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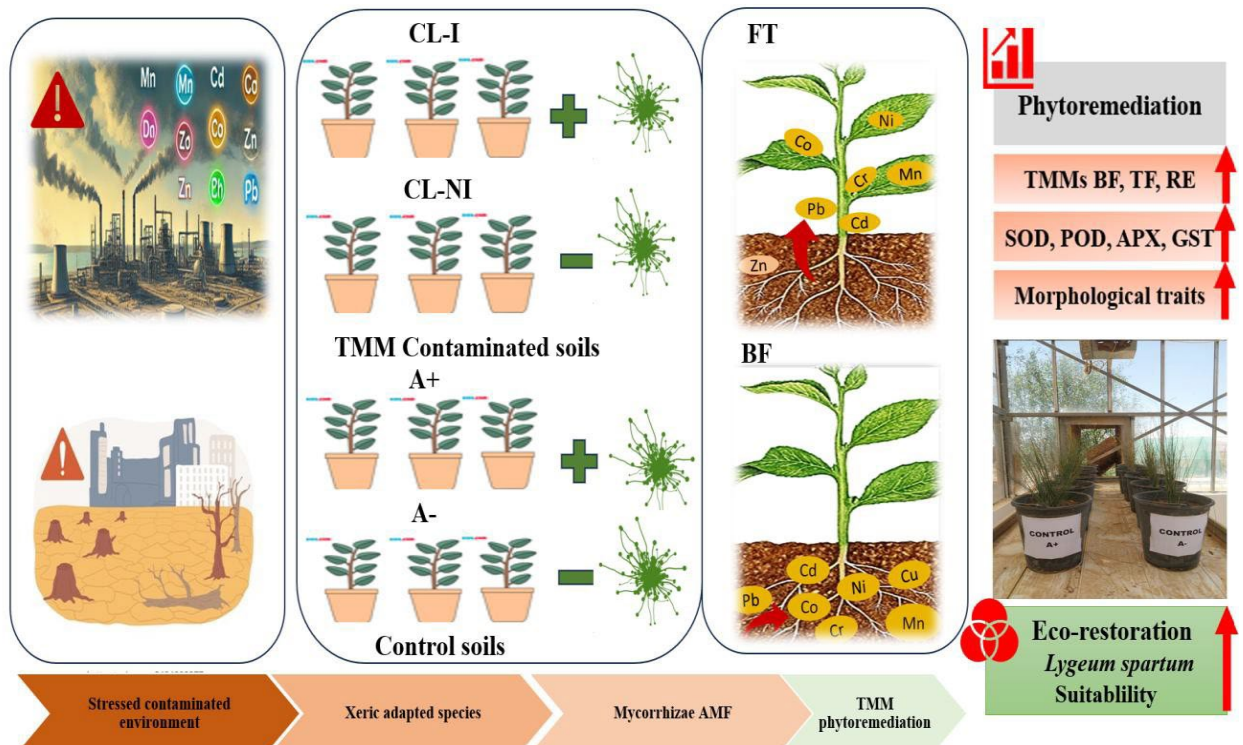
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Mycorrhized *Lygeum spartum* is Effective for the Eco-Restoration and Phytoremediation of Metal-Contaminated Soils in Arid Landscapes

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Abstract

Plant species inhabiting industrial areas have evolved resilience in stressed environments and represent potential valuable resources for remediating polluted sites. However, data describing the potential of xeric plant species in sustainable green applications remain scarce. This study aims to evaluate the effectiveness of mycorrhiza-amended xeric plant species *Lygeum spartum*, in coping with arid conditions for phytoremediation and ecological restoration of degraded and contaminated lands. A six-month controlled pot experiment was conducted to assess leaf length, enzymatic activities, and secondary metabolites. Toxic metals and metalloids (TMMs) fate was evaluated using translocation factor (TF), bioaccumulation factor (BF) and removal efficiency (RE). Fungal inoculation positively impacted growth, increasing leaf size by 64.6 % and enhancing chlorophyll content - *Chl a* (0.73 mg g⁻¹ DW) and *Chl b* (0.64 mg g⁻¹ DW). Root uptake of Cd, Ni, Zn, Cu, Co, Cr and Pb was significantly enhanced in mycorrhizal plants ($p < 0.05$), and inoculation improved translocation of Zn, Cr and Co, with $TF_{Zn} = 1.67$, $TF_{Cr} = 1.11$ and $TF_{Co} = 1.05$ respectively. Secondary metabolites included flavonoids, total phenols, glutathion-S-transferase, carotenoids, and antioxidants such as peroxidase (POD, 4.25 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ proteins), ascorbate peroxidase (APX, 2.14 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ proteins) and superoxide dismutase (SOD, 17.04 66 U mg⁻¹ protein). However, no improvement was observed in catalase activity or free radical scavenging potential. Therefore, xeric plant species amended with mycorrhiza are effective in TMM phytoremediation in arid environments and can be used for the restoration of degraded lands. Further, mycorrhiza could be used as sink cultures for the Phyto management and sustainable eco-restoration.

Key words: Phytoremediation, xerophytes, toxic metals uptake, mycorrhization, arid polluted lands.

47 Abbreviations list

48 AMF : Arbuscular Mycorrhizal Fungi

49 APX : Ascorbate Peroxydase

50 BF : Bioaccumulation Factor

51 CAT : Catalase

52 GST : Gluthatione S-Transferase

53 POD : Peroxidase

54 RE : Removal Efficiency

55 SOD : Superoxide dismutase

56 TF : Translocation Factor

57 TMM : Toxic Metal and Metalloids

58

1. Introduction

Toxic metal and metalloid (TMM) contamination is of global concern due to detrimental health effects on the various components of the environment. The accumulation of TMMs in soils can pose a serious threat to vegetation (Asif et al., 2025; Wang et al., 2025). In drylands, which occupy nearly 41% of the Earth's land surface and provide multiple ecosystem services, TMM contamination exacerbates vulnerabilities to global change and adds edaphic stress (Zhu et al., 2024). Therefore, strategic initiatives for integrative research on sustainable management are imperative. Excess TMMs in plants produces physiological stress affecting nutrient homeostasis, gas exchange, enzymatic reactions, and photosynthetic pigments (Tripathi and Nema, 2024). These stresses result in mortality or altered plant morphology in roots, leaves, and/or stems, as well as diminished plant growth and imbalanced functioning of the photosynthetic apparatus. In particular, the photosynthetic system is impaired on the primary and secondary photosystems PSI and PSII (Chen et al., 2025; Nguyen et al., 2023). Excess TMMs at the soil-plant interface triggers a defensive response (Rath and Das, 2023). Plants can inhibit uptake by releasing defensive root border cell traps (Huskey et al., 2018) to sequester TMMs outside of the root and isolate them from metabolic pathways, or transform them to a less toxic species (Hammond et al., 2018). Once bound to the cell wall, TMMs can induce a perturbation of the oxidation-reduction equilibrium, which leads to reactive oxygen species (ROS) generation (Zahra et al., 2024). At the cellular level, lipid peroxidation, perturbation of enzymatic functioning, and protein alteration could occur (Qu et al., 2024). Adapted plant species react by scavenging or eradicating ROS, activating a defense system involving a wide range of enzymatic and cellular processes (Narayanan and Ma, 2023). A dual cellular system based on enzymatic antioxidants drives plant-producing ROS (Zahra et al., 2024). The first mechanism gathers the enzymes superoxide dismutase (SOD), guaiacol ascorbate/peroxidase (APX/GPX), glutathione-S-transferase (GST) and catalase (CAT). The

second mechanism combines non-enzymatic reactions through metabolites like flavonoids, phenolic compounds, carotenoids, reduced glutathione GSH, organic acids, vitamins, and α -tocopherol (Narayanan and Ma, 2023; Terwayet Bayouli et al., 2020).

To alleviate the harmful effect of TMM contamination, optimized soil-plant-microorganism interaction has been explored for the acceleration of phytoremediation process (Asif et al., 2025; Lan et al., 2024; Yang et al., 2024; Honeker et al., 2019; Narayan et al., 2023). Once associated with plant roots, mycorrhizal fungi acquire special morphological and physiological properties allowing TMM transfer or immobilization (Tan et al., 2023). Arbuscular mycorrhizal fungi (AMF) belonging to the kingdom of Glomeromycota are nearly ubiquitous in all types of soils showing tolerance to contaminants and capacity to form beneficial symbiotic relations with most plant species (Huang et al., 2021). Their hyphae act as extensions of the root system, increasing the contact surface volume of the rhizosphere, which enables a greater absorption capacity of nutrients from the soil (Luo et al., 2025). *Glomus mosseae* when tested on Vetiver grass decreased the concentration of TMMs compared to non-treated plants and improved Cd translocation (Khafil et al., 2019). The inoculation of *Glomus intraradices* on *Monarda citriodora* Cerv. exLag (lemon beebalm) alleviated the negative effects of Ni, Pb and As (Luxmi et al., 2023).

