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Synergistic role of PGPR and fungal isolates in mitigating As and Pb stress and promoting growth of *Arachis hypogaea* L.

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Abstract Heavy metals, also known as toxic metals, are major environmental pollutants that adversely impact all forms of life, disturbing soil ecology and reducing agricultural productivity. Certain indigenous microbes possess remarkable tolerance to these metals and play a vital role in restoring contaminated soils. In the present study, plant growth-promoting rhizobacteria and fungi were isolated from the rhizosphere and evaluated for their metal tolerance potential. Among the bacterial isolates (*Pseudomonas alcaliphila* PAS1, *Pseudomonas aeruginosa* PAS2, *Pseudomonas toyotomiensis* PTS3, *Bacillus subtilis* BSS1) expressing resistance to arsenic and lead which observed up to concentrations of 100–1200 ppm on nutrient agar. The fungal isolate *Aspergillus japonicus* (AJ01) exhibited tolerance up to 200 ppm of Pb and 500 ppm of As. Pot culture experiments were using *Arachis hypogaea* grown under As and Pb stresses with treatments of PGPR, PGPF, Compost and chemical control. *Pseudomonas alcaliphila* PAS1 was significantly enhanced plant growth by 100% and *Pseudomonas aeruginosa* PAS2 by 60% in contaminated soil, indicating strong tolerance and growth-promoting potential. *Pseudomonas alcaliphila* PAS1 increased chlorophyll content by 92.3% under As stress as compared to the control, while *Pseudomonas toyotomiensis* PTS3 showed a 70% increased, demonstrating high metal tolerance. In contrast, As and Pb stressed plants showed adverse effects. These findings highlighted the potential of selected PGPR and fungi as eco-friendly alternatives for the remediation and recovery of heavy metal-contaminated soils in *Arachis hypogaea* cultivation.

Keywords: *Pseudomonas*, *Arachis hypogaea*, Chemical fertilizer, PGPF, Arsenic, Lead

Introduction

The excessive use of chemical fertilizers and pesticides, driven by rapid industrialization and diverse anthropogenic activities, has caused severe ecological disturbances and human health issues worldwide (Zhou *et al.,* 2025). Among the various environmental pollutants, heavy metal contamination of soil

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has emerged as one of the most critical global challenges (Gupta *et al*., 2024). Heavy metals such as arsenic (As) and lead (Pb) are persistent, non-biodegradable, and tend to accumulate in the soil, resulting in long term environmental toxicity. Arsenic (As), a Group I carcinogen, is a naturally occurring but highly toxic and persistent element (Nigra *et al.,* 2022). It enters agricultural soils mainly through industrial effluents, mining, and contaminated irrigation water. Excessive arsenic uptake disrupts plant growth, reduces chlorophyll content, and damages cellular components such as DNA and proteins, ultimately impairing metabolism and causing plant death (Rai *et al*., 2022). Lead (Pb), a persistent and non-essential metal, readily accumulates in plants, disrupting metabolic and enzymatic activities. In humans, chronic exposure causes anemia, kidney damage, and neurological disorders, particularly in children (Aslam *et al*., 2021). In plants, Pb toxicity leads to stunted growth, chlorosis, oxidative stress, and impaired seed germination due to disrupted protein and starch metabolism (Srivastava *et al*., 2023; Talha *et al*., 2023).

Phytoremediation is a sustainable approach for detoxifying contaminated soils through plant-mediated absorption, stabilization, and transformation of toxic metals. The rhizosphere plays a vital role in this process by supporting beneficial microbes such as plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF), which enhance nutrient uptake, hormone balance, and stress tolerance. PGPR aid heavy metal detoxification through nutrient solubilization, nitrogen fixation, phytohormone regulation, siderophore production, and enzymatic activity (Li *et al*., 2023; Sheng *et al*., 2025, Sridhar *et al*., 2025). Fungi play a vital role in metal contaminated ecosystems through diverse biochemical and ecological detoxification mechanisms. Their extensive mycelial networks immobilize or transform heavy metals via ion efflux, chelation, enzymatic conversion, and vacuolar sequestration. The synthesis of melanin, organic acids, and metal binding proteins further enhances their metal tolerance. Thus, both PGPR and PGPF act synergistically to promote plant growth under metal stress and support effective soil bioremediation (Akpasi *et al.,* 2023; Amin *et al.,* 2024). The present study was designed to utilize PGPR, PGPF as compared to chemical control for heavy metal mitigation. The study evaluated the tolerance levels of isolated bacterial and fungal strain against toxic metals (As and Pb) and determined their maximum tolerance capacities. Furthermore, comparative pot culture experiments were conducted using *Arachis hypogaea* plants treated with PGPR, PGPF, and chemical control. This comparative approach aimed to identify efficient microbial and organic treatments capable of sequestering arsenic and lead, thereby serving as a sustainable bioremediation strategy and contributing to the advancement of bioremediation and mycoremediation sciences.

