



Evaluation of heavy metal contamination in landfills and role of multi-heavy metal-resistant rhizobacteria in heavy metal mobilization

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Received: 3 April 2025 / Revised: 29 June 2025 / Accepted: 22 July 2025 / Published online: 19 August 2025
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Abstract

This study assessed heavy metal contamination levels associated with the soils in municipal solid waste landfills and heavy metal accumulation found in plants native to the area. Furthermore, the characterization of new isolates of plant growth promoting rhizobacteria (PGPR) which are resistant to heavy metals was carried out. Results found that in all soil samples tested, cadmium, copper, lead, and zinc could be found. Cadmium had the greatest contamination factor, indicating a very high level of contamination. The cadmium and lead concentrations were highest in the shoots of *Urena lobata* L., while shoots of *Brachiaria mutica* Forssk. and *Celosia argentea* L. contained the greatest copper and zinc concentrations, respectively. There were forty-eight isolates of rhizobacteria, nine of which were resistant to cadmium, copper, and lead. Two of these isolates, designated as Cd3A7 and Cd2ES7, produced elevated levels of indole-3-acetic acid and were identified to be *E. cloacae* and *K. pneumoniae*. Both PGPR strains demonstrated the ability to enhance root and shoot growth in *Helianthus annuus* L. under conditions of heavy metal contamination. They were also found to colonize the interior tissues of the roots as well as the surface of the *H. annuus* roots. The plants successfully survived in heavy metal-contaminated landfill soil, enhancing the bioavailability of cadmium and lead. In conclusion, these PGPR exhibited characteristics making them candidates for application in heavy metal phytoremediation at landfill sites.

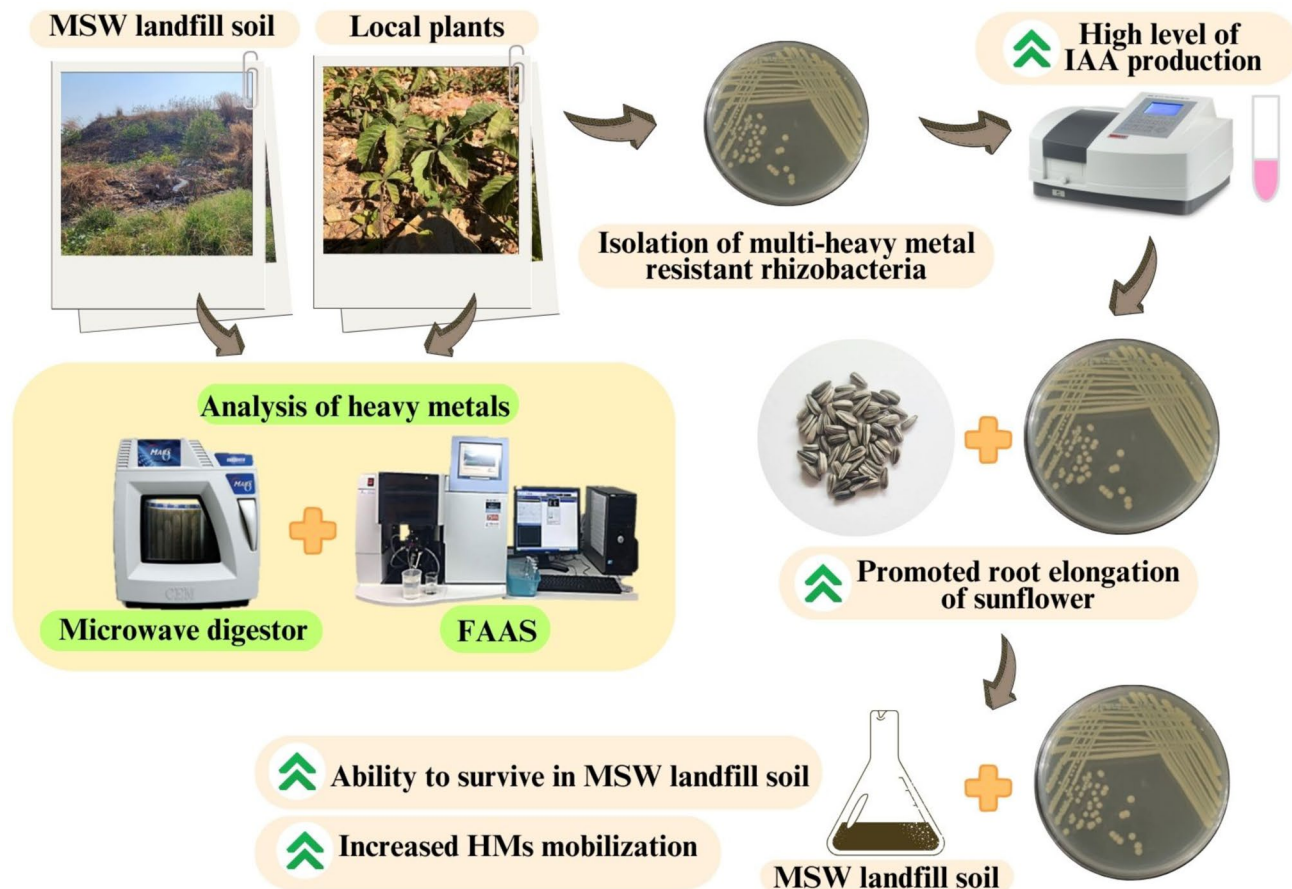
Editorial responsibility: S. Mirkia.