Although mycorrhizal fungi have been investigated in grass and wetlands (Liu et al., 2024; Yang et al., 2024), studies of their role in arid environments are scarce, and especially so in polluted localities. Plants that successfully colonize TMM contaminated soils in industrialized areas are likely tolerant species and could be used as potential sinks for pollutants. Plant species surviving these environments can manifest greater capacity to develop coping mechanisms and ecological adaptations. Xerophytes are particularly endowed with distinct tolerant mechanisms and special morphologic structure and forms enabling tolerance to xeric habitat (Zhu et al., 2024). However, xeric plant species grown in polluted areas and

their interaction with micro-organisms are understudied and less valorised for ecorestoration (Liu et al., 2022). Recent sustainability-focused ‘green’ strategies orient towards phytomanagement that relies on tolerant species and their associated microbiome (Terwayet Bayouli et al., 2021). The *L. spartum* species was identified as a potential TMM accumulator species when grown in arid and polluted areas (Terwayet Bayouli et al., 2020). This xeric species has a developed root system that enables robust mycorrhizal association and has promising biomaterial production potential such as craft, textiles and building blocks (Terwayet Bayouli et al., 2023). In arid polluted environments, mechanistic studies of mycorrhization impact on xeric tolerant species and their potential use in phytoremediation are still lacking. This study hypothesizes the suitability of xeric tolerant species assisted with mycorrhization for phytoremediation and ecorestoration of TMMs polluted arid soils. The objectives of this study are (i) to reveal the fate of eight TMM elements: Cu, Mn, Zn, Co, Cr, As, Ni and Pb in the *L. spartum* rhizosphere when mycorrhized through translocation and bioaccumulation factors, (ii) to determine morphological traits, such as leaf length, in response to mycorrhization and (iii) to quantify the flavonoids, total phenols, carotenoids and enzymatic activities of *L. spartum* for testing the antioxidative response to the synchronized effects of TMMs and mycorrhization to better exploit valuable species surrounding industrial areas and their associated microorganisms under stressed environments for phytoremediation applications, thereby adapting phytoremediation methods to increased global aridity.

2. Material and methods

2.1. Sampling and soil properties determination

The young plants of *L. spartum* were excavated with their underground parts from two sites. A site in close proximity to the Gabès cement plant (150 m) (33°52'07.5"N 9°59'50.1"E), which is considered the contamination source, and a control site located 25 km (33°82'07.5"N,

9°84'775.1''E) from the Gabès cement plant. The cement factory is situated approximately 10 km northwest of the city center of Gabès, Tunisia. The surrounding region spans 7.166 km², accounting for about 7.9 % of southern Tunisia's total area. Geographically, it lies in the Mediterranean basin, in the far southeastern part of the country. The landscape is notably marked by a coastal oasis, which dominates the local vegetation. The area is subject to a dry climate, with minimal and erratic rainfall ranging between 88 and 230 mm per year. It is also characterized by significant daily and seasonal temperature variations, with an average annual temperature of around 18.4 °C and a prolonged, hot summer season. Soil was collected by a random sampling method in the contaminated and control sites. Both soils were alkaline, pH > 7, with sandy loam texture that remained unaffected by land use and subjected to consistent meteorological conditions. Soil physicochemical properties are given in Table 1. Soil organic matter content (OM) was determined by Anne method (X31-109), (AFNOR, 1985). The pH and electrical conductivity (EC) were determined following the NF ISO (AFNOR, 1994). Briefly, 7 g of each soil sample were hydrated with distilled water (50 mL). The mixture was then shaken at 250 rpm, decanted and re-shaken manually for 5 min and the pH and EC were measured directly using daily-calibrated electrodes (Mettler Toledo EF20, Columbus, OH, USA). The water holding capacity of soil (WHC) was determined by dry sieved soil at 105 °C for 24 h, then weighing the water saturation capacity after placing a Keen-box in Petri dishes filled with water. Total concentrations of Cr, Mn, Co, Ni, Cu, Zn, As, Cd and Pb were evaluated following the NF 11464 protocol and compared to the control soil. Samples were dried at 60 °C and dissolved in an acidic mixture of HF (75 %, 400 µL), HNO₃ (37 %, 6 mL) and H₂O₂ (30 %, 6 mL), following NF11164 standards using a Microwave Sample Preparation system (MSP 1600 W, CEM Corporation, Matthews, NC, USA; 20 min). The Microwave is equipped with real-time temperature and pressure feedback controllers, ensuring precise control of digestion conditions throughout the process. Digestions were performed using a closed-vessel

system, consisting of 12 high-pressure Teflon vessels, each with an inner volume of 100 mL, specifically designed to withstand high temperatures and pressures. This setup allows for uniform heating, minimizing the risk of contamination and analyte loss. The temperature and pressure within each vessel were monitored continuously during the digestion cycle (20 min) to ensure complete and safe sample breakdown, especially for trace metal analysis. Metal and metalloid concentrations were determined by ICP-MS (Agilent 7600, USA). Mobile fractions were determined in a CaCl_2 extraction using 0.5 g of soil in 50 mL of 0.01 M CaCl_2 mixed for 3 h that was filtered through a 0.22 μm cellulose membrane. Experiments were conducted with six replicates and serial blanks used.

2.2. Pot experiment

Seedlings of *L. spartum* were transplanted into 7.6 L (25 x 19 x 20 cm) greenhouse pots filled with 5 kg of soil each sampled from the contaminated and control site described in the section 2.1. Soils used in pot experiments were autoclaved at 105 °C for 1 h three days prior to fungal inoculation (Boldt-Burisch and Naeth, 2017). The seedling pot cultivation ran for 6 months (April-September). Pots were maintained at ambient temperature, radiation and humidity to mimic the natural environmental conditions with an average temperature ranging from 18 to 20 °C, a 50–60 % of relative humidity, 5–7 $\text{kWh m}^{-2} \text{d}^{-1}$ of natural solar irradiance, and regular watering (1000 mL total). Pot moisture was controlled to ensure 65 % of field capacity by weighing. Plants were treated with Symbivit© (Symbiom LTD, Cze) inoculum, which consisted of six species of *Glomus* genus (spores, fragments of colonized roots and mycelium fragments), natural support material (humates, terrestrial minerals, extracts from marine organisms) with a clay base (act as inoculum carrier). Twenty five g of inoculum were thoroughly mixed with the planting substrate, following the manufacturer guidelines, to ensure direct contact between mycorrhizal fungi and plant roots. Inoculum viability was tested by

staining roots of inoculated plants after four weeks (Phillips and Hayman, 1970). Pots were divided into four batches; (1) contaminated substrate receiving fungal inoculation (CL-I); (2) contaminated soil with no inoculation (CL-NI); (3) positive control receiving fungal inoculation (A+) and (4) negative control that was an untreated control soil (A-).

2.2.1. Toxic metal and metalloid uptake evaluation

The concentrations of Cr, Mn, Co, Ni, Cu, Zn, As, Cd and Pb were evaluated in plants according to the method described in the section 2.1. Plant material was dried at 37 °C for 24 h, then dissolved in an acidic mixture of HF (75 %, 400 µL), HNO₃ (37 %, 6 mL) and H₂O₂ (30 %, 6 mL) (Terwayet Bayouli et al., 2021). Concentrations were determined using an ICP-MS (Agilent 7600, USA).

Bioaccumulation (BF) and translocation (TF) factors were determined for both treated and untreated plants as indicated in Eqs 1-2:

$$BF = C_i \text{ metal roots} / C_i \text{ metal soils} \quad (1)$$

$$TF = C_i \text{ metal leaves} / C_i \text{ metal roots} \quad (2)$$

With C_i = concentration of element i per unit mass of the tested material.

Removal efficiency was measured as indicated in Eq 3:

$$[C_i \text{ shoot} + C_i \text{ root}] / [C_i \text{ soil}] \quad (3)$$

2.2.2. Growth and chlorophyll content

To reveal the growth response to fungal inoculation of young *L. spartum*, the aerial growth (leaves) of all transplanted plants were initially cut to the same length, 13 ± 0.01 cm (Jones et al., 2017). Plant growth was estimated by monthly evaluation of leaf length expressed in cm (Clifton Brown et al., 2002). The Inskeep and Bloom (1985) method was applied for

chlorophyll *a* and *b* detection. A sample of 0.5 g of leaves was ground in 10 mL acetone 85 %. The cellular debris was then removed by 10 min centrifugation at 3000 rpm. Detection was achieved with absorbance measurements at 647-665 nm (Jenway 6400 spectrophotometer, London, UK). The chlorophyll contents were expressed by Eqs 4 and 5:

$$Chl\ a = 12.70 \times D_{\lambda\ 665} - 2.79 \times D_{\lambda\ 647} \quad (4)$$

$$Chl\ b = 20.70 \times D_{\lambda\ 647} - 4.62 \times D_{\lambda\ 665} \quad (5)$$

Where $D_{\lambda\ 665}$ and $D_{\lambda\ 647}$ are absorbance measures respectively at 665 nm and 647 nm.

2.2.3. Antioxidative response of *Lygeum spartum*

2.2.3.1. Secondary metabolites

Samples were collected from pots, cut leaves were placed in tubes and kept cool in an ice cooler. Leaves were then extracted in 80 % methanol for assessing secondary metabolites and antioxidant activity. Briefly, 1 g of fine powdered sample was added to methanol (80 %, 10 mL), stirred for 30 min, stored for 24 h at 4 °C in the dark and then filtered. A rotary evaporator was used to condense and dry the extract, which was stored at 4 °C until analyses. Methanolic extract served for quantifying total polyphenols using Folin-Ciocolteu reagent, reduced by the action of polyphenols in tungsten and blue-colored molybdenum following Dewanto et al. (2002). Optical density was read at 760 nm. Standards were prepared with gallic acid at concentrations ranging from 0 to 500 mg L⁻¹. Total polyphenol concentrations were reported as mg EAG g⁻¹ DW. Flavonoids concentrations were measured by crushing 1 g plant material and dissolving it in methanol (80 %, 100 mL). After mixing and stirring, the extract was added to 100 mL of Neu reagent following Hairi et al. (1991). Absorbance was determined at 404 nm and compared to the standard quercitol (0.05 mg mL⁻¹) containing the Neu reagent. Dosing carotenoids was carried out by mixing 1 mL acetone 80 % with 0.2 g of dry plant

matter. The mixture was preserved for 24 h at 4 °C and then centrifuged. The supernatant was read at 663, 647 and 470 nm.

