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# The Role of Cadmium-Resistant Bacterial Application and Compost in Promoting Water Spinach Growth and Reducing Cadmium Uptake

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#### ABSTRACT

The continuous use of chemical fertilizers and pesticides in soil can result in the presence of cadmium (Cd) residues that may interfere with plant growth and pose a risk of uptake by plants. The issue of soil contamination by Cd can be addressed through soil bioremediation, which involves the use of Cd-resistant bacteria and compost. The objective of this study was to analyze the impact of Cd-resistant bacteria and compost application on the growth and Cd uptake of water spinach. The research design was a completely randomized design with seven treatments: (1) control, (2) 5 mL Cd-resistant bacteria consortium, (3) 10 mL Cd-resistant bacteria consortium, (4) 10 tons/ha compost, (5) 20 tons/ha compost, (6) 5 mL Cd-resistant bacteria consortium +10 tons/ha compost, and (7) 10 mL Cd-resistant bacteria consortium +20 tons/ha compost. The results demonstrated that the application of Cd-resistant bacteria and compost did not notably impact the growth of water spinach. However, it did significantly influence the reduction of Cd uptake in water spinach. The application of the Cd-resistant bacterial consortium and compost was effective in reducing the Cd uptake of the water spinach plants in all treatments. The combination of 10 mL of bacterial isolate and 20 tons/ha of compost demonstrated the greatest reduction in Cd uptake by water spinach, reaching 73%.

## 1. INTRODUCTION

The Indonesian Fertilizer Producers Association (APPI) has announced that the use of chemical fertilizers is on the rise. From 2017 to 2021, the use was approximately 10-11 million tons per year. In the first half of 2022, it reached 48.18% of the previous year's total. By the end of 2023, there was a further increase due to the use of chemical fertilizers in January-June 2023 reaching 5.1 million tons (APPI, 2023). The extensive use of chemical fertilizers and pesticides in Indonesia has resulted in a number of adverse effects, including deterioration of soil quality, contamination of water and air, and toxicity, carcinogenicity, and mutagenicity in surrounding organisms (Ogura et al., 2021). A significant challenge currently facing intensive agricultural operations is soil contamination by heavy metals. One of the environmental consequences of the use of chemical fertilizers and pesticides is acidification caused by heavy metals in these products. The content of heavy metals in fertilizers and pesticides, including cadmium (Cd), mercury (Hg), chromium (Cr), lead (Pb), and arsenic (As), has been identified in agricultural products (Ahemad, 2019). Cadmium is a particularly harmful heavy metal, even at low concentrations. Cadmium is one of the three most hazardous heavy metals to human health (Kusumaningrum et al., 2012).

Cadmium has been identified in a number of agricultural chemical products, including chemical fertilizers and chemical pesticides. The Cd content in phosphate fertilizers ranges from 30 to 60 mg/kg, while in nitrogen fertilizers it ranges from 0.05 to 8.5 mg/kg, and in insecticides and fungicides it ranges from 0.04 to 0.5 mg/kg (Mahendra et al.,

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2018; Dewi et al., 2022). The continuous and high-dose use of agricultural chemical products has resulted in the accumulation of Cd in the soil. This finding is corroborated by the research conducted by Dewi et al. (2022), which indicates that the cadmium concentration in soil with extensive agricultural land use ranges from 1.01 to 1.46 mg/kg. The findings of Dewi et al. (2022) also indicate that the Cd content in soil where intensive shallot farming is practiced ranges from 1.01 to 1.46 mg/kg. Research by Charles & Rini (2018) also indicated that certain regions in Indonesia have been contaminated with Cd originating from the use of TSP fertilizer, with soil Cd concentrations ranging from 0.62 to 2.89 ppm (e.g., in Karawang, Subang, Indramayu, and Majalengka districts in West Java Province). The Cd content in the soil has exceeded the safe threshold, as defined by the KLH-Dalhousie University (1992), which is 0.5 mg/kg. Soil contamination with Cd has a detrimental impact on plant growth. Cadmium toxicity in the soil leads to oxidative stress, manifesting as symptoms of leaf wilting and chlorosis. This impairs plant growth and productivity (Mariem, 2022). Cadmium contamination in the soil also allows cadmium to be absorbed by plants and accumulate in plant tissues, which presents a health hazard to humans and animals that consume it (Li et al., 2021).

