Bioremediation of Leachate Using Heavy Metal-Tolerant Plant Growth Promoting Bacteria Isolates from Iron Sand Soils

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Abstract

The objectives of the study were to determine the tolerance of plant growth-promoting bacterial isolates from iron sand soil to Pb, Cd, and Hg, and the ability of bacterial consortium and incubation times to bioremediate of Pb, Cd, and Hg in leachate. Thirty bacterial isolates from iron sand soils were tested for tolerance to Pb and Cd at concentrations of 5, 25, and 100 ppm, and Hg at concentrations of 0.005, 0.1, and 0.5 ppm. Increasing metal concentrations inhibited bacterial growth; however, 15 isolates remained viable at 100 ppm Pb or Cd and 0.5 ppm Hg. The three consortia significantly reduced Pb, Cd, and Hg concentrations ($P < 0.05$), with reductions of 33.13–65.54%, 45.39-92.28%, and 68.33-90.70%, respectively. Treatment of incubation times reduced heavy metals by amounts of 52.50-71.46% for Pb, 30.16-66.25% for Cd, and 57.36-79.71% for Hg. The bacterial population in remediated leachate after 7 days of incubation was 2.99 log CFU.mL^{-1} for control and 4.97-5.71 log CFU.mL^{-1} for bacterial consortia. The pH, BOD, TDS, and C/N ratio of leachate following 7 days incubation were 9.32-9.49, 3.67–5.20 ppm, 7863.33-8453.33 ppm, and 0.36-0.56, while for the controls were 10.36, 0.73-0.97 ppm, 13,400-13,900 ppm, and 1.17, respectively.

Keywords: bioremediation; heavy metals; leachate; plant growth-promoting bacteria.

I. INTRODUCTION

Banyumas Regency operates an environmentally oriented and educational final waste disposal site (TPA BLE) in Wlahar Wetan Village, Kalibagor District, which employs the sanitary landfill method. The decomposition of waste at the site generates leachate, a complex liquid waste formed when water percolates through the waste mass and dissolves various organic and inorganic substances, including heavy metals (Lesmana & Apriyani, 2019). Leachate may contain dissolved organic compounds, suspended solids, acids, toxic metals, and microorganisms, making it a potential source of environmental pollution and a threat to soil quality and human health if inadequately managed. At TPA BLE Banyumas, leachate is currently treated through sedimentation and aeration. However, these processes have not been sufficient to fully meet applicable quality standards, particularly with respect to heavy metal concentrations. Heavy metals such as Pb, Cd, Zn, Cu, Cr, As, and Ni have been reported in leachate (Karamina *et al.*, 2021; Riana *et al.*, 2024). When present at concentrations above permissible limits, these metals can persist in the environment, accumulate in soil and biological systems, and cause adverse effects on ecosystems and human health.

To reduce the risk of environmental pollution, leachate generated during waste decomposition can be treated through bioremediation using microorganisms, particularly bacteria. A total of 62 bacterial isolates exhibiting tolerance to various heavy metals and acidic conditions, as well as plant growth-promoting properties, have previously been isolated from rhizosphere and iron-sand soils along the southern coast of Central Java (Oedjijono *et al.*, 2023; Oedjijono *et al.*, 2025). The ability of these bacteria to survive under the environmental stress characteristic of coastal iron-sand soils suggests that they may have potential for application in heavy metal bioremediation. Coastal soils are exposed to environmental stresses such as high salinity, heavy metal accumulation, and soil structural instability

(Sudarmaji *et al.*, 2020). Therefore, microorganisms that persist in these environments are likely to possess adaptive mechanisms that enable them to tolerate metal-induced stress (Wuryanto *et al.*, 2015). These characteristics make bacterial isolates from iron-sand environments promising candidates for the bioremediation of heavy metal-contaminated leachate.

The effectiveness of bacterial bioremediation largely depends on the ability of microorganisms to interact with and detoxify heavy metals. Bacteria can reduce heavy metal toxicity through various mechanisms, including biosorption, bioaccumulation, biotransformation, and precipitation (Alkasem *et al.*, 2023). However, the effectiveness of a single bacterial strain may be limited by its tolerance to specific metals and environmental conditions. Therefore, bacterial consortia comprising compatible strains with complementary metabolic capabilities may provide greater stability and broader bioremediation potential than individual isolates. Under mixed-culture conditions, interactions among bacterial strains can enhance microbial survival, metabolic activity, and heavy metal removal, making bacterial consortia potentially more suitable for practical and field-scale applications (Tarekegn *et al.*, 2020). For a consortium to function effectively, its constituent isolates must be compatible and capable of utilizing available nutrients without exhibiting antagonistic interactions. Synergistic interactions among the isolates may further enhance heavy metal detoxification by allowing the strains to complement one another's metabolic activities (Rifai *et al.,* 2020).

Bacteria can remove heavy metals through several complementary mechanisms. During biosorption, metal ions bind to functional groups on the bacterial cell surface or extracellular polymeric substances. Some metals may subsequently be taken up and accumulated within bacterial cells, a process known as bioaccumulation. Metals can also undergo biotransformation through enzymatic reactions, including oxidation, reduction, methylation, and demethylation, which may alter their toxicity and mobility (Emenike *et al.,* 2018; Fabian *et al.*, 2019). In addition, bacteria can facilitate bio-precipitation by converting dissolved metal ions into less soluble forms, thereby reducing their mobility in the environment (Sreedevi *et al.*, 2022). Conversely, bioleaching involves the mobilization of metals through the production or release of metal-binding compounds, such as organic acids, cyanides, thiosulfates, and phenolic compounds, which form soluble complexes with metal ions (Tarekegn *et al.*, 2020).