Materials and method

Assessment of potential plant growth promoting rhizobacteria

PGPR biostimulant strains selected as candidate organisms for the experimental design were *Pseudomonas alcaliphila, Pseudomonas aeruginosa, Pseudomonas toyotomiensis,* and *Bacillus subtilis*, which were previously isolated from rhizospheric soil. The physicochemical properties of the soil were analyzed. The rhizospheric soil was aseptically collected, serially diluted, and appropriate dilutions were spread onto Luria–Bertani (LB) medium. The plates were incubated at 30 °C for 24–48 h (Sathya *et al.,* 2024). Bacterial colonies exhibiting distinct morphological characteristics were serially purified and subsequently identified through 16S rRNA gene sequencing. The identified strains were deposited in the culture collection of the Soil Biology and PGPR Laboratory, Department of Botany, Periyar University, Salem, Tamil Nadu, India.

Molecular profiling of a PGPR biostimulant strain

Genomic DNA was extracted from each isolate, and its quality was verified using 0.8% agarose gel electrophoresis. The universal 16S rRNA gene region was amplified via PCR employing primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) following the method described by Giongo *et al*. (2010). The purified PCR amplicons were sequenced using the BigDye Terminator 3.1 sequencing kit (Applied Biosystems, USA). Sequence homology was determined using BLASTn (Basic Local Alignment Search Tool) against the GenBank database of the National Center for Biotechnology Information (NCBI). Phylogenetic relationships of the 16S rRNA sequences were analyzed and aligned using Molecular Evolutionary Genetics Analysis software (MEGA version 11) (Tamura *et al.,* 2021). A phylogenetic tree was constructed by the Neighbor-Joining method with 1000 bootstrap replicates, and the resulting sequences were submitted to the NCBI GenBank database (Figure 1).

Heavy metal stress tolerance test of PGPR biostimulant strains

Agar dilution method was used to analyze the arsenic and lead tolerance of bacterial isolates (Cervantes-Vega *et al*., 1986). Freshly prepared nutrient agar (NA) plates were supplemented with different concentrations (100, 250, 500,

800, and 1200 ppm) of arsenic trioxide and lead acetate as sources of heavy metals. Control plates contained NA without any metal supplementation. The plates were then incubated at room temperature for 24–48 hours. After incubation, bacterial tolerance to arsenic and lead was assessed based on the presence or absence of growth.

Assessment of potential plant growth promoting fungi

Heavy metal tolerant fungus *Aspergillus japonicus* isolate AJ01 was provided by Prof. Dr. Kasem Soytong (KMITL), Bangkok, Thailand. Prior to the experiments, a single-spore culture of the fungal strain was grown on potato dextrose agar (PDA) for 7 days (López *et al*., 2020).

Molecular profiling of fungal isolates

Genomic DNA from the fungal isolate was extracted using the cetyltrimethylammonium bromide (CTAB) method (White *et al*., 1990). The 18S ribosomal RNA (rRNA) gene was amplified through polymerase chain reaction (PCR) using specific forward and reverse primers. The PCR reaction mixture contained 1 µL of genomic DNA, 1.5 µL of Taq DNA polymerase, 5 µL of dNTPs, 10× polymerase buffer, and 1 µL of each primer. The amplification was carried out in a thermocycler programmed as follows: initial denaturation at 94°C for 5 min, followed by 32 cycles of denaturation at 94 °C for 40 s, annealing at 57 °C for 40s, and extension at 72 °C for 40s. The PCR products were subsequently sequenced, and the resulting sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) available in the NCBI database for identification.

Heavy metal stress tolerance assay of PGPF isolates

The minimum inhibitory concentration (MIC) of metals that suppressed fungal growth was evaluated using the agar dilution method. Different concentrations of As and lead Pb salts, ranging from 100 to 1200 ppm, were incorporated into Potato Dextrose Agar medium. The control plates contained PDA without any metal supplementation. Each plate was inoculated with the fungal isolate in triplicate to assess growth responses. The cultures were incubated at 24 ± 2 °C for one week, and the MIC values for both metals were subsequently determined following the method of Akbar *et al*. (2022).

Evaluation of plant growth promoting efficacy under heavy metal stress in groundnut

The present study investigated the influence of plant growth-promoting bacterial and fungal strains on the development of *Arachis hypogaea* seedlings exposed to arsenic (As) and lead (Pb) stress. Agricultural soil collected from Bangkok, Thailand, was air-dried, sieved, and analyzed for its physicochemical characteristics, including organic matter content, electrical conductivity, total nitrogen, available potassium and phosphorus, pH, and texture. The soil was artificially amended with 200 ppm concentrations of both arsenic trioxide and lead acetate. For each treatment, pots were filled with 2 kg of soil, and one seeds were sown per pot three replicates. After 30 DAI, plants were carefully uprooted, washed, and assessed for parameters such as root and shoot length, and fresh and dry biomass to determine the growth promoting potential of the microbial inoculants under heavy metal stress.

Pot experiment for arsenic stress on *Arachis hypogaea* was designed in Completely Randomized Design (CRD) with 3 replications. Treatments were *Pseudomonas alcaliphila, Pseudomonas aeruginosa, Pseudomonas toyotomiensis, Bacillus subtilis, Aspergillus japonicus,* compost:soil (a ratio of 1:10) and chemical fertilizer (15-15-15) at 5 g/pot. Loamy soil was prepared, sterilized and mixed arsenic at 200 ppm in each pot before planting the seed of peanut (*Arachis hypogaea*).