Soil contamination by Cd due to the use of chemical fertilizers and pesticides needs to be properly managed. Efforts that can be made to overcome this problem are through soil bioremediation, which is an action taken to convert hazardous compounds into harmless ones by using living organisms (Zhang et al., 2020), such as bacteria. Bacteria have the ability to bind heavy metals by using their cell walls (biosorption), accumulate heavy metals in their cell cytoplasm (bioaccumulation), and mineralize heavy metals into unavailable forms in soil (biomineralization), so they can be used as heavy metal bioremediators (Zhang et al., 2020). A previous study reported that the bacterial isolates (Bal sp.1 and Bal sp.2) isolated from laboratory waste can reduce Cd by 75.61-87.80% (Bahri et al., 2018). Bacterial isolates EK2 and EK4 from gold mining in Palu, South Sulawesi reported that they can reduce Cd by 99.57% and 99.59% (Fahruddin et al., 2020). The performance of bacteria as bioremediation can be maximized by adding organic materials (e.g., compost) that act as an energy source for bacteria and also act as heavy metal chelators because their surface is composed of negative charges, so they can bind positively charged Cd (Khan et al., 2017). Alfandi (2018) reported that the application of compost at a dose of 15 tons/ha can reduce the Cd content in the roots and shoots of plants by 31.37%. Moreover, the addition of compost can increase the height of plants and reduce the absorption of Cd in water spinach (Lestari & Aji, 2020).

It is anticipated that the combination of bacterial and compost applications will yield the most effective results in addressing the impact of soil contamination by Cd, which is a consequence of the use of chemical fertilizers and pesticides. The objective of this study is to analyze the impact of the application of Cd-resistant bacteria and compost on the growth and cadmium absorption of water spinach. The combination of Cd-resistant bacteria and compost application in soil bioremediation has not been widely studied in water spinach. This study aims to analyze the impact of cadmium-resistant bacteria and compost application on the growth and Cd uptake of water spinach plants, with the goal of providing valuable insights for future research and applications in this field.

## 2. MATERIAL AND METHODS

#### 2.1. Study Site

This research was conducted from March to June 2024 in a greenhouse of the Faculty of Agriculture, Brawijaya University, Jatimulyo Village, Lowokwaru District, Malang City, and in the Soil Chemistry and Biology Laboratory of the Faculty of Agriculture, Brawijaya University.

#### 2.2. Research Design

The soil was collected from a paddy field in Telukjame Village, East Telukjambe Subdistrict, Karawang Regency, West Java Province (6°20'33.709"S and 107°19'18.318"E). The soil Cd content was 1.22 ppm, the soil pH was 6.12, the soil organic carbon was 1.3%, and the total soil nitrogen was 0.125% Cd-resistant bacterial consortium (Shewanella decolorationis, Chryseobacterium cucumeris, and Aeromonas hydrophila) polybag (25 cm x 25 cm), 2 N HCl solution, Whatman 42 mm filter paper, TSP fertilizer, insecticide (Marshal® 200 EC; Carbosulfan), compost, water spinach seed, plastic sample, scales, rulers, stationery, autoclaves, soil grinder, sieves, shakers, and AAS (atomic absorption spectrophotometer). This study employed a completely randomized design (CRD) with a total of 28

experimental units, comprising seven treatments and four replications. Table 1 outlines the combination of treatments utilized in this study.

Table 1. Research Treatment

No	Treatment Code	Description			
1	K	Control			
2	TB1	5 mL Cd-resistant bacterial consortium			
3	TB2	10 mL Cd-resistant bacterial consortium			
4	TO1	10 ton/ha compost			
5	TO2	20 ton/ha compost			
6	TBO1	5 mL Cd-resistant bacterial consortium +10 ton/ha compost			
7	TBO2	10 mL Cd-resistant bacterial consortium +20 ton/ha compost			

## 2.3. Bacteria Preparation

## 2.3.1. Consortium Preparation of Cd-Resistant Bacteria

The bacterial **isolates** were prepared in nutrient broth with a cell density of 10<sup>8</sup> CFU/g for each isolate. The suspension is then placed in a sterile bottle in a ratio of 1:1:1 and shaken until the volume 300 mL. The isolates were mixed using shaker to ensure homogeneity.

## 2.3.2. Preparation of Planting Media

The soil was allowed to air-dry for a period of 4–5 days and then passed through a 2 mm sieve in order to ensure uniformity of all soil particles used. Subsequently, the soil was sterilized using an autoclave to eliminate any bacteria present. The planting medium was prepared by filling sterilized soil into polybags (up to 4 kg per polybag) and adding TSP fertilizer and insecticide to address Cd contamination in the soil. The addition of compost to the polybags, in accordance with the specified treatment, was conducted concurrently with the application of TSP and insecticide.

#### 2.3.3. Planting, Maintenance and Harvesting of Water Spinach

The bioremediation process was evaluated based on several parameters, including plant growth and the Cd content in harvested water spinach. The plant growth was evaluated based on the number of leaves and the height of the plant over a five-week period (one, two, three, four, and five weeks after planting). The results of measuring the height and number of plant leaves were not only used as plant growth parameters but also to calculate the relative growth rate (RGR), which was used to observe the impact of heavy metal contamination in the soil on plant growth (Nainggolan *et al.*, 2022). The following equation was used to calculate the RGR:

RGR = 
$$\frac{Wt - W0}{W0 x t} x 100\%$$
 (1)

where Wt and W0 is the average plant height at the end of observation the beginning of observation, respectively, and t is observation duration (days).

## 2.3.4. Preparation of Plant Samples and Analysis of Cd Uptake of Water Spinach

The harvested water spinach was cleaned of residual soil and weighed to determine its fresh weight. Subsequently, the water spinach was placed in a paper envelope and dried in an oven for a period of 3-4 days at an oven temperature of 70-80°C. The dried water spinach was then weighed (dry biomass), ground, and filtered using a 0.5 mm sieve. The water spinach samples were used to calculate the Cd content in the plant according to the wet digestion method (Eviati et al., 2023), using AAS at a wavelength of 228.8 nm.

## 2.4. Data Analysis

The data were analyzed for normal distribution using the Shapiro-Wilk test, then subjected to an analysis of variance (ANOVA) at the 5% level using R Studio. This was followed by a post-hoc test using the Honest Significant Difference test at the 5% level.

#### 3. RESULT AND DISCUSSION

#### 3.1. Growth of Water Spinach

The water spinach growth was evaluated through plant height, number of leaves and relative growth rate (RGR) for five weeks from 0 days after planting (DAP) to 35 days after planting. The results of observations on plant height ware presented in Table 2. The results of the analysis of plant growth observed based on the height of the water spinach plants for 35 days after planting (DAP) showed that there was no significant effect of each treatment on the height of the water spinach plants with the height of the plants at 35 DAP ranging from 49-51 cm. The RGR can be interpreted as the increase in organic matter per day which is calculated to determine the impact of heavy metal contamination in the soil on plant growth (Nainggilan *et al.*, 2022). The RGR value of plant height ranged from 7.5-11.8% each day, with the treatment with the highest RGR value being TBO1, namely the addition of 5 mL of Cd-resistant bacterial isolates and 10 tons/ha of compost. These results showed that the application of Cd-resistant bacterial consortium and compost gives a higher growth rate of water spinach plants than without the application (control).

Table 1. The average plant height 7 to 35 day after planting

No	Treatment	Plant Height (cm)					- RGR of Plant Height (%)	
		7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	- KOK of Flant Height (78)	
1	K	15.08	27.67	31.00	39.33	49.08	82	
2	TB1	13.92	28.00	31.92	37.67	47.92	91	
3	TB2	16.25	32.67	38.25	43.25	51.21	77	
4	TO1	15.17	31.08	35.25	42.33	49.17	75	
5	TO2	11.83	29.75	33.83	41.83	49.08	80	
6	TBO1	15.58	25.25	30.33	36.67	50.38	118	
7	TBO2	14.85	30.25	35.75	41.50	49.00	77	

Table 2. The average number of water spinach leaves

No.	Treatment	Average Number of Leaves					RGR
		7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	(%)
1	K	7.67	11.08	13.75	10.08	19.38	5.7
2	TB1	7.17	10.58	12.75	10.67	15.00	3.9
3	TB2	7.58	11.33	13.83	12.00	21.42	6.4
4	TO1	7.25	11.08	13.75	11.58	16.63	4.7
5	TO2	7.67	11.00	13.92	10.75	17.25	4.5
6	TBO1	6.83	10.17	12.42	10.75	17.00	5.5
7	TBO2	7.67	11.42	13.50	11.33	18.08	4.9

The analysis of the number of leaves of the water spinach plants revealed that the treatment had no significant impact on the number of leaves, with an average of 15-21 strands per plant at 35 days after planting (DAP) (Table 3). The RGR value for the number of plant leaves ranged from 3.9% to 6.4% each day, with the highest RGR value observed in the TB2 treatment, which involved the addition of 10 mL of a Cd-resistant bacterial isolate per polybag. The lack of a significant treatment effect on water spinach growth, as indicated by plant height and leaf number, may be attributed to inadequate nutrient provision by the planting medium, particularly nitrogen (N). The total soil nitrogen in polybags was found to be only around 0.124-0.160% in all treatments, indicating that the nitrogen content in the soil was low (Eviati *et al.*, 2023). The soil in the polybag, which serves as the water spinach planting medium, is not fertilized with nitrogen and is only provided with phosphate fertilizer (P) and insecticides due to Cd contamination. This results in a relatively low nitrogen (N-total) content in the soil in the polybag. The lack of nitrogen addition may be the reason for the insignificant effect of the treatment on plant height. Nitrogen plays a crucial role in the vegetative phase of plants, including the formation of stems, leaves, and roots (Suhastyo & Raditya, 2019).

#### 3.2. Cadmium Uptake of Water Spinach

The analysis of Cd uptake in plants revealed a notable impact of the treatment on Cd uptake in water spinach plants (Figure 1). The uptake of Cd by plants was found to decrease significantly, ranging from 19% to 73% compared to the control. The control treatment yielded the highest Cd uptake in plants at 0.057 g/plant, while the TBO2 treatment demonstrated the lowest Cd uptake at 0.015 g/plant. The results demonstrated that the application of Cd-resistant bacteria and compost could effectively reduce the uptake of Cd by water spinach. This is due to the ability of bacteria and compost to bind Cd, rendering it unavailable and preventing its uptake by water spinach. In line with the previous result by Alfandi (2018), organic matter has the potential to enhance the soil's capacity to adsorb Cd from an available to an unavailable form. The majority of the Cd is adsorbed in a non-exchangeable form, indicating the presence of a robust organic complex. The presence of organic matter derived from organic fertilizers can reduce the bioavailability of Cd by retaining the metal through the formation of complexes with phosphate, aluminum, and organic minerals derived from the organic matter itself.

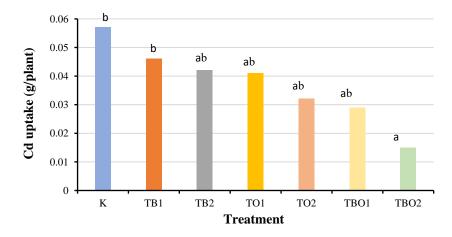


Figure 1. Cadmium uptake of water spinach [same letters indicate no significant difference according to HSD test at the 5% level].

Bacteria have the ability to synthesize metals through a number of processes, including biosorption, bioaccumulation, biodegradation, biotransformation, and biomineralization (Zhang et al., 2020). Urease-producing bacteria are capable of breaking down urea into carbonate, which can then be used to precipitate Cd with the urease they produce. Some sulfate-reducing bacteria produce H<sub>2</sub>S, which forms intracellular or extracellular CdS deposits (Xia et al., 2021). The bacterial species (Shewanella decolorationis, Chryseobacterium cucumeris, and Aeromonas hydrophila) utilized in this study are capable of producing H<sub>2</sub>S and urease enzymes that facilitate the formation of insoluble Cd salts. Shewanella decolorationis is capable of producing H<sub>2</sub>S from thiosulfate and reducing nitrate, nitrite, ferric materials, and thiosulfate with lactate or acetate as electron donors (Lemaire et al., 2019; Wang et al., 2021). Furthermore, Chryseobacterium cucumeris is capable of producing β-galactosidase, urease, gelatinase, and indole (Jeong et al., 2017), while Aeromonas hydrophila is able to produce indole from the amino acid tryptophan through the enzyme tryptophanase and produce H<sub>2</sub>S (Arwin et al., 2016).

#### 4. CONCLUSIONS

The application of cadmium-resistant bacterial consortium and compost with different doses had an impact on significantly reducing the Cd uptake of water spinach plants reached 73% compared to the control, after the application of 10 mL of Cd-resistant bacteria +20 tons/ha of compost. The provision of Cd-resistant bacterial consortium and compost also gave a better relative plant growth rate than the control, although between treatments was not significantly different. This occurs because of the low nitrogen content in the soil so that plant growth is not optimal.

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