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Potential of *Bacillus* sp. and FOBIO Biopesticide with Mustard Greens (*Brassica rapa* L. var. *parachinensis*) as Bioremediation Agents for Cadmium

Dykha Maretha Setyawan[1], Endang Tri Wahyu Prasetyawati[1, ✉], Sri Wiyatiningsih[1]

[1] Department of Agrotechnology, Faculty of Agriculture, UPN "Veteran" Jawa Timur, Surabaya 60294, INDONESIA.

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Corresponding Author:
✉ endang_tp@upnjatim.ac.id
(Endang Tri Wahyu Prasetyawati)

ABSTRACT

Cadmium (Cd) contamination in agricultural land due to excessive use of fertilizers and pesticides has detrimental effects on soil quality, plant growth, and human health. Bioremediation offers a simple and environmentally friendly approach to address this issue. This study aimed to evaluate the potential of Bacillus sp., FOBIO biopesticide, and green mustard (Brassica rapa L. var. parachinensis) as bioremediation agents for Cd-contaminated soil. The experiment was conducted using a Completely Randomized Design (CRD) with seven treatments and four replications. Observed parameters included Cd concentration in the soil before and after remediation, as well as the growth and physiological responses of mustard plants. The application of FOBIO biopesticide without plants resulted in the highest Cd reduction (4.57 mg/kg), while the combination of Bacillus sp. and mustard plants reduced Cd by 4.11 mg/kg, and mustard plants alone reduced Cd by 2.40 mg/kg, outperforming the control. FOBIO biopesticide application significantly enhanced leaf number, plant height, and fresh weight compared to other treatments. These findings indicate that Bacillus sp., FOBIO biopesticide, and green mustard have strong potential as bioremediation agents for Cd-contaminated soils.

1. INTRODUCTION

Cadmium (Cd) is a non-essential heavy metal that is highly toxic in agricultural systems. Due to its high mobility and solubility, cadmium is easily absorbed by plants (Meriem, 2022). Elevated Cd levels in soil are often the result of intensive use of chemical fertilizers and pesticides (Mulyani *et al*., 2023). According to (Sutrisno & Kuntyastuti, 2015) intensive agricultural regions such as Pengalengan, Karawang, Cirebon, Brebes, Subang, Tegal, Majalengka, and Indramayu are major centers of rice and vegetable production in Indonesia. In these areas, continuous farming without fallow periods and excessive use of agrochemicals have led to the accumulation of heavy metals particularly cadmium (Cd), lead (Pb), and copper (Cu) in agricultural soils.

Efforts to restore heavy metal-contaminated soils are necessary to ensure safe and sustainable agricultural use. Physical and chemical remediation methods such as ion exchange, evaporation, precipitation, chemical reduction, and reverse osmosis are available (Liwun *et al*., 2021), but these approaches are costly and may pose environmental risks. Bioremediation presents a more environmentally friendly and cost-effective alternative. This method involves the use of microorganisms, plants, or biocatalysts to transform hazardous pollutants into non-toxic substances.

The use of *Bacillus* sp., FOBIO biopestiside, and hyperaccumulator plants like green mustard (*Brassica rapa* L. var. *parachinensis*) offers a promising strategy for the bioremediation of cadmium-contaminated agricultural soils. Maulana *et al*. (2017) reported that *Bacillus* species were effective as bioremediation agents for heavy metals. The *Bacillus* Ba 13 strain used in this study was also proven to reduce mercury (Hg) levels (Purkan *et al*., 2017). Through the biosorption process, *Bacillus* produced enzymes that absorbed heavy metals such as Cd into the bacterial cells, which helped decrease metal toxicity in the environment (Aznur *et al*., 2022).

The FOBIO biopesticide formula is an organic pesticide based on microorganisms that function as biological agents, decomposers, and Plant Growth Inhibiting Bacteria (PGPR). In addition, the microorganisms contained in the FOBIO biopesticide formulation have shown potential for reducing heavy metals in the soil. This was proven by Widya *et al*. (2022), where weekly routine application of FOBIO biopesticide significantly reduced Pb concentrations in the soil, demonstrating its broader potential in bioremediation applications, including for cadmium (Cd).