Pot experiment for lead stress on *Arachis hypogaea* was designed in Completely Randomized Design (CRD) with 3 replications. Treatments were *Pseudomonas alcaliphila, Pseudomonas aeruginosa, Pseudomonas toyotomiensis, Bacillus subtilis, Aspergillus japonicus,* compost:soil (a ratio of 1:10) and chemical fertilizer (15-15-15) at 5 g/pot. Loamy soil was prepared, sterilized and mixed arsenic at 200 ppm in each pot before planting the seed of peanut (*Arachis hypogaea*).

Assessment of plant growth parameters

After 30 DAI of growth, the plants were harvested and thoroughly rinsed with deionized water to remove any adhering soil particles. The growth parameters, including shoot length, root length, and fresh weight, were measured immediately. The plant samples were then oven-dried at 60 °C for 72 hours to obtain the dry weight. Subsequently, the dried shoots and roots were ground into fine powder and sieved through a 2 mm mesh for further analysis.

Estimation of total chlorophyll

The total chlorophyll content (chlorophyll a and b) was determined following Arnon's method. Fresh leaf tissue (1 g) was ground in 80% acetone to extract the pigments, and the homogenate was centrifuged at 10,000 rpm for 10 minutes at 4 °C. The absorbance of the clear supernatant was recorded at 645 nm and 663 nm, and the concentrations of chlorophyll a and chlorophyll b were calculated using Arnow's equations**.**

$$\text{Total chlorophyll (mg/g)} = \frac{20.2\ (A_{645}) - 8.02\ (A_{663}) \times v}{1000 \times FW}$$

Physiochemical analysis of soil samples

After completing the 30-day pot culture experiment using *Arachis hypogaea* plants, soil samples from all treatments were collected and subjected to physicochemical and nutrient analyses. Basic soil properties such as pH and EC were determined. Macro-nutrients, including nitrogen, phosphorus, and potassium, as well as micro-nutrients such as iron, manganese, zinc, and copper, were analysed using standard procedures. Additionally, the applied heavy metals arsenic, cadmium, mercury, and lead were quantified to assess their residual levels and potential uptake by the plants metals As, Cd, Hg, Pb were measured to evaluate their residual levels and possible uptake by plants.

Statistical analysis

All experiments were performed in triplicate, and the results are expressed as mean ± SD. Statistical analysis was conducted using one-way ANOVA in SPSS. Differences were considered statistically significant at $P < 0.05$.

Results

Molecular profiling of rhizobacterial isolates

The 16S rRNA gene of the bacterial isolates was amplified by PCR using universal primers, and the products were analyzed through agarose gel electrophoresis, yielding amplicons of approximately 1 kb in size. The obtained 16S rRNA gene sequences were subjected to BLASTn analysis for molecular identification of the isolates and showed high similarity with available sequences

in the GenBank database (Table 1). A phylogenetic tree was constructed based on the 16s rRNA gene sequence using the neighbor-joining method in MEGA version 11.0. Phylogenetic analysis revealed that isolate PAS1 corresponded to *Pseudomonas alcaliphila*, PAS2 to *Pseudomonas aeruginosa* PTS3 to *Pseudomonas toyotomiensis*, and BSS1 to *Bacillus subtilis* (Figure 1). The 16S rRNA gene sequences of these biostimulant strains were deposited in the GenBank database (NCBI).

Table 1. 16S rRNA gene-based identification of biostimulant isolates

Isolate code	Identified species	Similarity	Gene accession number
PAS1	*Pseudomonas alcaliphila*	100 %	OQ804624
PAS2	*Pseudomonas aeruginosa*	99.34%	OQ804631
PTS3	*Pseudomonas toyotomiensis*	100 %	OQ804642
BSS1	*Bacillus subtilis*	99.79 %	OQ804638

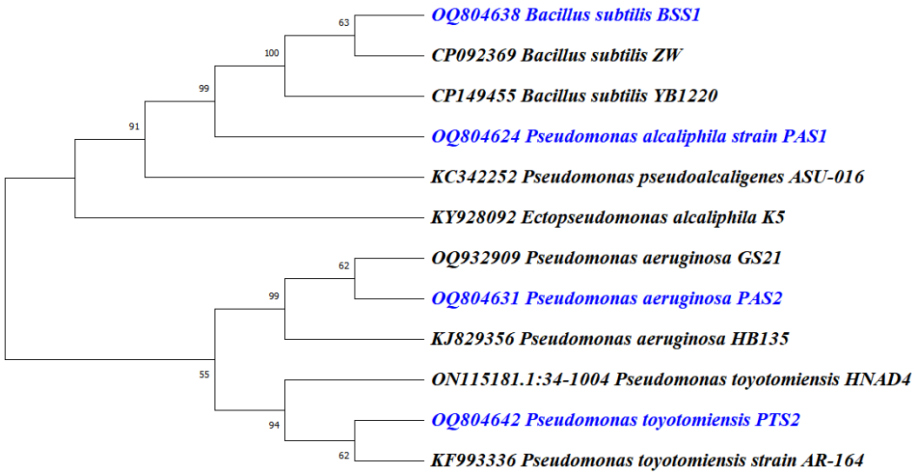


Figure 1. A phylogenetic tree (A) *Pseudomonas alcaliphila (OQ804624) (B) Pseudomonas aeruginosa* (OQ804631) (C) *Bacillus subtilis* (OQ804638) (D) *Pseudomonas toyotomiensis* (OQ804642) was constructed using partial 16S rRNA nucleotide sequences and the clustering algorithm

