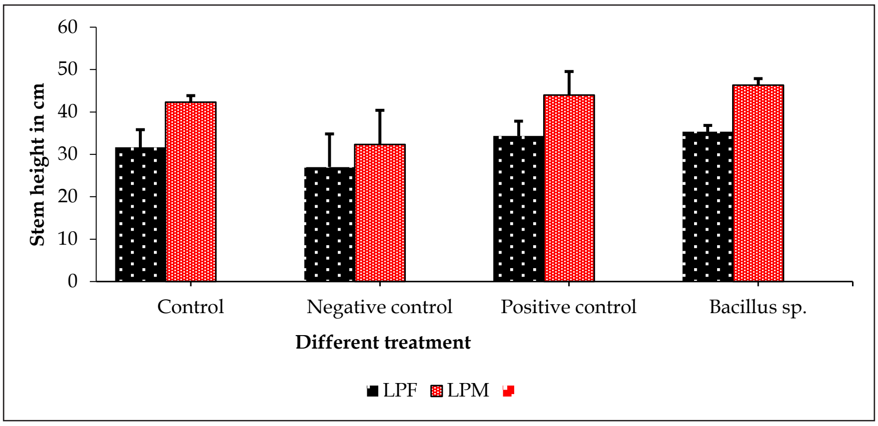

Fig. 1. Influence of the applied treatments on stem height growth.

Branching

The results, illustrated in Fig. 2, show that the number of branches per plant did not differ significantly among the various treatments. However, a general trend toward a moderate increase in the number of branches was observed in the *Bacillus* sp.-treated group, with respective values of 2.33 ± 0.57 and 2 ± 1, as well as in the positive control (2 ± 1; 2.33 ± 0.57; 2.33 ± 0.57). These results also indicate a certain homogeneity within these two groups. In contrast, the negative control (untreated) exhibited a lower number of branches, with values of 1 ± 1, 0.66 ± 0.57, and 1.66 ± 1.52, suggesting less favorable vegetative development in the absence of treatment. The combined analysis of variance for branch number revealed a significant effect of the treatments, following an ascending trend: T^{a} < C^{-a} < *Bacillus* sp.^{ab} < C^{+ab}. This indicates that the positive control (C^{+}) and *Bacillus* sp. induced the highest branching response, whereas the untreated plants (T) showed the lowest values. This trend may reflect a potential positive effect of *Bacillus* sp. on branching, warranting further investigation to confirm its extent and the underlying physiological mechanism.

Leaf area

The results illustrated in Fig. 3, show a non-significant (Student's *t*-test) increase ($p > 0.05$) in the leaf area of *Vicia faba* under controlled conditions, in the presence of EDTA, *Bacillus* sp. and copper. Nevertheless, the analysis of variance consistently showed that the C^{-} and *Bacillus* sp. treatments exhibited higher mean surface areas compared to the T and C^{-} groups (T^{a} < C^{-a} < *Bacillus* sp.^{a} < C^{+a}), different superscripts suggest significance differences highlighting their positive effect on plant growth. The leaf area values measured in the three pots of the *Bacillus* sp.-treated group were 17.00 ± 6.24 cm^2, 16.89 ± 3.38 cm^2, and 21.46 ± 4.46 cm^2. These results, although showing some variability, are comparable to those of the positive control (17.00 ± 7.78 cm^2, 15.13 ± 6.28 cm^2, and 17.20 ± 3.01 cm^2) and higher than those of the negative control, whose values