2.2.3.2. Free radicals scavenging

The 2,2-diphenyl-1-picrylhydrazyl (DPPH, $C_{18}H_{12}N_5O_6$, MW = 394.33) assay was used to evaluate radical scavenging antioxidant activity (Yu et al., 2002). The DPPH assay produces a violet solution in ethanol when free radicals are reduced upon exposure to an antioxidant. The DPPH solution (0.2 mM, 250 μ L in methanol) was introduced to a methanolic extract (10, 20, 30, 40 and 50 μ g mL⁻¹ methanol). The mixture was kept at 37 °C for 30 min in the dark. Readings were at 517 nm against a DPPH control solution.

2.2.3.3. Enzymatic activities

The plant material of *L. spartum* was crushed under liquid N₂. The ground samples were retrieved in 3 mL of potassium phosphate buffer solution. Protein content in enzyme extracts was measured using the Bradford assay (Bradford, 1976) with Bovine Serum Albumin (BSA) as the standard. Absorbance was measured at 595 nm. Enzymatic activities were assessed by preparing a mixture containing the enzymatic extract 0.5 mL; phosphate buffer solution (0.1 M; 2 mL, pH = 6.7) and pyrogallol solution (0.01 M, 1 mL); H₂O₂ (0.05 M, 1 mL) supplemented with H₂SO₄ (1 mL), according to Terwayet Bayouli et al. (2020), allowing determination of POD activity against a blank. After 5 min of incubation at 20 °C, optical density was read at 420 nm allowing determination of peroxidase activity against a blank. CAT (EC1.11.1.6) activity was quantified by blending 0.29 mL of the plant extract with phosphate buffer (0.1 M; 0.1 mL; pH = 7) and H₂O₂ (20 mM; 0.1 mL). CAT absorbance readings were taken at 240 nm. Activity of APX (EC 1.11.1.11) was determined in 40 μ L of the plant extract solution was reacted with 4 μ L of H₂O₂ (50 mM), then added to a phosphate buffer (50 mM; 956 μ L, pH = 7; ascorbate 0.5 mM) and measured at 290 nm every 3 min. GST was quantified

by a mixture of phosphate solution buffer (0.1 M; 0.1 M; pH = 6.5, 2.8 mL for the blank), glutathione (50 mM, 0.1 mL) to which CDNB (40 mM; 0.1 mL prepared in 95 % ethanol) and plant extract (0.5 mL) were added. Absorbance readings were made at 340 nm.

2.3. *Quality control and quality assurance*

To ensure the reliability and accuracy of the analytical procedures, stringent quality control measures were implemented. These included the use of multiple procedural blanks to monitor potential contamination throughout sample preparation and analysis. For the quantification of TMMs, both reagent blanks and a certified reference material (INCT-MPH-2, a standard plant matrix) were processed using the same digestion and analytical protocols as the experimental samples. This allowed for validation of the method's accuracy and recovery efficiency. Standard calibration curves for each target element demonstrated excellent linearity, with correlation coefficients (R^2) consistently ranging between 0.9998 and 0.9999. The precision of the analytical measurements was confirmed by maintaining a relative standard deviation (RSD) of less than ± 0.5 %. All measurements were conducted in six biological replicates per treatment, each analyzed in duplicate. Instrument performance was routinely verified through daily calibration and drift checks. Method detection limits (MDLs) and quantification limits (LOQs) were established for each element based on standard protocols.

2.4. *Statistical analysis*

To reveal the impact of mycorrhization on *L. spartum*, data were first tested for Shapiro-Wilk normality, variance analysis between inoculated, non-inoculated and control plants was carried out by one way ANOVA, supplied with adhoc Tukey HSD test; probability value (p) was estimated at 95 % confidence ($p < 0.05$). All measurements were conducted in six replicates and were pooled for statistical analysis and are presented as mean $\pm$ standard deviation. To maximize the variance, Principal Component Analysis (PCA) was performed for

testing the variables and observations. Graphs were displayed by vectors representing distance biplot at an automatic coefficient. No missing data was accepted for data treatment.

3. Results

3.1. *Lygeum spartum* TMMs uptake and translocation

3.1.1. Toxic metals and metalloids concentrations

Chemical properties showed higher organic matter (OM) and WHC in the control compared to the contaminated soil while EC was lower in the control site compared to the contaminated soils. The lower OM content in the contaminated pots ($16.12 \pm 0.02 \text{ mg g}^{-1}$) compared to the control soil ($24.22 \pm 0.02 \text{ mg g}^{-1}$) could potentially affect metal binding and complexation, which may influence TMM immobilization. The WHC was also higher in the control soil ($40.71 \pm 0.01\%$) than in the contaminated soil ($38.23 \pm 0.02 \%$). EC in the contaminated soil ($0.62 \pm 0.01 \text{ dS m}^{-1}$) was nearly double the control soil ($0.37 \pm 0.01 \text{ dS m}^{-1}$), consistent with higher ionic strength soil solution in the former, potentially favoring solubility and desorption of certain TMMs. Alkaline pH (> 7) characterized both sites. The elevated TMM concentrations in contaminated soils, along with reduced OM and higher EC, are associated with increased extractable fractions, which may contribute to enhanced TMM mobility and bioavailability. These differences suggest a potential increase in environmental risk due to higher TMM extractability, emphasizing the importance of monitoring and remediation, highlighting the need for targeted remediation strategies to stabilize TMMs and restore soil health. The TMM behaviour between both sites remains unaffected by soil alkalinity with non-significant differences. Total and CaCl_2 -extractable concentrations of TMMs (Table 1) were notably higher in the soils sampled in close vicinity to the cement plant compared to the control soil ($p < 0.05$), emphasizing the impact of altered soil chemistry on TMM mobility and bioavailability. In the control site, total TMM concentrations were ordered as follows $\text{Mn} > \text{Pb} > \text{Cr} > \text{Co} > \text{Zn} - \text{Cu} > \text{As} > \text{Ni} > \text{Cd}$. Concentrations in the contaminated

300 soil were ranked $\text{Pb} > \text{Mn} > \text{Cr} > \text{Co} > \text{Zn} > \text{Ni} > \text{Cu} > \text{As} > \text{Cd}$. Extractable TMMs (in 0.01
301 M CaCl_2) showed a significant difference ($p < 0.05$) between control and contaminated sites
302 for Mn, Cr, Co, Zn, Ni, Cu, with, e.g., an 8-fold increase in extractable Pb in contaminated soil
303 compared to control soil.

304

Table 1. Toxic metal concentrations in contaminated and control soils. Results are given in mean $\pm$ SD, a, b, A and B represent homogenous groups displayed by paired Student *t*-test.

[TMM]	Total		Extracted -CaCl ₂		Chemical properties		
$\mu\text{g g}^{-1}$ DW	Contaminated	Control	Contaminated	Control	Contaminated	Control	
	(n=6)	(n=6)	(n=6)	(n=6)	(n=6)	(n=6)	
Pb	100.59 $\pm$ 0.20 ^a	43.01 $\pm$ 0.03 ^b	0.80 $\pm$ 0.03 ^A	0.01 $\pm$ 0.001 ^A	pH	8.02 $\pm$ 0.01 ^a	8.00 $\pm$ 0.01 ^a
Mn	79.11 $\pm$ 0.11 ^a	56.20 $\pm$ 0.10 ^b	0.35 $\pm$ 0.07 ^A	0.25 $\pm$ 0.02 ^B	OM (mg g ⁻¹)	16.12 $\pm$ 0.02 ^b	24.22 $\pm$ 0.02 ^a
Cr	57.64 $\pm$ 0.36 ^a	9.05 $\pm$ 0.03 ^b	0.21 $\pm$ 0.03 ^A	0.05 $\pm$ 0.01 ^B	WHC (%)	38.23 $\pm$ 0.02 ^b	40.71 $\pm$ 0.01 ^a
Co	31.86 $\pm$ 0.30 ^a	8.11 $\pm$ 0.01 ^b	0.40 $\pm$ 0.02 ^A	0.03 $\pm$ 0.01 ^B	EC (dS m ⁻¹)	0.62 $\pm$ 0.01 ^a	0.37 $\pm$ 0.01 ^b
Zn	27.11 $\pm$ 0.06 ^a	2.17 $\pm$ 0.06 ^b	0.50 $\pm$ 0.03 ^A	0.03 $\pm$ 0.02 ^B	Texture	Sandy loam	Sandy loam
Ni	25.80 $\pm$ 0.09 ^a	2.14 $\pm$ 0.09 ^b	0.80 $\pm$ 0.01 ^A	0.20 $\pm$ 0.01 ^B			
Cu	23.25 $\pm$ 0.05 ^a	2.17 $\pm$ 0.06 ^b	0.60 $\pm$ 0.03 ^A	0.26 $\pm$ 0.01 ^B			
As	7.29 $\pm$ 0.03 ^a	2.21 $\pm$ 0.01 ^b	0.32 $\pm$ 0.08 ^A	0.03 $\pm$ 0.01 ^A			
Cd	0.83 $\pm$ 0.05 ^a	0.22 $\pm$ 0.01 ^b	0.07 $\pm$ 0.01 ^A	0.03 $\pm$ 0.01 ^B			

^{AB} $p < 0.05$ = variance is significant following Student paired *t*- test. ^{AA} $p > 0.05$ = variance is not significant following Student paired *t*-test.