The objectives of the study were to determine the tolerance of plant growth-promoting bacterial isolates from iron sand soil to Pb, Cd, and Hg, as well as the capability of bacterial isolate consortia and the effect of incubation time on the bioremediation of Pb, Cd, and Hg in leachate.

II. METHOD

Materials

Thirty bacterial isolates (from the culture collection of the Microbiology Laboratory, Faculty of Biology, Jenderal Soedirman University), Nutrient Agar (NA), Nutrient Broth (NB), distilled water, physiological saline; leachate collected from TPA BLE Banyumas;, Erlenmeyer flasks, large containers (gallon-sized), and an aerator.

Assay of the tolerance of bacterial isolates to Pb, Cd, and Hg at various concentrations (Abidin *et al.*, 2019)

Thirty bacterial isolates from iron sand soils were screened for their tolerance to Pb, Cd, and Hg. The concentration variations used were 5, 25, and 100 mg/L for Pb; 5, 25, and 100 mg/L for Cd; and 0.005, 0.1, and 0.5 mg/L for Hg. A 100 mL volume of bacterial culture with a density of 10^8 CFU/mL was inoculated into 25 mL of NB medium containing each metal concentration. Each treatment was prepared in duplicate and incubated at room

temperature for 48 h. Bacterial growth was evaluated based on culture turbidity by measuring the optical density at 600 nm (OD_{600}) using a spectrophotometer. Isolates capable of maintaining growth in the presence of the tested heavy metal concentrations were considered tolerant.

Bacterial compatibility and consortium preparation (Sari *&* Lucyana, 2021)

Isolates showing tolerance to high concentrations of Pb, Cd, and Hg were evaluated for compatibility using a paired-streak assay. Two bacterial isolates were streaked on NA medium in close proximity and incubated at room temperature for 24 h. The interaction between isolates was assessed based on the formation of a clear inhibition zone at the point of contact. The absence of a clear zone indicated a compatible or synergistic interaction, whereas the presence of a clear zone indicated antagonism.

Compatible isolates that exhibited tolerance to all three heavy metals were selected for consortium development. Three consortia, each consisting of five bacterial isolates, were prepared. Each selected isolate was inoculated into 10 mL of physiological saline and incubated for 18 h to obtain cells in the exponential growth phase. The bacterial suspensions were adjusted to approximately (10^8) CFU/mL using a 0.5 McFarland standard and OD600 measurement. Equal volumes of the standardized suspensions were subsequently mixed according to the consortium composition. Consortium 1 consisted of isolates CS3C7, PT6C2, KT2P3, PT4C5, and KT8A6; Consortium 2 consisted of KT10P2, KT1Y5, PT6P1, PT7X1, and CT5C1; and Consortium 3 consisted of PS2P1, KT5P1, PS2Y2, KT6A5, and KS1C4.

Assay of the capability of bacterial consortia in the bioremediation of heavy metals in leachate (Permono *et al.*, 2024)

Leachate used in the experiment was obtained from the BLE Banyumas landfill. Ten liters of leachate were placed into an 18-liter plastic container, followed by the addition of 500 mL (5% v/v) of a bacterial consortium inoculum with a density of 10^8 CFU/mL. Aeration was provided using an aerator. The cultures were continuously aerated using an aerator and incubated at room temperature for 3, 5, or 7 days, according to the respective incubation treatments (Figure 1).



Figure 1. Layout of the leachate bioremediation experiment using bacterial consortia.

This study was conducted experimentally using a completely randomized design with a factorial pattern. The treatments consisted of two factors: consortium type and incubation time. The consortium type factor comprised four levels (Control, Consortium 1, Consortium 2, and Consortium 3), while the incubation time factor comprised three levels (3 days, 5 days,

and 7 days). Each treatment was performed in triplicate. The primary parameter measured was the heavy metal concentration in the leachate. Data were analyzed using analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) at a significance level of 5%.

Other measured parameters included pH, BOD, TDS, the C/N ratio, and the total bacterial population in the leachate. Heavy metal concentrations in the leachate were measured prior to the addition of the consortium to establish baseline levels; subsequent measurements were conducted based on incubation periods (3, 5, and 7 days) and the type of consortium (three varieties). Heavy metal concentrations were determined using an Atomic Absorption Spectrophotometer (AAS) (Riana *et al.*, 2024). Leachate pH was measured using a pH meter. BOD and TDS measurements were conducted in accordance with SNI 6989.72-2009 and SNI 06-6989.3-2019, respectively.

The C/N ratio was measured (Pandi *et al.*, 2023) by trasferring a 1 mL leachate sample into a 100 mL volumetric flask, adding 1 N $K_2Cr_2O_7$ solution and concentrated H_2SO_4 to the mixture, and allowing it to stand for 30 minutes while homogenizing the mixture every 15 minutes. The solution was diluted with distilled water, cooled, and made up to the final volume. The sample was then incubated for 24 hours. Following incubation, the absorbance was measured at a wavelength of 570 nm using a spectrophotometer. Carbon standard solutions at concentrations of 0 and 250 ppm were prepared and treated using the same procedure as the samples. The resulting absorbance values were used to determine the organic carbon content of the samples based on the following equation:

$$\%C = \text{ppm curve} \times \left(\frac{\text{mL extract}}{1000\ \text{mL}}\right) \times \left(\frac{100}{\text{mg sample}}\right)$$

Where:

%C	=	organic carbon content
ppm curve	=	sample concentration obtained from the calibration curve
mL extract	=	flask volume
mg sampel	=	mass of sample.