Molecular phylogeny of Aspergillus japonicus

Molecular phylogenic identification was confirmed the species level. *Aspergillus japonicus* isolate AJ01 found to be closely related to AJB76880 *Aspergillus japonicus*, KX575852 *Aspergillus japonicus* isolate A1ON532708 *Aspergillus japonicus* isolate 2B6, JF770435 *Aspergillus japonicus* isolate AJP01 which compared to U38558 *Fusarium solani* was outgroup (Figure 2).

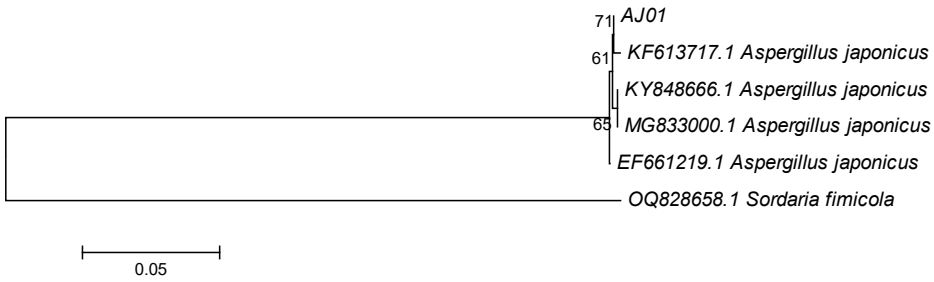
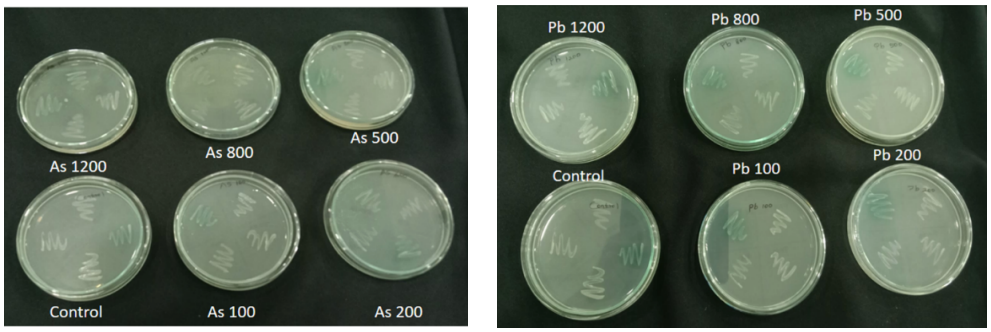


Figure 2. Phylogenetic tree of *Aspergillus japonicus* isolate AJ01 from GenBank with 100% bootstrap value constructed after the distance-based analysis of the universal primer

Determination of bacterial isolates resistance to heavy metals on agar plates

The four bacterial isolates demonstrated tolerance to varying concentrations of arsenic (As) (Figure 3A) and lead (Pb) (Figure 3B). When tested for metal resistance, all isolates were able to grow at concentrations ranging from 100 to 1200 ppm of the respective metals. The minimum inhibitory concentration (MIC) values for isolates PAS1, PAS2, PTS3, and BSS1 against arsenic and lead were recorded as 100, 200, 500, 800, and 1200 ppm, respectively. After seven days of incubation, colony diameter measurements revealed that maximum growth occurred at 500 ppm of metal concentration, while a gradual decline in colony size was observed with increasing concentrations. The smallest colony diameters were noted at 1200 ppm.



A **B**

Figure 3. PGPR strains PAS1, PAS2, PTS3, and BSS1 isolates were grown under 100 to 1200 ppm (A) Arsenic stress (B) Lead stress

Assessment of the minimum inhibitory concentration and tolerance index of the fungal strains

The fungal isolates were evaluated for heavy metal resistance by determining their Minimum Inhibitory Concentration (MIC) and Tolerance Index (TI). The isolated fungal strain *Aspergillus japonicus* AJ01 exhibited varying levels of tolerance to arsenic (As) and lead (Pb). *Aspergillus japonicus* AJ01 was most tolerant to As with an MIC of 500 ppm, and least tolerant to Pb with an MIC of 200 ppm (Figure 4).