3.1.2. Toxic metals and metalloids uptake

Mycorrhizal contribution to the phytoextraction efficiency and viability of the fungal inoculum was evaluated by BF and TF ratios (Fig. 1). Statistical analysis showed significant variation ($p < 0.05$) in TMM accumulation between mycorrhized and non-mycorrhized plants except for Pb with BF ($1.18 < 1.32$). This variation was most notable for TF between the two modalities of tested plants. The highest TF was registered for Zn and Cr with respective TFs of 1.67 and 1.11. The highest BFs were from BF_{Cd} in mycorrhized versus non-mycorrhized samples ($1.88 > 1.57$). The presence of mycorrhizal hyphae and spores may contribute to the observed $BF > 1$ in most tested TMMs, suggesting a potential enhancement in root uptake mechanisms. However selective accumulation in aboveground biomass was observed, with translocation appearing more efficient for specific elements such as Zn, Cr, and Ni.

Mycorrhized plants showed a slightly higher root uptake of Cd, Ni, Zn, Cu, Co, Mn and Cr than non-inoculated plants with respective $BF_{Cd} = 1.88$; $BF_{Ni} = 1.35$; $BF_{Zn} = 1.15$; $BF_{Cu} = 1.20$; $BF_{Co} = 1.09$; $BF_{Cr} = 1.03$. Root uptake of Pb was higher in non-mycorrhized plants than mycorrhized. However, the translocation of Ni, Cd, Co and Mn was higher in mycorrhized plants compared to non-mycorrhized with respective TFs ($TF_{Cr} = 1.11 > 0.75$; $TF_{Co} = 1.05 > 1.02$; $TF_{Zn} = 1.67 > 0.61$; $TF_{Cu} = 1.03 > 0.94$). Therefore, mycorrhization was associated with increased translocation of Ni, Cd, Co, and Mn compared to non-inoculated plants, although the extent of such increase varied.

Non-mycorrhized plants showed higher root accumulation of some metals, but this was not consistently accompanied by increased translocation to aerial parts. The RE values showed a statistically significant increase in AMF-inoculated plants compared to controls, with improvements ranging from 8 % to 20 %, suggesting that mycorrhizal symbiosis may enhance phytoextraction efficiency for certain metals. RE values ranged from 1.35 (As) to 2.35 (Co) in

mycorrhized plants, with statistically significant increases observed for most elements tested. The mean RE was 1.98 ± 0.03 for inoculated plants versus 1.80 ± 0.02 for non-inoculated ones ($p < 0.05$), indicating an overall improvement of approximately 10 %. Specifically, mycorrhizal inoculation significantly improved the removal efficiency of Cd, Ni, Zn, Co, Mn, Cr, and As, with increases ranging from 8 % to 20 % depending on the element. For example, the RE for Co increased from 2.24 ± 0.04 in the control and to 2.35 ± 0.04 in inoculated plants, while As increased from 1.20 ± 0.01 to 1.32 ± 0.1 . These results suggest that AMF inoculation can contribute positively to the phytoextraction of multiple metal(loid) contaminants, though the extent of improvement varies by element.

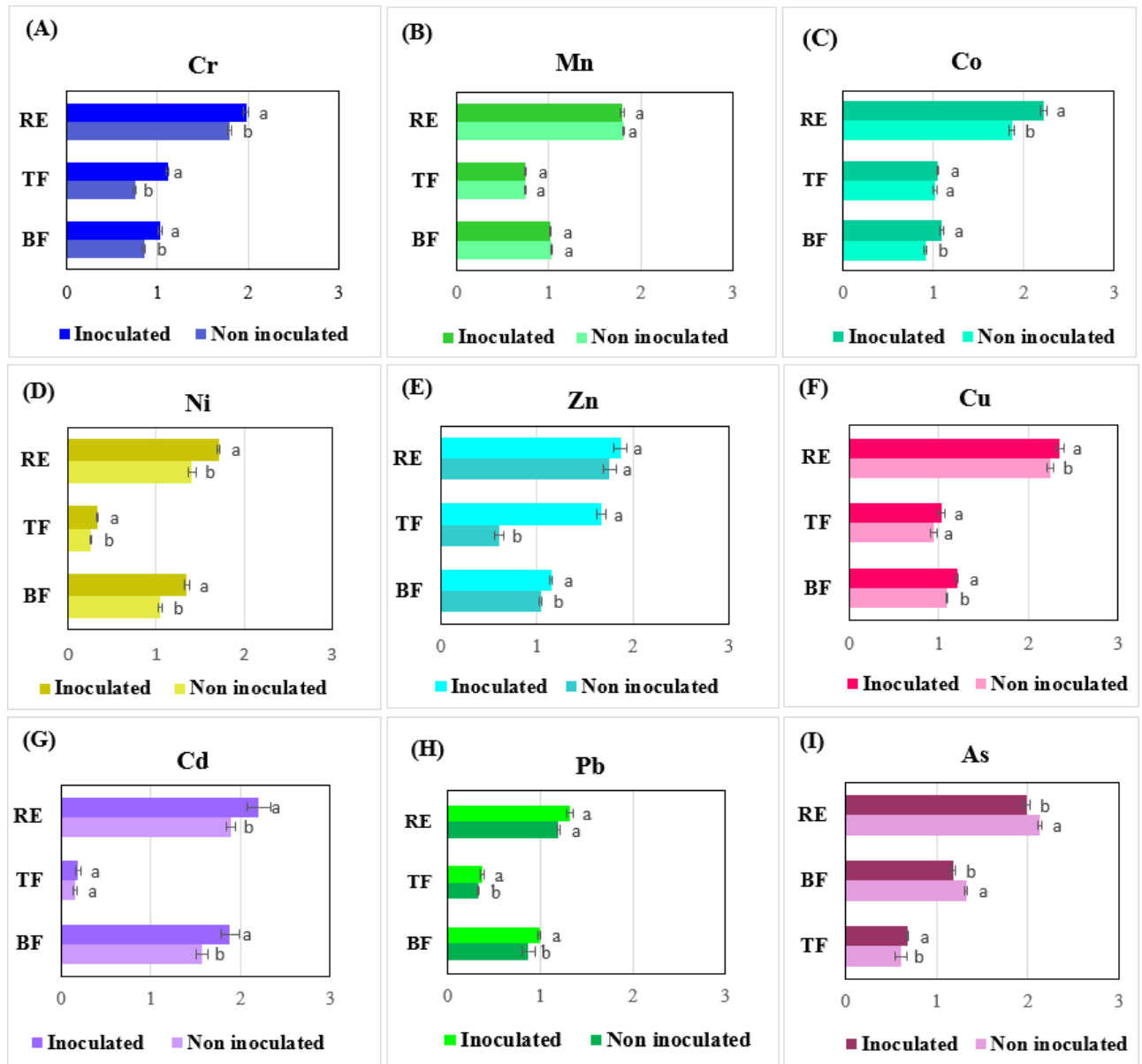


Figure 1. Bioaccumulation factor (BF), translocation factor (TF), and removal efficiency (RE) in inoculated and non-inoculated plants of *Lygeum spartum* (mean $\pm$ SD) for (A) Cr; (B) Mn; (C) Co; (D) Ni; (E) Zn; (F) Cu; (G) Cd; (H) Pb; and (I) As; a and b represent homogenous groups identified by Student paired *t*-test.

3.2. Plant growth and photosynthetic activity of *Lygeum spartum*

3.2.1. Leaf length

The length of leaves of *L. spartum* is provided in Table 2. The leaves grew about 8.4 cm with the inoculated plants in the pots containing contaminated soils CL-I. The growth of non-inoculated plants CL-NI was about 5.8 cm during six-months, while those in the pots containing uncontaminated and no inoculation control A- showed growth comparable to that of control plants ranging from 13.7 cm at an early stage to 22.34 cm in the sixth month (M6). For testing the viability of the inoculum, the positive control A+, exhibited a maximum of leaf length over untreated control A- with respective values (e.g., 25.04 ± 0.38 vs. 22.34 ± 0.12 at M6) which suggests a possible contribution of *Glomus* colonization to improved leaf development under control conditions. The A+ achieved the highest growth (25.04 ± 0.38 at M6), followed by the inoculated treatment (CL-I, 21.40 ± 0.20 at M6) with 64.62 % of increase over 37.46 % in CL-NI, supporting the potential effectiveness of the inoculum in promoting plant development. This difference suggests the inoculum's efficacy in enhancing plant growth under controlled conditions especially at the later stages of mycorrhization M4, M5 and M6. Non-inoculated plants exhibited slower and steadier growth, with A- outperforming CL-NI, suggesting additional environmental or soil-related factors. Fungal inoculation influenced positively the leaf length. Leaf growth in inoculated plants (CL-I) was statistically comparable to that of the uninoculated control (A-), based on Tukey's test. The mycorrhization reduced the effect of the TMMs on the leaf growth, which may be linked to the known roles of AMF in enhancing water uptake, nutrient availability, and photosynthetic activity, though these mechanisms were not directly assessed in this study.

Table 2. Leaf length (mean $\pm$ SD) of *L. spartum* inoculated (CL-I), non-inoculated (CL-NI); inoculated control *L. spartum*. Control (A+) and control (A-) over 6 months; a, b, c and d represent homogenous groups identified by Tukey test.

