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Bioremediation of Several TPS3R Compost Types Using Bacillus Thuringiensis and Analysis of Compost Effects on the Growth and Yield of Land Water Spinach *(Ipomea reptans Poir.)*

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ABSTRACT: Environmental pollution is a change in environmental conditions caused by human activities that lead to a decline in environmental quality. Waste accumulation is one of the most common problems faced worldwide. The Province of Bali faces significant challenges in waste management due to population growth, economic expansion, and tourism activities. Waste generation in Bali has reached 3,367 tons/day (SIPSN, 2023). Previous studies show that many landfill and temporary waste collection sites contain high levels of heavy metals (Ovianka et al., 2021). Analysis results indicate that heavy metal concentrations exceed the standards set by the Soil Research Institute (Balitan, 2005). Bioremediation is a remediation method using plants to absorb and transform heavy metal contaminants. This research used a Completely Randomized Design (CRD) factorial using compost from TPS3R (Kesiman, Jagapati, Darmasaba, Belega) with Bacillus thuringiensis at 5 g and 10 g dissolved in 50 ml water. Parameters measured included plant height, leaf number, fresh weight, dry weight, as well as compost pH, temperature, color, and moisture content.

KEYWORDS: Bioremediation, Bacillus bacteria, Soil pH, Biochar, Completely Randomized Design

I. INTRODUCTION

Heavy metal contamination poses serious risks due to its non-degradable properties and ease of absorption by living organisms. Although the short-term effects may not be severe, long-term exposure can be fatal (Irhamni, 2009). Heavy metal contamination in waste must be addressed urgently. Several remediation techniques include vermiremediation (earthworms), bioremediation (bacteria), and phytoremediation (plants).

Bioremediation is a biological waste treatment method using indigenous bacteria naturally present in the waste (Munawar et al., 2006). Waste characteristics in Indonesia vary by region. In Bali, organic waste composition is very high at 70.87% due to remnants of Hindu ceremonial offerings. Bacillus thuringiensis is a gram-positive bacterium containing peptidoglycan, teichoic acid, and teichuronic acid in its cell wall (Khaeruni, 2012). This bacterium performs metabolic reactions including anabolic and catabolic processes mediated by enzymes (Mastang, 2007).

II. METHODS

This study used a factorial Completely Randomized Design (CRD) with compost samples collected from four TPS3R locations in Bali: Kesiman Kertalangu, Jagapati, Darmasaba, and Belega. Treatments included Bacillus thuringiensis at
50 g and 100 g mixed with 100 ml water. A total of 36 experimental units and 12 treatment combinations were used

Measured variables included compost pH, moisture content, temperature, color, odor, and structure. Plant observations included number of leaves, height, fresh and dry weight. The study began with compost sampling, followed by laboratory heavy metal analysis, and plant cultivation in the greenhouse of the Faculty of Agriculture, Udayana University.

III. RESULTS

A. Bioremediation factor

Compost pH initially ranged from acidic to neutral, but gradually increased to near-neutral (6.5–7.5), which is optimal for Bacillus thuringiensis activity. Color darkened due to humus formation, while odor remained stable due to volatilization of organic compounds during early composting at TPS3R sites.

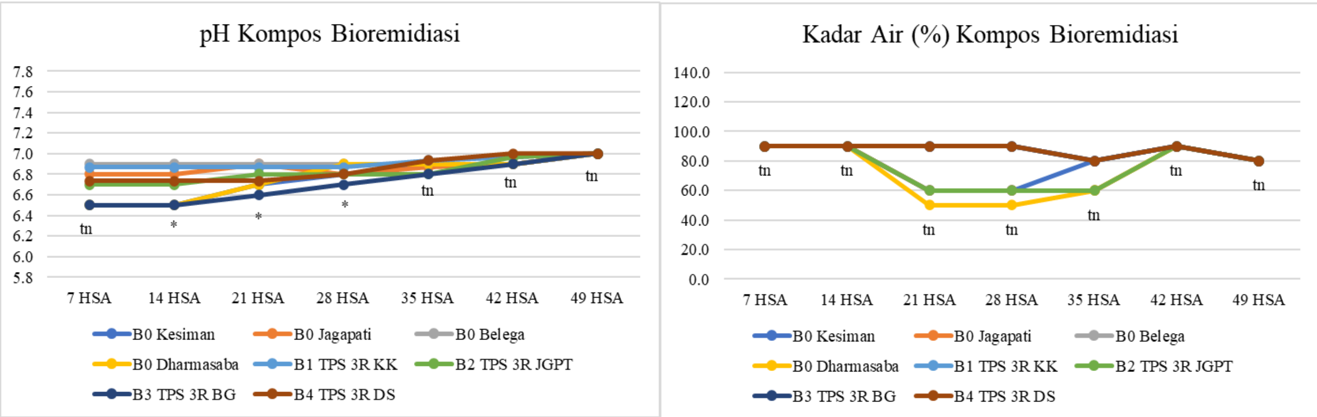

Figure 1. Grafik pH dan Kadar Air Kompos Bioremidiasi mengunakan bakteri bacillus Thureingensis

At the beginning of the process, the pH value of the compost tended to range from acidic to neutral. As the bioremediation process progressed, the pH increased toward neutral (6.5–7.5), which is the optimal range for microbial growth, including *Bacillus thuringiensis* used in this study. This increase in pH is presumed to result from the decomposition of organic compounds by microbes.