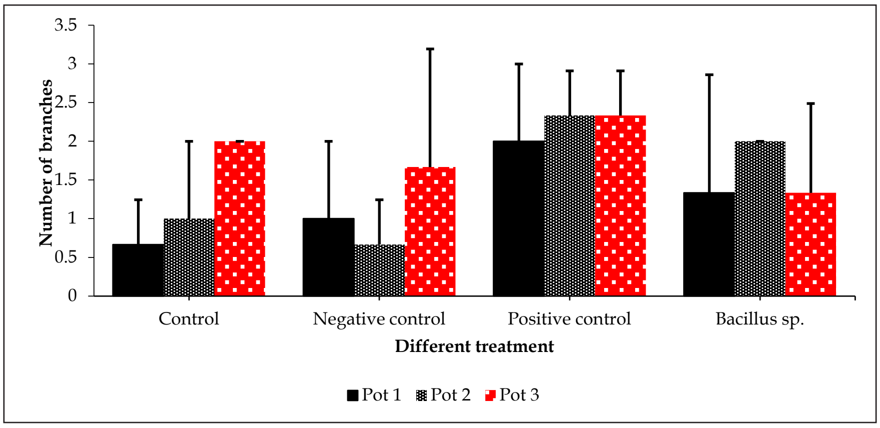

Fig. 2. Effects of experimental treatments on branch development.

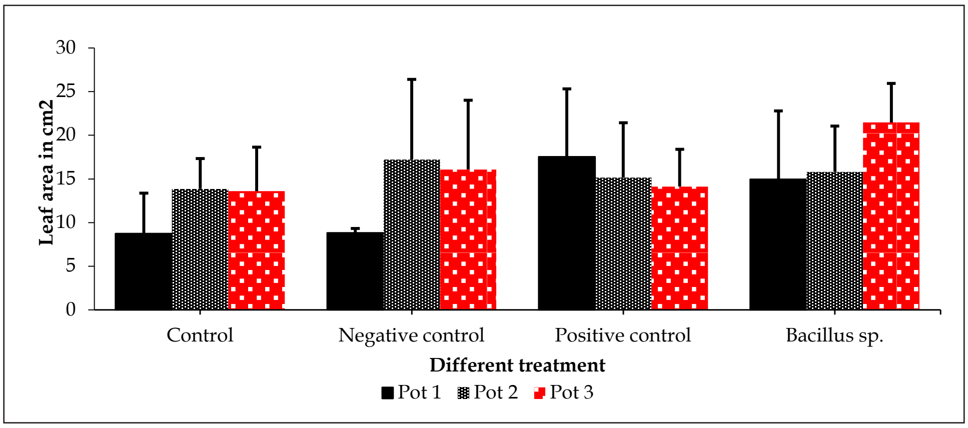

Fig. 3. Effects of different treatments on leaf area.

were 8.11 ± 1.05 cm², 13.65 ± 3.19 cm², and 13.08 ± 7.70 cm².

Total chlorophyll content

The results for total chlorophyll content reveal a highly significant difference ($p \leq 0.01$ Student's *t*-test) over the three observation weeks the week preceding flowering, the flowering week and the maturation week in *Vicia faba* plants grown in the presence of copper and treated either with the bacterium *Bacillus* sp. or with EDTA (standard chelating agent).

The ANOVA results further revealed a highly significant difference ($p < 0.01$) among treatments, with the following ascending order: C^{-a} < C^{+b} < *Bacillus* sp.^{b} < T^{c}, indicating a progressive improvement in plant response under the different treatments.

During the first week (Figure 4), total chlorophyll content was similar between plants treated with *Bacillus* sp. (50.63 ± 8.78 CCI) and those of the positive control (44.93 ± 3.49 CCI), both lower than that of the unexposed control (69.29 ± 4.14 CCI) but clearly higher than that of the negative control, treated only with copper (29.96 ± 1.95 CCI). These differences were statistically significant ($p \leq 0.05$).

In the second week, a highly significant decrease ($p \leq 0.01$) in chlorophyll content was observed in the *Bacillus* sp.-treated group (38.40 ± 2.51 CCI) and in the EDTA group (33.53 ± 4.14 CCI), values which nevertheless remained close to that of the unexposed control (44.26 ± 2.51 CCI) and much higher than that of the negative control (24.43 ± 3.46 CCI).