Leaf length (cm)	Contaminated (n=6)		Control (n=6)	
Time	CL-NI	CL-I	A+	A-
(Month)	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
M6	17.87 $\pm$ 0.09 ^d	21.40 $\pm$ 0.2 ^c	25.04 $\pm$ 0.38 ^a	22.34 $\pm$ 0.12 ^b
M5	17.02 $\pm$ 0.03 ^d	19.13 $\pm$ 0.05 ^c	25.00 $\pm$ 0.94 ^a	20.67 ^b $\pm$ 0.03
M4	15.9 $\pm$ 0.08 ^d	17.89 $\pm$ 0.06 ^c	23.21 $\pm$ 0.05 ^a	19.62 $\pm$ 0.02 ^b
M3	14.59 $\pm$ 0.28 ^c	17.33 $\pm$ 0.06 ^b	19.5 $\pm$ 0.03 ^a	19.25 $\pm$ 0.06 ^a
M2	13.70 $\pm$ 0.04 ^d	15.29 $\pm$ 0.04 ^c	16.18 $\pm$ 0.03 ^b	16.43 $\pm$ 0.06 ^a
M1	13.20 $\pm$ 0.08 ^b	13.60 $\pm$ 0.02 ^a	13.67 $\pm$ 0.02 ^a	13.71 $\pm$ 0.01 ^a
T0	13 $\pm$ 0.00 ^a	13 $\pm$ 0.00 ^a	13 $\pm$ 0.00 ^a	13 $\pm$ 0.00 ^a

3.2.2. Chlorophyll content

The evaluation of *Chl a* and *Chl b* on a dry weight basis of *L. spartum* plants showed significant ($p < 0.05$) variations between both groups of plants (Fig. 2). An increase of *Chl a* (0.73 mg g⁻¹ DW) and *Chl b* (0.64 mg g⁻¹ DW) contents were observed in inoculated plants with an increase of 17.9 % against 15.2 % for CL-NI that showed lower levels of *Chl a* (0.65 mg g⁻¹ DW) and *Chl b* (0.57 mg g⁻¹ DW). The reduced chlorophyll content in non-inoculated plants may be attributed to the antagonistic effects of TMMs on chlorophyll biosynthesis and

photosynthetic functioning. Mycorrhization may enhance nutrient availability (e.g., phosphorus uptake) and help mitigate TMM-induced oxidative stress which neutralizes their effect on CL-NI. In control soil, the absence of contamination conferred higher chlorophyll concentrations. The A+ marked the highest chlorophyll efficiency, with *Chl a* reaching $\sim 1.1 \text{ mg g}^{-1} \text{ DW}$ and *Chl b* $\sim 1.0 \text{ mg g}^{-1} \text{ DW}$. The A- also reached high levels with $\sim 1.0 \text{ mg g}^{-1} \text{ DW}$ and *Chl b* levels of $\sim 0.9 \text{ mg g}^{-1} \text{ DW}$. Increased root growth and microbial activity in the mycorrhized rhizosphere (A+) may support enhanced chlorophyll synthesis, indicating improved plant physiological status. Inoculation strengthened *Chl a* and *Chl b* in both contaminated and positive control soils. However, the positive effect of inoculation on chlorophyll content was more pronounced under uncontaminated conditions. In the control soil, inoculation modulates the TMM stress but cannot completely restore photosynthetic pigment levels to those found in the control. AMF functions as stress mitigators in degraded soils and can support plant growth even under optimal conditions. However, greater variability was observed in CL-NI with the wider distribution in response to TMM and metabolic activity. The observed increases in chlorophyll content suggest an enhanced photosynthetic potential, reflecting the resilience of *L. spartum* under both contaminated and arid conditions, highlighting the potential of the symbiosis with *Glomus* fungi to contribute to phytoextraction and revegetation in contaminated environments.

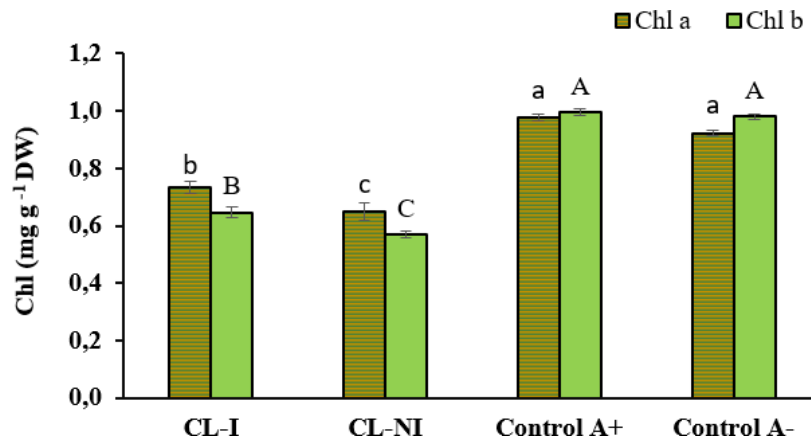


Figure 2. Chlorophyll content (mean $\pm$ SD), (n = 6) in *L. spartum* inoculated (CL-I), non-inoculated (CL-NI); inoculated control *L. spartum* (Control A+) and (Control A-), a, b and c represent homogenous groups identified by Tukey test.

3.3 Effect of mycorrhization on the xeric *L. spartum* antioxidative response

3.3.1. Enzymatic activities

Antioxidative response was evaluated by enzymatic activities, summarized in Table 3. Enzymatic response to mycorrhization varied. However, a significant increase of POD, APX and SOD was observed relative to control plants ($p < 0.05$). The observed increase in antioxidant enzyme activities in contaminated soils (Table 1) may reflect a defense response to TMM-induced stress. The CL-I SOD revealed markedly higher activity (17.04 U mg⁻¹ protein) compared to CL-NI (12.66 U mg⁻¹ protein), indicating a stronger superoxide dismutase response in mycorrhizal plants under TMM stress. This elevated activity may reflect an enhanced antioxidative defense potential conferred by mycorrhization.

On a similar trend, peroxidase (POD) and ascorbate peroxidase (APX), strengthened the CL-I defense mechanism of *L. spartum* with respective POD: 4.25 vs. 3.42 $\mu\text{mol min}^{-1} \text{mg}^{-1}$

protein; APX: 2.14 vs. 1.88 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein). These enzymes are often implicated in degrading H_2O_2 into water (H_2O) and oxygen (O_2). This enhanced enzyme activity may be linked to the role of AMF in promoting nutrient uptake in plant roots, particularly phosphorus. This could be reached by the extended hyphae in the soil compartment expanding the effective root surface area for absorbing essential nutrients particularly P that has a primordial role in energy metabolism and production of antioxidants stress defense molecules. Improved nutrient availability, particularly P uptake facilitated by AMF, may contribute to increased antioxidative enzyme activity. Mycorrhized *L. spartum* alters root exudates which can promote release of organic acids and phenolics that can chelate metals and reduce the reactive oxidative species ROS, thereby indirectly supporting antioxidant defenses. The response could, therefore, affect indirectly the antioxidative enzymatic system by reducing its burden on the plant.

The lower antioxidant enzyme activities observed in control soils may reflect the absence of TMM-induced stress in reference to Table 1. Also, non-significant variation between the A⁺ and A⁻ is registered, suggesting that AMFs impact on the antioxidative defense system of *L. spartum* is relevant only under TMM stress. Furthermore, In A⁻, SOD is slightly higher ($7.31 \text{ U mg}^{-1} \text{ protein}$) > ($5.42 \text{ U mg}^{-1} \text{ protein}$) than in A⁺, suggesting that the fungal improvers are more effective under contaminated conditions while *L. spartum* natural antioxidative system remains relatively stable in non-stressed environments. POD and APX were also found slightly univariable in A⁺ and A⁻, confirming that regardless of mycorrhization, in the absence of toxic metal stress, antioxidative enzyme levels remain relatively consistent between treatments. The results suggest that fungal inoculants may enhance plant resilience and antioxidative capacity under toxic metal stress, supporting their potential utility in phytoremediation strategies.

Table 3. Enzymatic activities (mean $\pm$ SD) of *L. spartum* as a function of inoculation in contaminated soils CL-I (inoculated); CL-NI (non-inoculated); A⁺ (positive control); A⁻ (negative control) uppercases

Enzymatic activity	Contaminated (n=6)		Control (n=6)	
	CL-I	CL-NI	A+	A-
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
SOD ¹	17.04 $\pm$ 0.02 ^a	12.66 $\pm$ 0.05 ^b	5.42 $\pm$ 0.02 ^c	7.31 $\pm$ 0.01 ^d
POD ²	4.25 $\pm$ 0.03 ^a	3.42 $\pm$ 0.03 ^b	1.42 $\pm$ 0.01 ^c	1.44 $\pm$ 0.02 ^c
APX ²	2.14 $\pm$ 0.02 ^a	1.88 $\pm$ 0.01 ^b	0.62 $\pm$ 0.01 ^c	0.64 $\pm$ 0.01 ^c
GST ²	1.28 $\pm$ 0.02 ^a	1.36 $\pm$ 0.03 ^b	0.58 $\pm$ 0.01 ^c	0.59 $\pm$ 0.01 ^c
CAT ²	0.31 $\pm$ 0.03 ^a	0.31 $\pm$ 0.01 ^a	0.21 $\pm$ 0.01 ^b	0.25 $\pm$ 0.02 ^b

¹ units of (U mg⁻¹ protein), where unit (U) is the amount of enzyme that catalyzes the conversion of 1 μ mol of substrate per min; ² units of (μ mol min⁻¹ mg⁻¹ protein); ^a, ^b, ^c and ^d represent homogenous groups identified by Tukey test.