The color of the compost gradually changed from lighter to darker (score 3–1), in line with the increase in humus content and the formation of complex organic compounds. The odor of the compost also did not undergo significant changes. From the initial direct observations at each TPS3R location, all compost samples were odorless. When the bioremediation process began and the compost was treated, it still showed no detectable odor. This is likely due to the volatilization of easily evaporated organic compounds that had already occurred during the early composting stages at the TPS3R facilities. As a result, odor-producing compounds such as ammonia, volatile organic acids, and sulfur compounds had already evaporated before the remediation treatments were applied, leaving the compost medium free of strong odors during the study.

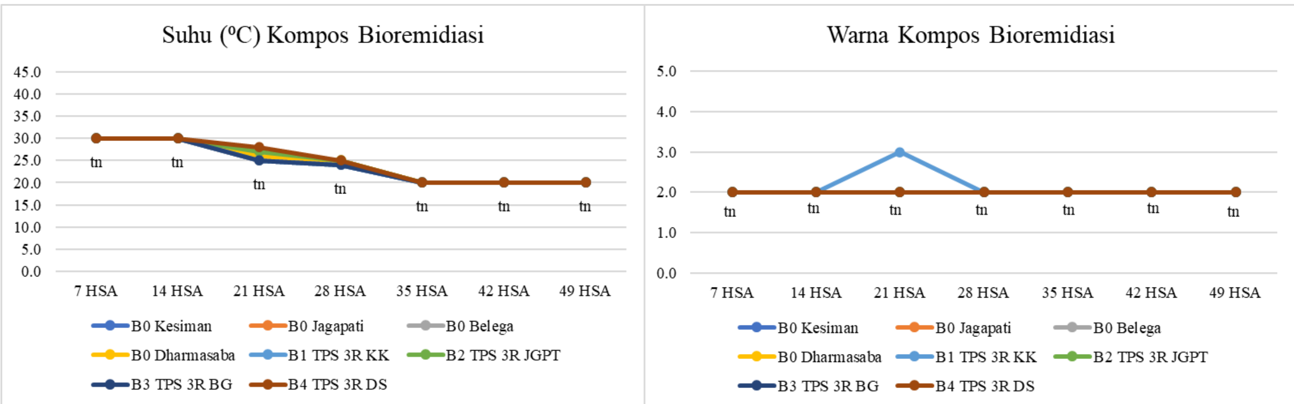

Figure 2. Graph of Compost Temperature and Color

The results of the decomposition level measurements in the figure above show that the bioremediation treatment combined with the addition of biochar significantly increased the efficiency of organic matter decomposition. Treatment groups B1 to B4 exhibited decomposition percentages of 85–95%, which were higher than the control (B0), which reached only about 65%.

The gradual color change of the compost from light to darker shades serves as a key indicator of compost maturity and stability. The darker color reflects the formation of complex organic compounds such as humic and fulvic acids, which are the final products of organic matter decomposition by microorganisms. This process is known as humification, in which simple organic compounds are transformed into more stable humus structures that are resistant to further decomposition.

B. Analysis of Heavy Metal Content

Based on the test results, it is evident that the bioremediation process, particularly when combined with the use of biochar and microbial agents such as *Bacillus thuringiensis*, is capable of significantly reducing heavy metal concentrations. The following are the results of the arsenic (As) heavy metal analysis.

Table 1. Analysis of Heavy Metal Content

Treatment	First & Second	Bioremediation
		As (ppm)
K0 Kesiman	202	1.70
K0 Jagapati	836	1.57
K0 Belega	177	1.26
K0 Darmasaba	134	1.16
K1 TPS 3R Kesiman	202	1.70
K2 TPS 3R Jagapati	836	1.57
K3 TPS 3R Belega	177	1.26
K4 TPS 3R Darmasaba	134	1.16
	SNI: As max 13 ppm	

Bioremediation treatment using *Bacillus thuringiensis* resulted in a significant reduction of arsenic levels. The Jagapati TPS recorded a decrease from 836 ppm to 1.57 ppm, while the Kesiman TPS decreased from 202 ppm to 1.70 ppm.

The primary mechanism of bioremediation by *Bacillus thuringiensis* in reducing heavy metal content, particularly arsenic (As), involves biological transformation and the formation of organic complexes. *Bacillus* has the ability to convert arsenic from As(V) (arsenate) to As(III) (arsenite) through the enzymatic activity of reductase.