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Graphical abstract



Keywords Heavy metal contamination · *Helianthus annuus* L. · Indole-3-acetic acid · Municipal solid waste landfill · Plant-growth promoting rhizobacteria

Introduction

Landfilling has been widely applied for disposing of municipal solid waste (MSW) in various nations, including China, Iran, Malaysia, and Thailand (Lu and Feng 2020; Sheijany et al. 2020; Hussein et al. 2021; Thongyuan et al. 2021). Its prevalence is attributed to its low cost, ease of maintenance, and simplicity compared to other MSW disposal techniques (Mandal 2019). However, landfilling has several environmental drawbacks, for example, foul odors, greenhouse gas emissions, and soil and groundwater pollution. The mismanagement of MSW segregation, collection, and disposal can cause the release of highly contaminated leachates, thus serving as a potential source of heavy metal soil contamination (Alam et al. 2020; Hussein et al. 2021). Therefore, MSW landfill sites are identified as significant repositories and point sources of heavy metal contamination (Gujre et al. 2021). Several investigators reported that

the soil around MSW disposal sites was contaminated with potentially harmful heavy metals (Sheijany et al. 2020; Gujre et al. 2021).

This heavy metal accumulation in the soil can lead to long-lasting toxicity affecting living organisms (Li et al. 2021). These toxic heavy metals can find their way into both wild and cultivated plants surrounding the landfill sites, ultimately contaminating food sources for human consumption, and potentially acting as a human health hazard (Guan et al. 2023). Owing to the toxic and non-biodegradable characteristics of heavy metals, global attention has been drawn to the issue of heavy metals released from MSW landfills (Yao et al. 2019). Therefore, there is an urgent need to explore appropriate solutions to mitigate the impact of heavy metals in MSW landfill sites. Conventional remediation methods, including soil washing, solidification, incineration, and excavation, are widely used; however, their application is often limited by high operational costs, substantial infrastructure



requirements, and the risk of secondary environmental impacts (DalCorso et al. 2019; Yan et al. 2020). As a sustainable alternative, phytoremediation employs plants to immobilize or extract pollutants and is recognized for its cost-effectiveness and environmental compatibility in the In situ remediation of heavy metal-contaminated soils (Madhav et al. 2024). This approach is non-invasive, aesthetically favorable, and well-suited for large-scale ecological restoration efforts (Garbisu et al. 2020; Teng et al. 2022). Therefore, phytoremediation is suggested as an effective strategy for the rehabilitation of landfill sites (Kanwar et al. 2023).