3.3.2. Secondary metabolites

Total polyphenols (Fig. 3A) were higher in CL-I versus CL-NI, reaching an optimum (11.06 mg EAG g⁻¹ DW). These phenolic compounds contribute to the plant's antioxidant defense system, indirectly supporting photosynthetic function under stress. Flavonoids (Fig. 3B) varied significantly ($p < 0.05$) upon AMFs inoculation. The CL-I (6.86 μ g EQ g⁻¹ DW) and A⁺ were more concentrated, whereas they were markedly lower in CL-NI. The observed increase in flavonoids may be linked to AMF-induced stimulation of biosynthetic pathways, such as the phenylpropanoid pathway, which includes enzymes like phenylalanine ammonia-lyase (PAL) or the activation of stress-response pathways (see sec 3.3.1). The elevated carotenoid content (Fig. 3C) in inoculated plants (2.48 mg g⁻¹ DW) may suggest a response to oxidative stress mitigation under TMM exposure and AMF influence ; the control batch was significantly lower (0.68 mg g⁻¹ DW). Carotenoids play a crucial role in photosynthesis and

photoprotection, and their increased production under inoculation may result from enhanced photosynthetic efficiency and improved plant health. AMFs are known to influence carotenoid biosynthesis, potentially through improved nutrient uptake or phytohormone signaling, although this was not directly assessed in the current study. In comparison, (CL-NI), (A+) and (A-) showed significantly reduced carotenoid levels, suggesting the possible role of mycorrhization in stimulating these biochemical pathways.

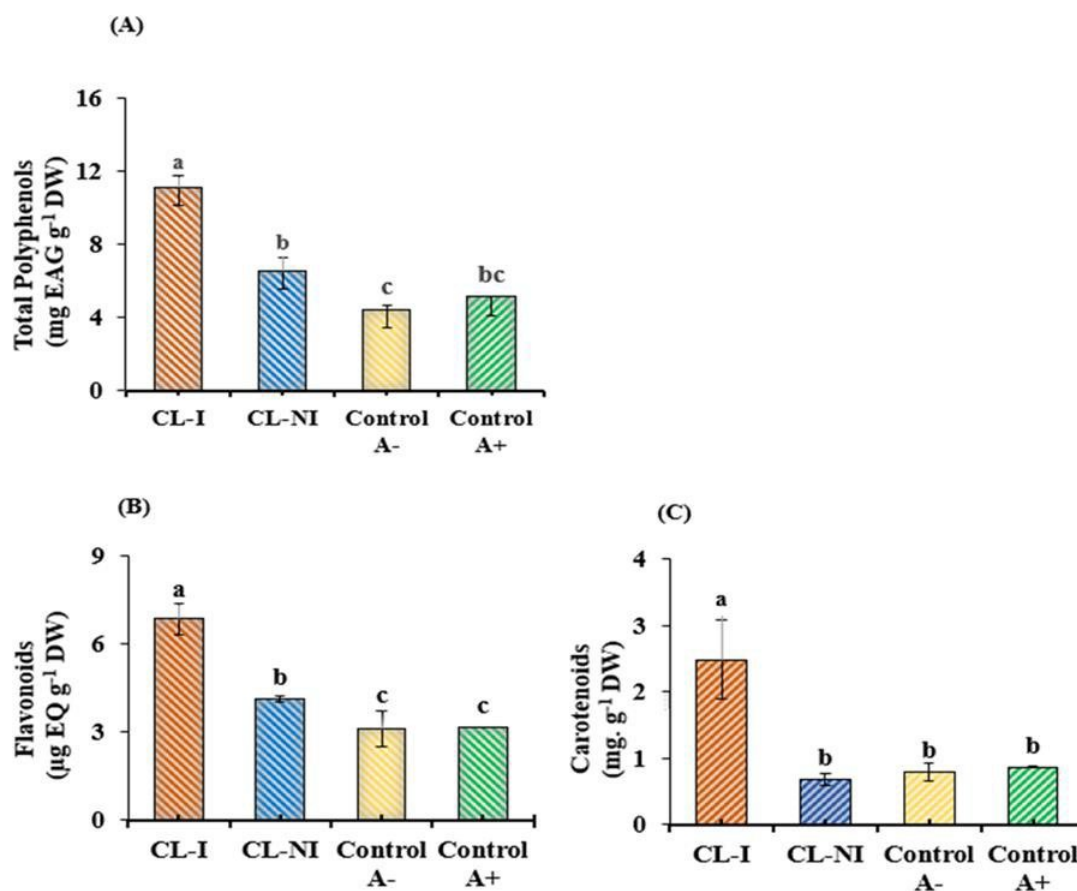


Figure 3. Contents (mean $\pm$ SD) of (A): total polyphenols, (B): flavonoids, (C): carotenoids of *L. spartum*; a, b, bc and c represent homogenous groups identified by Tukey test.

3.3.3. Free radical scavenging potential DPPH

The antioxidant activity is shown (Fig. 4). Non-inoculated plants presented a higher inhibition percentage; however, the fungal inoculated plants were distinguished by a lower half-maximal inhibitory concentration (IC_{50}) = 11.73 $\mu\text{g mL}^{-1}$. This trend may be associated with mycorrhization, which is known to influence secondary metabolism and antioxidant profiles. The analysis of variance (ANOVA) revealed a significant difference ($p < 0.05$) among the treatment pots in terms of antioxidant enzyme activity. CL-NI plants showed greater inhibitory concentration (IC_{50} = 21.10 $\mu\text{g mL}^{-1}$) explained by the neutralization of free radicals. This may reflect a higher level of oxidative stress in non-inoculated plants, leading to increased production of antioxidants as a defense response. However, CL-I plants exhibited a lower half-maximal inhibitory concentration, suggesting that the antioxidants produced were more potent or efficient, as less was required to achieve 50 % inhibition. Mycorrhized *L. spartum* may exacerbate the production of antioxidant secondary metabolites such as phenolics, flavonoids, and carotenoids. An alteration of the metabolic pathways and stress responses could contribute to the observed differences in antioxidant activity, even considering the low inhibition percentage. The lower IC_{50} values in mycorrhized plants put forth potentially more efficient antioxidant compounds, although further studies are needed to confirm changes in stress signaling or metabolic redirection.

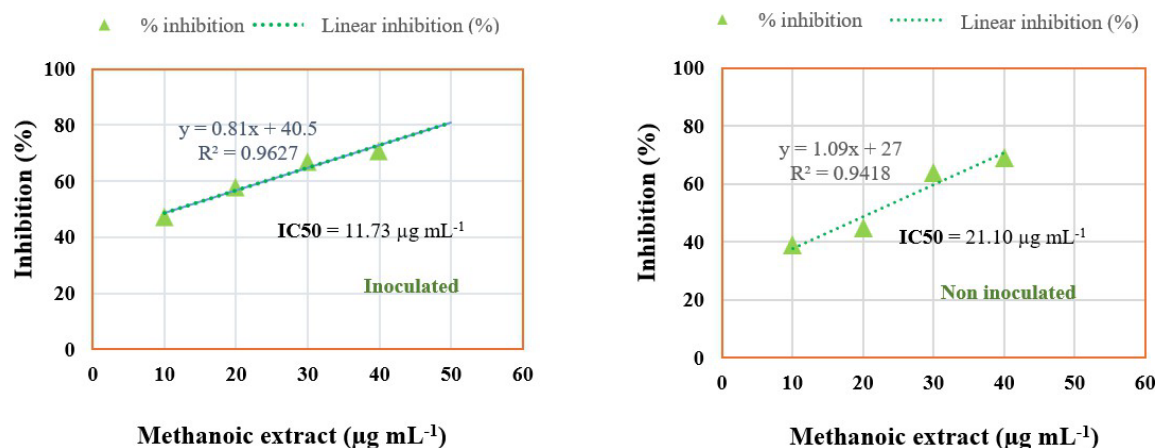


Figure 4. Inhibition percentage curves with IC₅₀: Inhibition concentration at 50 % of inoculated plants (a) and inoculated plants non-inoculated (b) in contaminated *L. spartum*.

3.4 Linking AMF inoculation to toxic metal(loid) uptake and antioxidative enzyme responses

Principal component analysis (PCA) is shown (Fig. 5). PC_1 was primarily associated with biochemical markers, while PC_2 grouped variables related to morphological traits and TMM uptake. Two main variable clusters were observed in the PCA biplot, the first gathers all enzymatic activities correlated with the component PC_1 : POD, SOD, APX, GST CAT, flavonoids, carotenoids, total phenols, RE and FB. The second group of variables was correlated to PC_2 assembling *Chl a*, *Chl b*, and FT and leaf length. Both FB and RE were positively correlated to APX, POD, SOD, flavonoids, carotenoids and polyphenols. However, *Chl a*, *Chl b* and leaf length were negatively correlated to all enzymatic activities, secondary metabolites, FB, and RE. Mycorrhization was associated with increased removal efficiency of TMMs, as shown by positive correlations in the PCA (Fig. 5). This improvement in removal efficiency of TMMs was associated with a heightened antioxidative response, notably through the accumulation of non-enzymatic antioxidants such as flavonoids, carotenoids, and polyphenols. Also, an excessive response of SOD, POD, APX and GST in response to TMMs tracks the

adaptability of *L. spartum* to TMMs stress. Taken together, these findings suggest that *L. spartum*, in symbiosis with mycorrhizal fungi, holds potential for contributing to the phytostabilization and ecological restoration of heavy metal-contaminated soils.

