Synergistic application of *Bacillus subtilis* IAGS174 and thiamine to mitigate salinity and lead stress in *Helianthus annuus* (#113498)

First revision

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Synergistic application of *Bacillus subtilis* IAGS174 and thiamine to mitigate salinity and lead stress in *Helianthus annuus*

Sidra Fatima [1], **Waheed Ullah Khan** [2], **Rehana Sardar** [Corresp., 3], **Bareera Munir** [1], **Areeba Rehman** [1], **Waheed Akram** [4], **Iqra Munir** [5], **Nasim Ahmad Yasin** [Corresp. 6]

[1] College of Earth and Environmental Sciences, University of the Punjab, Lahore 54590, Pakistan, Lahore, Pakistan

[2] College of Earth and Environmental Sciences, University of the Punjab, Lahore 54590, Pakistan, L, Pakistan

[3] Department of Biological and Environmental Sciences, Emerson University, Multan pakistan, Multan, Pakistan

[4] Department of Horticulture,, University of the Punjab, Lahore, Pakistan

[5] University Institute of Medical Laboratory Technology, Faculty of Allied Health Sciences, The University of Lahore, Lahore, Pakistan

[6] Department of Horticulture, University of the Punjab, Lahore, Pakistan

Corresponding Authors: Rehana Sardar, Nasim Ahmad Yasin
Email address: rehana.sardar@eum.edu.pk, nasimhort@gmail.com

The increasing soil contamination with salinity and heavy metals poses serious threats to the cultivation of economically and ornamentally important plants such as *Helianthus annuus* (Sunflower). *Bacillus subtilis* IAGS174 and thiamine are well known for their role in increasing plant stress tolerance levels by multiple mechanisms. The present research aimed to assess the effect of *B. subtilis* IAGS174 and thiamine on *H. annuus* under salinity and lead stress by analyzing the growth parameters, physiological markers, and biochemical assays. In a pot experiment, *B. subtilis* IAGS174 and thiamine were supplied to *H. annuus* plants grown in soil subjected to 500 mg/kg of salt and 150 mg/kg of lead. *H. annuus* plants exhibited a significant reduction in growth attributes and photosynthetic machinery under single and combined stress of Pb and salinity. The combined stress of Pb and salinity declined the root length, shoot length, root fresh weight, shoot fresh weight, chlorophyll *a*, and chlorophyll *b* of *H. annuus* by 49%, 61%, 48%, 39%, 53%, and 55%, respectively, as compared to the control. Moreover, under stress, H. annuus plants exhibited higher levels of antioxidant enzymes, phenol, flavonoid and proline content. Nevertheless, the combined effect of *B. subtilis* IAGS174 and thiamine improved the fresh weight of shoot and roots, chlorophyll *a*, chlorophyll *b* and carotenoids by 34%, 38%, 15%, 18% and 16%, respectively, under combined stress of salt and Pb to their respective controlled conditions. Supplementation of *B. subtilis* IAGS174 and thiamine significantly increased the antioxidative enzymes (superoxide dismutase, catalase, and peroxidase) and non-enzymatic antioxidants (phenol, flavonoids & proline) in sunflower under combined and individual stress of Na and Pb. Nevertheless, inoculation of *B. subtilis*

IAGS174 accelerated the translocation of Pb and Na, while thiamine application reduced the uptake of these metals. Conclusively, single and combined application of PGPR (Plant growth promoting rhizobacteria) and thiamine proved a sustainable and effective option to improve plant tolerance against salt and Pb stress and offer new avenues for suitable agricultural practices in heavy metal and salt-contaminated soil

Synergistic Application of *Bacillus Subtilis* IAGS174 and Thiamine to Mitigate Salinity and Lead Stress in *Helianthus annuus*

Sidra Fatima[a], Waheed Ullah Khan[a], Rehana Sardar [b], Bareera Munir[a], Areeba Rehman[a], Waheed Akram [c], Iqra Munir [d], Nasim Ahmad Yasin[c]

a. College of Earth and Environmental Sciences, University of the Punjab, Lahore 54590, Pakistan

b. Department of Biological and Environmental Sciences, Emerson University Multan, Pakistan.

c. Department of Horticulture, University of the Punjab, Lahore 54590, Pakistan

d. University Institute of Medical Laboratory Technology, Faculty of Allied Health Sciences, The University of Lahore

Corresponding authors;

Nasim Ahmad Yasin, Email: nasimhort@gmail.com

Rehana Sardar, Email: rehana.sardar@eum.edu.pk

ABSTRACT

The increasing soil contamination with salinity and heavy metals poses serious threats to the cultivation of economically and ornamentally important plants such as *Helianthus annuus* (Sunflower). *Bacillus subtilis* IAGS174 and thiamine are well known for their role in increasing plant stress tolerance levels by multiple mechanisms. The present research aimed to assess the effect of *B. subtilis* IAGS174 and thiamine on *H. annuus* under salinity and lead stress by analyzing the growth parameters, physiological markers, and biochemical assays. In a pot experiment, *B. subtilis* IAGS174 and thiamine were supplied to *H. annuus* plants grown in soil subjected to 500 mg/kg of salt and 150 mg/kg of lead. *H. annuus* plants exhibited a significant reduction in growth attributes and photosynthetic machinery under single and combined stress of Pb and salinity. The combined stress of Pb and salinity declined the root length, shoot length, root fresh weight, shoot fresh weight, chlorophyll *a*, and chlorophyll *b* of *H. annuus* by 49%, 61%, 48%, 39%, 53%, and 55%, respectively, as compared to the control. Moreover, under stress, H. annuus plants exhibited higher levels of antioxidant enzymes, phenol, flavonoid and proline content. Nevertheless, the

combined effect of *B. subtilis* IAGS174 and thiamine improved the fresh weight of shoot and roots, chlorophyll *a*, chlorophyll *b* and carotenoids by 34%, 38%, 15%, 18% and 16%, respectively, under combined stress of salt and Pb to their respective controlled conditions. Supplementation of *B. subtilis* IAGS174 and thiamine significantly increased the antioxidative enzymes (superoxide dismutase, catalase, and peroxidase) and non-enzymatic antioxidants (phenol, flavonoids & proline) in sunflower under combined and individual stress of Na and Pb. Nevertheless, inoculation of *B. subtilis* IAGS174 accelerated the translocation of Pb and Na, while thiamine application reduced the uptake of these metals. Conclusively, single and combined application of PGPR (Plant growth promoting rhizobacteria) and thiamine proved a sustainable and effective option to improve plant tolerance against salt and Pb stress and offer new avenues for suitable agricultural practices in heavy metal and salt-contaminated soil

Keywords; Antioxidants; PGPR; Stress tolerance; Salinity; Sunflower; Thiamine

Introduction

One of the major problems agricultural land faces is the continuous increase in salinity. Around the world, salinity affects 20% of irrigated land and 7% of all arable land (Liu et al., 2024). India, China, the United States, Pakistan, Sudan, and Turkey have major salinized lands, and it is spreading in other countries (Singh, 2022). About 6.3 million hectares of land have been affected by salinity in Pakistan and approximately 4000 hectares of fertile soil have been deteriorated with salinity every year (Alobaid et al. 2025).

The major causes of soil salinization are the weathering of rocks and climate change-induced evaporation, extensive groundwater extraction, and subsequent flooded irrigation leading to the accumulation of soluble salts in the land (Maryam et al., 2023). Anthropogenic activities that cause an increase of lead content in soil and water are industrial processes, transportation, fertilizer, pesticide use and irrigation of soil with industrial effluent (Ijaz et al., 2020). Nations, including Pakistan, have stated that lead concentrations in the soil have risen drastically over the last few decades and are a contributing factor to decreased crop yields (Altaf et al., 2021). This high salinity and Pb level have an unfavorable influence on germination, plant development, and production of reactive oxygen species (ROS), which results in plant death by negatively affecting several metabolic processes like photosynthesis, respiration, transpiration, membrane characteristics, cellular hemostasis, hormone balance, (Patwa et al., 2024; Naz et al., 2025). High salt

concentration also has an osmotic and ion-specific effect on plant growth, oxidative stress tolerance and yield. Since most metals have negative effects at low doses (between 1 and 10 mg/mL), heavy metal and salinity pollution have become a serious concern for plant growth (Rojas-Solis et al., 2023).