During the third week, chlorophyll content continued to decline in all treatments. Plants in the *Bacillus* sp. group still showed a value (19.06 ± 0.90 CCI) relatively close to that of the positive control (27.93 ± 0.20 CCI), in contrast to the negative control, which recorded the lowest value (14.43 ± 2.66 CCI). These findings suggest that *Bacillus* sp., similarly to EDTA, may help

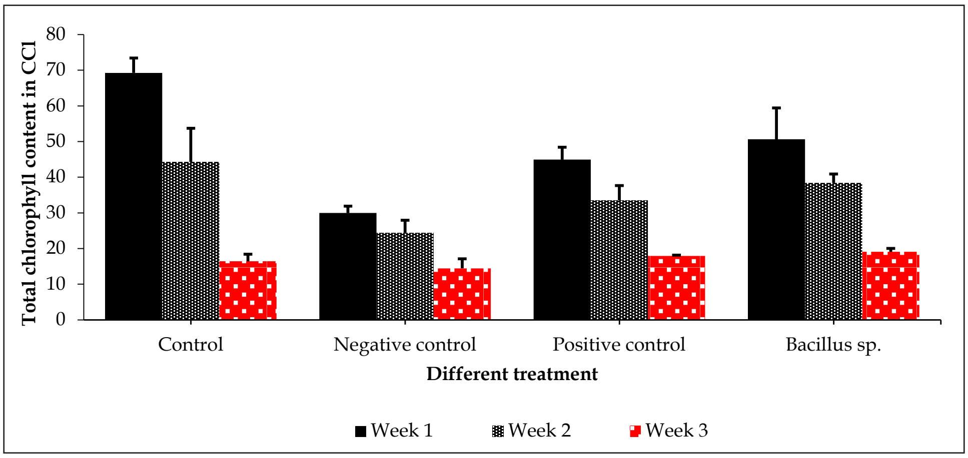

Fig. 4. Effect of different treatments on total chlorophyll content.

limit copper-induced chlorophyll degradation by partially preserving the integrity of the photosynthetic system.

The aim of this study was to evaluate the bioremediation effect of bacteria belonging to the genus *Bacillus* sp. on the growth of faba bean (*Vicia faba* L.) cultivated in the presence of copper, by comparing their performance with that of a reference chelating agent (EDTA). The results obtained highlight a marked improvement in several morphological and biochemical parameters in plants treated with *Bacillus* sp., compared to the negative control exposed solely to copper.

Exposure of plants to heavy metals is known to induce a wide range of physiological and biochemical disturbances, including reduced growth, morphological abnormalities, and impaired photosynthesis (Kopyra *et al.,* 2006). In our study, stem height was significantly improved in plants treated with *Bacillus* sp., reaching values comparable to those obtained with EDTA. This finding confirms the potential effectiveness of *Bacillus sp*. as a bioremediation agent capable of mitigating copper toxicity. Previous studies have shown that Plant Growth-Promoting Rhizobacteria (PGPR) can stimulate plant growth in hostile environments through various mechanisms, including phosphate solubilization, biological nitrogen fixation, and the production of phytohormones such as indole-3-acetic acid (Rehman *et al*., 2020; Sellal *et al*., 2024a).

Regarding branching, a moderate but non-significant increase in the number of branches was observed in both the *Bacillus sp*.-treated and EDTA-treated groups compared to the negative control (Müller and Leyser, 2011). Although the difference was not statistically significant, repeating the experiment on a larger scale could help confirm this effect.

At the leaf level, a slight, non-significant increase in leaf area was recorded in *Bacillus* sp.-treated plants, which is consistent with the observations of Ferhat *et al*. (2014), who reported improved morphological parameters in wheat under saline conditions after *Bacillus* sp. inoculation. These morphological improvements could be linked to better nutrient uptake, particularly nitrogen and phosphorus, whose availability is often reduced in polluted soils (Müller and Leyser, 2011; Sellal *et al*., 2024a).

Total chlorophyll content displayed an interesting dynamic pattern: an initial increase during the first week, followed by a gradual decline during flowering and maturation. However, values consistently remained higher than those of the negative control, indicating that *Bacillus* sp. helps to limit copper-induced chlorophyll degradation. The observed decline in the presence of copper could be explained by the inhibition of key enzymes involved in chlorophyll biosynthesis, such as aminolevulinic acid dehydratase, Rubisco and chlorophyll synthase, as well as disruptions in electron transport within the photosystem (Chen *et al*., 2022). PGPR may mitigate these effects by enhancing the uptake of essential nutrients and stimulating the production of metabolites that support photosynthesis (Kanika *et al.,* 2019).

Overall, our results confirm that *Bacillus* sp. has a potential comparable to that of EDTA in improving growth and partially preserving the integrity of the photosynthetic system under metal stress conditions. This potential likely relies on a combination of mechanisms: reduction of copper bioavailability through complexation, hormonal stimulation, improved nutrient uptake, and mitigation of oxidative stress. Further research, including analyses of antioxidant enzyme activity and the actual bioavailability of copper in soil and plant tissues, would be necessary to confirm these hypotheses and to better elucidate the modes of action of the studied strain (Sellal and Belattar, 2023; Sellal and Belattar, 2024; Sellal *et al*., 2016).

Conclusions

In this study, we evaluated the bioremediation effect of *Bacillus* sp. strains on selected morphological and biochemical parameters of *Vicia faba* L. grown in copper-contaminated soil. The experiments, conducted under controlled greenhouse conditions, revealed that bacterial inoculation mitigates the negative impact of metal stress and stimulates plant growth by improving stem height, branch number, leaf area, and total chlorophyll content. These findings highlight the potential of *Bacillus* sp. as both bioremediation agents and plant growth promoters, paving the way for agronomic applications aimed at rehabilitating degraded or polluted soils. Future perspectives include the identification and characterization of the bacterial metabolites involved, the enhancement of crop protection against trace metal toxicity and the optimization of cultivation conditions for sustainable production while reducing reliance on chemical inputs.

Conflict of Interest

All authors declare no conflict of interest in this study. Moreover, the corresponding author declares that this study is the original work.

References

Ahmedi, W.N.H., Lenchi, N., Sellal, A., Belattar, R. and Bencheikh, A. 2025. Biodecolorization and degradation of aniline blue and neutral red dyes through bacteria isolated from polluted water. *Bioremediation Journal* (in press). https://doi.org/10.1080/10889868.2025.2520211

Ali, H., Khan, E. and Sajad, M.A. 2013. Phytoremediation of heavy metals—Concepts and applications. *Chemosphere* 91(7): 869-881. https://doi.org/10.1016/j. chemosphere. 2013.01.075

Alobaidi, K.H., Oraibi, A.G., Yahya, H.N. and Al-Obaidi, J.R. 2020. Phytoremediation of zinc ion using faba bean Plant *Vicia faba in vitro. Indian Journal of Ecology* 47(4): 965-968.

Belattar, R. and Sellal, A. 2020. Study of the chelating ability of hexane, chloroform, ethyl acetate and methanolic root extracts from Algerian *Phragmites australis* species. *Journal of Drug Delivery and Therapeutics* 10(4): 87-92. https://doi.org/10.22270/jddt.v10i4.4210

Bencheikh, A., Belattar, R., Sellal, A., Mouffok, A., Mamache, W. and Rouag, N. 2025. Deep eutectic solvents and heavy metals as protective wheat seed and seedlings treatments against *Fusarium culmorum*. *Archives of Phyto Pathology and Plant Protection* 58(17): 966-980. https://doi.org/10.1080/03235408.2025.2558876

Chen, G., Li, J., Han, H., Du, R. and Wang, X. 2022. Physiological and molecular mechanisms of plant responses to copper stress. *International Journal of Molecular Sciences* 23: 12950. https://doi.org/10.3390/ijms232112950

Erneste, H., Bhople, B., Kumar, A., Byiringiro, E., Mugabo, J. and Kumar, A. 2017. Soil pollution – Major sources and types of soil pollutants. *Soil Pollution and Phytoremediation. Environmental Science & Engineering* 2(11): 55-65.

Ferhat, M., Ghorab, H., Laggoune, S., Ghannadi, A., Sajjadi, S.E., Touzani, R., Kabouche, A. and Kabouche, Z. 2014. Composition and antioxidant activity of the essential oil of *Thymus dreatensis* from Algeria. *Chemistry of Natural Compounds* 50(4): 919-921.