Nonetheless, utilizing plants to rehabilitate soil contaminated with heavy metals has its constraints. This approach is primarily effective for treating soil with minimal heavy metal contamination and typically demands an extended period for restoration because plants tend to grow rather slowly (Montreemuk et al. 2024). In instances of substantial heavy metal pollution, plants undergo stress and display inhibited growth as a consequence of the toxic effects exerted by heavy metals. To improve plant-mediated remediation effectiveness, rhizobacteria are vital in supporting plant survival and growth under toxic heavy metal conditions (Mendoza-Hernández et al. 2019). Certain bacteria have demonstrated resistance to heavy metals and the ability to enhance plant growth (Kumar et al. 2021; Ketaubon and Prapagdee 2023). Additionally, some bacterial products, for example, biosurfactants, exopolymers, and siderophores can increase heavy metal bioavailability within the soil (Prapagdee and Khonsue 2015; Das et al. 2017; Roskova et al. 2022), thereby facilitating phytoremediation efforts.

PGPR (plant growth-promoting rhizobacteria) are able to promote the development of plants by residing either in soil surrounding the roots or inside the interior tissues of the plant itself (Montreemuk et al. 2024). PGPR is able to support both the growth of plants and the efficiency of heavy metal phytoremediation through multiple pathways (Manoj et al. 2020). Some PGPR produce indole-3-acetic acid (IAA) which exhibits crucial activity in various functions of the plant tissue, particularly those crucial for promoting seed germination, enhancing root and cell elongation, and fostering favorable conditions for plant growth and maturity (Ratnaningsih et al. 2023). Key properties of bacteria that facilitate heavy metal phytoremediation are their heavy metal resistance and their potential to promote plant growth (Siripan et al. 2018; Saravanan et al. 2024). Consequently, employing potent heavy metal resistant PGPR is a critically important approach to enhance heavy metal phytoremediation effectiveness.

However, limited research efforts have been directed toward isolating and characterizing multi-heavy metal-resistant PGPR found in MSW landfill sites. For instance, Sahu et al. (2022) were recently able to isolate phosphate solubilizing bacteria from an MSW dumping site at Guwahati,

India. Hence, the isolation of new PGPR strains from native plants grown at MSW landfill sites has been deemed necessary for application in heavy metal phytoremediation within landfill areas. Considering that MSW landfill sites are known contributors to heavy metal contamination, our research focused on quantifying toxic heavy metal concentrations in landfill soil and local plants at a landfill in Thailand. The isolation and screening of indigenous rhizobacteria capable of resisting heavy metals and producing IAA were conducted. In addition, the effectiveness of selected IAA-producing rhizobacteria or PGPR in the root elongation and root colonization of *Helianthus annuus* L., along with their ability to mobilize heavy metals, was examined. *H. annuus* exhibits a strong capacity for the uptake and accumulation of heavy metals in its roots and shoots, contributing to the phytoremediation of contaminated soils (Kumar et al. 2021). Additionally, its aesthetic appeal can enhance the visual landscape of landfill sites. The findings from this study suggest these multi-heavy metal-resistant PGPR might be used to enhance heavy metal phytoremediation efficiency by ornamental plants in MSW landfills.

Materials and methods

Description of MSW landfill locations

The MSW sanitary landfill site is located at the Comprehensive Waste Management Center (CWMC) in Rayong, Eastern Thailand, with its precise UTM coordinates being 47P 742963.85, 1410503.75 (Fig. 1). It has been managed by the Rayong Province Administrative Organization, Thailand since 2001. The total area of this center is approximately 70 hectares with an allocated landfill area of about 40 hectares. The CWMC receives approximately 700–800 tons daily of municipal solid waste from a total of 61 municipalities and sub-district areas in Rayong Province as reported by the Pollution Control Department, Thailand.

Soil sampling and quantification of heavy metals

Soil samples from landfill areas, ranging in depth from 0 to 20 cm, were gathered from sixteen designated sampling locations as shown in Fig. 1 through the use of a manual soil drilling tool and a small trowel. The samples of soil collected from the covered landfill were air-dried, screened to remove small stones and other large contaminants, and then sieved using a 2-mm mesh. Homogeneous soil samples were used for analysis. Analyses of the overall (acid-digested form) and bioavailable (DTPA-extractable form) concentrations of Cd, Cr, Cu, Pb, and Zn were performed following respective processes explained by McGrath and Cunliffe (1985), and Faust and Christians (2000). The determination of the



