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Bioremediation of TPS3R Compost with Various Levels of Earthworms Supplemented with Biochar on the Growth and Yield of Land Water Spinach (*Ipomea Reptans* Poir)

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ABSTRACT: Waste is one of the most complex global issues faced by nearly every country in the world. The management of organic waste in Bali through the Reduce-Reuse-Recycle Waste Processing Facility (TPS3R) produces compost with potential use as a planting medium. However, compost derived from household waste is often contaminated with heavy metals such as arsenic (As), mercury (Hg), and manganese (Mn) that exceed the threshold limits set by SNI 19-7030-2004, thereby requiring bioremediation treatment before being agronomically utilized. This study aims to assess the effectiveness of a vermiremediation approach using earthworms combined with biochar in reducing heavy metal content in TPS3R compost and its impact on the growth and yield of water spinach (Ipomoea reptans Poir). The experiment was conducted using a factorial Completely Randomized Design (CRD) with two factors: compost sources from four TPS3R facilities (Kesiman, Jagapati, Belega, Darmasaba) and treatment combinations of earthworms (50 g, 100 g) with biochar (250 g). The results showed that the combination of earthworms and biochar significantly reduced heavy metal concentrations, such as arsenic from 836 ppm to 18.65 ppm, mercury from 22 ppm to 1.27 ppm, and manganese from 1289 ppm to 135.54 ppm. Additionally, this treatment improved plant growth and yield parameters, including plant height, number of leaves, fresh weight, and dry weight. Although the improvements were not statistically significant across treatments, they were evident in the media treated with bioremediation. These findings indicate that the combination of earthworms and biochar is a promising strategy to improve compost quality and support sustainable agriculture on drylands.

KEYWORDS: Bioremediation, Vermiremediation, Biochar, Heavy metals, TPS3R, upland water spinach.

INTRODUCTION

Proper waste management can bring significant benefits, as reflected in the Balinese proverb *"Sampah metemehan amerta"* (waste brings great value), while unmanaged waste will lead to *"metemahan wesia"* (disaster). One emerging solution is community-based waste management systems, such as the 3R Waste Processing Facility (TPS3R), which emphasizes the principles of Reduce, Reuse, and Recycle in managing household waste. The high proportion of organic waste in Bali Province presents an opportunity to utilize it as compost in support of Bali's vision as an Organic Island.

According to a 2023 study by Bappenas, 70.87% of the waste composition in Denpasar City is organic, consisting of 38.12% garden and ritual waste, and 32.75% food waste. Several studies have shown that waste in landfills (TPA) and TPS often contains high levels of heavy metals [1]. Bioremediation involves the use of microorganisms or other organisms, such as earthworms, to decompose organic materials into more environmentally friendly elements. Earthworms are known for their ability to accelerate the decomposition of organic waste and produce compost rich in nutrients beneficial for plant growth. Earthworm castings (vermicompost) are rich in nutrients and calcium, and contain a mixture of soil minerals and decomposed organic compounds [2]. Earthworms can increase the availability of nutrients such as nitrogen (N), phosphorus (P), and potassium (K) through their activity, which alters the environmental conditions of the vermicompost substrate and stimulates microbial growth [3]. On the other hand, the use of biochar as an additive in organic waste processing also demonstrates great potential. Biochar, produced through the pyrolysis of organic materials, can improve soil fertility, reduce greenhouse gas emissions, and adsorb heavy metal elements. According to [4], the term "biochar" is used to avoid misinterpretation. Charcoal is commonly used as fuel, whereas biochar is generally used as an adsorbent.

Bioremediation of TPS3R Compost with Various Levels of Earthworms Supplemented with Biochar on the Growth and Yield of Land Water Spinach (Ipomea Reptans Poir)

This study aims to explore the effect of combining various dosages of earthworms supplemented with biochar in the bioremediation of organic waste at TPS3R, as well as its impact on the growth and yield of water spinach (Ipomoea reptans Poir). Water spinach was chosen due to its fast-growing nature. By understanding the role of earthworms and biochar in enhancing the quality of compost derived from organic waste, this research is expected to provide a more efficient and environmentally friendly solution to support sustainable agriculture and improved waste management practices in Indonesia.

RESEARCH METHODS

This study employed a factorial Completely Randomized Design (CRD) with treatments involving waste sources from four TPS3R facilities located in three different regencies, where compost samples were collected compositely from each region. The treatments were structured as follows: Factor I (Compost Source): K1: TPS3R Kesiman (Denpasar), K2: TPS3R Jagapati (Badung) ,K3: TPS3R Belega (Gianyar), K4: TPS3R Darmasaba (Badung).Factor II (Combination of Earthworms and Biochar): V0: Without earthworms and biochar, V1: 50 g earthworms and 250 g biochar, V2: 100 g earthworms and 250 g biochar. The total number of experimental units was 36, derived from 12 treatment combinations replicated three times. This study, which focuses on reducing heavy metal content using water spinach (Ipomoea reptans Poir), will be conducted at the Experimental Farm of the Faculty of Agriculture, Udayana University (KPFP) from September 2024 to April 2025. The variables in this study include environmental parameters during vermiremediation, heavy metal content after vermiremediation, and plant growth parameters. The research will be carried out in several stages:1. Survey and compost sampling from each TPS3R, 2.Earthworm maintenance,3. Experimental setup, 4.Preparation of the planting medium, 5.Plant maintenance and observation

Compost samples were collected from four TPS3R locations across three regencies: TPS3R Kesiman Kertalangu (Denpasar), TPS3R Jagapati (Badung), TPS3R Belega (Gianyar), and TPS3R Darmasaba (Badung). Earthworm maintenance involved managing the rearing medium, feeding the worms with tofu residue, and maintaining proper moisture levels.

The planting medium consisted of TPS3R compost. The medium was placed into polybags measuring 15 cm in diameter and 30 cm in height, and fertilizers were mixed into the medium according to the respective treatments. The experiment was conducted in a greenhouse, requiring preparation such as clearing the area of weeds and debris, and organizing tools and materials including polybags, compost, biochar, earthworms, and water spinach seedling tools.

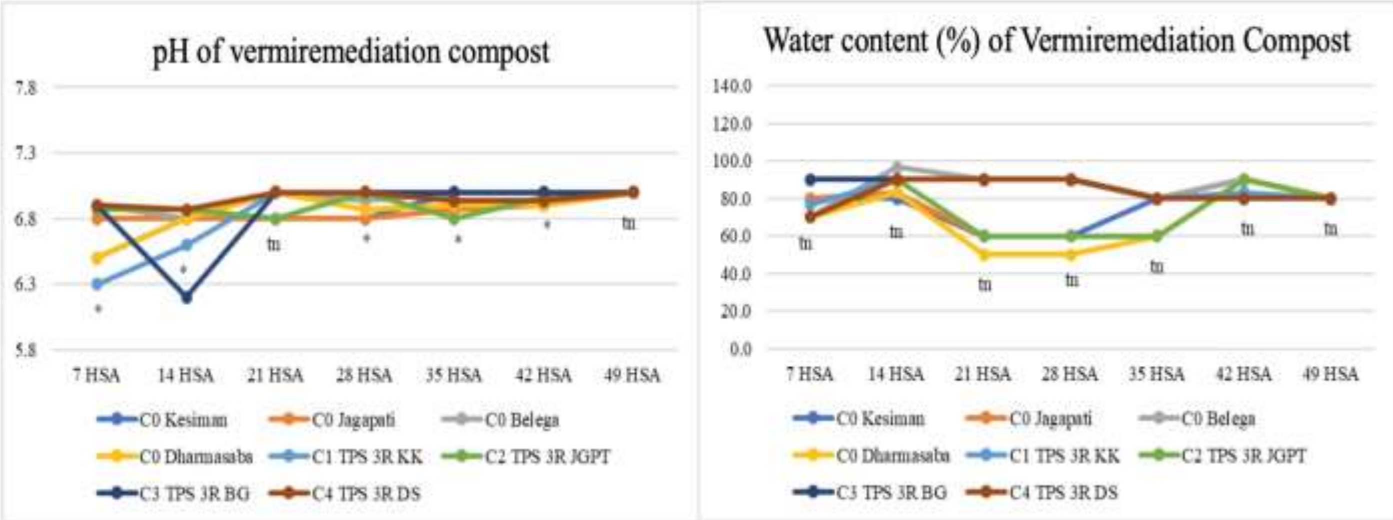
Plant maintenance activities included watering, fertilizing, weeding, staking, pruning, and pest control. Watering was carried out daily in the afternoon throughout the growing period. Pest control was done manually, by removing any visible pests by hand. Harvesting was conducted at 30 days after planting, by uprooting the entire plant. Harvest-ready water spinach is characterized by thick stems and broad leaves.

The observed growth parameters included: Plant height: Measured from the base of the stem to the growing tip, taken every 7 days. Number of leaves: Counted weekly, including only fully developed leaves. Fresh weight: Determined by weighing the entire fresh plant at harvest. Dry weight: Obtained by placing the fresh plant shoot in an oven at 50°C until reaching constant weight. Data were analyzed using Analysis of Variance (ANOVA) with SPSS software. If a significant effect was observed, further analysis was performed using the Least Significant Difference (LSD) test at a 5% significance level. Correlation analysis was also conducted to determine the relationships between treatments and observed variables.

RESULTS

Efforts to Reduce Heavy Metal Content through Vermiremediation Treatments

Observations indicated that vermiremediation treatments combined with biochar at TPS3R facilities resulted in changes in several key environmental factors, namely pH, moisture content, temperature, color, and odor of the compost (Figure 1). At the beginning of the vermiremediation process, the compost pH was generally slightly acidic to neutral; as decomposition progressed, the pH increased and approached a neutral range (6.5–7.5). The tendency of the pH to stabilize toward the end of the process indicates a balance between microbial activity and the role of earthworms in breaking down organic matter.

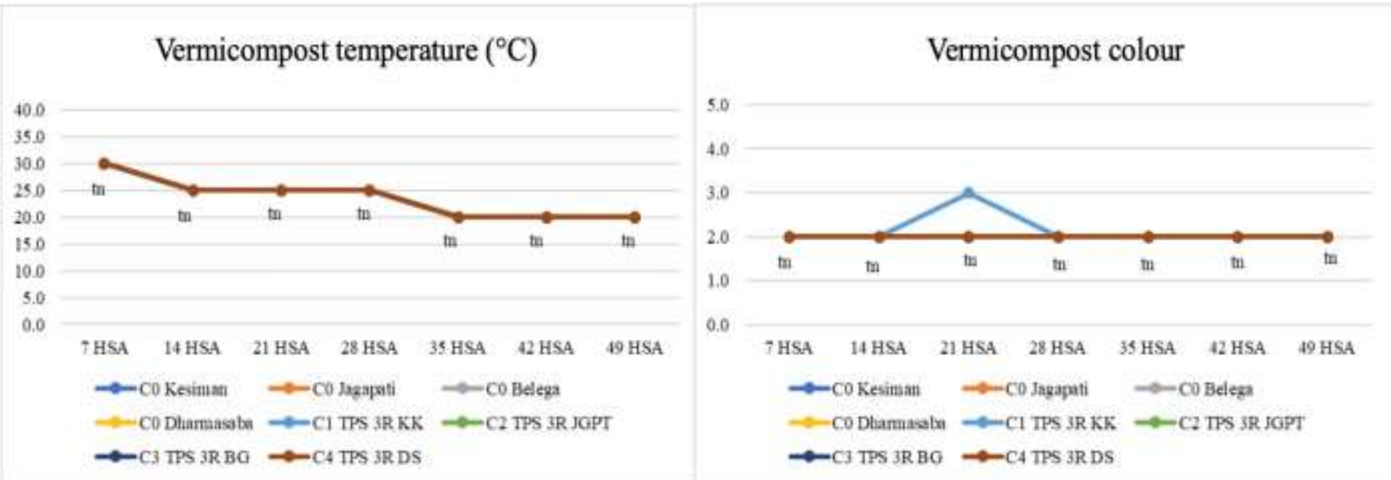


Picture 1. Vermiremediation Compost pH and Water Content Graph

The displayed graphs illustrate changes in compost pH and moisture content during the vermiremediation process under various treatments. In the pH graph, treatment C3 TPS3R BG showed relatively stable pH with a slight initial decrease, whereas C0 Kesiman exhibited a significant pH drop at the beginning of the measurement period, followed by a steady increase. Treatments C0 Jagapati and C0 Dharmasaba displayed greater pH fluctuations at several measurement points, with some points marked with asterisks (*), indicating significant differences between treatments.

In the moisture content graph, C0 Kesiman showed higher initial moisture content, which gradually decreased over time. Treatments C0 Jagapati and C0 Dharmasaba demonstrated sharper declines in moisture content at several time points, while C1 TPS3R KK maintained relatively higher and more stable moisture levels. C2 TPS3R JGPT also showed slight decreases at several measurement points.

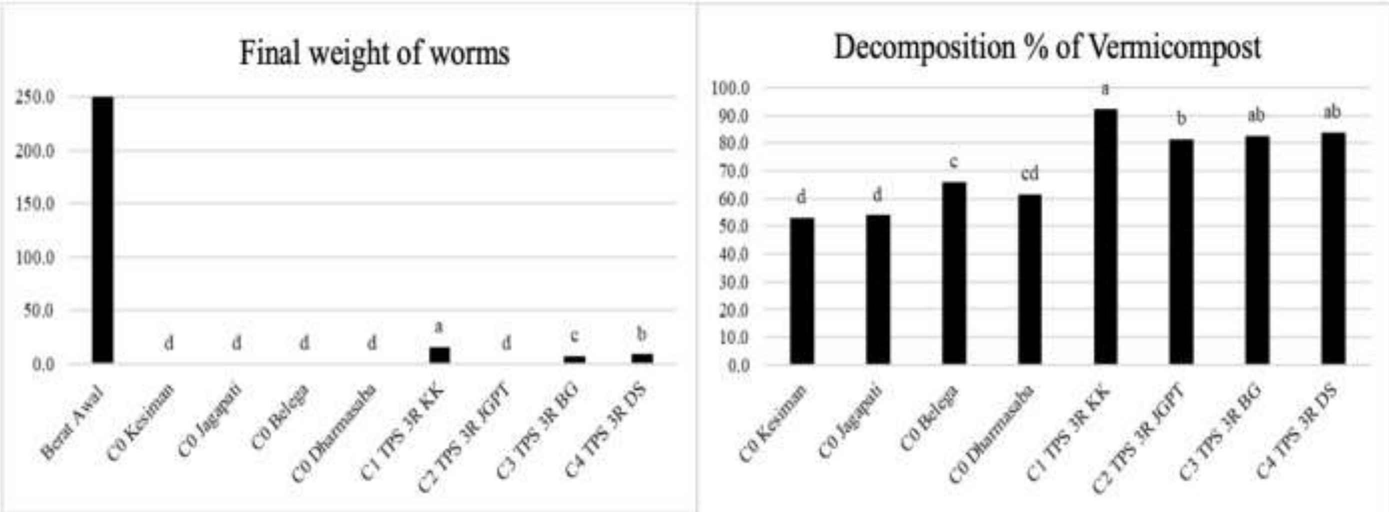
Overall, both graphs indicate that different treatments influenced the pH and moisture content of compost throughout the vermiremediation process, with some treatments demonstrating better stability than others. These findings are important for compost quality, as they affect soil fertility and the effectiveness of the vermiremediation process in improving soil quality.



Picture 2. Temperature and Color of Vermiremediation Compost

Based on the displayed graphs, changes in compost temperature and color during the vermiremediation process can be observed. The compost temperature across various treatments tended to remain stable, averaging around 25°C throughout the observation period, with slight fluctuations indicating that the vermiremediation process did not cause significant temperature changes. Treatments such as C0 Kesiman and C0 Dharmasaba maintained stable temperatures ranging from approximately 25°C to 30°C, while C3 TPS3R BG and C4 TPS3R DS showed slight temperature decreases at several observation points but remained within the lower range. Other treatments, such as C1 TPS3R KK and C2 TPS3R JGPT, also exhibited stable temperatures, although minor variations were noted at several time points.

Meanwhile, changes in compost color showed more distinct variations. Compost color was assessed qualitatively using a scale where: very dark (1), dark (2), light (3), and very light (4). The C0 Kesiman treatment showed a significant change from dark to light, with the most noticeable color shift observed on Day 28 After Treatment (DAT), indicating a faster decomposition process. Treatments C0 Dharmasaba and C3 TPS3R BG demonstrated more stable color transitions, with gradual changes from dark to light throughout the observation period. In contrast, C1 TPS3R KK and C4 TPS3R DS exhibited slower color changes, with compost color remaining darker compared to other treatments. The C2 TPS3R JGPT treatment showed a more rapid change toward lighter coloration, particularly on Day 14 and Day 21 After Treatment, indicating the effectiveness of this treatment in altering compost color characteristics in a shorter timeframe.



Picture 3. Chart Final Weight and Decomposition Vermicompost

Based on the displayed figures, two graphs illustrate the experimental results related to the final weight of earthworms and the decomposition process of TPS3R compost during the vermiremediation process.

In the first graph, which shows the final weight of earthworms (in grams), it is evident that most treatments resulted in very low final earthworm weights, with significant differences observed between treatments. Treatments C0 Kesiman, C0 Jagapati, C0 Belega, and C0 Dharmasaba all showed nearly identical results, with final worm weights approaching zero. In contrast, treatment C1 TPS3R KK exhibited a notable increase in final worm weight, producing better results compared to other treatments, although the values remained relatively low. This indicates substantial variation in the worms' responses to the given conditions, with the C1 TPS3R KK treatment demonstrating a more favorable effect on earthworm biomass.

HEIGHT OF WATER SPINACH PLANTS

Table 1. Interaction of Plant Height 7 & 14 Dap

Treatment	7 DAP	14 DAP
VO	27,53 b	32,37 a
V1	27,9 a	32,2 a
V2	27,67 b	32,3 a
BNT5%	**0,39**	**ns**
K1	21,07 a	24,6 a
K2	20,67 b	24,2 b
K3	20,57 b	24,07 b
K4	20,8 b	24 b
BNT5%	**0,31**	**0,38**

Description: Numbers followed by the same notation in the same treatment and column indicate no significant difference in the least significant test (LSD) at the 5% level.

Based on the analysis results at 7 days after planting (DAP), treatment V1 showed a significant interaction effect on the plant height variable. Treatment V1 resulted in an average plant height of 27.9 cm, which was significantly higher compared to treatments V0 and V2. Treatment V2 did not differ significantly from V0. At 14 DAP, the analysis of treatments V0, V1, and V2 showed no significant differences; however, V0 recorded the highest average plant height among the three.

At the same observation point (14 DAP), treatment K1 showed a significantly higher average plant height compared to the other treatments. Treatments K2, K3, and K4 did not differ significantly from one another in terms of plant height. Treatment K1 had the highest plant height at 24.6 cm, which was significantly greater than that of groups K2, K3, and K4. Meanwhile, treatments K2, K3, and K4 did not show significant differences in plant height among them.

Table 2. Interaction of Plant Height 21 DAP

21 DAP	K1	K2	K3	K4
VO	11.16 a	11.20 a	11.00 b	11.00 b
V1	11.2 a	11.00 b	11.00 b	11.00 b
V2	11.00 b	11.00 b	11.00 b	11.00 b
LSD 5%		**0,076**		

Description: Numbers followed by the same notation in the same treatment and column indicate no significant difference in the least significant test (LSD) at the 5% level.

Based on the results in Table 2, at 21 days after planting (DAP), treatment K2 showed the highest plant height at 11.20 cm, compared to treatment K1 with a plant height of 11.16 cm. The plant height observed in treatment K3 was not significantly different from that in K4. Furthermore, the V1K1 treatment showed the highest plant height at 11.2 cm, while treatment V0K2 also recorded one of the highest values across all categories for water spinach plant height.

Table 3. Interaction of Plant Height 28 DAP

28 DAP	K1	K2	K3	K4
VO	20 b	19.43 c	19.4 c	19.00 d
V1	20.17 ab	19.33 d	19.00 d	19.00 d
V2	20.50 a	19.0 d	19.0 d	19.0 d
LSD 5%		**0,227**		

Description: Numbers followed by the same notation in the same treatment and column indicate no significant difference in the least significant test (LSD) at the 5% level.

Based on the results in Table 3, the interaction effect on plant height was observed in treatment K1, which showed the highest value compared to treatments K2, K3, and K4. In terms of treatment interaction, V2 resulted in the highest plant height compared to treatments V0 and V1. Treatment V1 did not differ significantly from V0.

Number of leaves of water spinach plant

Table 4. Average number of leaves in compost and vermiremediation treatments

Treatment	Number of leaves (strands) at observation age (DAP)			
	7	14	21	28
K1	7,67	7	14,22	22,56
K2	7	6,56	13,56	21,89
K3	7,33	6,33	13,67	21,78
K4	6,33	6,22	13,56	21,89
LSD 5%	ns	ns	Ns	ns
V0	17,00	17,67	18,22	29,67
V1	20,33	17,67	18,56	29,33
V2	21,70	17,67	18,22	29,11
LSD 5%	ns	ns	ns	ns

Description: Numbers followed by the same notation in the same treatment and column indicate no significant difference in the least significant test (LSD) at the 5% level.

The presented table shows the progression of leaf number (in units of leaves) at 7, 14, 21, and 28 Days After Planting (DAP) under various treatments, namely K1 to K4 and V0 to V2. In general, the number of leaves increased with plant age across both treatment groups K and V. However, at 7 DAP, the number of leaves in treatments K1–K4 remained relatively low, ranging from 6.33 to 7.67 leaves, while treatments V0–V2 showed higher values, ranging from 17.00 to 21.70 leaves.

At 14 DAP, the V group maintained a stable leaf count at around 17.67 leaves, whereas the K group experienced a slight decrease. At 21 DAP, an increase in leaf number was observed across all groups, with treatment V1 recording the highest value at 18.56 leaves, although the differences were not statistically significant. By 28 DAP, the V treatments demonstrated higher leaf numbers compared to the K group, with the highest value observed in treatment V0 (29.67 leaves), but again, the differences were not statistically significant.

DISCUSSION

At the beginning of the vermiremediation process, compost pH was generally slightly acidic or neutral; as decomposition progressed, the pH increased and approached the neutral range (6.5–7.5). The trend toward pH stabilization at the end of the process indicates a balance between microbial activity and the role of earthworms in breaking down organic matter [5]. The observed moisture content ranged between 60–80 percent, as presented in Table 5.4, which is considered optimal for maintaining both earthworm viability and microbial activity [6]. Based on the measurements, the maximum compost temperature reached only about 30°C, which falls within the mesophilic phase range (25–40°C), where microbial activity is still ongoing but does not generate high heat as in the thermophilic phase. This condition may be influenced by the small size of the compost piles, open aeration systems, and insufficient moisture to retain heat [7].

Changes in color and odor also serve as qualitative indicators of composting success. Initially lighter (or varied) compost color gradually darkened as humus content increased. In qualitative assessments, compost that was originally light in color (value 3–4) gradually shifted to dark or very dark (value 1–2), while the odor did not show significant changes based on direct observation at each TPS3R site until the end of the vermiremediation period. Based on this study, the combination of earthworms, local microorganisms, and biochar can accelerate decomposition, enhance compost stability, and reduce the potential for heavy metal contamination. The mature compost produced had appropriate physical characteristics (dark color, minimal odor) and chemical properties (near-neutral pH, maintained moisture content), making it safe for agricultural application [8].

The initial weight of earthworms used in the study was approximately 250 g; however, the final weight of the earthworms in all treatments tended to decrease significantly. Treatments C1 TPS3R KK, C2 TPS3R JGPT, C3 TPS3R BG, and C4 TPS3R DS each showed relatively higher final earthworm weights compared to the 50-gram group (C1 Kesiman, C1 Jagapati, C1 Belega, and C1 Darmasaba). Nevertheless, the final weights remained far below the initial weight, indicating possible stress or suboptimal environmental conditions for earthworm growth [9]. Furthermore, the presence of earthworm carcasses at the end of the treatment period may serve as an additional source of nitrogen and carbon for heterotrophic microorganisms, thereby enhancing enzymatic activity and accelerating decomposition [10].

The role of biochar as an Mn^{2+} cation adsorbent and the contribution of earthworms in enhancing the activity of metal-binding microbes are key factors [11]. Biochar has the capacity to bind heavy metals in contaminated soils, significantly reducing heavy metal uptake by plants, while also improving the physical, chemical, and biological properties of the soil [12][13].

In the treatment of water spinach plant height, interaction effects were observed at multiple stages of plant growth (DAP). [14] reported that the application of compost improved the growth and yield of water spinach compared to plants grown without compost as a planting medium. This aligns with the findings of [15], which stated that compost application influences leaf number. The number of water spinach leaves at all growth stages showed that treatments did not have a statistically significant effect. In this case, genetic factors may have contributed to the uniformity in leaf number at the observed growth stages, resulting in similar leaf development across treatments.

CONCLUSIONS

The utilization of compost from TPS3R facilities holds potential as a planting medium; however, this compost often still contains heavy metals that exceed the safety threshold for agricultural use. Bioremediation using earthworms and biochar has proven effective in reducing heavy metal content. In organic compost from TPS3R. The combination of earthworms and biochar provides the best results in reducing harmful contaminants. Planting media resulting from vermiremediation (bioremediation with earthworms), especially when combined with biochar, significantly enhances the growth and yield of water spinach (Ipomoea reptans), including plant height, number of leaves, fresh weight, and dry weight.

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