INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print); 2222-5234 (Online)
Website: https://www.innspub.net
Email contact: info@innspub.net
Vol. 28, Issue: 3, p. 1-12, 2026

RESEARCH PAPER

OPEN ACCESS

Comparative assessment of phytoextraction and antioxidant responses in rice varieties under Cd and As stress mediated by *Bacillus subtilis*

J. Sujatha, R. Rajakumar*

PG and Research Department of Biotechnology, Maruthupandiyar College, Thanjavur,
Affiliated to Bharathidasan University, Tamil Nadu, India

Key words: *Bacillus subtilis*, Cadmium, Arsenic, *Oryza sativa*, Antioxidant, Phytoextraction

Received: February 14, 2026 **Accepted:** February 26, 2026 **Published:** March 03, 2026

DOI: https://dx.doi.org/10.12692/ijb/28.3.1-12

ABSTRACT

The present study evaluated the role of *Bacillus subtilis* in mitigating cadmium (Cd) and arsenic (As) stress in three rice (*Oryza sativa* L.) varieties ADT 36, CO 51, and TPS 5 under 100 mM metal concentration. Heavy metal stress significantly increased oxidative damage; however, microbial inoculation enhanced both enzymatic (SOD, CAT, POD, PPO, PAL, APX) and non-enzymatic (ascorbic acid, glutathione, phenols, and proline) antioxidant activities across varieties. Among the genotypes, ADT 36 exhibited the highest antioxidant enzyme activities, particularly under *B. subtilis* + As treatment, indicating superior oxidative stress management. Phytoaccumulation studies conducted at 30, 60, and 90 days after treatment revealed significantly higher metal accumulation in roots than shoots, with maximum accumulation at 60 days. Microbial inoculation markedly increased root Cd and As concentrations in all varieties. Bioconcentration Factor (BCF) and Bioaccumulation Factor (BAF) values exceeded unity under *B. subtilis* treatments, demonstrating enhanced metal uptake efficiency. However, Translocation Factor (TF) and Level 2 mobility values remained below 1.0, indicating restricted metal translocation to aerial parts and suggesting a phytostabilization tendency rather than complete phytoextraction. The results highlight the synergistic interaction between metal-tolerant genotypes and plant growth-promoting rhizobacteria in alleviating heavy metal stress.

***Corresponding author:** R. Rajakumar ✉ biotechrajakumar@gmail.com
* https://orcid.org/0000-0002-5565-7128

INTRODUCTION

Heavy metal contamination of agricultural soils has emerged as a serious environmental and public health concern worldwide. Among toxic metals, cadmium (Cd) and arsenic (As) are particularly hazardous due to their high mobility, persistence, and bioaccumulation potential in food crops (Alloway, 2013). Rice (*Oryza sativa* L.), a staple food for more than half of the global population, is highly susceptible to Cd and As accumulation because of its cultivation under flooded conditions that enhance metal bioavailability (Meharg and Zhao, 2012). The entry of these metals into the food chain poses significant risks to human health, including renal dysfunction, skeletal damage and carcinogenic effects. Cadmium and arsenic toxicity in plants results in impaired germination, reduced growth, chlorophyll degradation, nutrient imbalance, and metabolic dysfunction (Benavides, Gallego, and Tomaro, 2005). One of the primary consequences of heavy metal exposure is the excessive generation of reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals. Elevated ROS levels lead to oxidative stress, causing lipid peroxidation, protein denaturation, membrane disruption and DNA damage (Gill and Tuteja, 2010).

Plants possess an efficient antioxidant defense system to mitigate oxidative stress. The enzymatic antioxidant machinery includes Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POD), Ascorbate Peroxidase (APX), Polyphenol Oxidase (PPO) and Phenylalanine Ammonia Lyase (PAL), which function collectively to detoxify ROS (Mittler, 2002). In addition, non-enzymatic antioxidants such as ascorbic acid, glutathione (GSH), phenolic compounds, and proline play critical roles in maintaining cellular redox balance. Glutathione is particularly important as a precursor for phytochelatin synthesis, facilitating heavy metal chelation and vacuolar sequestration (Cobbett and Goldsbrough, 2002). Proline accumulation further contributes to osmotic adjustment and stabilization of cellular structures under stress (Hayat *et al.,* 2012).

Recent studies have highlighted the role of plant growth-promoting rhizobacteria (PGPR) in alleviating heavy metal stress. Species such as *Bacillus subtilis* enhance plant tolerance through mechanisms including biosorption, siderophore production, ACC deaminase activity, and stimulation of antioxidant enzymes (Glick, 2012). Microbial inoculation not only improves plant growth but also enhances phytoremediation efficiency under contaminated conditions (Rajkumar *et al.*, 2012).

Phytoremediation is considered an eco-friendly and cost-effective strategy for the removal of heavy metals from contaminated soils (Ali *et al.*, 2013). The efficiency of metal uptake and translocation is commonly evaluated using quantitative indices such as Bioconcentration Factor (BCF), Bioaccumulation Factor (BAF), Translocation Factor (TF), and Mobility Index (MI). A BCF value greater than one indicates strong accumulation potential, while TF reflects the efficiency of metal movement from roots to aerial parts (Yoon *et al.,* 2006; Mellem *et al.*, 2009). Although several investigations have examined heavy metal stress in rice, comprehensive comparative studies involving multiple varieties, temporal antioxidant profiling at different growth stages, and bacterial inoculation under combined Cd and As stress remain limited. Therefore, the present study was undertaken to evaluate the antioxidant defense responses, metal accumulation patterns, and phytoextraction efficiency of three rice varieties (ADT 36, CO 51, and TPS 5) under cadmium and arsenic stress, with particular emphasis on the mitigating role of a heavy metal-tolerant bacterial strain.