Total nitrogen content (%N) was determined using the Kjeldahl method. A 1-mL liquid sample was transferred into a 100-mL Kjeldahl flask, followed by the addition of 20 mL of concentrated H_2SO_4. The sample was digested at 350°C for approximately 2 h until the solution became clear. After digestion, the solution was allowed to cool to room temperature, and 20 mL of distilled water was added. Subsequently, 60 mL of 40% NaOH was added, and the sample was distilled for 10 min. The distillate was collected in 10 mL of 1% boric acid containing an appropriate indicator as the receiving solution. The condenser tip was then rinsed with distilled water, and the resulting distillate was titrated with 0.1 N HCl. A blank determination was performed using the same procedure without the sample. The total nitrogen content was calculated using the following equation:

$$\%N = \left(\frac{(\text{V HCl-V Blank}) \times \text{N HCl} \times 14{,}007 \times \text{DF}}{\text{Sample weight (mg)}}\right) \times 100$$

Where: %N: total nitrogen; DF: dilution factor.

The value of the C/N ratio is calculated using the ratio of total organic carbon to total nitrogen obtained from the analysis data. The C/N ratio is calculated using the following formula:

$$\text{C/N ratio} = \frac{\text{C}}{\text{N}}$$

Where: C: organic carbon value; N: total nitrogen value.

The total bacterial population in the leachate was measured as follows: a 1.0 mL leachate sample was serially diluted up to 10^{-6}. One milliliter from the 10^{-5} and 10^{-6} dilutions was inoculated onto NA medium in duplicate using the pour plate method. Total bacterial counts

were determined at the beginning (day 0) and the end (day 7) of the study using the total plate count (TPC) method. The number of colonies was calculated using the following formula:

$$\text{CFU's/mL} = \text{average number of colonies} \times \frac{1}{\text{DF}} \times \frac{1}{\text{PP}}$$

Note: CFU'S/mL: Colony Forming Units per mL; DF: Dilution factor; PP: Pour plate.

III. RESULTS AND DISCUSSIONS

A. Results

1. Tolerance of bacterial isolates from iron sand soils to heavy metals Pb, Cd, and Hg

Heavy metal tolerance test results for the bacterial isolates, based on OD values, indicated that increasing concentrations of Pb, Cd, and Hg in the medium resulted in reduced growth or the death of the isolates (Table 1). Regarding tolerance to Pb, all 30 isolates were able to tolerate 5 ppm, 29 isolates tolerated 25 ppm, and 26 isolates tolerated a concentration of 100 ppm. The results of the cadmium (Cd) tolerance test showed that 29 isolates were tolerant at a concentration of 5 ppm, 28 isolates at 25 ppm, and 22 isolates at 100 ppm. Meanwhile, regarding mercury (Hg), 28 isolates were tolerant at 0.005 ppm, 26 isolates at 0.1 ppm, and 22 isolates at 0.5 ppm. Based on the tolerance responses of each bacterial isolate to exposure to Pb, Cd, and Hg at the highest tested concentrations, and the results of the synergism assay yielded 15 isolates that demonstrated compatibility. These isolates were subsequently combined to form mixed cultures. The isolates were organized into three consortium groups, with each group comprising five isolates. Consortium 1 consisted of CS3C7, PT6C2, KT2P3, PT4C5, and KT8A6. Consortium 2 comprised KT10P2, KT1Y5, PT6P1, PT7X1, and CT5C1, while Consortium 3 consisted of PS2P1, KT5P1, PS2Y2, KT6A5, and KS1C4.

Table 1. Growth of bacterial isolates exposed to heavy metals Pb, Cd, and Hg in an *in vitro* tolerance test, based on OD values

Isolate Code	Control (0 ppm)	Optical Density (OD) Value								
		Pb (ppm)			Cd (ppm)			Hg (ppm)		
		5	25	100	5	25	100	0.005	0.1	0.5
KT6A5	1.765	1.540	1.320	1.110	1.428	1.420	0.515	0.825	0.708	0.657
KT10P2	1.830	1.675	0.980	0.564	1.258	1.065	0.492	0.755	0.559	0.552
KT6P1	1.432	0.980	0.544	0.237	0.022	0	0	0.474	0.107	0
KT11P1	1.560	0.777	0.245	0.142	0.633	0.361	0.038	0.532	0.156	0
KT7Y7	1.534	0.657	0.355	0	0.151	0.019	0	0.622	0.215	0
KT8K3	1.456	0.789	0.457	0.162	0.116	0.096	0.062	0.725	0.322	0.116
KT8K4	1.987	0.438	0.336	0.116	0.396	0.017	0	0.566	0.197	0.004
PT4C5	1.889	1.642	1.579	1.498	1.577	1.563	1.489	0.844	0.794	0.452
CT6P1	1.789	1.567	1.006	0.352	1.420	0.464	0.202	0.123	0.097	0
CT5C1	1.888	1.725	1.442	0.723	1.633	1.333	0.531	0.934	0.876	0.573
PT3P1	1.930	1.830	0.425	0.224	1.808	0.259	0	0.478	0.145	0.003
KT4A2	0.985	0.543	0.315	0.178	0.490	0.212	0.006	0.325	0.114	0.087
PS1Y3	0.869	0.245	0.154	0	0.029	0.006	0	0.534	0	0
PS2Y2	1.446	1.260	0.714	0.619	0.663	0.862	0.312	0.943	0.633	0.391
PS2P2	1.713	1.201	0.467	0.214	1.617	0.185	0.020	0.589	0.232	0.112
CS2Y10	0.985	0.415	0.103	0.007	0.796	0.369	0	0.462	0	0
CS3C7	1.973	1.789	1.043	0.899	1.878	0.900	0.570	1.127	0.847	0.696
CS2C8	1.876	1.483	0.670	0.267	1.394	0.361	0.264	0.745	0.132	0
PT6C1	1.379	1.102	0.815	0.120	1.017	0.862	0.056	0.687	0.223	0.183
PT6C2	1.989	1.900	1.567	1.342	1.853	1.489	1.262	0.910	0.675	0.324
KT2P3	1.982	1.561	1.367	0.675	1.525	1.339	0.486	1.182	0.843	0.612
KT1Y5	1.502	1.167	1.109	0.498	1.104	0.981	0.344	1.124	0.935	0.732
KT8A6	1.867	1.780	1.660	0.623	1.720	1.647	0.652	0.895	0.762	0.442
PT6P1	1.476	1.225	0.710	0.558	1.235	0.798	0.330	1.114	0.893	0.346