This As(III) form then interacts more readily with dissolved organic compounds (DOC) produced during the decomposition of organic matter, forming relatively insoluble and stable arsenic–organic complexes.

C. Results of Water Spinach Growth Analysis

In general, the number of leaves tended to increase as the plants aged across all treatments. However, the effect of the treatments on leaf number was only significant during the early to mid-growth stages (7, 19, and 22 days after planting), whereas at the later stages of observation (25–31 days after planting), the differences among treatments were no longer significant

Table 2. Analysis of Water Spinach Plant Growth

Days After Planting	F count	Sig.	description
7	5.600	0.000	Significantly different
10	1.174	0.354	Not significantly different
13	0.971	0.497	Not significantly different
16	1.290	0.288	Not significantly different
19	3.636	0.004	Significantly different
22	2.645	0.022	Significantly different
25	2.026	0.072	Tidak berbeda nyata
28	0.552	0.848	Not significantly different
31	0.824	0.619	Not significantly different

The results of the analysis of variance for the number of leaves at various observation periods are presented in Table 4.2. Based on the one-way ANOVA test with a 5% significance level (α = 0.05), it was found that the treatments had a significant effect on the number of leaves at several observation times, specifically at 7, 19, and 22 days after planting (DAP). Meanwhile, at 10, 13, 16, 25, 28, and 31 DAP, the treatments did not have a significant effect on the number of leaves.

Table 3. Average Height of Water Spinach Plants

Treatment	Average Plant Height (cm)
K2B0	23.57
K4B0	24.67
K3B0	26.00
K1B0	26.40
K2B1	27.97
K4B2	30.57
K3B1	30.83
K4B1	31.83
K3B2	32.73
K1B1	33.73
K1B2	34.00
K2B2	**34.90**

In general, the test results showed that plant height increased with age across all treatments. However, certain treatment combinations produced superior results at different growth stages:

1. **K1B1** had the most beneficial effect during the early growth phase” (7 HST),
2. **K1B0** produced the best results during the mid-growth phase (19 HST), dan
3. **K2B2** showed the highest results during the late vegetative phase (31 HST).

This indicates that the right combination of treatments can influence the efficiency of plant growth at each developmental stage. The differences in responses between treatments are likely affected by nutrient availability, the effectiveness of root absorption, and the ability of each variety to utilize nutrients and light for stem growth.

IV. DISCUSSION

The pH observations showed that the combination of bioremediation and biochar in TPS 3R compost resulted in significant changes to the compost’s physical–chemical conditions, particularly pH, moisture content, temperature, color, and odor. At the beginning of the process, the compost pH tended to be acidic, but it gradually increased toward neutral (6.5–7.5) as bioremediation progressed. This pH increase reflects microbial activity—including *Bacillus thuringiensis*—which decomposes organic matter and produces basic ions such as ammonium. Biochar also helps neutralize acidity, allowing the compost stabilization process to proceed more quickly and effectively.

The color of the bioremediated compost shows that all compost samples maintained stable color values in the range of 2–3 throughout the 7–49 days after treatment (HSA) observation period. Only the B0 Kesiman sample experienced slight color changes (becoming lighter) at 21 HSA before returning to the initial condition. The other samples remained at values around 2, indicating a dark brown color. Darker compost color reflects compost maturity and stability as a result of humification, the formation of complex organic compounds such as humic and fulvic acids. The consistency of color in most samples indicates that the compost was already mature from the beginning, so the bioremediation treatment did not greatly influence this parameter. The slight fluctuation in the B0 Kesiman sample was likely due to temporary microbial activity. Once the process stabilized again, the compost color darkened, indicating balanced humification.

For mercury (Hg), concentrations decreased drastically, for example from 22 ppm to <0.06 ppm in TPS Belega. The bacteria produced the enzyme mercury reductase, which can convert Hg^{2+} into a less harmful form. Meanwhile, Mn levels also experienced a reduction through ionic bonding with the surface of biochar and increased pH, although the decrease was not as drastic as that of arsenic or mercury.

V. CONCLUSION

Bioremediation using *Bacillus thuringiensis* and biochar has been proven effective in reducing heavy metal content (As, Hg, and Mn) in TPS 3R compost. In addition to improving compost quality, *Bacillus thuringiensis* also plays a positive role in supporting the growth of water spinach plants. This is evident from the leaf number observations, where the treatments showed significantly different effects at several observation times, specifically at 7 and 22 days after planting (DAP).

For the plant height parameter, observations showed that at 7 DAP, the K1B1 treatment produced the highest plant height at 7.07 cm, while the K3B0 treatment had the lowest plant height at 6.80 cm. These findings indicate that treatment combinations involving biochar and *Bacillus thuringiensis* can enhance the growth efficiency of water spinach during the early stages of development.

VI. ACKNOWLEDGMENT

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