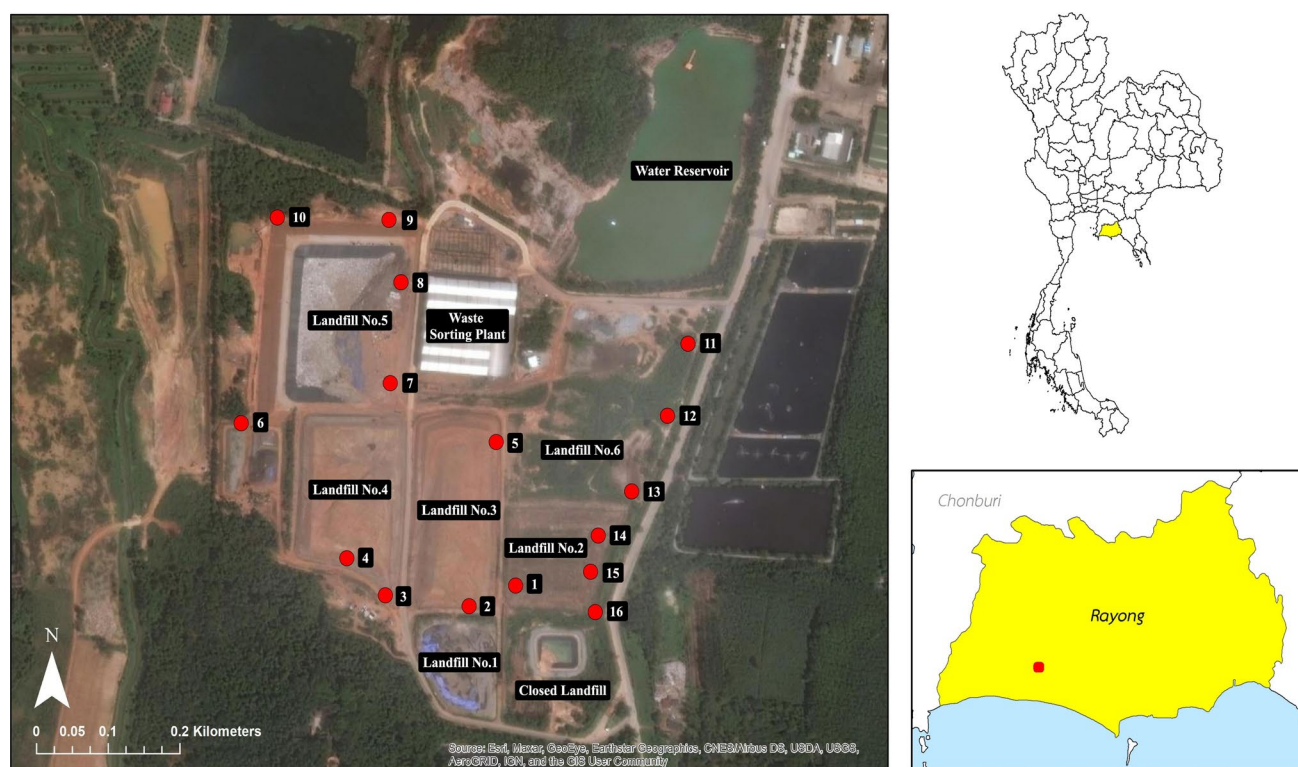


Fig. 1 Location maps of soil sampling sites at MSM landfill area

concentrations of heavy metals in covered landfill soil, following digestion and extraction procedures, utilized Flame Atomic Absorption Spectrophotometry (FAAS) (Model 240FS AA, Agilent, USA). The respective detection limits for Cd, Cr, Cu, Pb, and Zn standards were 1, 3, 1, 15, and $1 \mu\text{g kg}^{-1}$. The analysis was conducted on all samples in triplicate, incorporating certified soil (ERM-CC141, European Reference Material, Belgium) along with a blank reagent which had no soil to ensure a precise and accurate analytical process. Each heavy metal concentration in landfill soil was used to calculate the contamination factor (CF) as presented in Eq. 1 (Thongyuan et al. 2021).

$$\text{CF} = \frac{\text{Concentration of HM in soil}}{\text{Reference concentration of HM}} \quad (1)$$

The respective reference concentrations of Cd, Cu, Pb, and Zn were 0.01, 2.3, 4.9, and 3.6 mg kg^{-1} (Zarcinas et al. 2004). CF classification levels were divided as: $\text{CF} < 1$: low level; $1 \text{ to } < 3$: moderate level; $3 \text{ to } < 6$: considerable level; and $\text{CF} \geq 6$: very high level (Thongyuan et al. 2021).