PT7X1	1.666	1.539	1.462	0.598	1.384	1.302	0.566	1.457	1.112	0.897
PS2P1	1.171	1.130	0.404	0.391	0.996	0.814	0.334	0.998	0.782	0.581
KT5P1	0.934	0.879	0.778	0.385	0.674	0.536	0.321	0.885	0.642	0.458
PS1Y3	1.579	0.067	0.043	0	0.029	0.006	0	0	0	0
KS1C4	1.460	1.246	1.101	0.543	1.127	0.803	0.438	1.112	0.850	0.631
KT6C1	1.630	0.031	0	0	0	0	0	0	0	0

2. The capability of bacterial consortia in the bioremediation of heavy metals (Pb, Cd, and Hg) in leachate at different incubation times

Heavy metal analysis of the leachate following bioremediation, conducted using AAS, demonstrated that the bacterial consortia reduced levels of Pb, Cd, and Hg. Consortia 1, 2, and 3 proved effective in reducing heavy metal concentrations in the leachate, although the extent of reduction varied. The ANOVA results showed that the consortium treatment and incubation time had a significant effect ($P<0.05$) on the reduction of Pb, Cd, and Hg levels in the leachate; conversely, the interaction effect between the consortium type and incubation time was not significant (Table 2).

Table 2. Results of the two-way ANOVA analysis regarding the effects of consortium type and incubation time on the reduction of Pb, Cd, and Hg in leachate

Metal	Source of Diversity	Degrees of Freedom	Sum of Squares	Mean Square	F Calculated	F Table 5%
Pb	Consortium	3	13235.66	4411.89	68.13**	3.28
	Time	2	9868.68	4934.34	76.20**	3.28
	C*T	6	1257.20	209.53	3.24	3.28
	Error	24	1554.08	64.75		
	Total	35	25915.63			
Cd	Consortium	3	30494.89	10164.96	108.17**	3.28
	Time	2	2156.93	1078.47	11.48**	3.28
	C*T	6	372.74	62.12	0.66	3.28
	Error	24	2255.33	93.97		
	Total	35	35279.89			
Hg	Consortium	3	22343.26	7447.75	286.85**	3.28
	Time	2	3010.52	1505.26	57.98**	3.28
	C*T	6	224.76	37.46	1.44	3.28
	Error	24	623.13	25.96		
	Total	35	26201.67			

The results of the DMRT analysis of the consortium treatments in the reduction of heavy metals (Pb, Cd, and Hg) showed that Consortium 1, Consortium 2, and Consortium 3 had a highly significant effect on reducing heavy metal levels compared to the control (Table 3). This demonstrates that the bacterial isolates constituting the consortia were capable of reducing heavy metal content in the leachate. Among the consortium treatments, Consortium 1 and Consortium 2 resulted in a greater reduction of heavy metals compared to Consortium 3. Consortium 2 achieved the best heavy metal reduction, although the difference was not statistically significant compared to Consortium 1. The bacterial isolates comprising Consortium 2 were KT10P2, KT1Y5, PT6P1, PT7X1, and CT5C1, whereas those comprising Consortium 1 were CS3C7, PT6C2, KT2P3, PT4C5, and KT8A6.

Table 3. Average percentage reduction of Pb, Cd, and Hg in leachate on the effect of consortium types

Metal	Treatment	Average metal reduction (%)
Pb	Control	16.32 a
	Consortium 3	33.13 b
	Consortium 1	58.47 c
	Consortium 2	63.54 c
Cd	Control	22.33 a
	Consortium 3	45.39 b
	Consortium 2	87.72 c
	Consortium 1	92.28 c
Hg	Control	28.52 a
	Consortium 3	68.33 b
	Consortium 1	88.98 c
	Consortium 2	90.07 c

Note: Values followed by the same letter do not differ significantly according to DMRT at the 95% confidence level.

DMRT analysis regarding the effect of incubation time on the reduction of heavy metals (Pb, Cd, and Hg) showed that the percentage of heavy metal reduction increases with longer incubation times (Table 4). However, there was no significant difference in the percentage reduction of Cd between the 3-day and 5-day incubation periods. The highest percentage reduction for the heavy metals was observed at an incubation time of 7 days, with amounts of 71.46% for Pb, 66.25% for Cd, and 79.71% for Hg.

Table 4. Average percentage reduction of Pb, Cd, and Hg in leachate on the effect of incubation times

Metal	Incubation time (day)	Average metal reduction (%)
Pb	3	52.50 a
	5	62.21 b
	7	71.46 c
Cd	3	32.19 a
	5	30.16 a
	7	66.25 b
Hg	3	57.36 a
	5	69.86 b
	7	79.71 c

Note: Values followed by the same letter do not differ significantly according to DMRT at the 95% confidence level.