MATERIALS AND METHODS

Experimental site and seed collection

The pot experiment was conducted under controlled conditions at Biotechnology Laboratory, Maruthupandiyar College, Thanjavur, Tamil Nadu. Three rice (*Oryza sativa* L.) varieties such as ADT 36, CO 51 and TPS 5 were used in this study. Certified seeds were obtained from Tamil Nadu Rice Research Institute, Aduthurai, Thanjavur District, Tamil Nadu, India.

Soil preparation

Top soil was collected from an uncontaminated agricultural field, air –dried, sieved (2mm) and homogenized. They was collected in a plastic container, sealed and stored at 4 °C until further use.

Physico chemical properties of soil

The physic chemical properties of the soil such as pH, EC, organic carbon and available nutrient such as nitrogen, phosphorus and potassium were analysed by standards protocols (APHA, 2005).

Treatment conditions

The experiment consists of 12 pots using three rice varieties: ADT 36, CO 51, and TPS 5. Each variety is treated with two types of heavy metals—Cadmium ($CdCl2$) and Arsenic ($As2O3$), both at a concentration of 100 mM. For each metal treatment, plants are either exposed to a bacterial suspension or not. Specifically, Pots 1 and 2 contain ADT 36 treated with $CdCl2$, without and with bacterial suspension respectively; Pots 3 and 4 contain ADT 36 treated with $As2O3$, again without and with bacterial suspension. Similarly, Pots 5 and 6 include CO 51 treated with $CdCl2$, and Pots 7 and 8 include CO 51 treated with $As2O3$, each pair differing by the presence or absence of bacterial suspension. Finally, TPS 5 is used in Pots 9 and 10 for $CdCl2$ treatment and Pots 11 and 12 for $As2O3$ treatment, with and without bacterial suspension accordingly.

Seed germination

The healthy and uniform seeds were surface sterilized using 3.5% sodium hypochlorite solution for 10 minutes. The sterilized seeds were placed in a 9 cm-diameter Petri dish layered with Whatman No. 1 filter paper and 10 mL distilled water was added. The Petri dishes were sealed with Parafilm and placed inside a growth chamber for germination. After germination, seven-day-old seedlings were shifted to autoclaved sand in plastic pots (1kg sand per pot) and were grown for two weeks. All pots were placed in a greenhouse with average 29 ± 1°Cand 24 ± 1°C temperatures for day and night, respectively, with average humidity 70% throughout the experiment. The experiment was laid out in complete randomized design (CRD) with 12^th^treatments. According to the experimental design, 10mL bacterial suspension (108 CFU) was applied to each pot, whereas a $CdCl_2$ and $As2O3$ solution of 100mM concentration, with and without bacterial suspension, was applied to each pot of 21-day-old seedlings (Bal *et al.,* 2013).

Sample collection

Plants were harvested at 30th, 60th and 90th days of the experimental period. Roots and shoots were separated, washed thoroughly with distilled water, blotted dry and used for biochemical and metal analysis. Fresh samples were used for enzyme assays, while dried samples were used for metal estimation.

Antioxidant activity

Estimation of enzymatic antioxidant

Crushed plant parts were homogenized in a 100 mM phosphate buffer (pH6.8) and centrifuged at 12,000×g for 20 min. The supernatants were used to determine the enzyme activity levels. The whole procedure was carried out at 4°C. The activity of SOD was assayed spectrophotometrically by measuring its ability to inhibit the photochemical reduction of Nitro blue Tetrazoilum (Beauchamp and Fridovich, 1971). One unit of SOD is the amount of extracts that gives 50% inhibition in the rate of NBT reduction. Catalase activity (CAT) was determined by consumption of H_2O_2 and was monitored spectrophotometrically at 240 nm for 3 min (Luck, 1974). For Polyphenol oxidase activity, catechol was used and the activity was expressed as changes in absorbance at 495 nm min-1 g -1 fresh weight of tissue (Esterbauer *et al.,* 1977), For Peroxidase assay (POD) the increase in absorbance due to oxidation of guaiacol (extinction coefficient 26.6 mM^{-1} cm^{-1}) was monitored at 470 nm (Putter, 1974). Phenylalanine ammonia lyase activity was estimated by the method of Brueske (1980). Ascorbate Peroxidase (APX) activity was measured following Nakano and Asada (1981) by recording the decrease in absorbance at 290 nm. Enzyme activities were expressed as units mg^{-1} protein.

Estimation of non-enzymatic antioxidant

Ascorbic acid content was estimated using the 2,6-dichlorophenol indophenol method. GSH was estimated following Ellman's method (1959) using DTNB reagent. Total phenolic content was determined using the Folin–Ciocalteu method, expressed as gallic acid equivalents. Proline was analysed spectrophotometerically at 520 nm using toluene for a blank as per Bates *et al.* (1973).

The acid–ninhydrin method was used to determine the proline content.

Phytoaccumulation

Dried root and shoot samples were oven dried at 70 °C, powdered and digested using a HNO_3: $HClO_4$ (3:1) acid mixture. Cadmium and arsenic concentrations were analyzed in the rice varieties after experimental periods. After mineralization, the samples were diluted with deionized water to a volume of 10 ml. Concentration of Cd and As were measured using inductively coupled plasma-atomic emission spectroscopy. The concentration of various heavy metals were computed and expressed as mg Kg^{-1} Dry weight.

Phytoextraction

Bio concentration factor (BCF)

A plant's ability to accumulate metals from the soil can be estimated using the BCF. The BCF was calculated using the following equation (Yan *et al.,* 2020):

BCF= Metal concentration in plant parts/Concentration of metal in soil

The BCF value is a ratio of the metal content in different parts of the plant to that in the soil. A BCF value of greater than 1.00 suggests that the plant can accumulate metals, whereas a BCF value of 1.00 implies that the plant can only absorb heavy metals (Yan *et al.,* 2020). In other words, a BCF value of greater than 1.00 means that the plant can take up more metals than what is available in the soil, indicating that it has the ability to hyper accumulate heavy metals. However, a BCF value of 1.00 indicates that the plant cannot hyper accumulate metals, but only absorb them in proportion to their availability in the soil.

Bio accumulation factor (BAF)

The accumulation factor (AF) was considered to determine the quantity of heavy metals absorbed by the plant from soil. This is an index of the plant to accumulate a particular metal with respect to its concentration in the soil and is calculated using the formula (Ghosh and Singh, 2005; Yoon *et al.,* 2006):

BAF= Metal concentration in tissue of whole plant/ Initial concentration of metal in substrate (soil)

Translocation factor (TF)

To evaluate the potentiality of plant species for phytoextraction, the TF was considered. This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant (Mellem *et al.,* 2009). It is represented by the ratio:

TF = Metal concentration in stems +leaves/Metal concentration in roots

Mobility index (MI)

MI was considered to determine the bio mobility and transport of heavy metals in different plant parts. The whole experiment was divided into two categories: Level 1 (Soil – Roots) and Level 2 (Roots –leaves). It was calculated by the methods of Kumar *et al.* (2009).

MI = Concentration of metal in the receiving level /Concentration of metal in the source level

RESULTS

Physico chemical properties

The soil analysis revealed that the soil was slightly acidic in nature, with a pH of 6.77 ± 0.03, which falls within the optimal range for the growth of most agricultural crops. The EC was recorded as 0.53 ± 0.02 dS m^{-1}, indicating that the soil is non-saline and suitable for normal crop cultivation without salinity stress. The organic carbon content of the soil was 0.64 ± 0.05%, which is categorized as medium, suggesting moderate organic matter status and reasonable soil fertility. The available nitrogen content was found to be 276.2 ± 12.2 kg ha^{-1}, which is also rated as medium, indicating that nitrogen supplementation through fertilizers may be required for optimal crop productivity. The available phosphorus content was 20.6 ± 1.3 kg ha^{-1}, falling under the medium category, suggesting adequate but not excessive phosphorus availability (Table 1). In contrast, the available potassium content was 298.5 ± 12.3 kg ha^{-1}, which is classified as high, indicating sufficient potassium availability in the soil.

Table 1. Physico chemical properties of soil

Parameter	Value	Soil nature
pH	6.77±0.03	Slightly acidic
Electrical conductivity (dS m-1)	0.53±0.02	Non saline
Organic carbon (%)	0.64±0.05	Medium
Nitrogen (Kg ha-1)	276.2±12.2	Medium
Phosphorus (Kg ha-1)	20.6±1.3	Medium
Potassium (Kg ha-1)	298.5±12.3	High

Enzymatic antioxidant

A comparative evaluation of antioxidant enzyme activities clearly indicated significant varietal differences in response to Cd and As stress. Among the three varieties, ADT 36 exhibited the highest enzymatic antioxidant activities, particularly under combined *Bacillus subtilis* and metal treatments. For instance, under *B. subtilis* + As treatment, ADT 36 recorded maximum SOD (77.9 ± 2.7 Units mg^{-1} protein), CAT (67.9 ± 2.5 Units mg^{-1} protein), POD (60.6 ± 2.6 Units mg^{-1} protein), PPO (55.8 ± 3.4 Units mg^{-1} protein), PAL (72.1 ± 1.5 Units mg^{-1} protein) and APX (46.2 ± 2.8 Units mg^{-1} protein) activities, which were significantly higher ($p \leq 0.05$) than those observed in CO 51 and TPS 5. Under similar *B. subtilis* + As treatment, CO 51 recorded comparatively lower values of SOD (68.6 ± 2.3), CAT (56.2 ± 2.1), POD (66.6 ± 2.6), PPO (65.2 ± 3.2), PAL (67.1 ± 3.5), and APX (54.5 ± 2.8 Units mg^{-1} protein). Similarly, TPS 5 showed enhanced enzyme activities under *B. subtilis* + As treatment, with SOD (71.2 ± 2.5), CAT (60.2 ± 2.1), POD (58.3 ± 2.5), PPO (72.2 ± 3.6), PAL (57.1 ± 2.7), and APX (65.5 ± 2.7 Units mg^{-1} protein). Notably, TPS 5 recorded higher APX and PPO activities compared to the other varieties; however, its overall antioxidant profile was slightly lower than ADT 36 in terms of coordinated elevation of all major enzymes. Under Cd stress alone, a similar trend was observed, where ADT 36 recorded higher SOD (55.3 ± 2.1) and CAT (46.3 ± 1.7) activities compared to CO 51 (51.2 ± 2.5 and 34.3 ± 0.7) and TPS 5 (53.2 ± 1.0 and 36.3 ± 0.9 Units mg^{-1} protein), indicating better oxidative stress management. The superior tolerance of ADT 36 may be attributed to its stronger and coordinated enzymatic antioxidant defense system (Fig. 1).

Non-enzymatic antioxidant activity

Cadmium (Cd) and arsenic (As) stress significantly enhanced the accumulation of non-enzymatic antioxidants ascorbic acid, reduced glutathione (GSH), total phenols, and proline in all three rice varieties. The increase was consistently greater in plants inoculated with *Bacillus subtilis* than in metal-alone treatments ($p \leq 0.05$). In ADT 36, antioxidant levels increased markedly under Cd and As stress, with the highest values recorded under *B. subtilis* + As treatment (ascorbic acid: 2.82–3.25 mg g^{-1} FW; GSH: 3.34–3.82 µmol g^{-1} FW; phenols: 4.51–5.37 mg GAE g^{-1}; proline: 12.6–14.6 µmol g^{-1} FW). CO 51 and TPS 5 showed a similar trend, though the magnitude of increase was comparatively lower in CO 51. Under combined microbial and metal treatments, ascorbic acid and GSH levels in CO 51 and TPS 5 increased up to 3.12 mg g^{-1} FW and 3.87 µmol g^{-1} FW, respectively, while phenolic and proline contents also exhibited significant enhancement. Overall, ADT 36 displayed the highest accumulation of non-enzymatic antioxidants, followed by TPS 5, indicating superior tolerance to heavy metal stress. The pronounced elevation of ascorbic acid, GSH, phenols, and proline under *B. subtilis* inoculation demonstrates its role in reinforcing non-enzymatic antioxidant defense mechanisms and mitigating Cd- and As-induced oxidative stress in rice (Fig. 2).

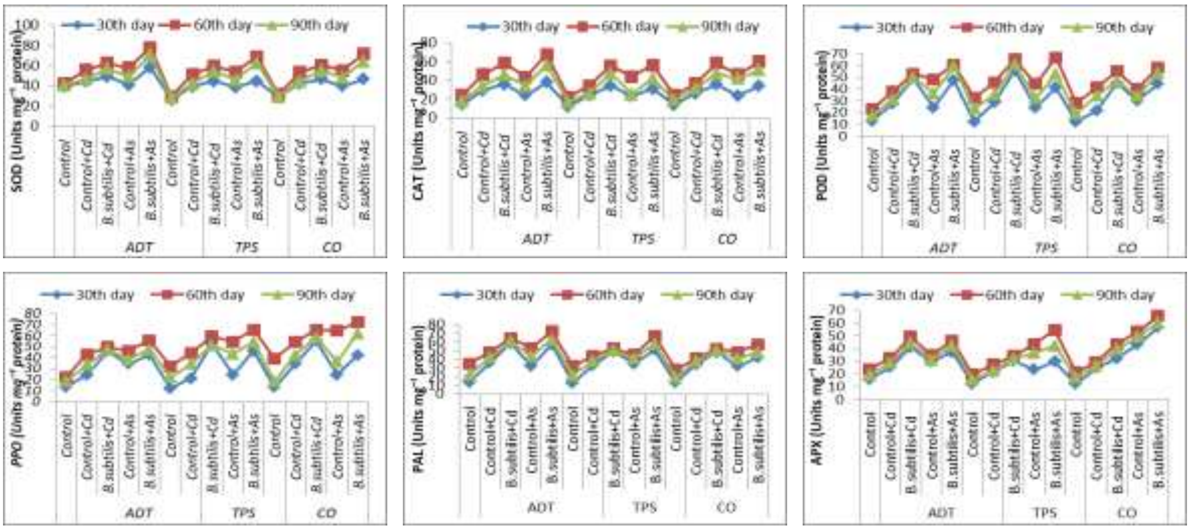

Fig. 1. Enzymatic anti-oxidant activity in different variety of rice under experimental condition during different growth period

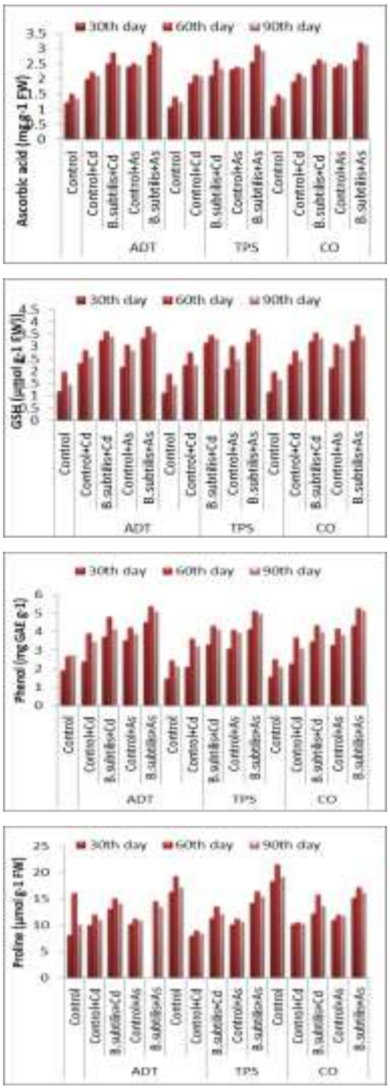

Fig. 2. Non-enzymatic anti-oxidant activity in different variety of rice under experimental condition during different growth period

Phytoaccumulation of heavy metals

The phytoaccumulation of Cd and As in three rice varieties (ADT 36, CO 51 and TPS 5) was evaluated at 30, 60 and 90 days after treatment under 100 mM concentration. In all varieties, metal accumulation was significantly higher in roots than shoots and was markedly enhanced by *Bacillus subtilis* inoculation. Cadmium (Cd) and arsenic (As) accumulation in roots and shoots increased progressively up to 60 days, followed by a slight decline at 90 days in all three rice varieties. Root accumulation was consistently higher than shoot accumulation. In ADT 36, Cd accumulation under Control + Cd increased from 28.2 to 43.2 mg kg^{-1} (30–60 days) before declining at 90 days, whereas *B. subtilis* + Cd treatment significantly enhanced root accumulation (47.1–63.1 mg kg^{-1}). Under As stress, Control + As recorded 35.2–51.7 mg kg^{-1}, while *B. subtilis* + As resulted in the highest values (67.4–72.4 mg kg^{-1}). Shoot accumulation followed a similar but lower trend. CO 51 showed comparatively lower accumulation. Under Cd stress, root values ranged from 29.2–38.2 mg kg^{-1} in Control + Cd and increased to 39.2–59.2 mg kg^{-1} with microbial inoculation. Under As stress, *B. subtilis* + As enhanced accumulation up to 69.5 mg kg^{-1}. TPS 5 exhibited a similar pattern, with peak accumulation at 60 days. Under *B. subtilis* + Cd, root Cd reached 62.2 mg kg^{-1}, while *B. subtilis* + As resulted in maximum accumulation (71.5 mg kg^{-1}).

Microbial inoculation significantly increased Cd and As accumulation in both roots and shoots ($p \leq 0.05$), with ADT 36 and TPS 5 showing higher accumulation than CO 51 (Table 2). The results indicate that *Bacillus subtilis* enhances metal uptake and accumulation, particularly at the active vegetative stage (60 days).

Table 2. Phytoaccumulation in different variety of rice under various experimental condition

Variety	Treatment	Conc.	Phytoaccumulation					
			30th day		60th day		90th day	
			Root (mg kg^{-1} dry weight)	Shoot (mg kg^{-1} dry weight)	Root (mg kg^{-1} dry weight)	Shoot (mg kg^{-1} dry weight)	Root (mg kg^{-1} dry weight)	Shoot (mg kg^{-1} dry weight)
ADT 36	Control	-	0.03±0.01^{a}	0.02±0.01^{a}	0.05±0.01^{a}	0.03±0.01^{a}	0.04±0.01^{a}	0.03±0.01^{a}
	Control+Cd	100 mM	28.2±1.2^{d}	15.2±0.2^{d}	43.2±1.8^{d}	27.2±0.8^{d}	36.2±1.5^{d}	19.2±0.5^{d}
	B. subtilis+Cd	100 mM	47.1±1.7^{c}	23.5±0.7^{c}	63.1±2.7^{c}	32.5±1.2^{c}	52.1±2.4^{c}	28.5±1.2^{c}
	Control+As	100 mM	35.2±1.4^{b}	17.1±0.5^{b}	51.7±2.4^{b}	27.1±1.5^{b}	43.7±1.9^{b}	22.1±1.5^{b}
	B. subtilis+As	100 mM	67.4±2.1^{e}	25.2±1.2^{e}	72.4±3.1^{e}	35.2±1.7^{e}	69.1±2.5^{e}	31.2±1.7^{e}
CO51	Control	-	0.02±0.01^{a}	0.02±0.01^{a}	0.04±0.01^{a}	0.02±0.01^{a}	0.03±0.01^{a}	0.02±0.01^{a}
	Control+Cd	100 mM	29.2±1.2^{d}	11.2±0.2^{d}	38.2±1.5^{d}	19.2±0.3^{d}	32.2±1.2^{d}	16.2±0.2^{d}
	B. subtilis+Cd	100 mM	39.2±2.1^{c}	17.5±1.1^{c}	59.2±2.2^{c}	26.5±1.1^{c}	46.2±2.0^{c}	22.5±1.1^{c}
	Control+As	100 mM	45.1±2.3^{b}	21.1±1.4^{b}	53.7±2.5^{b}	28.1±1.8^{b}	49.9±2.4^{b}	25.1±1.4^{b}
	B.subtilis+As	100 mM	56.5±2.5^{e}	29.2±1.6^{e}	69.5±2.8^{e}	32.2±1.9^{e}	62.5±2.5^{e}	31.2±1.6^{e}
TPS 5	Control	-	0.03±0.01^{a}	0.02±0.01^{a}	0.05±0.01^{a}	0.02±0.01^{a}	0.04±0.01^{a}	0.02±0.01^{a}
	Control+Cd	100 mM	32.1±1.3^{c}	17.1±0.4^{c}	41.1±1.6^{c}	24.1±0.6^{c}	35.1±1.4^{c}	21.1±0.5^{c}
	B. subtilis+Cd	100 mM	56.2±2.1^{d}	19.4±1.2^{d}	62.2±2.5^{d}	29.4±1.7^{d}	59.2±2.2^{d}	24.4±1.4^{d}
	Control+As	100 mM	45.2±1.9^{e}	23.1±1.5^{e}	52.2±2.3^{e}	26.1±1.5^{e}	48.2±2.0^{e}	26.1±1.5^{e}
	B. subtilis+As	100 mM	67.5±2.4^{b}	30.2±1.6^{b}	71.5±2.9^{b}	34.2±1.9^{b}	69.5±2.6^{b}	32.1±1.7^{b}

Table 3. Phytoextarction efficiency in different variety of rice

Variety	Treatment	Conc.	Phyto extraction		
			BCF	BAF	TF
ADT 36	Control	-	0.01±0.00^{a}	0.01±0.00^{a}	0.36±0.02^{a}
	Control+Cd	100 mM	0.91±0.04^{c}	0.87±0.04^{c}	0.45±0.03^{c}
	B.subtilis+Cd	100 mM	1.27±0.06^{d}	1.21±0.07^{d}	0.47±0.02^{d}
	Control+As	100 mM	0.89±0.05^{b}	0.92±0.04^{b}	0.39±0.02^{b}
	B.subtilis+As	100 mM	1.14±0.04^{e}	1.17±0.07^{e}	0.44±0.02^{e}
CO 51	Control	-	0.01±0.00^{a}	0.01±0.00^{a}	0.26±0.01^{a}
	Control+Cd	100 mM	0.82±0.03^{c}	0.72±0.04^{c}	0.38±0.02^{c}
	B.subtilis+Cd	100 mM	1.16±0.04^{e}	1.11±0.04^{e}	0.41±0.03^{e}
	Control+As	100 mM	0.78±0.02^{d}	0.81±0.06^{d}	0.33±0.02^{d}
	B.subtilis+As	100 mM	1.09±0.03^{b}	1.10±0.05^{b}	0.39±0.03^{b}
TPS 5	Control	-	0.01±0.00^{a}	0.01±0.00^{a}	0.32±0.01^{a}
	Control+Cd	100 mM	0.87±0.04^{c}	0.79±0.03^{c}	0.41±0.03^{c}
	B.subtilis+Cd	100 mM	1.21±0.05^{d}	1.17±0.07^{d}	0.46±0.04^{d}
	Control+As	100 mM	0.81±0.03^{b}	0.86±0.06^{b}	0.35±0.02^{b}
	B.subtilis+As	100 mM	1.12±0.04^{e}	1.12±0.06^{e}	0.40±0.03^{e}

Phytoextraction efficiency

Phytoextraction efficiency under 100 mM Cd and As stress was evaluated using Bioconcentration Factor (BCF), Bioaccumulation Factor (BAF), and Translocation Factor (TF).

In all three rice varieties, *Bacillus subtilis* inoculation significantly enhanced BCF and BAF compared to metal-alone treatments ($p \leq 0.05$). In ADT 36, BCF and BAF increased from negligible control values (0.01) to 0.91 and 0.87 under Cd stress, and further to 1.27 and 1.21, respectively, with microbial inoculation. Under As stress, BCF and BAF similarly exceeded 1.0 with *B. subtilis* treatment. CO 51 and TPS 5 showed comparable trends, with BCF and BAF values rising above unity under combined microbial and metal treatments, though to a lesser extent than ADT 36. Translocation Factor (TF) remained below 1 in all treatments (0.26–0.47), indicating limited transfer of metals to shoots and predominant root retention. In this study, *B. subtilis* enhanced metal uptake and accumulation (BCF and BAF > 1), particularly in ADT 36, which exhibited the highest phytoextraction potential (Table 3). However, low TF values suggest a phytostabilization tendency rather than effective metal translocation to aerial parts.

Metal mobility

Metal mobility was evaluated using Mobile Index Level 1 (soil–root) and Level 2 (root–shoot) under 100 mM Cd and As stress. In all three rice varieties, *Bacillus subtilis* significantly enhanced Level 1 mobility compared to metal-alone treatments ($p \leq 0.05$). In ADT 36, Level 1 mobility increased from negligible control values (0.01) to 0.91 under Cd stress and further to 1.27 with *B. subtilis* inoculation. Under As stress, values increased from 0.89 to 1.20 with microbial treatment. Similar trends were observed in CO 51 (up to 1.18 under Cd and 1.15 under As) and TPS 5 (up to 1.24 under Cd and 1.18 under As). In contrast, Level 2 mobility remained below 0.50 in all treatments, ranging approximately from 0.38 to 0.47 under combined microbial and metal treatments, indicating limited root-to-shoot translocation (Fig 3). Overall, *B. subtilis* enhanced soil-to-root metal uptake (Level 1 > 1.0), particularly in ADT 36 and TPS 5, whereas restricted Level 2 mobility confirms predominant metal retention in roots, suggesting a phytostabilization tendency despite improved uptake.

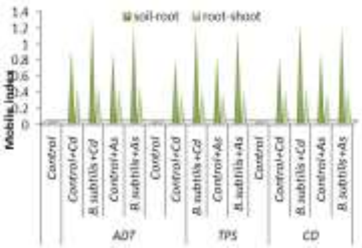

Fig. 3. Mobile index in different variety of rice under

DISCUSSION

Heavy metal stress is known to induce oxidative stress through enhanced production of reactive oxygen species (ROS) (Shahid *et al.,* 2014). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and ascorbate peroxidase (APX) are key components of the defense system that mitigate oxidative damage (Gill and Tuteja, 2010). In this study, all three varieties exhibited increased enzymatic activities under Cd and As stress, consistent with previous reports that heavy metals stimulate antioxidant defenses (Zhang *et al.,* 2013). However, inoculation with *Bacillus subtilis* further elevated these activities, especially in ADT 36, which recorded the highest SOD (77.9 ± 2.7), CAT (67.9 ± 2.5), POD (60.6 ± 2.6), PPO (55.8 ± 3.4), PAL (72.1 ±1.5), and APX (46.2 ± 2.8) under *B. subtilis* + As. This enhancement suggests that *B. subtilis* may act as an elicitor, inducing systemic antioxidant responses, a phenomenon previously demonstrated in plants under abiotic stress (Kumar *et al.,* 2016). ADT 36's superior enzymatic response implies better ROS detoxification capacity compared to CO 51 and TPS 5. Similar varietal differences in antioxidant enzyme induction under metal stress have been observed in rice and wheat, where tolerant genotypes showed stronger upregulation of SOD and CAT activities (Srivastava *et al.,* 2014).

Non-enzymatic antioxidants such as ascorbic acid, reduced glutathione (GSH), phenolic compounds, and proline play crucial roles in scavenging ROS and maintaining redox homeostasis (Noctor and Foyer, 1998). The present data show significant increases in these metabolites under Cd and As stress, augmented by *B. subtilis* inoculation. In ADT 36, ascorbic acid increased up to 3.25 mg g^{-1} FW and GSH reached 3.82 µmol g^{-1} FW under *B. subtilis* + As, which is consistent with microbial enhancement of non-enzymatic defenses reported in plants exposed to salt and metal stress. Proline accumulation under stress is widely recognized as an osmoprotectant and ROS scavenger (Szabados and Savouré, 2010). Increased proline levels in TPS 5 and ADT 36 reflect adaptive responses to maintain cell turgor and mitigate oxidative injury. Phenolic compounds, shown here to reach 5.37 mg GAE g^{-1} in ADT 36, also contribute to antioxidant capacity and metal chelation (Rice-Evans *et al.,* 1997). The overall trend confirms that tolerant varieties accumulate higher levels of non-enzymatic antioxidants, a pattern supported by studies in rice genotypes exposed to cadmium (Benavides *et al.,* 2005).

The significant enhancement of antioxidant systems due to *Bacillus subtilis* suggests that this plant growth-promoting bacterium (PGPB) confers stress tolerance through multiple mechanisms. PGPBs are known to improve nutrient uptake, produce phytohormones such as indole-3-acetic acid (IAA), and induce systemic tolerance via antioxidant priming (Vacheron *et al.,* 2013). *B. subtilis* is also reported to enhance metal uptake and sequestration, likely through modulation of metal chelators and root exudation patterns (Khan *et al.,* 2000). The present results align with these findings and highlight the potential of microbial inoculation as a sustainable strategy to reduce heavy metal toxicity in crops.

Across all treatments, metal accumulation was consistently higher in roots than in shoots, confirming restricted translocation and preferential metal sequestration in below-ground tissues. This pattern is widely reported in rice and other cereals exposed to heavy metals, where roots act as primary sites of metal binding and immobilization (Clemens, 2006; Shahid *et al.,* 2014). The significantly higher accumulation observed at 60 days in all varieties suggests that the vegetative growth phase is critical for metal uptake due to increased root surface area and metabolic activity (Kabata-Pendias, 2010). The enhancement of metal accumulation following microbial inoculation may be attributed to rhizosphere modifications such as increased metal solubilization, siderophore production, and root surface expansion (Khan *et al.,* 2000; Vacheron *et al.,* 2013). Plant growth-promoting rhizobacteria (PGPR) are known to alter soil pH and secrete organic acids, thereby increasing metal bioavailability (Ma *et al.,* 2016). The slight decline in accumulation at 90 days may be associated with dilution effects due to biomass expansion or activation of detoxification mechanisms limiting further uptake (Benavides *et al.,* 2005).

Bioconcentration Factor (BCF) and Bioaccumulation Factor (BAF) values greater than 1 in *B. subtilis*-treated plants indicate enhanced metal uptake from soil and accumulation within plant tissues. In ADT 36, BCF reached 1.27 ± 0.06 and BAF 1.21 ± 0.07 under *B. subtilis* + Cd treatment, demonstrating strong phytoextraction potential. Similar trends were observed in TPS 5 and CO 51, although the magnitude was comparatively lower. According to established phytoremediation criteria, plants with BCF > 1 are considered efficient accumulators, while TF values determine translocation efficiency (Yoon *et al.,* 2006). Root retention is considered a phytostabilization strategy, where metals are immobilized within root tissues or bound to cell walls, thereby preventing excessive translocation to edible parts (Clemens, 2006). Such restricted translocation may involve sequestration in vacuoles and binding with phytochelatins and metallothioneins (Shahid *et al.,* 2014).

The Mobile Index further confirmed these observations. Level 1 mobility (soil to root) exceeded 1.0 under *B. subtilis* treatments in all varieties, with the highest value recorded in ADT 36 (1.27 ± 0.06). This indicates improved metal uptake efficiency mediated by microbial inoculation.

PGPR-mediated enhancement of nutrient and metal uptake has been widely documented and is associated with improved root architecture and increased rhizospheric activity (Kumar *et al.,* 2016). However, Level 2 mobility (root to shoot) remained below 0.50 across treatments, reinforcing the observation of restricted translocation. Similar findings have been reported in rice, where Cd and As are largely confined to roots due to limited xylem loading and sequestration mechanisms (Ma *et al.,* 2016). Although enhanced uptake was observed, the consistently low TF values suggest that the studied rice varieties function more as phytostabilizers than hyperaccumulators.

CONCLUSION

The present investigation clearly demonstrates that cadmium (Cd) and arsenic (As) stress significantly affect physiological and biochemical responses in rice, leading to enhanced oxidative stress and metal accumulation. However, inoculation with *Bacillus subtilis* effectively mitigated heavy metal toxicity by strengthening both enzymatic and non-enzymatic antioxidant defense systems. The enhanced antioxidant activity indicates improved reactive oxygen species (ROS) scavenging capacity, thereby reducing oxidative damage under metal stress conditions.

Phytoaccumulation studies revealed that metal concentration was consistently higher in roots than shoots across all treatments and growth stages, with peak accumulation observed at 60 days after treatment. Microbial inoculation significantly increased metal uptake, as evidenced by higher Bioconcentration Factor, Bioaccumulation Factor and Level 1 mobility values (>1). This pattern suggests that the rice varieties exhibit a phytostabilization tendency rather than complete phytoextraction, with predominant metal retention in root tissues. Overall, the study concluded that the integration of *Bacillus subtilis* inoculation with metal-tolerant varieties such as ADT 36 offers a promising, eco-friendly, and sustainable strategy for cultivating rice in heavy metal-contaminated soils while minimizing the risk of metal translocation to edible plant parts.

ACKNOWLEDGEMENTS

The authors wish to thanks the Chairman, Maruthupandiyar College, Thanjavur for providing necessary facilities that successful completion of the work.

REFERENCES

Ali H, Khan E, Sajad MA. 2013. Phytoremediation of heavy metals—Concepts and applications. Chemosphere **91**(7), 869–881.

Alloway BJ. 2013. Heavy metals in soils (3rd ed.). Springer.

APHA. 2005. Standard methods for the examination of water and wastewater (21st ed.). American Public Health Association, American Water Works Association and Water Environment Federation.

Bal A, Singh P, Yadav A. 2013. Isolation and identification of *Bacillus subtilis* and its potential in bioremediation. International Journal of Environmental Science and Technology **10**(1), 1–8.

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil **39**(1), 205–207. https://doi.org/10.1007/BF00018060

Beauchamp C, Fridovich I. 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry **44**(1), 276–287. https://doi.org/10.1016/0003-2697(71)90370-8

Benavides MP, Gallego SM, Tomaro ML. 2005. Cadmium toxicity in plants. Critical Reviews in Plant Sciences **24**(1), 1–22.

Brueske CH. 1980. Phenylalanine ammonia-lyase activity in tomato roots infected and resistant to Meloidogyne incognita. Phytopathology **70**(10), 1017–1020.

Clemens S. 2006. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. New Phytologist **174**(1), 11–22.

Cobbett C, Goldsbrough P. 2002. Phytochelatins and metallothioneins. Annual Review of Plant Biology **53**, 159–182.

Ellman GL. 1959. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics **82**(1), 70–77. https://doi.org/10.1016/0003-9861(59)90090-6

Esterbauer H, Schwarzl E, Hayn M. 1977. A rapid assay for catechol oxidase and laccase using catechol as substrate. Analytical Biochemistry **77**(2), 486–494.

Ghosh M, Singh SP. 2005. A review on phytoremediation of heavy metals and utilization of its by-products. Applied Ecology and Environmental Research **3**(1), 1–18.

Gill SS, Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48(12), 909–930.

Glick BR. 2012. Plant growth-promoting bacteria: Mechanisms and applications. Scientifica **2012**, 963401.

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. 2012. Role of proline under changing environments: A review. Plant Signaling & Behavior*,* 7(11), 1456–1466. https://doi.org/10.4161/psb.21949

Kabata-Pendias A. 2010. Trace elements in soils and plants (4th ed.). CRC Press.

Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ. 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere **41**(1–2), 197–207. https://doi.org/10.1016/S0045-6535(99)00412-9

Kumar A, Singh R, Yadav A, Giri DD. 2016. Isolation and characterization of bacterial endophytes of rice with plant growth promoting potential. Frontiers in Plant Science **7**, 1279.

Kumar JIN, Soni H, Kumar RN, Bhatt I. 2009. Macrophytes in phytoremediation of heavy metal contaminated water and sediments in Pariyej community reserve, Gujarat, India. Turkish Journal of Fisheries and Aquatic Sciences **9**(2), 193–200.

Luck H. 1974. Catalase. In Bergmeyer HU (Ed.), Methods of enzymatic analysis (2nd ed., pp. 885–894). Academic Press.

Ma Y, Oliveira RS, Freitas H, Zhang C. 2016. Biochemical and molecular mechanisms of plant-microbe-metal interactions. Environmental and Experimental Botany **126**, 52–63.

Meharg AA, Zhao FJ. 2012. Arsenic and rice. Springer, Dordrecht.

Mellem JJ, Baijnath H, Odhav B. 2009. Translocation and accumulation of Cr, Hg, As, Pb, Cu and Ni by *Amaranthus dubius* (Amaranthaceae) from contaminated sites. Journal of Environmental Science and Health, Part A **44**(6), 568–575. https://doi.org/10.1080/10934520902719771

Mittler R. 2002. Oxidative stress and antioxidant defense in plants. Trends in Plant Science **7**, 405–410.

Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology **22**(5), 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Noctor G, Foyer CH. 1998. Ascorbate and glutathione: Keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology **49**, 249–279.

Pütter J. 1974. Peroxidases. In Bergmeyer HU (Ed.), Methods of enzymatic analysis (2nd ed., pp. 685–690). Academic Press.

Rajkumar M, Sandhya S, Prasad MNV, Freitas H. 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnology Advances **30**, 1562–1574.

Rice-Evans C, Miller N, Paganga G. 1997. Antioxidant properties of phenolic compounds. Free Radical Biology and Medicine **20**(7), 933–956.

Shahid M, Dumat C, Khalid S, Niazi NK, Antunes PMC. 2014. Cadmium stress in plants: Mechanisms of toxicity and tolerance. Journal of Environmental Management*, 146*, 407–418. https://doi.org/10.1016/j.jenvman.2014.08.030

Srivastava S, Srivastava AK, Suprasanna P D'Souza SF. 2014. Differential response of antioxidant enzymes in rice genotypes under cadmium stress. BMC Genomics, 15, 126. https://doi.org/10.1186/1471-2164-15-126

Szabados L, Savouré A. 2010. Proline: A multifunctional amino acid. Trends in Plant Science **15**(2), 89–97.

Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dyé F, Prigent-Combaret C. 2013. Plant growth-promoting rhizobacteria and abiotic stress tolerance in plants. Trends in Plant Science, 18(10), 489–499. https://doi.org/10.1016/j.tplants.2013.05.004

Yan A, Wang Y, Tan SN, Mohd Yusof ML, Ghosh S, Chen Z. 2020. Phytoremediation: A promising approach for revegetation of heavy metal-polluted land. Frontiers in Plant Science **11**, 359. https://doi.org/10.3389/fpls.2020.00359

Yoon J, Cao X, Zhou Q, Ma LQ. 2006. Accumulation of Pb, Cu and Zn in native plants growing on contaminated soils. Science of the Total Environment **368**(2–3), 456–464.

Zhang F, Wang Y, Lou Z, Dong J. 2013. Effect of heavy metal stress on antioxidant enzymes and lipid peroxidation in leaves and roots of two mangrove plant species. Chemosphere **93**(2), 203–210. https://doi.org/10.1016/j.chemosphere.2013.05.079