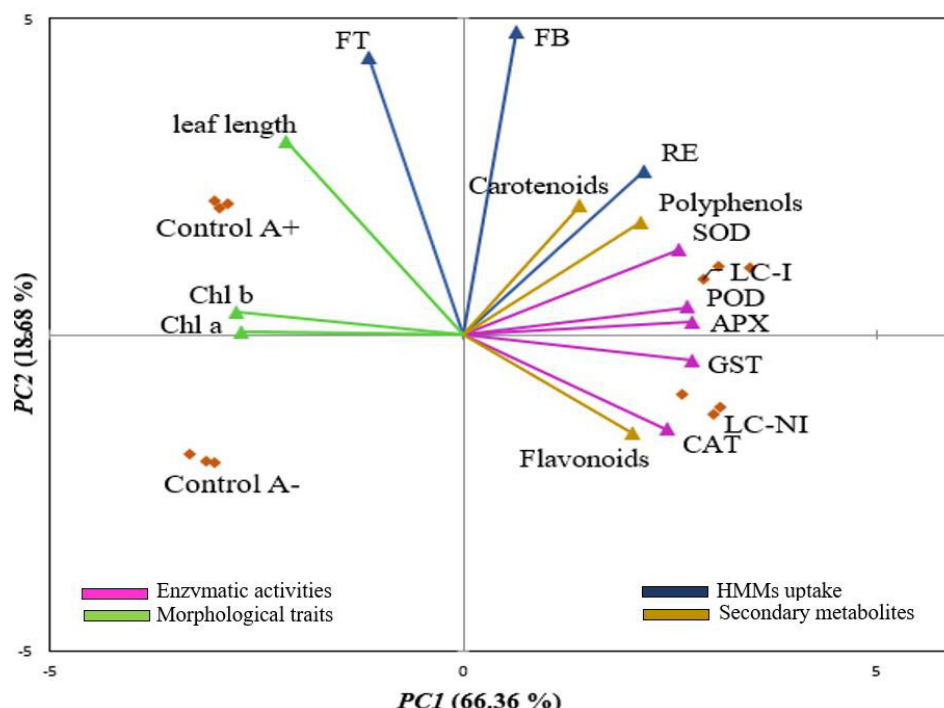


Figure 5. Biplot of principal components ($PC1$, $PC2$) depicting relationship between antioxidative response, toxic metals and metalloids uptake and fungal inoculation.

4 Discussion

4.1. Suitability of mycorrhized *Lygeum spartum* for phytoremediation

In addition to the elevated TMMs found in soils surrounding the study site, including Pb, Cr, Cd, Zn, Ni, and Co, the site's proximity to a cement plant (SCG) raises the possibility of exposure to additional atmospheric contaminants. These include cement kiln dust (CKD) containing particulate-bound trace metals (e.g., V, Se, Th, Al), and gaseous emissions such as CO_2 , NO_x , and SO_2 . While the alkaline pH (~ 8.0) of the soil, likely maintained by CKD deposits rich in $CaCO_3$ and CaO , buffers against the acidification from NO_x and SO_2 , these gases may

influence oxidative stress, nutrient cycling and rhizospheric microbial activity, including mycorrhizal colonization. NO_x and SO₂ are known to disrupt stomatal function and cellular redox balance in plants, which may help to contextualize the observed variations in chlorophyll content, photosynthetic performance, and enzymatic antioxidant responses (e.g., SOD, APX, POD) in non-inoculated plants. Moreover, CKD deposition may have influenced the availability of essential and non-essential elements, thereby affecting the bioaccumulation (BF), translocation (TF), and removal efficiency (RE) patterns observed. The improved physiological and biochemical resilience observed in mycorrhized *L. spartum* suggests that AMF colonization effectively buffered both edaphic and atmospheric stressors, supporting its potential relevance for phytoremediation in drylands affected by multi-contaminant industrial emissions.

The biochemical mycorrhization outcomes pertaining to TMMs accumulation rely on BF, TF and RE (Raklami et al., 2022; Yang et al., 2025). Previous field research conducted on plants growing in semi-arid polluted area by Terwayet Bayouli et al. (2020) indicated that *L. spartum* held potential for phytoremediation applications. In our current study, the BF average exceeded 1 for more than six TMMs, indicating that fungal inoculation improved Cu, Cd, Co, Ni, and Cr and Pb uptake to the root. Moreover, inoculation enhanced the translocation of Cd, Cu, Ni, Co and Cr, where higher shoot concentrations were found over non-inoculated plants reaching its highest in Zn. The increased TMMs uptake may be related to physiological interactions between root tissues, AMF, and TMMs. However, AMFs affinity to TMMs uptake remains controversial. Salazar et al. (2024) found a reduced Pb translocation; however, translocation was improved for Pb, Ni and As in *Monarda Citriodora* Cerv ex Lag inoculated with *Glomus fasciculatum* (Luxmi et al., 2024). In our findings, the CL-I batch at M6 reached 60 % of AMF colonization and 50 % of root colonization (see Fig. S2 supplementary material) boosting Ni, Cd, Zn, Cr and Mn translocation. Such patterns may be influenced by AMF-associated mechanisms reported in the literature, including TMM immobilization in fungal cell

walls, extracellular polymeric substance (EPS) binding, or vacuolar compartmentalization (Wahab et al., 2024; Teng et al., 2024). The interaction TMMs-AMFs underscores the sequestration through these advantageous mechanisms such as the TMMs extracellular sequestration in the fungal cells where walls immobilize TMMs. Hyphal sheath and polysaccharides therein could also assist. Polymeric substances are associated with several binding sites where metals may form complexes with hydroxyl, carboxyl, mycorrhiza groups as well as phosphate groups (Wahab et al., 2024). However, intracellular sequestration could be reached when TMMs enter the fungal cells. Phytochelatins vacuolar compartmentalisation eliminates TMMs through thiols to maintain cytoplasmic homeostasis (Teng et al., 2024). TMM translocation to aboveground parts could additionally be supported by increasing metal efflux from the intracellular compartment, particularly from the cytosol to other compartments, or even mobilization towards intracellular sequestration. TMM transport is facilitated by proteins such as permeases, P-ATPase, carrier proton-cation and cationic diffusion facilitators (Chandwani et al., 2023). While these mechanisms were not directly assessed in our study, they offer possible explanations for the increased metal accumulation observed in mycorrhized *L. spartum*. Further research combining molecular and physiological approaches will be needed to confirm these hypotheses. However, AMF mechanisms can potentially explain the increasing concentration in the root zone and the strengthened bioaccumulation process of mycorrhized relative to non-mycorrhized *L. spartum* plants, suggesting a suitability for mycorrhized *L. spartum* for TMM phytoremediation applications.

4.2. Enhanced morphological traits

The potentiality of the phytoremediation is discerned by the ability of a species to grow in contaminated sites (Yang et al., 2024). AMF inoculation promoted *L. spartum* leaf growth compared to non-inoculated plants, with differences becoming significant at later growth stages. Plant growth is fostered while connected to AMFs that form vesicles, arbuscules and

hyphae, as well as spores and hyphae crossing the rhizosphere (Fig. S1.a and S1.b supplementary material). Hyphal networks are known to enlarge root-soil interfacial areas, which may improve resource acquisition and support plant growth. Under contaminated conditions, the effect of inoculation (CL-I vs. CL-NI) shows a steady increase, with the highest percentage differences at M6 (+19.8 %) and M3 (+18.8 %), indicating that the impact of inoculation becomes more pronounced in later stages. In contrast, under control conditions (A+ vs. A-), the percentage differences are highest at M5 (+20.9 %) and M4 (+18.3 %), suggesting that the inoculation effect peaks earlier. Interestingly, slight decreases or negligible differences are observed at M2 and M1 in the control group, highlighting the diminished role of inoculation under less stressful conditions. Fungal inoculation appeared more beneficial under contaminated conditions, where its effects on enhancing growth or activity are more substantial and sustained over time. AMF are reported to influence micronutrient availability and translocation, which may contribute to observed growth trends (Bushra et al., 2024). Jin et al. (2024), found that *Rhizophagus irregularis* inoculation boosted *Cinnamomum camphora* leaf yield under Cd stress. In addition, root inoculation increased the height of switchgrass biomass by 92.4 % in saline alkali stress (Wen et al., 2024). Rhizospheric fungal network was also correlated to shoot biomass and Cd and Zn accumulation (Luo et al., 2025). Likewise, mycorrhization boosted the aerial biomass production of *L. spartum* during six months of experimentation.