Plant sampling and heavy metals analysis

Ten species of native plants were randomly collected in triplicate from the soil surrounding the landfills (at sampling points 8–9 as indicated in Fig. 1). The plants were

carefully washed in tap water before being immersed for the duration of 5 s in 0.01 M HCl (Ketaubon and Prapagdee 2023). The plants underwent rinsing twice with deionized water before being segregated into the root (below-ground) or shoot (above-ground) parts. After the oven drying for 72 h at a temperature of 60°C , the plants were milled into a fine powder. The homogenized plant samples were digested in a microwave digester using conc. HNO_3 . The various concentrations of Cd, Cu, Pb, and Zn in digested plants underwent analysis using FAAS. In addition, the assessment of a plant's ability to transfer heavy metals from roots to shoots was accomplished by determining the quotient concentration of heavy metals residing in the shoots relative to the corresponding root concentration known as the translocation factor (TF) (Mattina et al. 2003).

Isolation of multi-heavy metal resistant rhizobacteria

Rhizosphere samples of soil and plant roots were randomly gathered from the sampling points and placed in sterilized plastic bags. The numbers of viable bacteria were counted through the use of a standard plate count method on nutrient agar (Difco, USA) plates. Sucrose-minimal salts low phosphate buffer (SLP) (He et al. 2010), containing 2 mM of CuSO_4 , 2 mM of $\text{Cd}(\text{NO}_3)_2$, or 3 mM of $\text{Pb}(\text{NO}_3)_2$, was used

to treat rhizosphere soils and coarsely ground plant roots. Incubation of the soil suspension was carried out for 24 h at 28 °C and 150 rpm for 24 h before dilution and spreading on SLP agar amended with the same concentration of each heavy metal. Selection of the colonies appearing on SLP agar plates was made through consideration of colony morphology variations and these were re-streaked on SLP agar supplemented using each of the various heavy metals to obtain pure cultures with heavy metal resistant ability. Each rhizobacterium that was resistant to one heavy metal was cross streaked on SLP agar supplemented with other heavy metals to assess their resistance to additional heavy metals.

Screening of IAA production and identification

Isolated rhizobacteria resistant to multiple heavy metals were screened for IAA production by cultivation in Luria–Bertani (LB) broth (Criterion, USA) adjusted via 0.5 mg mL⁻¹ of L-tryptophan. Bacterial cultures were incubated for 24 h, harvested, and placed in a centrifuge for 20 min at 8000 rpm at a temperature of 4 °C. A supernatant free of cells was subsequently obtained, while IAA levels could be assessed via the approach proposed by Gordon and Weber (1951). To summarize, 1 mL of this resulting supernatant was combined with 2 mL of Salkowski's reagent before a 20-min reaction at room temperature took place, whereupon the absorbance was measured at 530 nm. For bacterial identification, the genomic DNA of the multi-heavy metal-resistant PGPR was extracted for PCR amplification of the 16S rRNA gene via 20F (5'-GAG TTT GAT CCT GGC TCA G-3') along with 1500R (5'-GTT ACC TTG TTA CGA CTT-3') primers (Brosius et al. 1981). Following sequencing of the 16S rRNA gene, the 16S rDNA sequence homology was analyzed utilizing BLASTn available through the GenBank database accessible via the National Center for Biotechnology Information (NCBI). Selected rhizobacteria that produced high IAA were selected as PGPR with multi-heavy metal resistance.

Measurement of IAA production, growth, and MICs of heavy metals

LB broth was used for culturing selected PGPR with multi-heavy metal resistance (amended using 0.5 mg mL⁻¹ of L-tryptophan for 96 h. Bacterial cultures at different growth periods were harvested to measure IAA levels as previously described above. The growth of the bacteria was assessed through measurements taken at a wavelength of 600 nm to evaluate cell turbidity. In addition, resistance to Cd, Cu, and Pb of the chosen PGPR with multi-heavy metal resistance was determined in terms of MICs (minimum inhibitory concentrations) through the method proposed by Kumar et al. (2021).