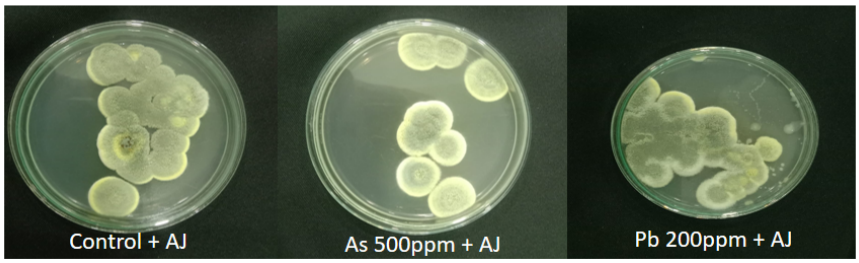


Figure 4. *Aspergillus japonicus* AJ01 showed growth on PDA containing 500 ppm As and 200 ppm lead Pb

Root and shoot length analysis in plants inoculated with growth promoting strains

After 30 (DAI) of *Arachis hypogaea* plant growth under heavy metal stress (200 ppm of As and Pb), the effects of different bacterial strains *Pseudomonas alcaliphila* (PAS1), *Pseudomonas aeruginosa* (PAS2), *Pseudomonas toyotomiensis* (PTS3), and *Bacillus subtilis* (BSS1) along with the fungal strain *Aspergillus japonicas* (AJ01), compost, and chemical fertilizer treatments were evaluated under pot culture conditions (Figures 5 and 6). Under As stress, bacterial treatments enhanced root length by 66%, 70%, 68.5%, and 68.4%, and shoot length by 42.8%, 60%, 55.5%, and 25% in the presence of PAS1, PAS2, PTS3 and BSS1, respectively. Similarly, under Pb stress, bacterial treatments also exhibited an increase in root length by 86.27%, 76.4%, 66.6%, and 74.5%, and in shoot length by 22.3%, 20.2%, 18.8% and 16% with PAS1, PAS2, PTS3, and BSS1, respectively (Figure 7). The PGP fungal strain *Aspergillus japonicus* AJ01 also demonstrated a significant positive effect on both root and shoot growth under As and Pb stress. Compost and chemical fertilizer treatments showed only moderate improvement compared to bacterial and fungal inoculations.

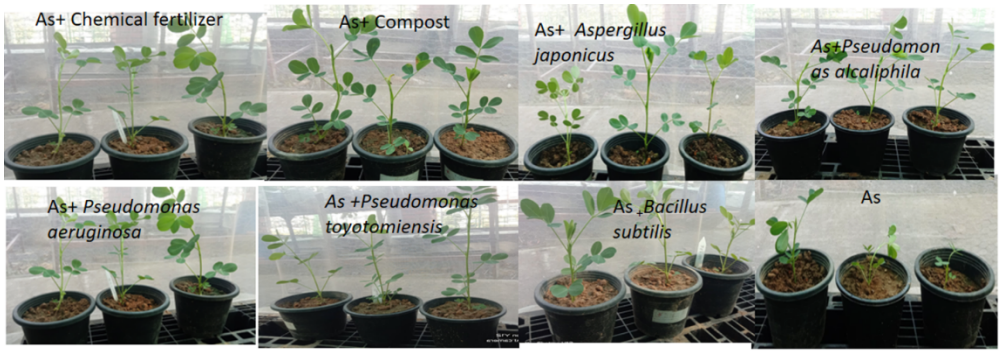


Figure 5. Pot culture studies on *Arachis hypogaea* plants under arsenic stress using PGPR, PGPF, compost, and chemical fertilizers

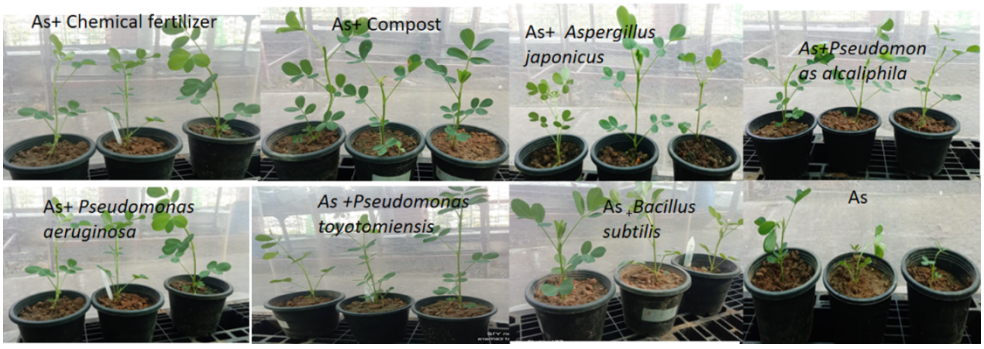
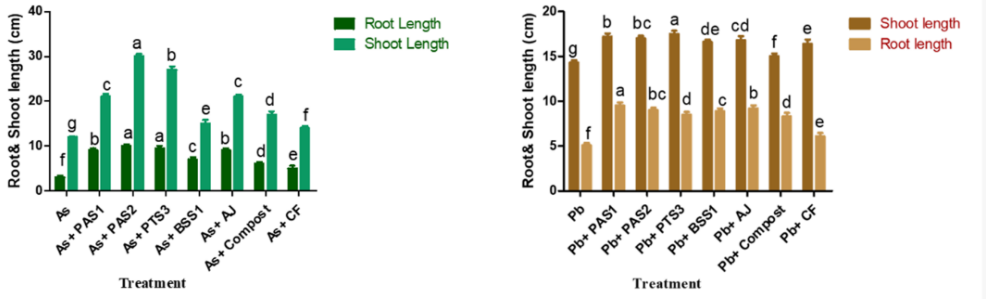


Figure 6. Pot culture studies on *Arachis hypogaea* plants under arsenic stress using PGPR, PGPF, compost, and chemical fertilizers

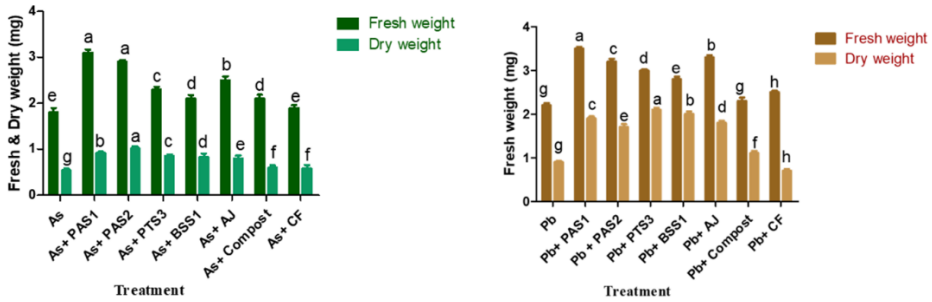


A B

Figure 7. Effect of PGPR (PAS1, PAS2, PTS3, BSS1) and PGPF (AJ01) on the Root and shoot length of *Arachis hypogaea* under As and Pb stress: (A) As and (B) Pb. Data represent mean ± SD (n = 3). Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$)

Effect of A. hypogea on plant weight under heavy metal stress using plant growth promoting strains

The bacterial strains PAS1, PAS2, PTS3, BSS1 and the fungal strain *Aspergillus japonicus* AJ01 were inoculated into As and Pb induced rhizosphere soils planted to *Arachis hypogaea*. Under As stress, compared with uninoculated control plants, the fresh and dry weight of PAS1-inoculated plants increased significantly by 72.2% and 40.4%**,** respectively (Figure 8). In contrast, *Aspergillus japonicus*-inoculated plants showed a greater increase in fresh and dry weight by 38.8% and 48.14%**,** respectively, compared with the control. Under Pb stress, PTS3-inoculated plants also exhibited significant increases in fresh and dry weight by 133% and 36.3%**,** respectively, relative to the uninoculated control. Similarly, *Aspergillus japonicus*-inoculated plants showed increases of 100% and 48.5% in fresh and dry weight, respectively. Compost-treated plants under As and Pb stress showed moderate improvements, with 16.6% and 11.1% increases in root length and 18.8% and 33.2% increases in shoot length, respectively, compared with control plants.



A B

Figure 8. Effect of PGPR (PAS1, PAS2, PTS3, BSS1) and PGPF (AJ01) on the fresh weight and dry weight of *Arachis hypogaea* under As and Pb stress: (A) As and (B) Pb. Data represent mean ± SD (n = 3). Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$)

Estimation of total chlorophyll contents in A. hypogaea plants under As and Pb stress

The total chlorophyll content was analyzed in *Arachis hypogaea* plants under As and Pb stress with inoculation of plant growth-promoting bacteria and fungi and compared with uninoculated control plants. Under As stress, total chlorophyll contents in PAS1, PAS2, PTS3, and BSS1 inoculated plants were significantly increased by 92.3%, 76.9%, 53.8%, and 46.15%, respectively,

compared with the uninoculated control (Figure 8). Similarly, *A. japonicas* inoculated under As stressed plants showed an 84.61% increase in chlorophyll content. Under Pb stress, total chlorophyll contents in PAS1, PAS2, PTS3, and BSS1 inoculated plants increased by 100%, 80%, 80%, and 66%, respectively (Figure 8). Likewise, *Aspergillus japonicas* inoculated Pb stressed plants showed a 66% increase in chlorophyll content compared with uninoculated plants. These results indicate that PAS1 inoculation significantly enhanced plant growth and chlorophyll accumulation under As and Pb stress. Chemical fertilizer and compost did not significantly enhance the chlorophyll content in *Arachis hypogaea* plants both As and Pb stress.

Soil chemical properties, nutrients and heavy metals after pot culture

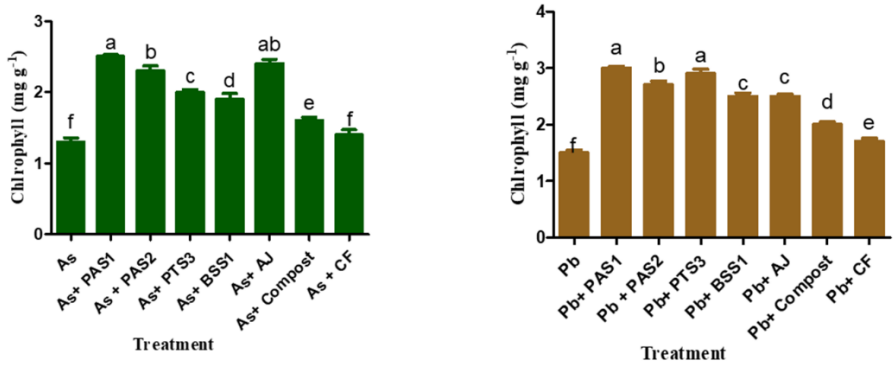
There was a significant variation in the physicochemical parameters of all soil samples collected from the eight different treatments after conducting the pot culture experiment using *Arachis hypogea*. The details are presented in Tables 2, 3. A comparison of arsenic- and lead-contaminated soils treated with PGPR, PGPF, compost, and chemical fertilizer is also analyzed.

Table 2. Effect of PGPR and PGPF on pot culture studies in *Arachis hypogea* under arsenic stress

Elements	**As**	**As+ compost**	**As+ Chemical Fertilizer**	**As+ AJ01**	**As+ PAS1**	**As+ PAS2**	**As+ PTS3**	**As+ BSS1**
Physicochemical analysis								
EC	4.20	2.0	3.2	1.1	1.8	2.3	0.6	2.7
pH	5.3	6.3	6.0	6.8	6.9	7.0	7.2	6.0
Macronutrients (mg/kg)								
N	6.6	25.8	20.5	19.0	46.6	18.3	18.5	15.8
P	0.21	0.58	0.11	0.88	0.52	0.60	0.42	0.33
K	0.7	0.66	0.24	0.36	0.72	0.55	0.43	0.44
Micronutrients (mg/kg)								
Ca	3.2	2.5	4.2	6.6	3.2	2.5	4.2	6.6
S	8.7	16.1	27.4	15.9	8.7	16.1	27.4	15.9
Mg	18	28.5	16.6	23.8	18	28.5	16.6	23.8
Cu	1.59	2.79	7.76	4.43	1.59	2.79	7.76	4.43
Mo	0.40	0.60	0.84	0.50	0.40	0.60	0.84	0.50
B	18	28.5	16.6	23.8	18	28.5	16.6	23.8
Zn	1.4	0.61	0.26	2.2	1.4	0.61	0.26	2.2
Fe	59	41	51.5	39	59	41	51.5	39
Heavy metals (mg/kg)								
As	74.5	62.1	55.2	45.1	33.6	31.8	30.4	30.6
Hg	0.01	0.02	0.01	0.03	0.01	0.01	0.01	0.01
Pb	0.07	0.03	0.05	0.01	0.02	0.01	0.01	0.01
Cd	0.03	0.04	0.05	0.04	0.06	0.05	0.06	0.05

Table 3. Effect of PGPR and PGPF on pot culture studies in *Arachis hypogea* under lead stress

Elements	As	As+ compost	As+ Chemical Fertilizer	As+ AJ01	As+ PAS1	As+ PAS2	As+ PTS3	As+ BSS1
Physicochemical analysis								
EC	4.20	2.0	3.2	1.8	1.8	2.3	1.6	1.7
pH	5.8	6.3	6.6	6.9	7.2	7.8	7.4	6.8
Macronutrients (mg/kg)								
N	7.6	55.8	30.4	24.0	46.6	21.3	43.5	33.8
P	1.21	4.58	3.11	5.88	4.52	6.60	3.42	4.39
K	0.7	0.66	0.24	0.36	0.72	0.55	0.43	0.44
Micronutrients (mg/kg)								
Ca	2.8	2.9	3.2	5.6	3.0	8.1	6.2	8.6
S	9.7	44.1	27.4	55.9	6.7	61.1	62.4	51.9
Mg	28	52.5	74.6	54.8	66	31.5	63.6	45.8
Cu	1.59	2.79	7.76	4.43	1.59	2.79	7.76	4.43
Mo	0.40	0.60	0.84	0.50	0.40	0.60	0.84	0.50
B	18	28.5	16.6	23.8	18	28.5	16.6	23.8
Zn	1.8	0.75	0.62	2.67	1.44	0.82	0.53	2.31
Fe	31	41	59.5	59	69	51	41.8	49
Heavy metals (mg/kg)								
As	1.5	0.71	0.81	0.53	0.46	0.88	0.43	0.64
Hg	0.15	0.07	0.03	0.05	0.06	0.09	0.11	0.14
Pb	61.7	42.3	31.5	26.1	45.2	25.1	11.6	33.1
Cd	0.06	0.03	0.03	0.04	0.05	0.01	0.02	0.04



A B

Figure 8. Effect of PGPR (PAS1, PAS2, PTS3, BSS1) and PGPF (AJ) on the Chlorophyll of *Arachis hypogaea* under As and Pb stress: (A) As and (B) Pb. Data represent mean ± SD (n = 3). Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$)