Plant growth is closely linked to chlorophyll content and photosynthetic efficiency, which are influenced by environmental and physiological factors with the chlorophyll content being used recently as an index for disturbance factors (Liang et al., 2024). The chlorophyll proteins are classified as *Chl a* and *Chl b* and are multi-factors functioning for photosystems PS I and PS II. These proteins bind to Chls and carotenoids where it indicates the photosynthetic potential. Inoculated plants showed increased *Chl a* and *Chl b* contents, with relative

improvements of approximately 50 % and 60 %, respectively, compared to non-inoculated plants. TMM interaction with the photosynthetic apparatus has been observed at different levels of organization either in the leaves, organs, tissues, cell, cytosolic enzymes and organics (Reddy et al., 2024). In our findings, the chlorophyll a/b ratio in inoculated *L. spartum* plants was approximately 1.14, which is lower than typical values reported in many mesophytic species, where *Chl a* is often 2–3 times more abundant than *Chl b*. This relatively low *Chl a/b* ratio suggests either a higher proportion of *Chl b* or a relative reduction in *Chl a*. In xerophytic species, such as those adapted to arid environments, shifts in pigment ratios have been reported as adaptive responses to highlight intensity and stress conditions. *Chl b* serves to broaden the light-harvesting capacity under suboptimal light or stress, possibly explaining its increased relative abundance in our experiment. Similar patterns were reported by Gunes et al. (2008) and Ullah et al. (2024) in drought-tolerant Mediterranean species, where the *Chl a/b* ratio declined under stress, reflecting adjustments in the light-harvesting complex (LHC) composition. Additionally, mycorrhizal symbiosis may modulate pigment biosynthesis and photoprotective mechanisms, contributing to the atypical ratio observed, although further investigation is required to confirm these mechanisms in *L. spartum* under TMM stress. Under drought conditions, the chlorophyll a/b ratio tends to decrease where the causes could be linked to a degradation of chlorophyll under drought conditions affecting the patterns of *Chl a* and *Chl b*. (Yang et al., 2021). This was also supported by Farghali et al. (2014) where a low chlorophyll a/b ratio was detected in the xerophyte *T. desertorum*. Potential causation was ascribed to the adaptation of plants in their natural habitats to enduring drought and high temperatures, resulting in decreasing chlorophyll a/b ratios balanced by an increased chlorophyll stability to heat.

The decrease of *Chl a* and *Chl b* under contaminated conditions may be explained by the decrease in leaf protein content, which contributes to the alteration of photosynthetic pigments

added to the structural chloroplast disruption. Among plant species, accumulators are endowed with several adaptative mechanisms against TMM stress. Few studies have inspected the coupled modalities of TMMs and AMFs in accumulator species. A study conducted by Wu et al. (2020) showed that *Chl a* and *Chl b* were found to be significantly higher ($p < 0.05$) in T1-treated AMF plant under As stress. *Chl* content enhancement could be explained by the up-regulated expression of transmembrane protein–pigment complexes CP43 (photosystem II) and FNR, ferredoxin-NADP⁺ oxidoreductase related to photosynthetic electron transport. It is noteworthy that photosynthesis and water vapor efflux are controlled by stomatal conductance (g_s) through the CO₂ influx that contributes to plant adaptation to xeric conditions. Under xeric conditions, the g_s declines as a result of reduced carbon assimilation rates and limited CO₂ availability in the mesophyll, thereby limiting the photosynthetic efficiency (Flexas et al., 2004). However, some studies highlighted the effect of microbial inoculants such as PGPB (Plant Growth Promoting Bacteria) and AMF in enhancing photosynthetic performance by optimizing stomatal regulation and maintaining favorable internal CO₂ levels (AlOtaibi et al., 2023; Ruiz-Lozano et al., 2016). These interactions highlight the role of microbial partners in modulating stomatal behavior to sustain carbon fixation under water-limited environments. In addition, AMF can improve physiological mechanisms by boosting the chlorophyll content, efficiency of light energy utilization, gas exchange, and rubisco activity under salinity stress (Sachdev et al., 2025). Such mechanisms may contribute to the improvement *Chl a* and *Chl b* contents in *L. spartum* that raised significantly ($p < 0.05$) in mycorrhized plants. AMF association was also found to enhance drought and salinity resistance (Zhu et al., 2024). The latter will foster mitigating the effect of aridity alongside developed biomass of the amended *L. spartum* that supports xeric plants thriving in stressed environments and enhances phytoremediation potential.

4.3 Magnified phytochemical antioxidants in mycorrhiza -*Lygeum spartum* consortia

In both vulnerable drylands and wetlands, the antioxidative potential of plant species to TMM contamination is a determinant for tolerance and adaptability in continuous global change. Enzymatic antioxidants have been demonstrated to be efficient in alleviating abiotic stresses such as those induced by drought, TMMs and salinity (Jahan et al., 2021). Boosted POD, SOD and APX activities in inoculated batches of *L. spartum* were observed in the current research (Table 3); however, there was no evidence of varied CAT activity. SOD, POD and APX were significantly affected by AMFs inoculation by 34.6 %, 24.3 %, and 13.8 %, respectively, compared to non-inoculated, which may indicate a potential antioxidative response to TMM-induced stress in inoculated plants. Conversely, under control conditions, enzymatic activities are either slightly reduced or unchanged, with notable decreases in SOD (-25.8 %) and catalase (CAT, -16 %) reflecting that TMM also induce the enzymatic cascade. This variation may suggest differential regulation or responsiveness of antioxidant enzymes to AMF colonization. The antioxidative performance varies also upon the stress induced. Similar to our findings, in wetlands AMF application magnified shoot SOD and POD by 39.6 % and 29.5 % of *I wilsonii* under Cr stress (Hu et al., 2020). Improved APX activity was observed in AMF support while no effect was detected for other enzymes, suggesting that enzymatic activity does not have the same affinity (Bushra et al., 2024), as also evidenced in our results. Under severe drought stress, SOD, CAT, POD, APX and GR activities were strengthened in *Zea mays* L. (Begum et al., 2019). It was reported that *Archaeospora* and *Scutellospora* occurrence in roots has positively influenced SOD, POD, and CAT activities, particularly, *Glomus*, *Scutellospora*, and *Claroideoglomus* applied on *Robinia pseudoacacia* L. in Cd stressed soils (Wang et al., 2022). However, decreased POD and CAT in shoots, and CAT in roots ($P \leq 0.05$) in accumulator genotype *Zea mays* were reported (Kuang et al., 2025). AMF have been reported to modulate enzymatic responses under specific stress conditions. The increased levels of phenolics, flavonoids, and carotenoids under mycorrhization may reflect an adaptive antioxidative strategy

in response to TMM stress. While these biochemical responses support the potential use of *L. spartum* in phytoremediation, further assessments of stress resilience, long-term survival, and contaminant stabilization under field conditions are essential.

Our study reveals boosted non-enzymatic phytochemicals (Figs. 3 and 4) as demonstrated by flavonoids, carotenoids, and polyphenols increases after fungal inoculation. Concentrated phenols was recorded in the xeric *L. spartum* with a respective increase by 72.1 % in CL-I. It is noteworthy that the use of methanol (80 %) as solvent, while widely adopted for extracting polyphenols and flavonoids due to its high polarity and efficiency, may influence the overall profile of extracted compounds. Specifically, methanol is less efficient in solubilizing certain non-polar compounds such as carotenoids, which may lead to their underestimation (Adugna et al., 2024). Although we observed enhanced carotenoid content in mycorrhized plants, future studies could benefit from complementing methanol with solvents of lower polarity (e.g., acetone or hexane) to better capture the full antioxidant profile. Other grass species such *Tamarix gallica* L.; *Commiphora leptophloeos* (Mart.) and *Zea mays* L. showed comparable enhancement of phenols with *Glomus* inoculation being *Glomus albida* and *Cladroiglomus etunicatum* under drought stress (Bencherif et al., 2019; Lima et al., 2017; Begum et al., 2019). The observed 59.0 % enhancement of flavonoids relative to CL-NI was also related to fungal supply hitting a maximum in CL-I. Subsequently, AMF effects on secondary metabolite production appeared more pronounced under stressed conditions rather than control A+ and A-. Although total phenols and flavonoids were amplified, our experiments revealed lower amplification than that reported by Matinizadeh et al., (2024) wherein *M. citriodora*, AMF increased flavonoids by 149 % for Pb contaminated soils, 136% for As contaminated soils, and 127 % for Ni contaminated soils (Matinizadeh et al., 2024). This may relate to the performance of AMF specific to the ecological e whether wetlands or drylands. Similarly, mycorrhized plants boosted flavonoids synthesis and their role in fertility and growth was demonstrated to be

important to the antioxidant activity linked to scavenging properties (Zhang et al., 2023). Flavonoid chelation of metal ions could also affect the antioxidant response induced by metal contamination in the current experiment using contaminated soils. Furthermore, AMF can play a dual role in sequestering toxic metals through the modulation of the phosphate transport pathway and enhanced secondary metabolites involved in the phenylpropane biosynthesis pathway, flavonoid biosynthesis pathway, and flavanols biosynthesis pathways (Wang et al., 2025).

5 Conclusions

This study demonstrated that *L. spartum*, a xeric plant species, in symbiosis with arbuscular mycorrhizal fungi (AMF), significantly enhances the phytoremediation potential in metal- and metalloid-contaminated arid environments. Mycorrhization improved biomass production, chlorophyll content, metal uptake, and translocation factors in soils contaminated with Cd, Zn, Cr, Co, and Ni. Additionally, inoculation stimulated enzymatic (SOD, POD, APX) and non-enzymatic antioxidant (polyphenols, flavonoids, carotenoids) responses, enhancing the plant's resilience to oxidative stress induced by TMM exposure. The improved physiological and biochemical resilience suggests that AMF colonization buffered both edaphic and atmospheric stressors, supporting its relevance for phytoremediation in drylands affected by industrial emissions. . These results support using mycorrhiza-assisted xerophytes as a promising eco-restoration strategy for degraded lands and highlight their potential for sustainable phytoremediation in areas affected by climate-induced aridity.

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Highlights

- Xeric plant species proved a phytoremediation potential of TMMs.
- Endophytic fungi enhanced TMMs root uptake and removal efficiency.
- Enhanced enzymatic and non-enzymatic antioxidants vs mycorrhization
- AMF boosted biomass and resilience of *Lygeum spartum*
- Xeric *Lygeum spartum* is suitable for arid environments phytoremediation.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: