

# Bioremediation potential of rhizosphere bacterial consortium in lead (Pb) contaminated rice plants

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**Abstract.** Ubaidillah M, Thamrin N, Cahyani FI, Fitriyah D. 2023. Bioremediation potential of rhizosphere bacterial consortium in lead (Pb) contaminated rice plants. *Biodiversitas* 24: 4566-4571. Lead (Pb) is one of the toxic heavy metals detected in various chemical fertilizers and pesticides used in Indonesia. These chemicals can increase plant lead (Pb) accumulation and affect other organisms through its circulation with food chain. Excess amounts of Pb in soil may be remediated using different approaches such as bioremediation. Rhizosphere bacteria can be used as bioremediation agents to reduce Pb accumulation in plants and also increase plant growth. *Actinomyces*, *Azotobacter* sp., *Azospirillum* sp., *Rhizobium* sp., *Pseudomonas* sp., *Lactobacillus* sp., *Bacillus* sp., and *Streptomyces* sp. are some of the several bacteria that can reduce lead (Pb) accumulation in plants by the ability to sequester Pb, change its oxidation state, or enable Pb to precipitate. This research was conducted to determine the ability of the rhizosphere bacterial consortium to prevent lead (Pb) accumulation in rice plants. This study confirmed that the application of rhizosphere bacterial consortium to rice plants contaminated with lead (Pb) can reduce the plant's lead (Pb) content by up to 45%. The use of a bacterial consortium did not significantly affect the growth parameters of the plant but increased root length by 41%, fresh weight by 32%, dry weight by 26%, and chlorophyll content by 25%, in rice plants contaminated with Pb.

**Keywords:** Bioaccumulator, bioremediation, rhizosphere bacterial consortium, rice

## INTRODUCTION

Rice is a major food crop in Indonesia, where its consumption increases every year following population growth, impacting the availability that should be maintained. However, several problems in cultivation, including environmental degradation and land stress, retard production (Basit et al. 2022). Lately, there has been an increase in toxic and hazardous substances caused by heavy metal pollution in rice fields. As a staple food, rice could be a major source of toxic metal intake, resulting in adverse human health effects.

Heavy metals enter the environment (water, air, soil, and food) through industrial waste, vehicle residues, chemical fertilizers, and pesticides. Heavy metals that have the potential to contaminate the environment include arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) (Shraim 2017). According to the Indonesian National Standardization (SNI), the maximum permissible limit of lead (Pb) in rice for consumption is 0.3 mg/kg. Previous research explained that lead (Pb) can be absorbed in rice plants at 0.89 mg/kg on land contaminated with heavy metals and is harmful to humans and the environment (Ihedioha et al. 2016). Heavy metals are non-biodegradable and persist for a long time in the environment and have the potential to impair the growth of plants by altering various aspects of plant metabolism including cell division, seedling growth, and photosynthesis (Khan et al. 2021). It can also accumulate in animals causing nervous system

disorders, kidney damage, paralysis, and premature death (Baker et al. 2019; Rinawati and Sofiatun 2019).

Lead (Pb) is known as being neither essential nor beneficial for any living organisms and is detrimental to plant growth, and productivity. Lead (Pb) can be found in chemical fertilizers and pesticide ingredients widely used in Indonesia, making it possible to accumulate in plants (Budianta et al. 2022). Its accumulation in the soil can reduce productivity and retard the growth of crops. Rice plants can absorb heavy metals from the soil, and retain it affect animals and humans when consumed (Sukarjo et al. 2018). Heavy metal contamination issues need to be addressed through efforts that can reduce the impact of heavy metal contamination (Ahmed et al. 2019). This mitigation can be in the form of bioremediation which could accumulate heavy metals, by utilizing microorganisms commonly referred to as bioremediation or phytoremediation.

Bacteria found in the rhizosphere could be used for heavy metals remediation and to promote plant growth (El-Meihy et al. 2019). Several bacteria, including *Azotobacter* sp., *Azospirillum* sp., *Lactobacillus* sp., *Bacillus* sp., and *Pseudomonas* sp. are known to reduce lead (Pb) content and stimulate plant growth. These bacteria can be found in liquid organic fertilizers and have the potential as a bioaccumulator because they can accumulate heavy metals in the soil. Rhizosphere bacteria still need to be studied, regarding its potential to accumulate lead (Pb) absorption and its impact on rice plants. Despite the complexity of the food system and diversification in the preference for

various foods, rice remains a staple food crop in Indonesia solely cultivated for human consumption. This research was conducted to determine the potential use of rhizosphere bacteria in reducing lead (Pb) accumulation in rice plants and the potential for rice plant growth in lead-contaminated areas.

## MATERIALS AND METHODS

### Experimental design

This research was conducted at the Agrotechnology Laboratory of Agriculture Faculty, University of Jember, Jember, Indonesia. The seeds of the IR64 rice variety were procured from Indonesian Center for Rice Research (BB Padi Subang). The seeds were soaked in distilled water, placed in a dark room for 3 days and then sown in pot trays filled with sand media. After the emergence of the roots, seedlings were irrigated with nutrient solution (ABMix). Rice seedlings at 7 days after planting (DAP) were treated with the bacterial consortium (*Actinomyces*  $1.4 \times 10^8$  cfu/mL, *Azotobacter* sp.  $3.4 \times 10^{10}$  cfu/mL, *Azospirillum* sp.  $2.7 \times 10^9$  cfu/mL, *Rhizobium* sp.  $6.15 \times 10^{11}$  cfu/mL, *Pseudomonas* sp.  $8.35 \times 10^{11}$  cfu/mL, *Lactobacillus* sp.  $1.0 \times 10^8$  cfu/mL, *Bacillus* sp.  $1.0 \times 10^8$  cfu/mL, *Cyrtophaga* sp.  $3.8 \times 10^8$  cfu/mL, *Streptomyces* sp.  $7.2 \times 10^5$  cfu/mL, *Saccharomyces* sp.  $9.0 \times 10^7$  propagule/mL, Cellulotic  $3.4 \times 10^{10}$  cfu/mL, BPF  $5.5 \times 10^7$  cfu/mL, *Mycoriza*  $2.8 \times 10^5$  cfu/mL and *Trichoderma*  $1.2 \times 10^4$  cfu/mL) in liquid organic fertilizer at a dose of 2 mL/L, 4 mL/L, and 6 mL/L then rested for 1 hour (Tian et al. 2022). The seedlings were then contaminated with heavy metal by adding lead (Pb) at a concentration of 800 mg/L in ABMix solution and observed for 7 days (El-Meihy et al. 2019).

### Observations

#### Rice plant morphology

Plant morphology including height, root length, fresh weight, and dry weight was observed based on samples collected 7 days after treatment (DAT). Plant height was measured from the base of the stem (at the soil surface) to the highest part of the seedlings, whereas root length was measured from the base to the tip of the roots. Fresh weight was determined by weighing fresh samples. Dry weight was obtained by drying samples in the oven at 70 degree Celsius temperature for 72 hours and then determined the weight using an analytical balance.

#### Total chlorophyll content

Leaf samples (100 mg) were crushed and homogenized with 5 mL of 95% ethanol solution then samples were centrifuged at 10.000rpm for 10 minutes. Total chlorophyll was measured by taking absorbance readings using UV-Vis spectrophotometer at 664 nm and 649 nm wavelengths then calculated using the appropriate extinction coefficient (Lichtenthaler 1987).

Chlorophyll a ( $\mu\text{g/mL}$ ) =  $(13.36 * \text{Abs. } 664) - (5.19 * \text{Abs. } 649)$

Chlorophyll b ( $\mu\text{g/mL}$ ) =  $(27.43 * \text{Abs. } 649) - (8.12 * \text{Abs. } 664)$

Total Chlorophyll ( $\mu\text{g/mL}$ ) =  $(5.24 * \text{Abs. } 664) - (22.24 * \text{Abs. } 649)$

#### MDA and ROS content

Total MDA content was determined by adding 5000  $\mu\text{L}$  0.1% trichloroacetic acid (TCA) solution to a homogenized sample of rice leaves (100 mg). The homogeneous sample was then transferred into a 1.5 mL tube and centrifuged at 12,000 rpm for 10 minutes at 4°C. Then 4000  $\mu\text{L}$  TBA was added to 1000  $\mu\text{L}$  supernatant and incubated at 90°C for 30 minutes. The supernatant absorbance value was read using a UV-Vis spectrophotometer at 532 nm, and the values corresponding to non-specific absorption (600 nm) were subtracted. Total ROS content was determined from homogeneous samples that were centrifuged at 12,000 rpm for 15 minutes. 0.5 mL supernatant was taken and then transferred to a 1.5 mL tube and added 0.5 mL of 10 mM phosphate buffer (pH 7) and 1 mL of 1 M potassium iodide were. Samples were then incubated at room temperature for 30 minutes. The absorbance value of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) content was measured using a UV-Vis spectrophotometer at 390 nm.

#### Lead (Pb) content

Lead (Pb) in rice plants was determined using Atomic Absorption Spectrophotometry (AAS) analysis. The AAS analysis method is used to calculate the amount of radiation energy absorbed by atoms that are at the fundamental energy level. AAS analysis in lead (Pb) was determined in 283.3 nm wavelength (Sylvia 2019).

#### Data analysis

Research data were analyzed statistically using Analysis of Variance (ANOVA) at 5% significant level. Significant differences between treatments were then analyzed using Duncan Multiple Range Test at a 5% significant level.

## RESULTS AND DISCUSSION

### Rice plant morphology

Plant morphologies were observed to determine the effect of Pb and bacterial consortiums on the growth of rice plants, including plant height, root length, fresh weight, and dry weight. Results showed lead (Pb) treatment did not significantly affect plant height, and the addition of several doses of the bacterial consortium to the plants also did not significantly affect plant height parameters (Figure 1A). Results regarding the root length of rice seedlings presented in Figure 1B showed lead (Pb) treatment significantly increased the root length compared to control. However, the addition of bacterial consortium in 6 mL/L had a significant effect by increasing 41% the root length compared to the control (Figure 1B). Fresh weight showed a significant effect on lead (Pb)-treated plants compared to the control. Each dose of bacterial consortium produced significant effects on Pb treatment compared to control (Figure 1C). Plants treated with rhizosphere bacterial consortium had about 27 to 32% increase in fresh weight

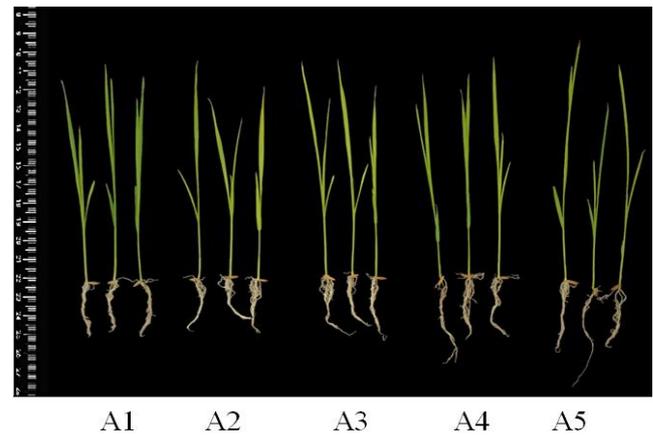
compared to the Pb treatment. The dry weight of Pb-treated plants showed significant differences as compared to the control. The addition of the bacterial consortium did not affect control plants but showed a significant effect on lead (Pb)-treated plants (Figure 1D). Rhizosphere bacterial consortium gives the highest increase of dry weight at 26% in 6 mL/L treatment.

The results in Figure 1 showed the influence of heavy metals and the addition of a consortium of rhizosphere bacteria on the morphological parameters of rice plants (plant height, root length, fresh weight, and dry weight). Plants that were exposed to lead (Pb) contamination showed decreased growth compared to control plants (without heavy metals).

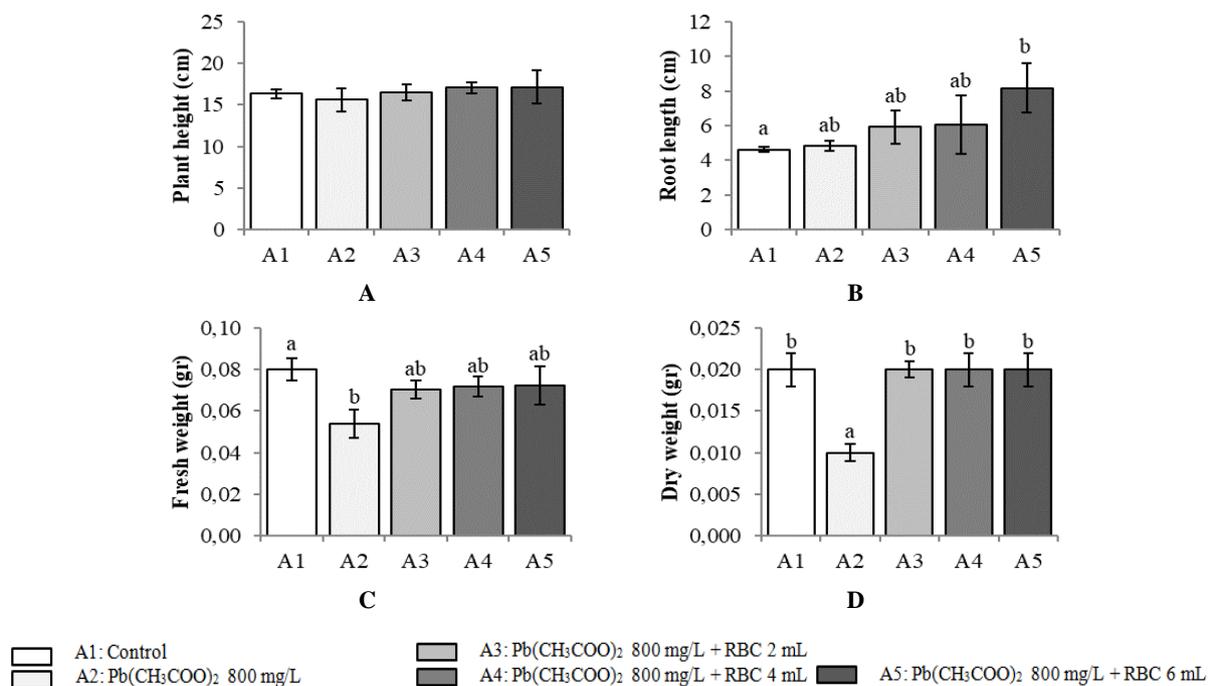
According to Chandwani et al. (2023), high levels of Pb decreases germination and limit plant physiological characteristics such as length, height, and biomass. These effects occurred because the heavy metal content received by plant roots through nutrition could reduce polysaccharides in the cell walls, therefore causing inhibition of cell elongation and plant growth and development (Keyster et al. 2020).

The addition of a rhizosphere bacterial consortium to lead (Pb)-contaminated rice plants resulted in a significant increase in root length and fresh weight as compared to control plants without heavy metals. This is because a consortium of rhizosphere bacteria can produce plant-growth-promoting substances in an environment stressed by heavy metals. Growth-promoting substances in the form of indole-3-acetic-acid (IAA) molecules are synthesized by a rhizosphere bacterial consortium and transported into

plant cells (Bahadur et al. 2017). The IAA molecule increase in lateral and adventitious rooting which leads to increased absorption of minerals and nutrients. This IAA molecule has a role in the activity of 1-aminocyclopropane-1-carboxyle (ACC) deaminase, which can reduce ethylene levels in plants. The deaminase activity can protect plants from inhibition of growth caused by ethylene stress from heavy metal lead (Pb) contamination (Gupta et al. 2022).



**Figure 2.** Rice plants after treatments. A1: Control, A2: Pb(CH<sub>3</sub>COO)<sub>2</sub> 800 mg/L, A3: Pb(CH<sub>3</sub>COO)<sub>2</sub> 800 mg/L + POC 2 mL, A4: Pb(CH<sub>3</sub>COO)<sub>2</sub> 800 mg/L + POC 4 mL, A5: Pb(CH<sub>3</sub>COO)<sub>2</sub> 800 mg/L + POC 6 mL



**Figure 1.** Rice morphology in different treatments. A: plant height, B: root length, C: fresh weight, D: dry weight. The mean  $\pm$  SD followed by different letters indicate significant difference according to Duncan's test at a significance level  $\alpha = 0.05$

### Chlorophyll content of rice

Chlorophyll converts sunlight into energy, which plants need, through the photosynthesis process. Lead (Pb) toxicity negatively impact on plants' photosynthetic pathways by altering the ultrastructure of chloroplasts and preventing the synthesis of critical pigments. All chlorophyll content shows a reduction due to lead (Pb) contamination compared to the control treatment (Figure 3).

Chlorophyll A content in rice plants treated with Pb showed no significant effect in the control treatment, whereas lead (Pb) treatment had a significant effect on plants treated with rhizosphere bacterial consortium (Figure 3A). Chlorophyll B and total chlorophyll did not show a significant effect in any treatments. The addition of rhizosphere bacterial consortium 2 mL/L, 4 mL/L, and 6 mL/L showed increased values of 25%, 24%, and 21% in chlorophyll A content compared to lead (Pb)-treated plants. The data shown in Figure 3 reveal that the chlorophyll content increased in plants treated with the rhizosphere bacterial consortium compared to those not treated with the bacterial consortium. This is in line with a study conducted by Manoj et al. (2020) showed a consortium of bacteria was capable of collecting heavy metals, increasing photosynthetic activity and chlorophyll content in plants, leading to an increase in plant growth.

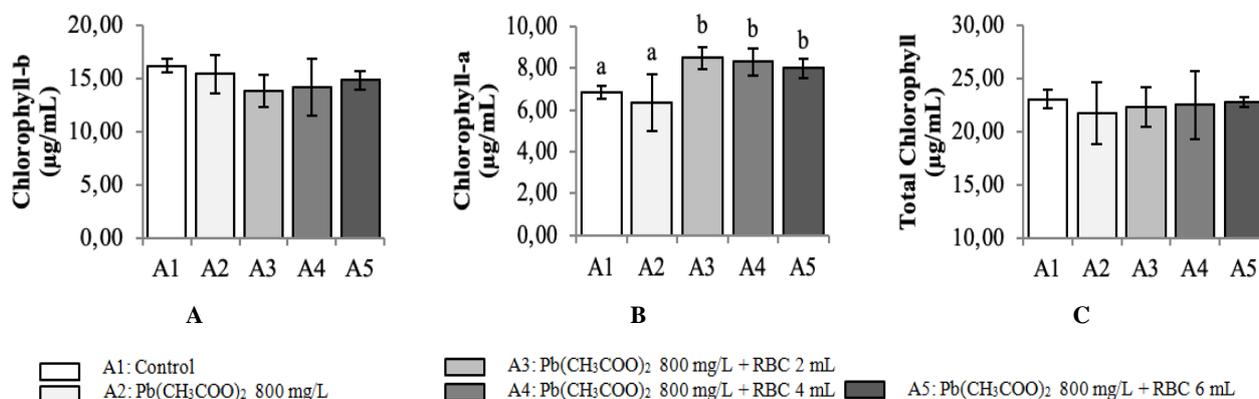
### MDA and ROS content

The analysis of malondialdehyde (MDA) and Reactive oxygen species (ROS) content in rice plants was used to determine plant response to the contamination. Rice plants contaminated with lead (Pb) showed increased MDA and ROS content (Figure 4). This represents distinct plant responses to abiotic stress caused by various defense mechanisms (Ubaidillah et al. 2019). The best result of MDA content (1.189 nmol/L) was found in the 6 mL/L treatment of bacterial consortium. This result showed that the bacterial consortium at 6 mL/L could reduce MDA content in lead (Pb) treated rice plants by up to 50% (Figure 4A). ROS content in rice plants treated with lead

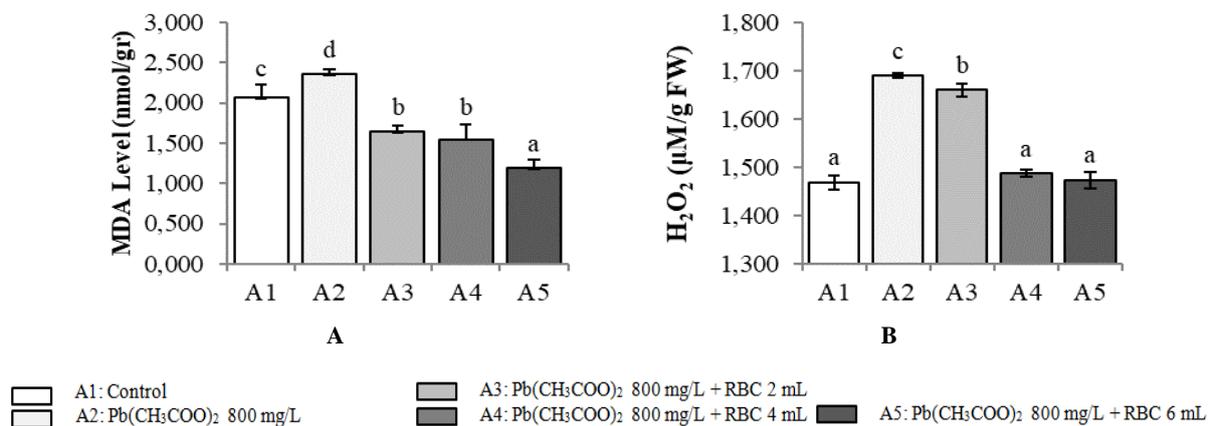
(Pb) showed significant results compared to the control treatment. The ROS content in the Pb treatment increased compared to the control. Figure 4B shows that 6 mL/L bacterial consortium treatment gave the best results in reducing 13% ROS content in rice plants.

Analysis of MDA and ROS content in rice plants are used to observe the oxidative damage in rice plants. Figure 4 shows that heavy metal contamination can increase the production of MDA and ROS in rice plants and has been confirmed by Khan et al. (2018). ROS accumulation in stress conditions causes by the reduction of CO<sub>2</sub> intake resulted in an accumulation of O<sub>2</sub> in plants, which can lead to severe damage in metabolic processes (Ubaidillah et al. 2023). In plants contaminated with heavy metals, ROS and MDA accumulation triggers the Haber-Weiss cycle which can produce lipid and pigment peroxidases and the production of hydroxyl radicals. This resulted in damage to the permeability and function of the membrane (Ghorbani et al. 2019).

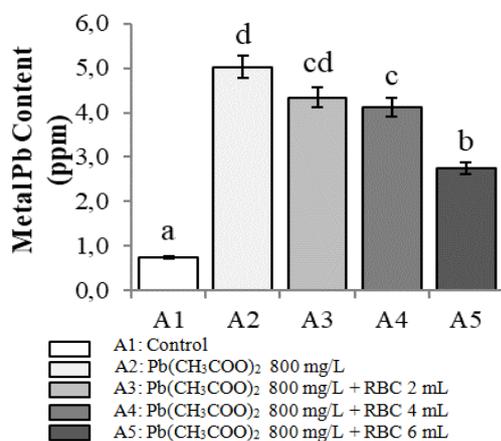
The MDA and ROS content in Figure 4 decreased in the plants with the rhizosphere bacterial consortium compared to the control plant. To reduce damage caused by MDA and ROS, plants need to have an antioxidant defense system by producing enzymes such as catalase, peroxidase, superoxide dismutase, polyphenol oxidase, and phenylalanine ammonia-lyase (Bano et al. 2012). These antioxidant enzyme productions can be triggered by the addition of rhizosphere bacteria. Plants under stress conditions can cause an increase in peroxidase enzymes and polyphenol oxidases to protect plants from stress-induced ROS and release phenols formed from these conditions. After that, there was a decrease in both enzymes and an increase in the enzyme phenylalanine ammonia-lyase which acts on the products of the two previous enzymes. The product can bind with proline and produces proline hydroxyl compounds to enter lignin so that it can help strengthen cell walls to reduce the transpiration process and reduce plant stress (Dong et al. 2021).



**Figure 3.** Chlorophyll content of rice plants in different treatments. A: Chlorophyll-a, B: Chlorophyll-b, C: Total Chlorophyll. The mean  $\pm$  SD followed by different letters indicates significant difference according to Duncan's test at a significance level  $\alpha = 0.05$



**Figure 4.** MDA content and ROS accumulation of rice plants in different treatments. A: MDA content, B: H<sub>2</sub>O<sub>2</sub> production. The mean  $\pm$  SD followed by different letters indicates a significant difference according to Duncan's test at a significance level  $\alpha = 0.05$



**Figure 5.** Lead (Pb) content (ppm) in rice plants in different treatments. The mean  $\pm$  SD followed by different letters indicate significant difference according to Duncan's test at a significance level  $\alpha = 0.05$

### Lead (Pb) content in rice plants

The content of heavy metals in rice plants produced a significant effect on the lead (Pb) treatment compared to the control. Plants treated with lead (Pb) showed a significant effect on the control and each dose of bacterial consortium (Figure 5). The analysis resulted in the highest lead (Pb) content found in the lead (Pb) treatment, which was 5.02 mg/L, while the lowest Pb metal content was found in treatment with 6 mL/L bacterial consortia, which had 2.75 mg/L lead (Pb) metal content. The lead (Pb) treatment increased 85% of the Pb content in rice plants compared to control plants. The present results showed that the addition of 6 mL/L bacterial consortia could decrease lead (Pb) by up to 45% in rice plants.

Figure 5 shows lead (Pb) accumulation in rice plants due to different treatments. According to Khan et al. (2018), rice plants are very susceptible to Pb contamination. The mechanism of heavy metal accumulation in plants involves transfer through nutrient absorption in the roots because metals can bind to the organic matter in the soil and be absorbed from the soil as

well as from the atmosphere by leaves with certain morphologies, such as downy leaves (Javaid et al. 2020).

The use of rhizosphere bacteria increases plant tolerance to toxic substances such as heavy metals. According to El-Meihy et al. (2019), certain bacteria may associate with plants and induce plant growth and health to speed up the process of remediation of heavy metal contamination. Figure 1 reveals that the heavy metals contained in plants decreased in the addition of a consortium of rhizosphere bacteria treatments. The rhizosphere bacteria consortium reduces heavy metal pollution in rice plants by absorbing heavy metals (Ahemad and Kibret 2013). The present results demonstrated that the rhizosphere bacteria consortium was effective in minimizing Pb and heavy metal pollution in rice plants.

In this study, the application of rhizosphere bacterial consortium to plants contaminated with lead (Pb) reduced the lead (Pb) content by up to 45% in rice plants. The use of a bacterial consortium in rice plants did not significantly affect the parameters of plant height. However, it can increase several growth parameters, such as root length by 41%, fresh weight by 32%, dry weight by 26%, and chlorophyll content by 25%, in plants contaminated with heavy metals. Therefore, adding a rhizosphere bacterial consortium could be a sustainable method for reducing lead (Pb) accumulation in rice plants. However, experiments at further growth stages, such as the reproductive stage of rice plants, are needed to provide complete information regarding this bioremediation agent's potential.

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