On day 0, the bacterial population in the consortium treatments ranged from 6.36 to 7.12 log CFU/mL. In contrast, the control leachate without a consortium showed a significantly lower population of 2.89 log CFU/mL. TPC results from days 5 and 7 indicate that the total bacterial populations for all three consortia in the leachate tended to decline (Figure 1). On day 3, the bacterial populations were relatively stable, with Consortium 3 exhibiting the highest population at 6.95 log CFU/mL, followed by Consortium 2 at 6.89 log CFU/mL and Consortium 1 at 6.50 log CFU/mL. On day 5, the population of Consortium 1 declined to 4.44 log CFU/mL, Consortium 2 reached 6.45 log CFU/mL, and Consortium 3 reached 4.41 log CFU/mL. On day 7, the population of Consortium 1 increased to 5.62 log CFU/mL, while Consortium 2 and Consortium 3 reached 5.71 and 4.97 log CFU/mL,

respectively (Figure 2). These results indicate that Consortium 1 and Consortium 2 maintained relatively more stable bacterial populations during the incubation period.

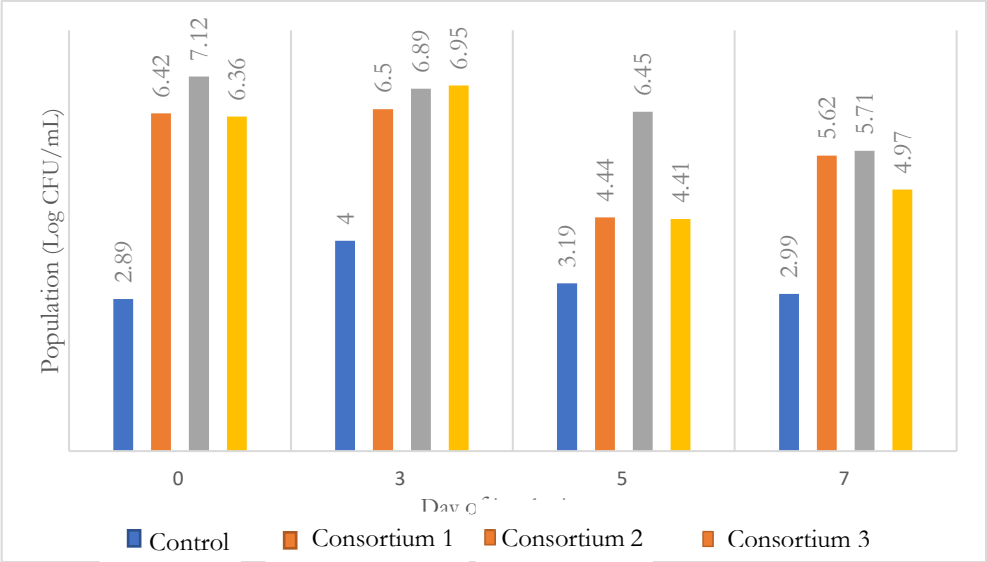

Figure 2. Total bacterial population in leachate at different incubation times.

The result of the pH measurement showed that the leachate from the BLE Banyumas landfill is alkaline (10.0–10.34), and pH values across all treatments increased on days 3 and 5. On day 7, the pH values decreased, particularly in the treatment involving the application of the consortium ranging from 9.32-9.47 (Figure 3). Meanwhile, the pH value of the control did not decrease by the end of the incubation period. The drop in pH observed in the consortium treatment on day 7 indicated a change in the chemical conditions of the leachate associated with bacterial activity during the bioremediation process.

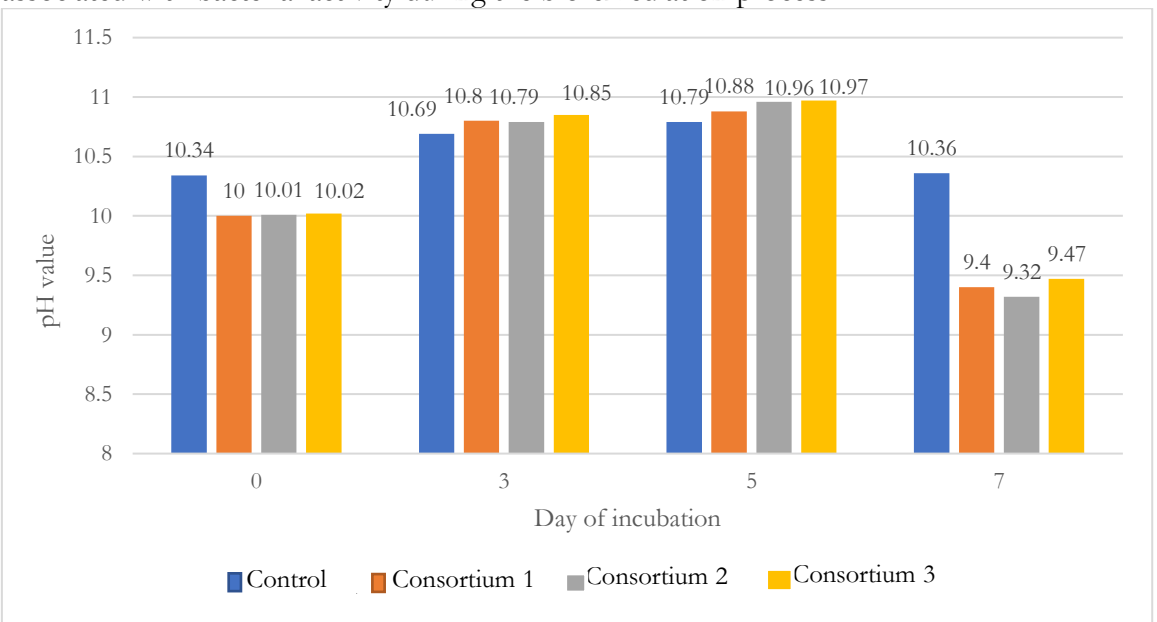

Figure 3. Leachate pH value during the bioremediation process with an incubation period of up to 7 days.

The results of TDS measurement showed a decrease as the incubation period progressed across all treatments. At the initial of incubation, the average TDS values for all treatments ranged from 13,400 to 13,900 ppm. The greatest reduction in TDS occurred on the seventh

day of incubation, with percentage decreases of 37.69%, 40.26%, 41.32%, and 41.15% for the control, Consortium 1, Consortium 2, and Consortium 3, respectively (Figure 4). The greatest percentage decrease was observed in Consortium 2, indicating that the microbial activity within this consortium was potentially more effective in utilizing or degrading certain dissolved substances during the bioremediation process. The reduction in TDS values across all treatments demonstrates a decrease in dissolved components during the incubation period.

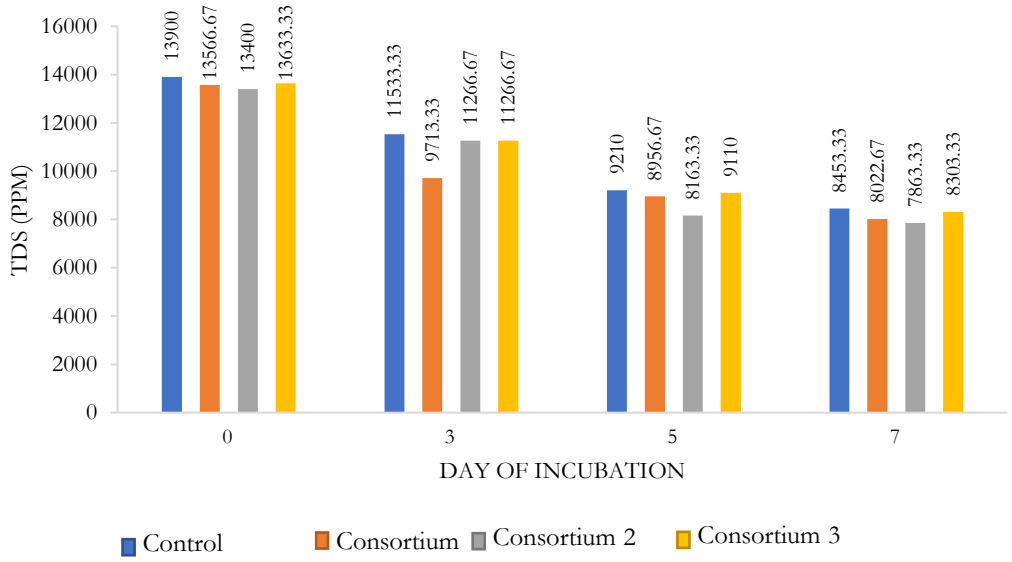

Figure 4. Measurement results of leachate TDS values during the bioremediation process.

Measurements of leachate organic C showed an initial value of 0.31%. Organic C values decreased in accordance with incubation, with more pronounced reductions observed in treatments treated with bacterial consortia. By the end of the incubation period, Consortium 2 and Consortium 3 exhibited the greatest reductions in organic C, reaching 0.08% each (Table 5).

The results of the total N measurement showed that values for all treatments tended to decrease as the incubation period progressed. The total N value of the leachate at the start of incubation was 0.30%. On day 3, the highest total N value was observed in the control treatment (0.28%), while on day 5, total N values across all treatments were relatively similar, ranging from 0.21% to 0.23%. Total N values for all treatments declined again by day 7, with the highest value recorded in the Consortium 2 treatment at 0.22% (Table 5).

The average C/N ratio of the leachate at the start of incubation was 1.03. After 3 days of incubation, the C/N ratios for the control and Consortium 1 decreased to 0.57 and 0.67, respectively, whereas the ratios for Consortium 2 and Consortium 3 increased to 1.24 and 1.16. By day 5 of incubation, the control's C/N ratio remained relatively stable; conversely, all consortium treatments showed a sharp decline leading up to day 7. Meanwhile, the C/N ratio for the control treatment increased to 1.17 by day 7 of incubation (Table 5).

Leachate BOD was measured on day 0 (initial) and day 7 (final). The measurement results showed that the initial BOD values for the control, Consortium 1, Consortium 2, and Consortium 3 were 0.77, 0.73, 0.80, and 0.97 ppm, respectively. After 7 days of incubation, the BOD values increased to 4.03, 4.37, 5.20, and 3.67 ppm, respectively. Consortium 2 exhibited both the highest BOD value and the greatest increase in BOD (Table 5).

Table 5. Organic C, total N, and C/N ratio in leachate during the bioremediation process

Treatment	Incubation time (day)	C organic (%)	N total (%)	C/N ratio	BOD (ppm)
Control	0	0.31	0.30	1.03	0.77
	3	0.16	0.28	0.57	
	5	0.12	0.21	0.57	
	7	0.21	0.18	1.17	4.03
Consortium 1	0	0.31	0.30	1.03	0.73
	3	0.18	0.27	0.67	
	5	0.19	0.23	0.83	
	7	0.10	0.18	0.56	4.37
Consortium 2	0	0.31	0.30	1.03	0.80
	3	0.26	0.21	1.24	
	5	0.17	0.23	0.74	
	7	0.08	0.22	0.36	5.20
Consortium 3	0	0.31	0.30	1.03	0.97
	3	0.29	0.25	1.16	
	5	0.13	0.21	0.62	
	7	0.08	0.17	0.47	3.67

B. Discussion

The ability of bacterial isolates to tolerate heavy metal exposure was analyzed by observing their growth in Nutrient Broth (NB) medium supplemented with Pb, Cd, or Hg. Assays for Pb and Cd were conducted at concentrations of 5, 25, and 100 ppm, while assays for Hg were performed at concentrations of 0.05, 0.1, and 5 ppm, representing low, medium, and high levels of exposure. Differences in adaptability among the isolates upon exposure to the respective metals were assessed based on Optical Density (OD) values. The OD values reflect the turbidity of the medium, which correlates with the bacterial cell count; thus, higher OD values indicate high bacterial growth and viability. The tolerance was evaluated by comparing the OD values of control cultures with those of cultures exposed to Pb, Cd, or Hg at various concentrations. Isolates exhibiting a relatively small decrease in OD value compared to the control demonstrate high tolerance, as they are able to maintain growth under heavy metal stress conditions. Conversely, a significant decrease in OD value indicates growth inhibition due to the toxic effects of heavy metals on the bacterial cells.

The results of the heavy metal tolerance test on the isolates indicate that increasing concentrations of Pb, Cd, or Hg in the medium were accompanied by a decline in the bacterial isolates' growth capacity (Table 1). This response suggests that exposure to higher metal concentrations increases toxic stress on the bacterial cells, thereby disrupting the physiological processes and metabolic activities required to maintain growth. Furthermore, growth responses among the isolates indicated that bacteria originating from iron sand soil exhibited varying levels of tolerance to exposure to Pb, Cd, and Hg. These differences were thought to be related to the physiological characteristics of each isolate regarding their ability to tolerate heavy metal stress. Certain bacterial genera were known to tolerate mixtures of heavy metals at concentrations of 150 ppm, depending on the species and the specific heavy metals involved (Abidin *et al.*, 2019).

The increase in Pb, Cd, and Hg concentrations tended to be accompanied by a decrease in bacterial optical density (OD) values, indicating a decline in physiological activity and cell growth. This phenomenon is attributed to the toxicity of heavy metals at high concentrations,

which can disrupt various cellular metabolic processes. Exposure to high concentrations of Pb, Cd, and Zn can inhibit bacterial metabolic activity by suppressing intracellular enzymatic systems (Fahrudin *et al.*, 2020). This disruption inhibits metabolic processes and the synthesis of cellular components, thereby reducing the bacterial growth rate and, at certain exposure levels, causing cellular damage and death.

The selection of the 15 isolates was based on their respective abilities to tolerate heavy metals, as indicated by relatively high OD values during exposure compared to other bacterial isolates. This capability is thought to be linked to the isolates' physiological and biochemical adaptations to their native habitat of iron sand soil, through mechanisms such as endospore formation and the production of extracellular polymeric substances (EPS) (Oedjijono *et al.*, 2025). PGPR isolates from coastal areas were considered potential bioremediation agents for heavy metal-contaminated environments due to their ability to modify metal availability (Tarekegn *et al.*, 2020). Continuous exposure to heavy metals in natural habitats such as iron sand soils is thought to exert selective pressure that favors the presence of microorganisms with high heavy-metal tolerance.

Testing for synergistic interactions among bacterial isolates is an important step in determining their compatibility for consortium formation. Isolates that are compatible with one another can coexist and potentially interact synergistically, allowing their complementary metabolic activities to enhance the removal or detoxification of Pb, Cd, and Hg from leachate. In a microbial consortium, individual strains may contribute different but complementary functions, such as heavy-metal transformation, immobilization, or detoxification. These complementary activities can broaden the functional capacity of the consortium and potentially improve its bioremediation efficiency compared with individual isolates. The effectiveness of microbial consortia in bioremediation depends on the ability of individual strains to perform specific and mutually supportive functions, including the detoxification and transformation of contaminants (Zhang & Zhang, 2022).

A total of 15 bacterial isolates were selected based on their ability to tolerate Pb, Cd, and Hg while maintaining relatively high viability under heavy-metal exposure. These isolates were subsequently evaluated for compatibility, and five compatible isolates were selected to form a bacterial consortium. The use of a consortium is expected to provide greater functional diversity than individual isolates because bacteria with different physiological characteristics may contribute complementary mechanisms for heavy-metal remediation. Such complementary activities may enhance the overall capacity of the consortium to immobilize, transform, or detoxify heavy metals and thereby improve the removal of Pb, Cd, and Hg from leachate.

The results showed that a 7-day incubation period produced the greatest reduction in Pb, Cd, and Hg concentrations in leachate treated with the bacterial consortia (Table 4). The higher removal efficiency observed after 7 days may indicated that this incubation period provided sufficient time for the bacteria to adapt to the leachate environment and establish active interactions with the heavy metals. During this period, bacterial biomass may increase, providing more binding sites for metal ions through biosorption. Heavy metals can bind to functional groups on bacterial cell surfaces and extracellular polymeric substances (EPS), thereby reducing the fraction of metals remaining dissolved in the leachate. Other processes, such as bioaccumulation and biotransformation, may also contribute to heavy-metal immobilization or changes in metal bioavailability. Prolonged contact between bacterial biomass and metal ions can facilitate these interactions and increase the amount of metal associated with the bacterial biomass. This interpretation is consistent with Jakovljević et al. (2022), who reported that heavy-metal biosorption by bacterial biofilms increased between days 5 and 10, highlighting the importance of sufficient contact time for effective metal-binding processes.

The reduction in heavy-metal concentrations in leachate following treatment with the bacterial consortium demonstrated the potential of the selected bacterial isolates to contribute to heavy-metal removal. This reduction may be associated with several microbial mechanisms for coping with heavy-metal stress, including biosorption of metal ions onto bacterial cell surfaces, bioaccumulation within bacterial cells, and the production of extracellular compounds capable of binding metal ions (Sreedevi *et al.,* 2022).

Bacteria play a role in the bioremediation of heavy metals (Pb, Cd, and Hg) in leachate through metabolic activities and mechanisms such as metal binding, transformation, and immobilization. Therefore, maintaining a sufficient population of viable and metabolically active bacteria is important for sustaining the bioremediation process. In this study, the bacterial populations in the three consortium treatments were monitored on days 0, 3, 5, and 7 using the TPC method to evaluate bacterial survival and population dynamics throughout the bioremediation process. The TPC results showed that the bacterial populations in all three consortia tended to decrease during incubation, with a more pronounced decline observed on days 5 and 7 (Figure 2). The decrease in bacterial population during prolonged incubation may have been associated with changes in the leachate environment. As incubation progressed, available nutrients may have been increasingly consumed to support bacterial growth and metabolic activity, resulting in reduced nutrient availability for maintaining the bacterial population (Ughy *et al*., 2022). In addition, the presence of heavy metals in the leachate may have imposed physiological stress on the bacterial cells, thereby limiting their growth and survival. These conditions may have contributed to the decline in bacterial populations observed at the later stages of incubation. Despite the observed decline, the ability of the bacterial consortia to remain viable throughout the treatment period is important for maintaining their bioremediation activity. Active bacterial cells are required to sustain the metabolic processes involved in heavy metal detoxification and removal. Consequently, a substantial reduction in the bacterial population may decrease the number of metabolically active cells and potentially reduce the efficiency of heavy metal bioremediation (Sardi, 2025). Thus, maintaining an adequate and active bacterial population throughout the treatment period is an important factor in achieving effective leachate bioremediation.

Total dissolved solids (TDS) were measured to monitor changes in the concentration of dissolved substances in the leachate during the bioremediation process. TDS represents the total concentration of dissolved inorganic and organic substances present in the aqueous phase. As shown in Figure 4, the TDS values decreased during bioremediation, indicating a reduction in the concentration of dissolved substances in the leachate. This decrease may be associated with microbial activity during the bioremediation process. Bacteria can utilize certain dissolved organic compounds as sources of carbon and energy, thereby reducing their concentrations through metabolic activity and biodegradation (Ilyas *et al*., 2013). In addition, microbial processes such as biosorption and biofilm formation may facilitate the binding or immobilization of dissolved compounds and ions on bacterial cell surfaces or within the extracellular polymeric matrix. These processes may reduce the amount of dissolved material remaining in the aqueous phase and consequently contribute to the observed decrease in TDS.

Organic carbon and total nitrogen are important nutrients that support microbial growth and metabolic activity during the bioremediation process. Organic carbon serves as a source of carbon and energy for bacterial growth and metabolism, whereas nitrogen is required for the synthesis of cellular components and microbial biomass. The availability and balance of these nutrients can influence bacterial activity and, consequently, the efficiency of contaminant transformation. The C/N ratio represents the relative availability of carbon and nitrogen in the leachate and can therefore be used as an indicator of the nutrient balance supporting microbial growth during bioremediation. The results showed that the C/N ratio

in the bacterial consortium treatments decreased throughout the incubation period. This decrease indicates a change in the relative availability of carbon and nitrogen during the bioremediation process. The reduction in the C/N ratio may be associated with the utilization of organic carbon by bacteria as a source of carbon and energy to support growth and metabolic activity (Narayana *et al.*, 2025).

Consistent with these findings, the 7-day treatment with bacterial consortia reduced the concentrations of Pb, Cd, and Hg and altered several physicochemical characteristics of the leachate. The initially highly alkaline pH decreased from 10 to 9, while BOD increased from 0.73–0.97 ppm to 3.67–5.20 ppm. In addition, TDS decreased by 37.69–41.32% from the initial range of 13,400–13,900 ppm, reaching approximately 8,000–8,300 ppm after 7 days. The decrease in the C/N ratio, together with the changes in BOD and TDS, suggests that microbial activity during bioremediation influenced the composition and availability of organic and dissolved constituents in the leachate. These findings indicate that bacterial consortium treatment not only promoted the removal of heavy metals but also contributed to changes in the physicochemical characteristics of the leachate, reflecting an overall improvement in leachate quality.

Leachate quality standards establish permissible pollutant concentrations for leachate discharged or released into water bodies from landfill operations (Ministry of Environment and Forestry Regulation, 2016). Leachate discharged into the environment must comply with these standards. According to the Ministry of Environment and Forestry (2016), the key quality parameters include a pH of 6–9, BOD of 150 mg/L, COD of 300 mg/L, TSS of 100 mg/L, total nitrogen of 60 mg/L, Hg of 0.005 mg/L, and Cd of 0.1 mg/L. The results of this study also indicated that bioremediated leachate has potential for use as liquid organic fertilizer for crop cultivation. According to Minister of Agriculture Decree No. 261/KPTS/SR.310/M/4/2019 on the minimum technical requirements for organic fertilizers, biofertilizers, and soil conditioners, the requirements include a minimum organic carbon content of 15%, a C/N ratio of 15–25, and maximum concentrations of Pb, Cd, and Hg of 50, 2, and 1 ppm, respectively. Based on these two regulatory standards, the bioremediated leachate partially meets the requirements for both leachate discharge and liquid organic fertilizer. However, some parameters remain below the required standards, particularly organic carbon content and the C/N ratio, which were substantially lower than the minimum requirements for liquid organic fertilizer (Table 5).

IV. CONCLUSION

It can be concluded that plant growth-promoting bacterial isolates from iron sand soil could tolerate Pb and Cd at 100 ppm and Hg at 0.5 ppm. Consortia 1 and 2 were significantly capable of bioremediating the leachate after 7 days of incubation.

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