Green mustard (*Brassica rapa* L. var. *parachinensis*) is known for its tolerance to heavy metal stress and its ability to absorb metals from soil. The plant's roots can extract heavy metals and translocate them to other parts such as stems, leaves, and even edible tissues, allowing for metal accumulation within its biomass (Panjaitan & Sidauruk, 2024). Therefore, the benefits of this research are expected to contribute to the development of sustainable agricultural technology regarding the potential of *Bacillus* sp. bacteria, FOBIO biopesticide, and green mustard plants as bioremediation agents for heavy metal cadmium (Cd).

2. MATERIALS AND METHODS

2.1. Materials and Tools

This research was conducted from August 2024 to May 2025 at the Plant Health Laboratory, Soil Resources Laboratory, and Greenhouse of the Veteran National Development University in East Java.

The tools used in the research are autoclave, LAF, petri dish, mortar and pestle, mesh, measuring cup, beaker glass, erlenmeyer, glass bottle, test tube, measuring flask, volume pipette, incubator, AAS spectrophotometer, and other supporting equipment. The materials used in the research are *Bacillus* sp. bacteria collection of Dr. Dra. Endang Triwahyu Prasetyawati, M.Si, NA and NB media, distilled water, soil, green mustard seeds, compost, heavy metal cadmium (Cd), FOBIO biopesticide, polybags, trays.

2.2. Research Design and Data Analysis

This study used a non-factorial Completely Randomized Design. The experiment involved 7 treatments replicated 4 times, resulting in a total of 28 experimental units. The observation data were analyzed using analysis of variance (ANOVA). If ANOVA showed significant differences between treatments, the analysis was continued with the Honestly Significant Difference (HSD) test at a significance level of 5% using SPSS software to identify differences between treatments in more detail.

Table 1. Research Treatments

Treatment	Healthy Soil	Contaminated Soil	*Bacillus* sp.	FOBIO Biopesticide	Green Mustard
P0		√			
P1		√			√
P2	√				√
P3		√	√		
P4		√	√		√
P5		√		√	
P6		√		√	√

2.3. Preparation

2.3.1. Preparation of stock solution

The 1000 ppm stock solution of $Cd(NO_3)_2$ was prepared by dissolving 2.107 grams of $Cd(NO_3)_2$ in 1000 ml of distilled water. Stock solutions with concentrations of 4 ppm, 8 ppm, 12 ppm, and 16 ppm were then prepared through a gradual dilution process of the stock solution (Kunsah *et al*., 2021).

2.3.2. *Bacillus* sp. Resistance Test to Cadmium

The resistance test in this study aimed to evaluate five *Bacillus* sp. bacterial isolates (Ba 1, Ba 2, Ba 11, Ba 12, and Ba 13) resistant to cadmium. Each isolate was inoculated into liquid media containing cadmium solution. Bacterial culture suspensions were obtained through a 10^{-5} dilution process. Then, 1 ml of *Bacillus* sp. culture suspension was grown in 5 ml of NB medium supplemented with 5 ml of cadmium (Cd) at concentrations of 4 mg/kg, 8 mg/kg, 12 mg/kg, and 16 mg/kg. The mixture was vortexed for 1 minute and incubated for 3 days at room temperature. After the incubation period, the turbidity of the medium was observed; the more turbid the medium, the more resistant the *Bacillus* sp. bacteria were to cadmium (Cd) (Ilmianti *et al*., 2025). Additionally, to confirm that the bacteria are resistant to heavy metal cadmium (Cd), the Total Plate Count (TPC) method was performed with a colony count range of 25–250 (Rizki *et al*., 2022). The number of *Bacillus* sp. colonies was converted into cfu/ml units using the formula (Azizah & Soesetyaningsih, 2020).

$$\text{Number of colonies (CFU/ml)} = \sum \text{breeding colony} \times \frac{1}{dilution\ factor} \quad (1)$$

Based on the test results, it was found that the *Bacillus* sp. isolate with the code Ba 13 had the highest colony density with a value of 8.2 × 106 at a cadmium concentration of 16 mg/kg. Ba 13 is the isolate most resistant to heavy metal cadmium among the five other bacterial isolates. Therefore, the *Bacillus* sp. isolate with the code Ba 13 was used in the subsequent bioremediation testing in this study.

2.3.3. Preparation of Planting Media and Application of Cadmium

The planting medium used was a mixture of soil and compost in a 3:1 ratio. The planting media for all treatments except P5 and P6 were sterilized using 5% formalin at a dose of 2.5 ml/kg. During the sterilization process, the soil was covered with plastic and left for 7 days. The planting medium was then air-dried for another 7 days (Mevianti *et al*., 2021). Meanwhile, the planting media for treatments P5 and P6 were sterilized using the FOBIO biopesticide according to the SOP. The FOBIO biopesticide was applied as a soil sterilizing agent to reduce pathogen inoculum and suppress soilborne pathogens (Wiyatiningsih *et al*., 2020). Soil sterilization with the biopesticide was carried out using a formulation of 2 ml/0.2 L per polybag (Widya *et al*., 2022). The sterilized media were then contaminated with Cd heavy metal at a concentration of 20 ppm.

2.4. Procedure

2.4.1. *Bacillus* sp. Application

The *Bacillus* suspension was mixed with NB media and shaken on a shaker at 150 rpm for 24 hours. The suspension was then diluted with 1000 ml of distilled water and applied at a rate of 100 ml per cadmium-contaminated polybag.

2.4.2. FOBIO Biopestiside Application

The application of FOBIO biopesticide was carried out according to the operational standards. The application on annual plants began one week after transplanting or when the plants were one week old. FOBIO liquid was sprayed once a week until the plants were one month old (Wiyatiningsih *et al*., 2020). In this study, the biopesticide formulation was applied weekly after planting at a rate of 0.59 ml/0.05 L per polybag (Widya *et al*., 2022).

2.4.3. Planting and Harvesting Mustard Greens

Mustard greens were transplanted into polybags when the seedlings were 14 days old or had developed 3 to 4 leaves. The transplanting was carried out in a growing medium containing heavy metals, *Bacillus* sp., and FOBIO biopesticide (Syaiffudin *et al*., 2022).

Harvesting was carried out when the mustard greens were 28 days old after planting. The plants were gently pulled out to avoid damage and then placed in a shaded area to maintain freshness. For storage, ventilated containers such as baskets, plastic boxes, or perforated cardboard were used to maintain air circulation and quality (Syaiffudin *et al*., 2022).

2.4.4. Sample Analysis

Soil samples for cadmium (Cd) analysis were collected at two stages: before treatment with *Bacillus* sp. and FOBIO biopesticide, and after harvest from each treatment unit. The samples were placed in sealed plastic bags and labeled according to the treatment type for subsequent laboratory Cd analysis (Widya *et al*., 2022).

2.5. Parameter

2.5.1. Cadmium (Cd) Level in Soil

Heavy metal levels were tested initially after adding cadmium to the growing medium and again after harvest. Cadmium levels were measured using the Atomic Absorption Spectrophotometer (AAS) method according to SNI 6989.16:2009 (Irawanto *et al*., 2015).

2.5.2. Plant Growth and Yield Parameters (Mahfudiawati *et al*., 2016):

Growth and yield parameters of the plant included plant length, number of leaves, fresh weight, and heavy metal symptom. Plant length and number of leaves was observed from week 1 to 4 WAP (weeks after planting). Fresh weight of mustard green was determined after harvest by weighing entire plants, including the roots. Heavy metal symptom was observed by examining and photographing symptoms of chlorosis and necrosis on the leaves and stems of mustard plants as indicators of cadmium accumulation during the cultivation period.

3. RESULTS AND DISCUSSIONS

3.1. Levels of the Heavy Metal Cadmium (Cd) in Soil

Table 2 shows Cd content in the soil before and after treatment application. The results of the study indicate that most differences in cadmium (Cd) concentrations among treatments were statistically significant. The application of the FOBIO biopestiside showed a substantial potential to reduce Cd levels in contaminated soil. Treatment P5 (FOBIO biopestiside without plants) resulted in the highest reduction of 4.57 mg/kg (from 10.18 to 5.61 mg/kg), followed by P6 (a combination of FOBIO biopestiside and mustard greens (Table 2), which showed a decrease of 3.01 mg/kg. The bioremediation efficacy of FOBIO biopestiside is supported by the synergistic activity of its microbial components, including *LactoBacillus* sp., *Rhizobium* sp., and phosphate-solubilizing bacteria (PSB), which act together to bind, precipitate, and reduce the bioavailability of heavy metals in the environment.

LactoBacillus sp. is known to produce exopolysaccharides (EPS) capable of binding Cd ions (Aminah & Nur, 2018). *Rhizobium* sp. has demonstrated superior effectiveness in reducing Pb concentrations in soil compared to conventional treatments using cow manure (Prasasti, 2019). Phosphate-solubilizing bacteria (PSB) produce organic acids that solubilize phosphate from soil minerals, allowing the formation of insoluble phosphate compounds with heavy metal ions (Pb, Cd, Zn), thereby reducing their availability in the soil (Han *et al*., 2020; Wan *et al*., 2020). This supports the multifunctional role of FOBIO biopestiside not only as a biofertilizer, biostimulant, and bioprotector but also as an effective bioremediation agent.

Table 2. Effect of treatments on heavy metal cadmium (Cd) levels in soil

Treatment	Heavy metal (mg/kg)		Decrease in the amount of cadmium	
	Before	After	mg/kg	%
P0	10.86 c	10.28 d	0.58	0.000058
P1	16.60 g	14.20 f	2.4	0.00024
P2	0.00 a	0.00 a	0.00	0.000000
P3	13.84 e	11.36 e	2.48	0.000248
P4	14.36 f	10.25 d	4.11	0.000411
P5	10.18 b	5.61 b	4.57	0.000457
P6	11.27 d	8.26 c	3.01	0.000301
HSD 5%	0.15	0.07	-	-

Note: Numbers followed by different letters indicate significant differences according to the 5% BNJ test.

A synergistic effect between *Bacillus* sp. and mustard green plants significantly enhanced Cd reduction. Treatment P4 showed a reduction of 4.11 mg/kg (from 14.36 to 10.25 mg/kg), whereas treatment P3 (*Bacillus* alone) resulted in a smaller decrease of 2.48 mg/kg (from 13.84 to 11.36 mg/kg) (Table 2). Under heavy metal stress, mustard greens activate a defense mechanism by synthesizing phytochelatins peptides derived from amino acids such as cysteine, glutamate, and glycine (Irawan, 2019). These phytochelatins chelate heavy metal ions via their sulfhydryl groups, forming stable complexes that neutralize metal toxicity (Mariwy *et al*., 2020).

Moreover, mustard green roots exude carbon and nutrients that support *Bacillus* sp. growth, promoting a mutualistic interaction that further enhances the bioremediation process (Hartono *et al*., 2024). This plant–microbe synergy strengthens the efficiency of soil heavy metal detoxification. Green mustard has shown high phytoremediation potential for cadmium-contaminated soils. The treatment with mustard plants (P1) reduced Cd concentrations by 2.40 mg/kg, substantially greater than the control without plants (P0), which showed only a 0.58 mg/kg reduction (Table 2). This highlights the plant's capability for phytoremediation.

Phytoremediation is an eco-friendly biotechnological approach that utilizes plants to reduce, stabilize, or degrade contaminants from soil, water, or air (Sukono *et al*., 2020). Mustard green performs phytoremediation through phytoextraction a process involving the uptake, accumulation, and translocation of heavy metal contaminants from the growing medium into plant tissues, particularly leaves and stems qualifying it as a hyperaccumulator species (Ramadaningrum, 2016).

3.2. Growth of Green Mustard Plants

3.2.1. Plant length

Observations of plant height over a four-week period revealed varying responses among treatments (P1, P2, P4, and P6). During the first and second weeks, no significant differences were observed. However, by the third week, treatments P6 (4.69 cm) and P2 (4.34 cm) exhibited significantly greater growth compared to P4 (3.51 cm) (Table 3), indicating that the application of bioremediation agents had begun to positively influence plant development. The effectiveness of FOBIO biopestiside in supporting plant growth aligns with findings by Laila *et al*. (2023), who reported a positive impact of FOBIO biopestiside on the height of green mustard.

In contrast, no significant differences were recorded among treatments in the fourth week, which was likely due to the plants reaching the late vegetative stage prior to harvest typically between 25 to 30 days after transplanting consistent with the growth characteristics of the Tosakan mustard variety (Sangadji, 2017). At this stage, active vegetative growth tends to decelerate as the plant shifts its physiological focus from expansion to stabilization (Auguestien, 2023).

Table 3. Effect of treatments on plant length of mustard greens at 1 to 4 WAP

Treatment	Plant length (cm)			
	Week 1	Week 2	Week 3	Week 4
P1	2.62 a	3.83 a	4.24 ab	3.37 a
P2	2.72 a	3.83 a	4.34 b	3.46 a
P4	2.79 a	4.43 a	3.51 a	2.40 a
P6	2.91 a	4.17 a	4.69 b	5.14 a
BNJ 5%	TN	TN	0.72	TN

Note: Numbers followed by different letters indicated significant differences according to the 5% HSD test. The notation "TN" indicated no significant difference based on the 5% HSD test.

3.2.2. Number of Leaves

The observations showed that there were no significant differences in the number of leaves among treatments in the first week, indicating that early vegetative growth was not yet affected. Significant differences appeared in the second and third weeks, with treatment P6 producing the highest number of leaves in both 2 WAP (2.95 leaves) and 3 WAP (3.07 leaves), which differed significantly from P4. Table 4 details the effect of treatments on the number of leaves of mustard greens at 1 to 4 WAP.

Table 4. Effect of treatments on the Number of Leaves of mustard greens at 1 to 4 WAP

Treatment	Number of leaves			
	Week 1	Week 2	Week 3	Week 4
P1	2.21 a	2.59 ab	2.45 ab	1.88 a
P2	2.27 a	2.69 ab	2.69 ab	2.04 a
P4	2.34 a	2.28 a	1.97 a	1.39 a
P6	2.17 a	2.95 b	2.86 b	2.72 a
HSD 5%	TN	0.39	0.75	TN

Note: Numbers followed by different letters indicated significant differences according to the 5% HSD test. The notation "TN" indicated no significant difference based on the 5% HSD test.

The increase in leaf number from week 2 to week 3 was assumed to be influenced by the effectiveness of the bioremediation agent in stimulating growth, as well as the application of NPK fertilizer, which supported leaf production as reported by Istiqomah & Serdani (2018). By week 4, although differences among treatments were still present, the average number of leaves tended to stabilize. This condition indicated that the plants had entered the final growth phase toward harvest, during which the formation of new leaves slowed, and the plants focused their energy on maintaining existing tissues (Frary, 2015).

3.2.3. Plant Fresh Weight

The results showed that treatment P6 produced the highest fresh weight of 5.08 g, while the other treatments (P1, P2, and P4) had lower fresh weights ranging from 2.50 to 2.80 g. This difference was assumed to be related to the role of FOBIO as a biological biopesticide functioning as a Plant Growth-Promoting Rhizobacteria (PGPR). FOBIO was able to support plant growth even under cadmium-contaminated soil conditions. This finding was consistent with Ariyono *et al*. (2021), who reported that FOBIO could enhance plant growth performance.

Table 5. Effect of treatments on the fresh weight of mustard greens

Treatment	P1	P2	P4	P6	HSD 5%
Fresh weight (g)	2.70	2.80	2.50	5.08	TN

Note: The notation "TN" indicated no significant difference based on the 5% HSD test.

The fresh weight of the plants was influenced by plant height and the number of leaves. This aligns with Rolanda *et al*. (2021), who explained that fresh weight was closely related to plant height and leaf area, where increases in these factors generally contributed to greater biomass. However, according to the 5% HSD test, all treatments showed the same notation, indicating that the differences in fresh weight were not statistically significant. Thus, although P6 had a higher value, the increase was not sufficient to produce a significant difference at the 5% significance level.

3.2.4. Heavy Metal Symptoms

Cadmium (Cd) was a non-essential heavy metal that could be readily absorbed by plants from the soil. The level of Cd uptake depended on the intrinsic physiological characteristics of each plant species. In this study, green mustard was recognized as a hyperaccumulator capable of absorbing and accumulating high concentrations of heavy metals, particularly in the leaves (Prasodjo *et al*., 2021). The accumulation of Cd generally caused toxicity symptoms such as growth inhibition and chlorosis due to disrupted chlorophyll synthesis and chloroplast damage (Gallego *et al*., 2012).

However, the observations showed that the mustard plants did not exhibit chlorosis from the first to the fourth week (Figure 1). This response was assumed to be related to the plant's ability to restrict Cd translocation from roots to leaves through metal sequestration in root tissues. In addition, plants had internal detoxification systems, including the production of phytochelatins and metallothioneins that bound Cd and stored it in vacuoles, thereby protecting photosynthetic pigments (Lux *et al*., 2011). Under low concentrations or short exposure durations, plants also maintained photosynthetic function by increasing the activity of antioxidant enzymes such as SOD, CAT, and POD, which reduced oxidative stress (Gupta *et al*., 2013). Thus, the visual symptoms of Cd toxicity depended strongly on the plant's genetic tolerance as well as the level and duration of Cd exposure.

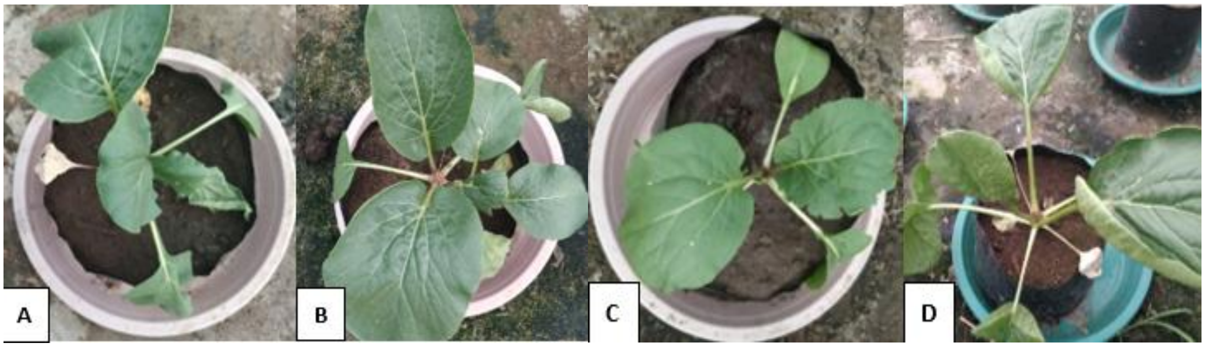

Figure 1. Symtoms of mustard plants contaminated with cadmium (Cd) in the fourth week: A = P1, B = P2, C = P4, D = P6

4. CONCLUSION

Bacillus sp. Ba 13, FOBIO biopesticide, and green mustard plants have the potential to be used as bioremediation agents, with the highest reduction in cadmium (Cd) content in the soil observed in the FOBIO biopesticide treatment. The growth of mustard plants was not affected by cadmium contamination. The treatment of heavy metal-contaminated growing media + FOBIO biopesticide + mustard plants had the best growth values with an average plant height of 5.14 cm, 2.72 leaves, and a wet weight of 5.08 g. Future studies are recommended to analyze cadmium levels in mustard plants to confirm their potential as hyperaccumulators and to improve the accuracy and completeness of the data.

AUTHOR CONTRIBUTION STATEMENT

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
DMS	✓	✓	✓		✓	✓		✓	✓	✓				
ETWP	✓						✓			✓				
SW							✓			✓				

C: Conceptualization	Fo: Formal Analysis	O: Writing - Original Draft	Fu: Funding Acquisition
M: Methodology	I: Investigation	E: Writing - Review & Editing	P: Project Administration
So: Software	D: Data Curation	Vi: Visualization	
Va: Validation	R: Resources	Su: Supervision	

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