For the soil remediation from these pollutants, many physical, chemical, and biological techniques are being used all across the world**.** Physicochemical methods are mostly costly, unfriendly, and impractical when handling larger amounts of soil. On the other hand, bioremediation techniques such as using growth-promoting rhizobacteria (PGPRs) in combination with plants is considered a feasible, economic and eco-friendly approach for the remediation of multi-contaminated soil (Sarwar et al., 2023). The PGPRs are found near plant roots and improve plant growth by providing nutrients and hormones (Shabaan et al., 2023). Hyper-accumulator plants can uptake 100 times more heavy metals from soil and are deemed a viable option for the decontamination of salt and Pb-polluted sites (Altaf et al., 2021). Sunflower (*H. annuus*) is a hyper-accumulator and the fourth largest global commercial oil seed crop. Over the past 20 years, its production has increased significantly, but since 2010, its production has decreased steadily due to different causes, including soil degradation with salinity and heavy metals (Aftab et al., 2020). According to many investigations, most PGPR strains like *Agrobacterium*, *Bacillus*, and *Rhizobium* species increase the host plants' ability to withstand heavy metal toxicity. Numerous procedures such as fixation of atmospheric nitrogen, the solubilization of phosphate, and the synthesis of phytohormones (such as gibberellin, abscisic acid, and indole 3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and exopolysaccharide (EPS), can be used by these PGPRs to improve plant growth and heavy metal stress tolerance. Furthermore, PGPRs increase the enzymatic and non-enzymatic antioxidative activity to confer salinity and Pb toxicity in plants (Hahm et al., 2017).

According to recent research, apart from plant growth regulators (PGRs), vitamins like Thiamine and ascorbic acid also help the plants to deal with the deadly consequences of both biotic and abiotic stressors by participating in energy-producing mechanisms like Kreb's cycle and Calvin cycle. The foliar application of thiamine was found to dramatically boost growth, photosynthetic pigments, and antioxidant activities in many plants grown under abiotic stressed regimes (Naheed et al., 2021). Thiamine contributes to the defensive network by acting as an antioxidant or

indirectly by supplementing NADH and NADPH in plants. Thiamine supplementation may boost the performance of an anti-oxidative defense system in plants to cope with salinity and Pb toxicity (Sanjari et al., 2019).Although different studies have identified the efficacy of these growth regulators on numerous plant species under stress conditions, there is a lack of comprehensive research on the co-application of PGPR and thiamine to *H. annus* (sunflower) subjected to salinity and Pb stress. The main objectives of this study were: (i) evaluation of the potential of *B. subtilis* IAGS174 and thiamine for improvement of growth, photosynthetic pigments, enzymatic and non-enzymatic antioxidants, and attenuation of lipid peroxidation in sunflower exposed to Pb and salinity stress and (ii) the assessment of *B. subtilis* IAGS174 and thiamine role in the translocation of sodium and Pb from soil to sunflower tissues. This study has shown insights into how these growth regulators improve stress tolerance, increase nutrient uptake, and confer oxidative damage in sunflower exposed to salinity and lead toxicity.

Material and methods

The pot experiment was conducted in the wirehouse of the College of Earth and Environmental Sciences, University of Punjab, Lahore (31.5204^0 N, 74.3587^0 E).

Inoculum Preparation and Soil Inoculation

To prepare inoculation, a loopful of rhizobacteria (*B. subtilis* IAGS174) was inoculated to Luria Bertani (LB) broth media, which was then inoculated in a mechanical shaker for 2 days at 27 ° C. The optical densities were then measured to establish a uniform population of 108-109 CFU mL-1. Approximately 8-10 mL of bacterial culture was applied to the soil with the help of a sterile syringe

Pot Experiment

A pot experiment was performed to determine the positive effect of rhizobacteria and thiamine on Pb and salt stress in sunflower plants. The plastic pots were filled with soil (1.5kg) obtained from the agricultural fields of the University of the Punjab, Pakistan. Salt and heavy metal stress was applied by amending sodium chloride (NaCl) (500 mg/kg soil) and lead (Pb $(NO_3)_2$ 150 mg/kg) in the soil. The pots were moistened up to field capacity and then kept for 14 days to blend up the pollutants in the soil. The sunflower seeds were procured from Roshan Seed shop in Lahore, Pakistan. For surface sterilization, seeds were immersed in 70% ethanol for 30 seconds and 1%

sodium hypochlorite for an additional 60 seconds, following multiple washes with distilled water. The healthy seeds (8-10) were planted in each pot, and thinning was performed after germination to keep 5 plants in each pot. After three weeks of planting, growth regulator (PGPR, Thiamine) treatments were applied. The bacterial inoculum (10 mL) was applied to plants by soil drenching and thiamine (100 ppm) was supplemented by foliar application to the sunflower plants. The experiment was done in a wirehouse. The environmental conditions in wirehouse were a temperature of 28-35 ^{o}C, a night/day period of 11/13 h, and humidity of 75 ± 5%. The plants were watered at regular intervals to keep optimum moisture in the soil. Each treatment was replicated thrice by a completely randomized design (CRD). A total of 48 pots for 16 treatments were planted with sunflower seeds, and these plants were uprooted after 120 days. Hence, total of 16 treatments was planned by using either single or combined treatments of both growth promoters (microbe, thiamine) and single and co-application of pollutants (Na, Pb) such as C; control, B; Bacteria, T; thiamine, B+T; Bacteria+ thiamine, S; Salinity, S+B; Salinity+ Bacteria, S+T; Salinity and Thiamine, S+B+T; Salinity and Thiamine+Thiamine, Pb; lead, Pb+B; Lead+ Bacteria, Pb+T; Lead+ Thiamine, Pb+B+T; Lead+Bacteria+Thiamine, Pb+S; Lead+ Salinity, Pb+S+B; Lead+ Salinity+Bacteria, Pb+S+T; Lead+ Salinity+ Thiamine, and Pb+S+B+T; Lead+ Salinity+Bacteria+Thiamine etc.

Assessment of photosynthetic pigments

The fully mature and widened leaves from the center of each plant were selected and removed. To estimate chlorophyll (Chl.) pigments, 0.25 g of fresh leaf tissues were blended with 80% acetone to make a plant extract. After extraction, the mixtures were centrifuged at 12,000×g for 10 min. The amount of Chl. *a* and Chl. *b* was measured by monitoring the absorbance of the supernatant at wavelengths of 663 nm and 645 nm, respectively, with the help of a spectrophotometer (UV/VIS, Cecil Aquarius CE 7200). The relative absorbance of the supernatant was obtained at 480 nm for examining carotenoid content (Liu et al., 2024). The formulas used for calculating the chlorophyll content are:

$$\text{Chl. `}a\text{' (mg g}^{-1}\text{ FW)} = \frac{(12.7 \times A663) - (2.69 \times A645) \times \text{Sample volume}}{1000 \times W}$$

$$\text{Chl.`}b\text{'(mg g}^{-1}\text{ FW)} = \frac{(12.7 \times A645) - (4.68 \times A663) \times \text{Sample volume}}{1000 \times W}$$

$$\text{Total chlorophyll (mg g-}^{1}\text{ FW)} = \frac{20(A645) + 8.02(A663) \times \text{Sample volume}}{1000 \times W}$$

$$\text{Carotenoids (mg g}^{-1}\text{ FW)} = OD480+0.114\ (OD663)-0.638(OD645)$$

Antioxidant enzymes analysis

Enzyme extract was obtained by adding 1 g of the plant's fresh leaves in a mortar and crushing by pestle by pouring liquid N_2. Afterward, the plant biomass was homogenized in 2.0 ml of an ice-cold 50 mM sodium phosphate buffer. The homogenous mixture was centrifuged at 10000×g for 20 minutes at 4 °C. The resulting supernatant was employed for the assessment of different antioxidative enzymes with the help of a spectrophotometer (Rao et al., 2000).

Superoxide dismutase (SOD) content was observed by pouring 0.1 mL of enzyme extract into 1.5 mL 50 mM sodium phosphate (pH 7.8), 0.300 mL 750 µM nitro blue tetrazolium (NBT), 0.300 mL 20 µM riboflavin, 0.3 mL 130 µM methionine, 0.3 mL 100 µM EDTA-N Spectrophotometer (UV/VIS, Cecil Aquarius CE 7200) was used to note reading at 560 nm after the reaction mixture was illuminated under light of 4000 flux for 20 min (Bin at al., 2010). POD activity was analyzed using 50 µL enzyme extract and 1.0 mL 0.3% H_2O_2, 1.0 mL 50 mM sodium phosphate (pH 5.5), and 0.95 mL 0.2% guaiacol. This was recorded at 470 nm using a spectrophotometer (UV/VIS, Cecil Aquarius CE 7200). A reaction mixture containing 500 µL 0.1 M H_2O_2, 3.0 mL 50 mM sodium phosphate (pH 7.8), 1.0 mL deionized water, and 200 µL enzyme extract was prepared for catalase (CAT) activity. A spectrophotometer (UV/VIS, Cecil Aquarius CE 7200) was used to note the absorbance at 240 nm (Rhaman et al., 2024).

Quantification of Hydrogen Peroxide (H_2O_2) and Malondialdehyde (MDA)

For malondialdehyde (MDA) determination, 0.2 g of plant leaf sample was crushed in 5 ml of 0.1% TCA and centrifuged for 5 minutes at 10,000g. The reaction mixture was made by adding 1 mL of the supernatant aliquot to 4 mL of 20% TCA with 0.5% thiobarbituric acid (TBA). The absorbance of the reaction mixture was estimated at 532 nm by spectrophotometer (UV/VIS, Cecil Aquarius CE 7200), and the reading for non-specific absorption at 450 nm and 600 nm was subtracted (Zhang et al., 2013). The MDA level (mol g^{-1} FW) was recorded using (= 155 mM^{-1} cm^{-1}). The H_2O_2 content was estimated by crushing 0.5 g of fresh plant leaf with 5 mL of trichloroacetic acid (TCA 0.1%) and then centrifuging at 12,000g at 4°Cfor 20 minutes. Afterward,

0.5 ml of supernatant liquor was homogenized with 0.5 mL of 10 mM KPO_4 buffer. The reading of absorbance was noted at 390 nm by a spectrophotometer (UV/VIS, Cecil Aquarius CE 7200) (Velikova et al., 2000).

Estimation of Flavonoid and Phenolic Content

A volumetric flask was filled with 1 mL of plant extracts and 4 mL of distilled water to determine the flavonoid concentration. The solution was mixed with 0.3 mL of 5% $NaNO_2$ after 5 minutes. 0.3 mL of 10% $AlCl_3$ was added to it. After 6 minutes, 2 mL of 1M NaOH was added to the solution. Distilled water was added to make the volume 10 mL. The absorbance of the solution was measured at 510 by spectrophotometer (UV/VIS, Cecil Aquarius CE 7200) (Zhishen et al., 1999). Phenol contents were determined by immersing 2 g of plant leaf in 10 mL of 80% methanol for 15 minutes at 65°C. 1 mL of plant extract was mixed with 250 µL of Folin-Ciocalteau reagent (1N) and 5 mL of distilled water. The reaction mixture was stored at 30 degrees Celsius. To identify the exact number of phenols, the absorbance at 725 nm was measured by spectrophotometer (UV/VIS, Cecil Aquarius CE 7200) and compared to the gallic acid curve (Zieslin and Ben-Zaken, 1993).

Proline Estimation

A sample of dried and prewashed leaf (100 mg) was placed into a flask containing 10 mL of sulfosalicylic acid (3%). The mixture was vortexed and then filtered through Whatman's no-filter paper. Then, 2mL of the filtrate was transferred to a glass tube containing as much glacial acetic acid as there was acid ninhydrin. It was placed in a water bath at 100°C for 0.5 hours. Then, 4 mL of toluene was added, and the chromophore was aspirated. This reaction mixture was incubated at 25°C for 0.5 hours, and its colorimetric value was recorded at 520 nm by spectrophotometer (UV/VIS, Cecil Aquarius CE 7200) and compared to the standard curve (Bates et al., 1973).

Plant Growth and Biomass Production Assessment

After 120 days, the plants were removed from the pots and properly rinsed with distilled water. Plant parts, including roots, shoots, and leaves, were separated. The lengths of the roots and shoots were measured. The values of fresh biomass production from root and shoot were also measured using an electrical balance. The root and shoot samples were placed in an oven at 700°C for one day. The root and shoot dry weights were also calculated.

Heavy Metal Analysis

The dried plant samples were ground into a fine powder, and 1 g was mixed with 10 mL of HNO_3 and 5 ml of $HCLO_4$. The mixture was put in a flask and transferred to the hot plate, which was then placed in the hot plate at 1500 C until the solution turned transparent and reduced to 5 mL. After the digestion, 45 mL of distilled water was mixed in this solution, making a solution of 50 mL. The solution was filtered twice by using the Whatman 42 filter paper. The filtrate was shifted to sample bottles, and distilled water was poured to make the sample up to 100 mL. The Pb quantity was estimated using an atomic absorption spectrophotometer (AAS; PerkinElmer AAnalyst 800), while Na was analyzed by a flame photometer (Model PFP7, 90-125V).

The metal tolerance index, translocation factor (TF) and bio-concentration factor (BCF) were calculated by the methodology proposed by Wu et al. (2028). The metal tolerance index was analyzed for the evaluation of the ability of the plant to grow in the presence of metal concentration by a formula:

$$\text{Metal tolerance index} = \frac{\text{Mass of treated plant}}{\text{Mass of control plant}} \times 100$$

The translocation factor was determined as;

$$\text{TF} = \frac{\text{or Na conc. in shoot}}{\text{Pbor Na conc. in root}}$$

The bio-concentration factor (BCF) was determined by:

$$\text{BC} = \frac{\text{Pb or Na conc. in shoot}}{\text{or Na conc. in soil}}$$

Statistical Analysis

The experiment was performed with three replicates of each treatment in a completely randomized design (CRD). The obtained results were demonstrated as mean $\pm$ standard deviation. For statistical analysis, a one-way analysis of variance (ANOVA) was performed with the help of DSAASTAT software. Significance among multiple treatments was checked by performing Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ significance level using DSAASTAT software.

Results

Effect *B. Subtilis* IAGS174 and thiamine on growth parameters of *H. annuus* subjected to salinity and Pb stress

The effects of different treatments were significant shoot length (SL), root length (RL), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW) of sunflower. Both salinity and lead stress and their combination significantly hampered shoot and root length, dry weight, and fresh weight of the shoot and root of sunflower compared to the control treatment. The individual application of PGPR and thiamine and their combined treatment enhanced the growth of sunflower plants under normal and stress conditions. B+ T treatment under lead and salt stress was found to be most effective and meaningfully improved shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight by 28.12%, 60%, 6.84%, 9.3%, 16.3% and 32.25% as compared to Pb+ S treatment (Table 1).

Influence *B. Subtilis* IAGS174 and thiamine on Photosynthetic attributes of *H. annuus* subjected to salinity and Pb stress

The application of PGPR and thiamine increased the Chl. *a*, Chl. *b*, total chlorophyll and carotenoid content in sunflower plants either alone or in combination as compared to the control. Both lead and salinity stress significantly reduced the Chl. *a*, Chl. *b*, total chlorophyll and carotenoid content in sunflower plants. Combined Pb+ S stress significantly (DMRT at $p \leq 0.05$) reduced Chl. *a* by 44.5%, Chl. *b* by 54.81%, total Chl. by 47.33%, and carotenoid by 45.21% as compared to the control. B+ T treatment under lead and salt stress was found to be most effective in improving the Chl. *a*, Chl. *b*, total chlorophyll and carotenoid content by 16.33%, 16.39%, 15.66%, and 14.28%, respectively, as compared to the Pb+ S treatment (Table 2).

Impact of *B. Subtilis* IAGS174 and thiamine on Antioxidant enzymes of *H. annuus* subjected to salinity and Pb stress

The physiological parameters like SOD, POD, and CAT showed a significant change in all the treatments. The amount of SOD, POD, and CAT showed a significant (DMRT at $p \leq 0.05$) increase in salt (220.5%, 226.58%, and 220%, respectively) and lead stress (412.8%, 358.38%, and 97.14%) as compared to the control. While combined Pb+ S treatment significantly (DMRT at $p \leq 0.05$) increased the antioxidant machinery of the sunflower plant by 6.62-fold in SOD, 5-fold on POD, and 3.2-fold in CAT as compared to the control. The application of PGPR to lead and salinity

stress soil further increased the antioxidative enzymes in sunflower, though the application of thiamine also augmented the antioxidant enzyme levels in sunflower plants compared to the relevant control but less than the Bacterial and B+T treatment. The B+ T treatment significantly (DMRT at $p \leq 0.05$) improved the activity of SOD, POD, and CAT in sunflower plants growing in lead and salt-stressed soil by 67.74%, 65.08%, and 34.82%, respectively, as compared to Pb+ S treatment (Figure 1).

Effect of *B. Subtilis* IAGS174 and thiamine on phenol, flavonoid and proline contents of *H. annuus* exposed to salinity and Pb stress

A similar trend was observed in the phenol, flavonoid, and proline content of sunflower plants. The application of PGPR and thiamine alone or in combination enhanced phenol, flavonoid, and proline content of plants as compared to the control. Phenol, flavonoid, and proline levels were also improved due to lead and salt stress, alone or in combination. Pb+ S significantly (DMRT at $p \leq 0.05$) increased phenol content by 84.13%, flavonoid content by 2.14-fold, and proline content by 3.31-fold. Combined application of B+ T. under stress conditions also resulted in a substantial (DMRT at $p \leq 0.05$) increase in phenol, flavonoid, and proline content as compared to the respective control (Table 2).

Influence of *B. Subtilis* IAGS174 and thiamine on Malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) levels of *H. annuus* exposed to salinity and Pb stress

Lead and salt stress significantly (DMRT at $p \leq 0.05$) increased the MDA and H_2O_2 levels in sunflower plants by 2.28-fold and 2.08-fold in the Pb+ S treatment compared to the control. The treatment, including PGPR and thiamine in combination, significantly (DMRT at $p \leq 0.05$) reduced the MDA and H_2O_2 levels in sunflower plants raised under lead and salt stress by 26.57% and 39.70%, respectively, as compared to the Pb+ S treatment (Figure 2).

Effect of *B. Subtilis* IAGS174 and thiamine on lead and sodium uptake in *H. annuus*

Under salt stress conditions, sodium uptake by the sunflower plant significantly increased. However, the PGPR inoculation enhanced the Na uptake, while thiamine application reduced the Na uptake. The amount of salt was much more when treated with PGPR than thiamine as PGPR acts as a phyto-extractant, which tends to absorb salt from the soil. Thiamine does phyto-

stabilization by keeping the metal-stabilized near roots, preventing them from entering the plants (Table 3).

A similar trend was observed with Lead (Pb) concentration in the root and shoot of the sunflower. Under thiamine influence, lead uptake was reduced by 28% in the shoot and 18.6% in the root of the sunflower. After PGPR inoculation, significantly (DMRT at $p \leq 0.05$) enhanced uptake was observed in the root and shoot of sunflower by 30.7% and 36.94%, respectively, as compared to the Pb treatment. The combined treatment (B+ T) showed high translocation values but less than PGPR-treatment. Similar results were observed in Pb+ S treatment after applying thiamine and PGPR alone or in combination. Table 3 shows the TF, BCF, and MTI values for thiamine and bacteria/PGPR strain (Table 3).

Principal component analysis

The biplot (loading and score) resulted from the principal component analysis (PCA) to evaluate the efficacy of PGPR strain and thiamine-induced ameliorative effects on the biochemical and physiological attributes of sunflower (*H. annuus*). PCA of plants exposed to lead and salt stress are given in Figure 3. The first two components of PCA, i.e., PC1 and PC2, showed maximum contribution and accounted for 86.5% of the total variance in the given database. The PC1 accounted for a 75.8% variance, while PC2 carried a 10.7% variance in the given dataset correspondingly. All 16 treatments were dispersed successfully by the first two principal components. The distribution pattern provided a clear indication that bacterial strain and thiamine supplementation under combined (lead and salt) stress had a prominent beneficial effect on various growth characteristics of sunflower than the control. The variables aligned with PC1 were correlated positively with each other, such as Root L, Shoot L, Root FW, Root DW, Shoot FW, Shoot DW, total carotenoids, total Chl., Chl. *a* and Chl. *b*. However, a highly negative relationship between the variables of PC1 and PC2 was observed: Shoot Pb; Root Pb; POD; SOD; CAT; Total flavonoid content; Total phenolic content; Proline; MDA; H_2O_2; Shoot Na and Root Na (figure 3).

Treatments under no stress exhibit scores clustered within a relatively small range, having values such as C (4.03, -0.04), B (6.16, 0.57), T (5.55, 0.41), and B+T (7.19, 0.72). However, treatments under salinity stress (S) exhibit scores clustered within a small range with positive PC1 scores and negative PC2 scores such as S (0.04, -2.38), S+B (0.90, -2.40), S+T (0.83, -1.33) and S+B+T (1.34,

-1.92). Treatment under lead (Pb) stress showed negative PC1 scores and positive PC2 scores such as Pb (-2.09, 1.23), Pb+B (-1.50, 2.52), Pb+T (-1.33, 1.36) and Pb+B+T (-1.15, 2.50). Meanwhile, treatment with combined lead and salinity stress (Pb+S) showed negative scores for PC1 and PC2. Pb+S (-4.93, -1.01), Pb+S+B (-5.33, -0.29), Pb+S+T (-4.72, -0.08) and Pb+S+B+T (-5.004, 0.14). All the treatments showed great variability in the PCA biplot **Correlation Analysis:**

A Pearson correlation matrix was performed among the studied parameters of sunflower (Figure 4) indicated by the color scale. Blue, showing a highly positive correlation, light yellow indicating no correlation between the parameters, and dark red showing a highly negative correlation between the parameters. Shoot length (shoot L) was negatively correlated with total phenolics content; total flavonoid content; proline content; SOD; CAT; POD; MDA; H_2O_2; Root Pb; Shoot Pb; Shoot Na, and Root Na. There was a slightly negative correlation between Root Na, Shoot Na, and Root Pb, Shoot Pb. On the other hand, Shoot Length (Shoot L) was positively correlated with root L, Shoot FW, Shoot DW; Root FW; Root DW; Chl. *a*; Chl. *b*; total Chl. and total carotenoids. All of these were significantly and positively correlated with each other as well. The treatments demonstrate (*) within rows that depict significance ($p \leq 0.05$) level.

Discussion

The increasing menace of salinity and heavy metal pollution in soil by natural and anthropogenic sources leads to the disturbance in the crop production system by disrupting soil quality. Many physical, chemical and biological remediation techniques are used to eliminate salt and Pb contamination from soil. One of the leading soil remediation techniques is Phyto-remediation. *B. subtilis* IAGS174 was checked for its potential to tolerate Pb and salt stress in this experiment. In our study, *B. subtilis* IAGS174 survived under different levels of NaCl. In addition, the isolated bacteria showed significant tolerance to Pb stress. In agreement with our results, (Daraz et al., 2023) isolated salt and Cd tolerant and observed that different bacteria might tolerate salt stress to varying levels like *H. bacillus* can tolerate up to 29% of NaCl.

Salinity and heavy metal contamination are the two most important threats to our environment, which are caused by natural and anthropogenic factors and pollute soil, water, and air. These pollutants affect soil fertility and can enter the food chain through crops. For the sustainable production of crops, there is a need to adopt more environmentally friendly remediation techniques

that can eliminate these pollutants or at least reduce their uptake in edible parts. During the current study, Pb/salt tolerant *B. subtilis* IAGS174 and thiamine were selected to reduce salinity and Pb toxicity in sunflower. The environmental variability and confounding factors may interfere with the study findings; hence, these were controlled by adapting a completely randomized research design, replication and precision during experimental setup such as treatment application as well as analysis to get valid and reliable results. Moreover, quality control was also exercised during chemical acquisition, sample preparation, plant analysis and data handling.

During the present study, the negative effects of Pb and salt on growth attributes and photosynthetic pigments of sunflowers relate to Pb and salt phototoxicity, ROS production, and disturbance in the metabolic functions of concerned plants. Disturbance in chlorophyll production and the electron transport chain with stomal closure also reduces plant growth and yield under stressed conditions. The deceleration of the Calvin cycle, impeding disorder in mitochondrial activity and plastoquinone production, and disruption in CO_2 assimilation as well as stomatal conductance during toxic regimes could all be contributing factors to the decrease in plant growth and biomass (Altaf et al., 2021) as *B. subtilis* IAGS174 secrete a variety of growth-promoting biochemicals that increase assisted plant growth and abiotic stress resistance in plants growing under stressed conditions. Previous studies proved that *B. subtilis* helps in the phytoextraction of heavy metals as it increases the stress tolerance of plants, which induces metal translocations in plant roots and shoots (Ayub et al., 2024). The bacteria perhaps improved the growth of the plant exposed to abiotic stress by producing 1-carboxylate (ACC) deaminase, auxin, siderophores, and Fe-proteins. The results of this experiment are in agreement with Ayub et al. (2024), who stated a similar increasing trend for growth increment in brassica by using a combined treatment of two Pseudomonas species (*Pseudomonas fluorescens* and *Pseudomonas gessardi*) with compost under Cd and salt. Moreover, salt-tolerant Bacillus strains NMCN1 and LLCG23 promoted the growth and photosynthetic activity of wheat plants subjected to salinity stress (Ayaz et al., 2022). Additionally, Pb-tolerant Bacillus strains (*Bacillus megaterium* N29 & *Bacillus safensis* N11) improved the growth, photosynthetic, and antioxidant capability of spinach plants grown in sewage water (Ahmad et al., 2021). Thiamine acts as a secondary metabolite and phytostabilizer because it regulates heavy metal translocation in plant roots and shoots. Ahmed & Sattar (2024) reported that thiamine dose enhanced the growth, photosynthetic pigments, and antioxidants in *Vicia faba* plants under saline conditions. Thiamine may have improved salinity and Pb toxicity resilience in

sunflower plants by promoting enzymatic and non-enzymatic activities, expressing stress-alleviating genes and accelerating photosynthetic machinery.

The results of our study depicted a noticeable decline in Chl. *a*, Chl. *b*, total chlorophyll and carotenoid contents in sunflowers under Pb and salinity stress. Heavy metals impede chlorophyll production by deactivating the enzymes involved and impairing their activities. Changes in pigment content under stressful conditions have a direct impact on plant disease and photosynthate production. Secondly, Pb stress inhibits the inclusion of iron (Fe) in the Phyto-porphyrin ring of chlorophyll, which reduces chlorophyll synthesis and ultimately causes damage to chlorophyll molecules (Bender et al., 2025). Under salt stress, plants show a similar trend of decreasing pigment as the photosynthetic rate and stomatal conductance decrease continuously. So, under the combined stress of Pb and salt conditions, our experiment showed the lowest contents of chlorophyll pigments in *H. annuus*. A similar depression for chlorophyll pigments in *Spinacia oleracea* grown under Cd toxicity was observed by Tanveer et al. (2022). Siddika et al. (2024) described that *B. subtilis* augmented the growth and photosynthetic pigments in rice plants grown in saline circumstances. During the present research, plants treated with thiamine and *B. subtilis* showed an increase in the biosynthesis of photosynthetic pigments because PGPR and thiamine can trigger plant defense systems against salt and Pb stress. Thiamine priming can activate the plant defense mechanism under abiotic stress. Thiamine is also known to scavenge or fight against ROS species and thus helps to sustain chlorophyll pigment levels in sunflower plants. Increased plant growth may have resulted from improved photosynthetic pigment production following rhizobacterial inoculation, which restored the plant's photosynthetic potential under Pb and salt stress. Jabeen et al. (2022) observed that thiamine application improved the growth, photosynthetic, and antioxidant parameters of *Brassica oleracea* exposed to arid conditions. The rhizobacteria improved the iron (Fe) uptake, which helped in the chlorophyll synthesis by increasing the leaf area and ultimately enhanced the photosynthetic activity of plants under a stressed environment (Khan et al., 2018).

A higher amount of ROS generated under toxic regimes induces oxidative stress, damaging cellular membranes and DNA in plants (Verma and Dubey, 2003). The results of our study depicted a substantial rise in ROS-initiated oxidative stress in *H. annuus* under Pb and salinity toxicity. The content of anti-oxidant enzymes, i.e., POD, SOD, and CAT, was found to be higher

in Pb and salt-stressed sunflower as compared to controlled conditions. Ahluwalia et al. (2021) observed that heavy metal stress increases the activity of different anti-oxidant enzymes in plants for capturing ROS. Moreover, the presence of *B. Subtilis* and thiamine also showed higher anti-oxidant enzyme production in stressed sunflowers. High amounts of antioxidants are produced to counter the increased content of ROS (reactive oxidative species) in stressed plants. Our results are in agreement with Ullah et al. (2024), who found that PGPRs and biochar boosted anti-oxidative enzymes in stressed plants. The increased activity of anti-oxidant enzymes by bacteria and thiamine decreased the negative effects of salt and lead on sunflower by scavenging ROS. Augmented rate of SOD in plants induced tolerance against pb and salt stress. It converts toxic O_2^- radicals to oxygen molecules. SOD also regulated the intercellular ROS and other physiological conditions in plants exposed to abiotic stress (Faize et al., 2011). Mohamed –Hussein & Orabi (2025) exhibited that thiamine supply diluted the salinity-induced oxidative impairments in sorghum plants by increasing the antioxidant enzymes such as SOD, CAT, APX, GR, and POD. Moreover, thiamine dose improved the enzymatic (SOD, CAT, POD) and non-enzymatic antioxidants (phenol, flavonoid, proline) in maize plants grown in As stress (Atif et al., 2022). Analogous to our investigations, Jamil *et al.* (2024) observed that inoculation of *B. cereus* and *B. aerius* augmented the antioxidant enzymes such as SOD, POD, and CAT in spinach plants grown under heavy metal stress.

During our study, phenol, flavonoid, and proline showed an increasing trend in *H. annuus* under lead and salt stress. Osmoprotectants including soluble sugars, proteins, phenol, flavonoids, proline, and amino acids, accumulate in plants exposed to abiotic stress (Awasthi et al., 2019). The increased rate of flavonoids, phenols, and proline demonstrates the ability of *H. annuus* to withstand Pb and salt stresses (El-Tayeh et al., 2023). Due to this, proline is considered an important osmoregulator for many plants to eliminate salt and Pb stress. Proline serves as the primary source of energy and nitrogen and stabilizes the macromolecular structure. In a similar study, an increased amount of proline was depicted in the canola plant under salt stress compared to non-stressed conditions (Vazayefi et al., 2024).

Using PGPRs and thiamine, sunflower plants may enhance stress tolerance due to up-regulation in soluble protein, proline, phenol, flavonoids, and antioxidant enzyme activity. Proline, phenol, flavonoids, and soluble carbohydrates contribute to membrane integrity and endow stress

resistance in plants. By preventing tissue damage, these systems enable the plant to grow and develop in saltwater and heavy-metal polluted environments. Our results are in agreement with Atif et al. (2024), who depicted that the combined effect of IAA and thiamine increased the amount of proline in stressed plants. Similarly, our study showed higher proline contents in plants under the combined effect of *B. subtilis* and thiamine. An exogenous supply of thiamine augmented stress tolerance in *Pisum stadium* by increasing growth, photosynthesis, antioxidant enzymes, phenolics, and proline (Kausar et al., 2023). By our findings, Khan et al. (2023) revealed that supplementation of *Pseudomonas fluorescens* (NAIMCC-B-00340) and *Azotobacter chroococcum* Beijerinck 1901 (MCC 2351) palliated salinity stress in *Pusa Jagannath* plants by increasing antioxidant enzymes, phenol, flavonoid, and proline contents.

In our study, sunflower plants exhibited higher contents of both H_2O_2 and MDA when exposed to Pb and salinity toxicity. The overabundance of these two biomolecules interfered with the functioning of the cell membrane (Bhat et al., 2022). In contrast, MDA and H_2O_2 levels significantly declined in *B. subtilis* and thiamine-supplied sunflowers under Pb and salt stress. Our results are in agreement with Ayub et al. (2024), who observed the reduced production of MDA, electrolyte leakage, and H_2O_2 in PGPR and compost-supplied plants under stress. The limited production of MDA and H_2O_2 is also associated with the reduced uptake of Pb and Na in thiamine-applied *H. annuus*. Plants treated with thiamine activated their defense mechanisms and restricted the amounts of both MDA and H2O2, thereby reducing the harmful stress effects. Analogous to our findings, Kaya et al. (2020) found that thiamine application significantly ameliorated boron-induced oxidative stress by decreasing H_2O_2, electrolyte leakage and MDA levels in pepper plants. Moreover, in line with our results, Sehrish et al. (2024) found that PGPR-assisted *Triticum aestivum* L. plants exhibited lower levels of MDA, electrolyte leakage, and H_2O_2 when raised under Cd stress.

During our study, it was noted that the amount of lead and Na concentration was increased in roots and shoots in *H. annuus* when treated with *B. subtilis* IAGS174. Our results are in agreement with those of Bender et al. (2025), who noticed the augmented accretion of the metal in microbe-assisted sunflowers under lead stress. Lead-tolerant rhizobacteria might decrease soil pH, which is important for the solubilization and bioavailability of metal. Through alterations in lead and Na availability and solubility as well as redox fluctuations in rhizosphere, *B. subtilis* IAGS174 assisted sunflower to exhibit higher Na and Pb uptake in its root and shoot tissues. In addition to acidifying

the soil, plant roots release protons and organic acids that reduce heavy metal adsorption and promote metal mobility in the rhizosphere. On the other hand, thiamine supply reduced the uptake of Na and Pb in the roots and shoots of sunflower plants. It may be assumed that thiamine adsorbed and immobilized the Na and Pb in roots and restricted the translocation to other plant parts.

In our study, the rate of translocation and bioconcentration factor varied by *B. subtilis* IAGS174 and thiamine application to *H. annuus* under lead and salt stress. Na and Pb-translocation increased in plants from roots to shoots when supplemented with *B. subtilis* IAGS174. The translocation of heavy metals in plants depends upon soil chemistry. So, the application of *B. subtilis* IAGS174 accelerated the extractable amount of Pb and salt in the soil, which led to augmented uptake in roots and shoot tissues of sunflower plants. A higher amount of Pb and salinity was found in the shoots than in the roots. Nevertheless, *B. subtilis* IAGS174-assisted plants exhibited an increased rate of bioconcentration and translocation of Na and Pb. The metal accumulation was more in shoots than roots, corroborating with the study of Atif et al. (2022), which showed more translocation of Arsenic in shoots. Conversely, a decrease in bioconcentration and translocation rate was observed in sunflower plants treated with thiamine. Thiamine as a phytoregulator improved the tolerance capacities of plant roots and immobilized Na and Pb in roots, making it impossible to translocate to other parts of the plant. Similarly, thiamine application declined the Pb and Na accumulation in roots and shoots of *Lens culinaris* exposed to lead stress (Bouhadi et al., 2024). Moreover, Ahmed et al. (2024) reported that thiamine supply lowered the Na uptake in *Vicia faba* plants treated with salinity toxicity. Furthermore, thiamine supplementation reduced the uptake and translocation of Cd in the root and shoot of canola plants under Cd stress (Sanjari et al., 2019). The combined treatment of thiamine and bacteria showed an improved rate of translocation and bioconcentration in *H. annuus,* as the plant is also a hyper-accumulator and has a higher capability for metal uptake (Naheed et al., 2022).

Conclusion

Results of the current study revealed that the salt and Pb stress significantly decreased plant growth, biomass production and photosynthetic pigments. Moreover, salt and Pb stress significantly accelerated the levels of antioxidant enzymes, proline, flavonoids, phenolic contents, MDA and H_2O_2. On the other hand, supplementation of *B. subtilis* and thiamine significantly enhanced the growth, biomass and photosynthetic activity of *H. annuus* under salt and Pb stress

and endowed stress resilience in sunflower. Anti-oxidant enzymes, proline, flavonoid, and phenolic content also increased in bacteria and thiamine applied *H. annuus* exposed to Pb and salt stress. The application of *B. subtilis* IAGS174 and thiamine successfully reduced MDA and H_2O_2 contents in *H. annuus* plants under Pb and salinity stress, which might have palliated oxidative stress in plants. While bioconcentration and translocation of Na and Pb individually were highest in *B. subtilis* IAGS174 assisted *H. annuus* under salt and Pb stress while lower in thiamine-supplemented plants. The results advocated that single or co-application of *B. subtilis* IAGS174 and thiamine conferred Pb and salinity resilience in hyper-accumulator *H. annuus*. The synergistic supply of *B. subtilis* IAGS174 and thiamine may be a suitable, cheap and eco-friendly alternative for the cultivation of ornamental crops in multi-polluted soil. In the future, proteomics, genomics and metabolomics studies for understanding plant stress response and field trials may be exercised to explore more insights into sustainable and eco-green agriculture production under contaminated environments.

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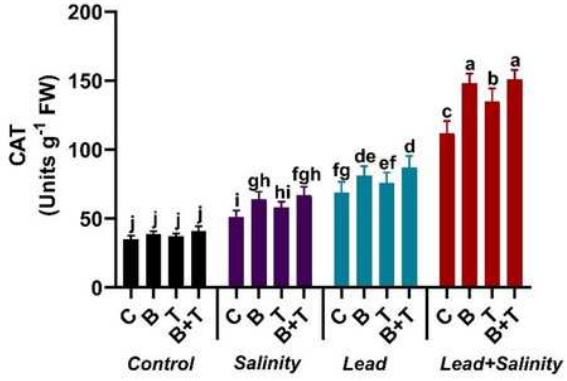
679 substances in peduncles of rose flowers.

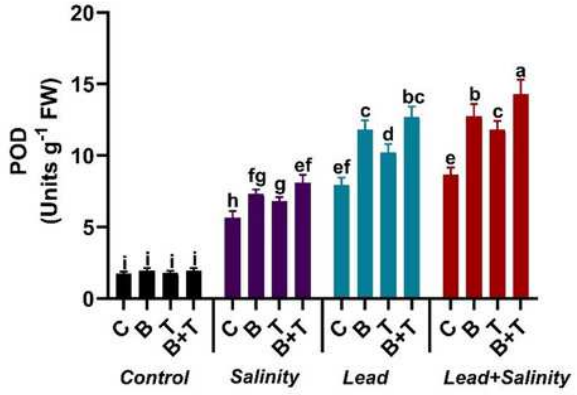
680

Figure 1

Effect of *Bacillus Subtilis* IAGS174 and Thiamine on antioxidant enzymes of *H. annuus* subjected to Pb and Salinity stress.

Values presented are means+SD (n= 3). The different letters in a column show significant difference among the treatments at p>0.05 (DMRT). C ; control, B; bacteria, T; thiamine.


CAT
(Units g-1 FW)
200
150
100
50
0
C B T B+T
Control
Salinity
Lead
Lead+Salinity


POD
(Units g-1 FW)
20
15
10
5
0
C B T B+T
Control
Salinity
Lead
Lead+Salinity

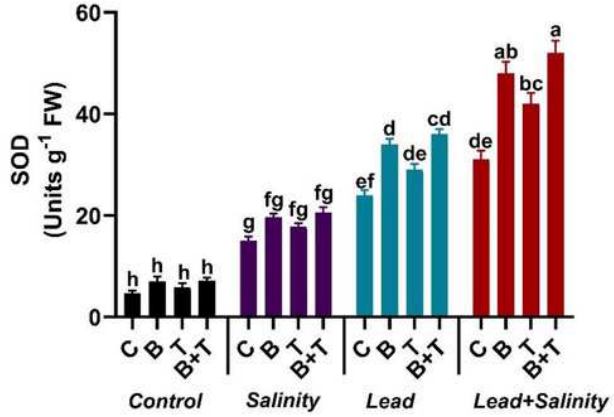
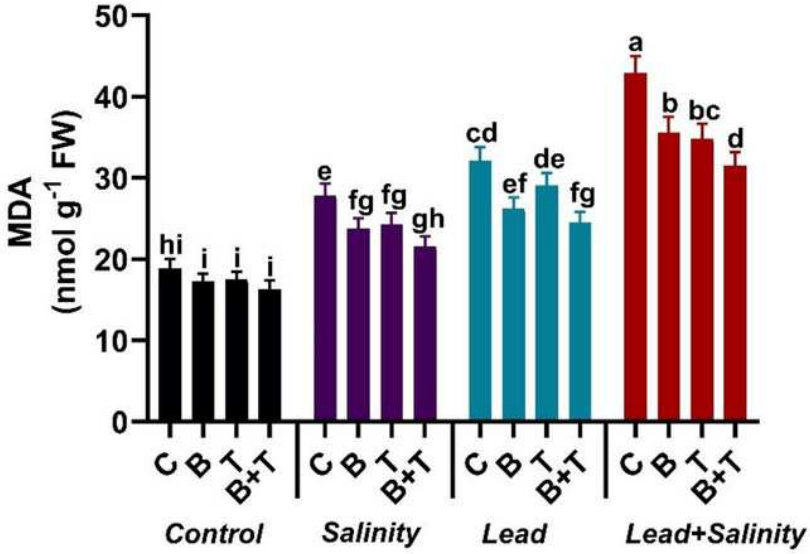

SOD
(Units g-1 FW)
60
40
20
0
C B T B+T
Control
Salinity
Lead
Lead+Salinity

Figure 2

Effect of *Bacillus Subtilis* IAGS174 and Thiamine on Malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) level of *H. annuus* subjected to Pb and Salinity stress.

Values presented are means+SD (n= 3). The different letters in a column show significant difference among the treatments at p>0.05 (DMRT). C ; control, B; bacteria, T; thiamine.


MDA
(nmol g-1 FW)
50
40
30
20
10
0
hi i i i
e fg fg gh
cd ef de fg
a b bc d
C B T B+T
Control
Salinity
Lead
Lead+Salinity

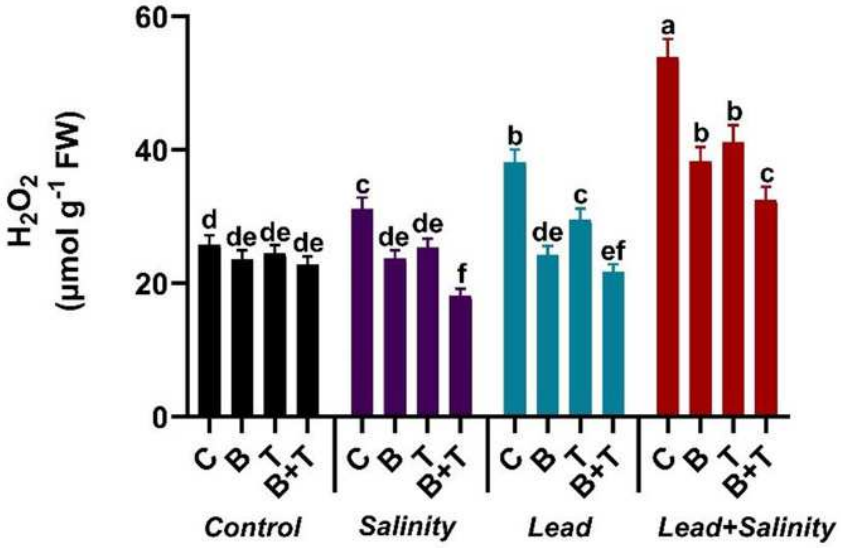

H2O2
(µmol g-1 FW)
60
40
20
0
d de de de
c de de f
b de c ef
a b b c
C B T B+T
Control
Salinity
Lead
Lead+Salinity

Figure 3

Principal Component Analysis of different attributes of *H. annuus* plant.

The abbreviations are as followed: Shoot length: Shoot L, Root length: Root L, Shoot fresh weight: Shoot FW, Root fresh weight: Root FW, Shoot dry weight: Shoot DW, Root dry weight: Root DW, Concentration of Lead in shoot: Shoot.Pb, Concentration of Lead in root: Root.Pb, Malondialdehyde: MDA, Hydrogen peroxide: H_2O_2, Peroxidase: POD, Superoxide dismutase: SOD, Catalase: CAT, Concentration of Sodium in shoot: Shoot. Na, Concentration of Sodium in root : Root. Na, Chlorophyll a: Chl. *a*, Chlorophyll b: Chl. *b*, and Total Chlorophyll: Total Chl., C ; control, B; bacteria, T; thiamine.

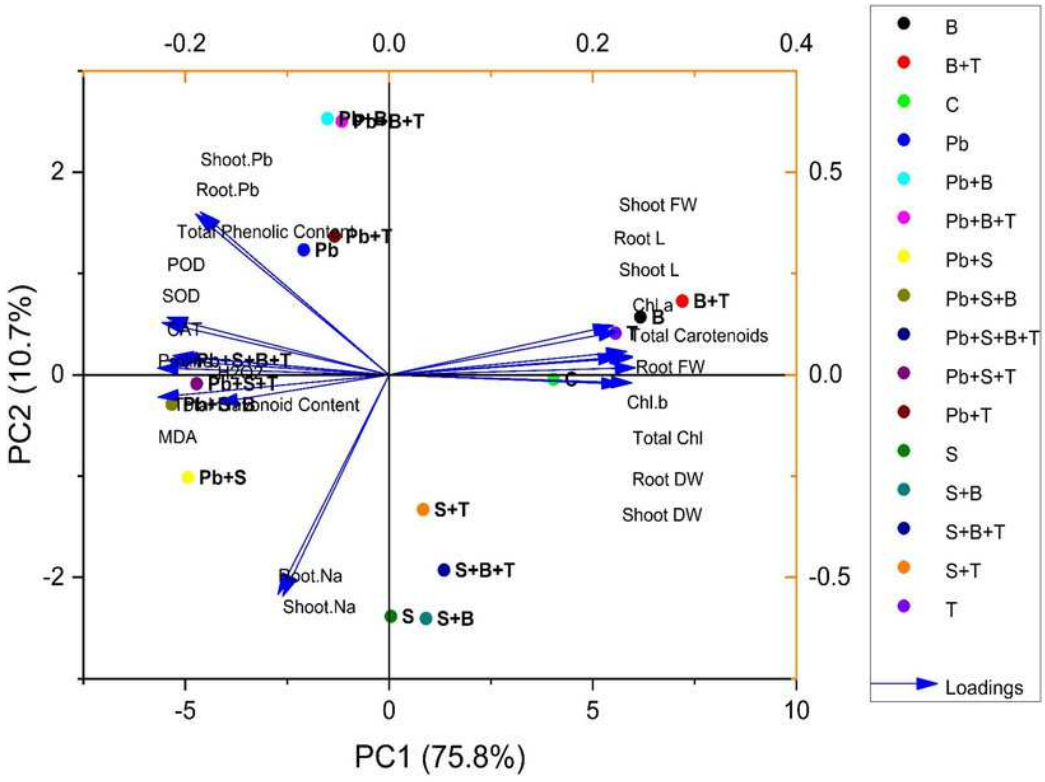

-0.2
0.0
0.2
0.4
2
0
-2
0.5
0.0
-0.5
-5
0
5
10
PC1 (75.8%)
PC2 (10.7%)
Shoot.Pb
Root.Pb
Total Phenolic Content
POD
SOD
CAT
MDA
Pb+S
Root.Na
Shoot.Na
Shoot FW
Root L
Shoot L
Chl.a
Total Carotenoids
Root FW
Chl.b
Total Chl
Root DW
Shoot DW
B
B+T
C
Pb
Pb+B
Pb+B+T
Pb+S
Pb+S+B
Pb+S+B+T
Pb+S+T
Pb+T
S
S+B
S+B+T
S+T
T
Loadings

Figure 4

Correlation matrix representing correlation among different attributes of H. annuus plant.

The abbreviations are as followed: Shoot length: Shoot L, Root length: Root L, Shoot fresh weight: Shoot FW, Root fresh weight: Root FW, Shoot dry weight: Shoot DW, Root dry weight: Root DW, Concentration of Lead in shoot: Shoot.Pb, Concentration of Lead in root: Root.Pb, Malondialdehyde: MDA, Hydrogen peroxide: H_2O_2, Peroxidase: POD, Superoxide dismutase: SOD, Catalase: CAT, Concentration of Sodium in shoot: Shoot. Na, Concentration of Sodium in root : Root. Na, Chlorophyll a: Chl. *a*, Chlorophyll b: Chl. *b*, and Total Chlorophyll: Total Chl., C ; control, B; bacteria, T; thiamine.

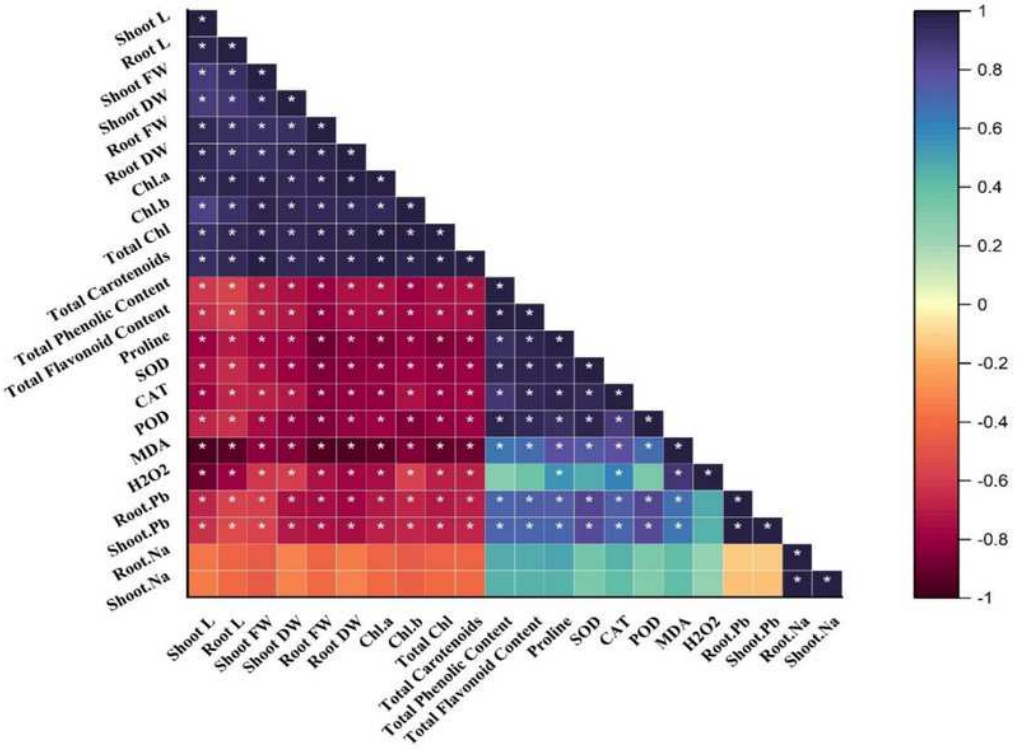

Shoot L
Root L
Shoot FW
Shoot DW
Root FW
Root DW
ChL.a
ChL.b
Total Chl
Total Carotenoids
Total Phenolic Content
Total Flavonoid Content
Proline
SOD
CAT
POD
MDA
H2O2
Root.Pb
Shoot.Pb
Root.Na
Shoot.Na
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Table 1(on next page)

Impact of Bacillus subtilis IAGS174 and thiamine on growth attributes of H. annuus subjected to Pb and salinity stress.

Values are means ±SD of 3 replicates. Different letters in a column depict significant differences among treatments according to DMRT at $p \leq 0.05$. C ; control, B; bacteria, T; thiamine, Pb; Lead stress and S: Salt stress.

Table 1: Impact of *Bacillus subtilis* IAGS174 and thiamine on growth attributes of *H. annuus* subjected to Pb and salinity stress.

Treatments	Growth Attributes					
	Shoot L (cm)	Root L (cm)	SFW (g/plant)	SDW g/plant)	RFW (g/plant)	RDW (g/plant)
C	62±1.63bcde	9.1±1.63c	10.2±1.39cd	3.15±0.03d	1.78±0.06b	0.61±0.02d
B	68±0.85ab	12.2±1.59ab	14.5±1.23a	3.53±0.02b	1.92±0.06a	0.71±0.02b
T	65±1.63bc	11.5±0.66b	13.1±1.15b	3.43±0.02c	1.86±0.03ab	0.65±0.05c
B+T	72±2.45a	13.4±0.54a	15.3±1.01a	4.36±0.03a	1.97±0.02a	0.78±0.05a
S	47±1.63h	5.4±0.24fg	8.5±0.86efj	2.75±0.02f	1.34±0.04c	0.51±0.10h
S+B	60±2.45cdef	8.6±0.16cd	9.1±0.24cdef	2.88±0.02e	1.47±0.03c	0.57±0.04f
S+T	56±2.16ef	7.2±0.29de	8.9±0.08defg	2.85±0.06e	1.41±0.04c	0.54±0.02g
S+B+T	63±2.45bcd	9.4±0.29c	10.5±0.16c	2.9±0.05e	1.51±0.03c	0.59±0.07e
Pb	39±2.45ig	4.1±0.37gh	8.1±0.22efg	2.31±0.01g	1.13±0.02c	0.43±0.02k
Pb+B	54±2.16fg	8.3±0.43cd	9.2±0.17fcde	2.45±0.02g	1.25±0.02c	0.46±0.01j
Pb+T	49±2.94gh	6.8±0.37ef	8.7±0.16defg	2.39±0.02g	1.21±0.05c	0.45±0.02j
Pb+B+T	58±2.44def	8.9±0.67c	9.3±0.16fcde	2.53±0.01g	1.38±0.02c	0.49±0.02i
Pb+S	32±5.10j	3.5±0.33h	7.3±0.08g	2.15±0.02g	0.92±0.02c	0.31±0.02k
Pb+S+B	39±3.74ig	5.3±0.33fg	7.6±0.35fg	2.28±0.09g	0.98±0.08c	0.38±0.05k
Pb+S+T	37±5.09ig	4.7±0.24gh	7.3±0.16g	2.32±0.02g	0.96±0.04c	0.36±0.03k
Pb+S+B+T	41±3.74i	5.6±0.24fg	7.8±0.08efg	2.35±0.01g	1.07±0.18c	0.41±0.02k

Values are means ±SD of 3 replicates. Different letters in a column depict significant differences among treatments according to DMRT at $p \leq 0.05$. C; control, B; bacteria, T; thiamine, Pb; Lead stress and S: Salt stress.

Table 2(on next page)

Impact of Bacillus subtilis IAGS174 and thiamine on photosynthetic attributes (Chlorophyll a, b , total chlorophyll, and Carotenoids) and biochemical parameter (Phenol, Flavonoid, and Proline) of H. annuus subjected to Pb and salinity str

Values are means ±SD of 3 replicates. Different letters in a column depict significant differences among treatments according to DMRT at $p \leq 0.05$. C ; control, B; bacteria, T; thiamine, Pb; Lead stress and S: Salt stress.

Table 1: Impact of *Bacillus subtilis* IAGS174 and thiamine on photosynthetic attributes (Chlorophyll *a, b*, total chlorophyll, and Carotenoids) and biochemical parameter (Phenol, Flavonoid, and Proline) of *H. annuus* subjected to Pb and salinity stress

Treatments	Chl. *a*	Chl. *b*	Total Chl.	Carotenoids	Phenol	Flavonoids	Proline
	mg g^{-1} FW	mg g^{-1} FW	mg g^{-1} FW	mg g^{-1} FW	mg(GAE)/g FW	mg(GAE)/g FW	mmol kg-1 DW
C	2.76±0.10abcd	1.35±0.45abcd	4.12±1.68bc	1.15±0.47ab	37.2±1.7j	12.3±1.81j	12.65±0.51i
B	3.48±0.16ab	1.82±0.25ab	5.3±2.16a	1.64±0.67ab	44.5±2.3ij	14.1±0.68j	12.92±0.64i
T	3.29±0.17abc	1.73±0.71abc	5.05±2.06ab	1.53±0.62ab	42.7±2.1ij	13.4±0.71j	12.71±0.63i
B+T	3.75±0.16a	1.92±0.62a	5.69±2.31a	1.79±0.57a	46.1±2.2i	15.2±0.61j	13.54±0.65i
S	2.14±0.82abcd	0.92±0.36abcd	3.09±1.33d	0.91±0.29ab	54.8±2.4h	17.9±0.87i	27.25±1.31h
S+B	2.62±1.07abcd	0.98±0.75abcd	3.8±1.9bc	1.12±0.44ab	67.6±3.1ef	23.4±1.2fg	29.62±1.45gh
S+T	2.56±1.15abcd	0.93±0.37abcd	3.49±1.13cd	1.05±0.44ab	63.2±2.9fg	21.5±1.1gh	28.93±1.44gh
S+B+T	2.71±1.10abcd	1.06±0.03abcd	3.79±1.26c	1.18±0.59ab	71.5±3.5de	25.2±1.3ef	31.43±1.57fg
Pb	1.92±0.10bcd	0.75±0.31cd	2.68±1.10de	0.83±0.41ab	59.8±2.29gh	20.3±0.75hi	32.36±1.37fg
Pb+B	2.28±0.09abcd	0.84±0.36bcd	3.15±1.28cd	0.95±0.38ab	74.9±3.1cd	27.2±1.72de	36.82±1.50de
Pb+T	2.23±0.74abcd	0.81±0.40bcd	3.07±1.27cde	0.87±0.38ab	69.6±2.7def	23.4±1.1fg	34.97±1.75ef
Pb+B+T	2.36±0.76abcd	0.89±0.35abcd	3.31±1.36cde	0.98±0.49ab	78.3±3.4c	29.6±1.3d	39.81±1.79cd
Pb+S	1.56±0.61d	0.62±0.24d	2.17±0.76e	0.63±0.31b	68.5±3.2def	26.4±1.4e	41.95±2.01c
Pb+S+B	1.69±0.84cd	0.67±0.26d	2.39±0.83e	0.68±0.34b	85.2±4.1b	35.7±1.9b	63.74±3.187a
Pb+S+T	1.64±0.75cd	0.65±0.26fd	2.31±0.98e	0.65±0.32b	79.1±3.9c	32.6±1.7c	57.92±2.84b
Pb+S+B+T	1.79±0.73cd	0.73±0.28cd	2.51±1.02de	0.75±0.29ab	94.7±4.57a	38.3±1.8a	61.93±2.85a

Values are means ±SD of 3 replicates. Different letters in a column depict significant difference among treatments according to DMRT at $p \leq 0.05$. C: control, B: bacteria, T: thiamine, Pb: Lead stress and S: Salt stress.

Table 3(on next page)

Values are means ±SD of 3 replicates. Different letters in a column depict significant difference among treatments according to DMRT at $p \leq 0.05$. C : control, B: bacteria, T: thiamine, Pb: Lead stress and S: Salt stress. TF: Translocation factor; B

Influence of Bacillus subtilis IAGS174 and thiamine on Na and Pb uptake, Translocation factor, Bioconcentration factor and Metal Tolerance index of H. annuus plants subjected to Pb and salinity stress

Table 1: Influence of *Bacillus subtilis* IAGS174 and thiamine on Na and Pb uptake, Translocation factor, Bioconcentration factor and Metal Tolerance index of *H. annuus* plants subjected to Pb and salinity stress

Treatments	Sodium (Na) Uptake					Lead (Pb) Uptake				
	Root (Na) mg kg^{-1}	Shoot (Na) mg kg^{-1}	TF	BCF	MTI	Root (Pb) mg kg^{-1}	Shoot (Pb) mg kg^{-1}	TF	BCF	MTI
C	1.51±0.16e	1.72±0.11g	1.13cd	0.003de	100cd	ND	ND	-	-	-
B	1.69±0.22e	1.89±0.17g	1.12d	0.004d	110bc	ND	ND	-	-	-
T	1.48±0.22e	1.53±0.25g	1.03f	0.003de	89e	ND	ND	-	-	-
B+T	1.62±0.24e	1.71±0.23g	1.06ef	0.003de	99d	ND	ND	-	-	-
S	41±10.23bc	57±9.90cd	1.40ab	0.114ab	100cd	ND	ND	-	-	-
S+B	51±3.27a	73±3.74a	1.43a	0.146a	128a	ND	ND	-	-	-
S+T	29±4.55d	38±5.35f	1.31bc	0.076bc	67ef	ND	ND	-	-	-
S+B+T	48±3.74ab	65±5.1b	1.35b	0.13ab	114b	ND	ND	-	-	-
Pb	0.85±0.22e	0.93±0.22g	1.09e	0.002e	100cd	91±3.74c	157±2.94d	1.73b	1.05bc	100bc
Pb+B	1.02±0.25e	1.14±0.07g	1.12d	0.002e	122ab	119±4.55a	215±3.27a	1.81a	1.43a	137a
Pb+T	0.79±0.25e	0.82±0.08g	1.04f	0.002e	88e	74±2.94e	113±5.10f	1.53c	0.75d	72d
Pb+B+T	0.91±0.10e	0.98±0.36g	1.08ef	0.002e	105c	108±5.10b	191±4.55b	1.77ab	1.27ab	122ab
Pb+S	37±4.9c	51±4.55de	1.38ab	0.102b	100cd	83±4.55d	139±6.68e	1.68bc	0.93c	100bc
Pb+S+B	41±4.32bc	59±4.55bc	1.44a	0.118ab	116b	96±5.89c	168±5.89c	1.75ab	1.12b	121ab
Pb+S+T	27±5.1d	32±2.94f	1.19cd	0.064c	63f	71±3.74e	109±4.32f	1.54c	0.73d	78c
Pb+S+B+T	39±4.55c	48±4.55e	1.23c	0.096bc	94de	92±9.63c	158±4.24d	1.71bc	1.05bc	114b

Values are means ±SD of 3 replicates. Different letters in a column depict significant difference among treatments according to DMRT at $p \leq 0.05$. C: control, B: bacteria, T: thiamine, Pb: Lead stress and S: Salt stress. TF: Translocation factor; BCF: Bio-concentration factor; MTI: Metal tolerance index