Hashem, M.A., Nur-A-Tomal, M.S., Mondal, N.R. and Rahman, M.A. 2017. Hair burning and liming in tanneries as a source of pollution by arsenic, lead, zinc, manganese, and iron. *Environmental Chemistry Letters* 15(3): 501-506. https://doi.org/10.1007/s10311-017-0624-1

Kanika, K., Jamwal, V.L., Gandhi, S.G., Ohri, P. and Bhardwaj, R. 2019. Metal-resistant PGPR lowered Cd uptake and expression of metal transporter genes with improved growth and photosynthetic pigments in *Lycopersicon esculentum* under metal toxicity. *Scientific Reports* 9: 5855. https://doi.org/10.1038/s41598-019-42374-2

Kopyra, M. and Gwozdz, E.A. 2006. Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. *Plant Physiology and Biochemistry* 41(11-12): 1011-1017. https://doi.org/10.1016/j.plaphy. 2003.12.003

Laamari, M., Khelfa, L. and Cœur acier, A. 2008. Resistance source to cowpea aphid (*Aphis craccivora* Koch) in broad bean (*Vicia faba* L.) Algerian landrace collection. *African Journal of Biotechnology 7*(14): 2486-2490.

Müller, D. and Leyser, O. 2011. Auxin, cytokinin and the control of shoot branching. *Annals of Botany* 107: 1203-1212. doi:10.1093/aob/mcr069

Rehman, F., Kalsoom, M., Adnan, M., Toor, M.D. and Zulfiqar, A. 2020. Plant growth promoting rhizobacteria and their mechanisms involved in agricultural crop production: A review. *SunText Rev Biotechnol* 1(2): 110. https://doi.org/10.51737/2766-5097.2020.010

Sellal, A. and Belattar, R. 2023. Phyto-remediator effect of common reed species (region of Algeria) on the total organic load of wastewater. *International Journal of Environment and Pollution* 73(1/2/3/4): 193-204.

Sellal, A. and Belattar, R. 2024. The trace elements absorption, accumulation and translocation ability of Phragmites australis. *International Journal of Phytoremediation* 26(5): 618-625. https://doi.org/10.1080/15226514.2023.2258984

Sellal, A., Belattar, R. and Bouzidi, A. 2019a. Heavy metals chelating ability and antioxidant activity of Phragmites australis stem extracts. *Journal of Ecological Engineering* 20(2): 116-123. https://doi.org/10.12911/22998993/96276

Sellal, A., Belattar, R. and Bouzidi, A. 2019b. Trace elements removal ability and antioxidant activity of Phragmites australis (from Algeria). *International Journal of Phytoremediation*, 21(5): 456-460. https://doi.org/10.1080/15226514.2018.1537252

Sellal, A., Belattar, R., Khither, H., Boucheffa, S., Touati, N., Boussoualim, N. and Diafat A. 2024a. Total nitrogen, nitrate and ortho-phosphate elimination effect in common reed and *Typha angustifolia* from wastewater. *Asian Journal of*

Water, Environment and Pollution, 21(6): 31-37. DOI 10.3233/AJW240070

Sellal, A., Belattar, R., Mamache, W., Bencheikh, A. and Touati, N. 2024b. Mineral signatures in Oued K'sob River (Algeria): Highlighting geological variations and potential anthropogenic impacts. *Annals of Arid Zone* 63(3): 69-78. *doi.org/10.56093/aaz.v63i3. 149697*

Sellal, A., Melloul, D., Benghedfa, N., Belattar, R. and Bouzidi, A. 2016. Iron, zinc and copper chelation activity of Phragmites australis leaf extracts. *Advances in Environmental Biology* 10(1): 1-5.

Yruela, I. 2009. Copper in plants: Acquisition, transport, and interactions. *Functional Plant Biology* 36(5): 409-430. https://doi.org/10.1071/FP08288

Printed in December 2025