Discussion

Phytoremediation of metal-contaminated soils is often difficult because of the harsh conditions that plants must endure in such environments. The identification of heavy metal-tolerant PGPR and PGPF is of significant practical importance, as these microorganisms not only enhance plant growth and physiology but also contribute to improved metal tolerance, particularly under stressful environmental conditions (Alves *et al*., 2022). Assessing the tolerance levels of bacterial isolates is an essential step in understanding their adaptability to various heavy metals and in selecting suitable candidates for phytoremediation applications. The findings of this study revealed that the isolates exhibited strong resistance to high concentrations of heavy metals such as arsenic and lead. As reported by Oziegbe *et al.* (2021), indigenous bacteria from polluted environments often possess multi-metal resistance, developed through adaptive mechanisms such as intracellular and extracellular sequestration, bioaccumulation, bioaugmentation, efflux systems, enzymatic transformation, and reduced cell permeability (Jayaram *et al*., 2022). In the present study, bacteria belonging to the *Pseudomonas* genus, known for their exceptional metal resistance, were found to be prevalent in heavy metal-contaminated environments (Monsieurs *et al.,* 2011; Wei *et al*., 2020). The bacterial isolates were identified based on 16S rRNA gene sequencing. The identified isolates included *Pseudomonas alcaliphila* (PAS1), *Pseudomonas aeruginosa* (PAS2), *Pseudomonas toyotomiensis* (PTS3), and *Bacillus subtilis* (BSS1). Amplification and sequencing of the 16S rRNA gene is a widely employed method for bacterial identification (Naqqash *et al.,* 2022, Sridhar *et al*., 2025). The 18S rRNA analysis confirmed that the fungal isolates exhibited varying levels of resistance to heavy metals. Growth inhibition was observed at higher concentrations of arsenic (As) and lead (Pb), consistent with earlier reports on the toxic effects of elevated metal levels on fungi. The *Aspergillus* strain isolated in this study displayed strong metal-tolerance and uptake capacities, in agreement with previous findings under metal stress conditions (Iram *et al.,* 2012). In this study, PGPR, PGP fungi, compost and chemical fertilizer were used in pot culture experiments with *Arachis hypogaea* plants. After 30 days of DAI, plant biomass was analyzed, as biomass is a crucial indicator for assessing the impact of various stresses and plays a key role in phytoremediation strategies (Mousavi *et al*., 2022). Arsenic and lead stress significantly affected plant growth, as evidenced by reduced root and shoot fresh weight and dry weight. Under such stress, plants redirect their metabolic energy towards producing stress-related metabolites to enhance resistance, often at the expense of growth processes (Salam *et al*., 2025). Moreover, inhibition of mitosis, reduced synthesis of cell wall components, and

alterations in photosynthetic activity contribute to decreased biomass and overall development typical symptoms of As and Pb toxicity (Malaie *et al*., 2025). In the present study, the fresh weight of plants increased by 45% with BSS1 bacterial inoculation, while dry weight showed a 33% increase with fungal inoculation. Additionally, shoot length in PAS2-treated plants increased by 66%, and root length in BSS1-treated plants showed a 77% increase compared to control plants. Heavy metal stress significantly inhibits plant growth by inducing various toxic effects on physiological and biochemical processes, with photosynthesis being among the most sensitive. Photosynthetic efficiency is considered a key indicator of plant adaptability under abiotic stress conditions (Muhammad *et al.,* 2021). Studies have shown that arsenic and lead stress trigger excessive ethylene production, which interferes with photosynthesis. In plants treated with PGPR, yield increased by about 50%, while treatment with PGPF 30% compost enhanced yield by 35%. In contrast, chemical fertilizers showed only a minor improvement in plant growth (Singh *et al*., 2021). Arsenic toxicity adversely affects photosynthetic pigments, thylakoid membrane integrity, and Calvin cycle enzyme activities, indicating that such stress severely disrupts photosynthetic performance (Singh *et al*., 2020). Therefore, the application of As and Pb-tolerant PGPR and PGPF can enhance plant growth and phytoextraction potential in heavy metal-contaminated soils, contributing to soil restoration and environmental sustainability. The present study evaluated the potential of previously isolated plant growth-promoting rhizobacteria (PGPR) and PGPF for their tolerance to heavy metals and their role in enhancing plant growth under metal stress. Four PGPR strains: *Pseudomonas alcaliphila*, *Pseudomonas aeruginosa*, *Pseudomonas toyotomiensis*, and *Bacillus subtilis* along with the fungal strain *Aspergillus japonicus* were screened for resistance to As and Pb on NA and PDA media containing metal concentrations ranging from 100 to 1200 ppm. All bacterial isolates demonstrated tolerance up to 1200 ppm, while *A.japonicus* exhibited a maximum inhibitory concentration of 500 ppm against both As and Pb. Pot culture experiments further assessed the effects of As- and Pb-tolerant PGPR, plant growth-promoting fungi (PGPF), compost, and chemical fertilizer on the growth and phytoextraction efficiency of *A. hypogaea* grown in contaminated soil. The results revealed that the application of PGPR and PGPF significantly enhanced plant growth parameters and metal uptake, suggesting their potential use as sustainable and eco-friendly bioinoculants for soil restoration and environmental sustainability.

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