Assays for root elongation and colonization

Helianthus annuus seeds were surface-sterilized and soaked in bacterial suspensions for which the cell density measured OD₆₀₀ ~ 0.2 (the viable cell number is around 10⁷ CFU mL⁻¹) under shaking conditions for 2 h according to the methods of Khanthom et al. (2021). The control seeds were soaked for 2 h in sterile 50 mM phosphate buffer at a neutral pH. For in vivo root elongation, both bacterial-treated seeds and seeds serving as the control were separately added to sterilized petri-dishes in which there was also 10 mg L⁻¹ Cd, 25 mg L⁻¹ Cu, and 50 mg L⁻¹ Pb. After cultivation for one week, the lengths of shoots and roots were determined using ImageJ software for precise measurement.

For root colonization assay using a gnotobiotic system (Gao et al. 2016), single seeds underwent transfer aseptically to designated test tubes which contained 0.4% water-agar (Mafia et al. 2009) and maintained in dark condition until germination before placing under natural light. After one week of cultivation, a turbid zone surrounding the roots exhibited signs of root colonization in contrast to the uninoculated seeds. To confirm root colonization, roots were transferred to LB agar plate for incubation for a period of 24–48 h at a temperature of 28 °C in order to observe the colonies of bacteria. To determine endophytic colonization, root surfaces were sterilized and ground before suspending in sterilized normal saline. After vigorously mixing, root suspensions were spread on LB agar and incubated for 24–48 h to observe the endophytic bacterial colonies on LB agar plates.

Evaluation of bacterial viability and their performance on heavy metals mobilization in landfill soil

Autoclaving was employed to sterilize the landfill soil (from inside landfill No. 5, Fig. 1) for 30 min at a temperature of 121 °C on two successive days. The multi-heavy metal-resistant PGPR were individually inoculated into the sterilized soil from the landfill which was placed inside a sterile polypropylene flask. During incubation at around 30 ± 2 °C under conditions of darkness, soil aliquots were taken at intervals of 3, 7, 14, 21, and 28 days. Viable bacterial cell numbers in aliquots of landfill soil were measured via the standard plate count technique on LB agar plates. Soil samples were also analyzed for pH along with the overall concentrations and bioavailable concentrations of Cd, Cu, and Pb. Heavy metal mobilization was expressed in terms of the bioavailable concentration. The determination of non-bioavailable heavy metal concentrations involved the subtraction of bioavailable concentrations from the total concentrations.



Statistical analysis

The data were analyzed by calculating the mean and standard error. To compare the mean heavy metal concentrations, as well as plant growth under various heavy metal concentrations in *H. annuus*, a one-way analysis of variance (ANOVA) was performed at a 95% confidence level. The significance of differences between treatments was determined using Duncan's multiple range test (DMRT) at a significance level of $p < 0.05$.

Results and discussion

Contamination due to heavy metals in MSW landfill soils

Table 1 presents the total concentrations of Cd, Cu, Cr, Pb, and Zn, selected based on their prevalence and ecological relevance at the landfill sites, as supported by prior regional surveys (Ruchuwarak et al. 2019; Thongyuan et al. 2021) and baseline assessments. The table also reveals the bioavailable concentrations of these metals, which exhibit considerable variation across the samples. The highest total and bioavailable concentrations in soil was Pb, followed by Zn. The bioavailable concentrations of these heavy metals were less than 10% of the total. The bioavailable concentration of Cu was less than 2.3% of its total concentration. The assessment of bioavailable concentrations of heavy metals serves as a critical tool in understanding the potential risks posed by heavy metals to living organisms (Ramos-Miras et al. 2011). There are several factors affecting the bioavailable heavy metal concentrations in the soil, e.g., soil pH, organic matter, electrical conductivity, and the ability to exchange cations (Patra et al. 2020). All landfill soil samples revealed the existence of Cd, Cu, Pb, and Zn. Cr remained undetected within all samples (Table S1). The averages for overall heavy metal concentrations can be described as follows in descending order (mg kg^{-1}): Pb (59.36) > Zn (19.24) > Cu

(3.70) > Cd (0.49). The excessive deposition of MSW causes severe soil pollution and changes the physical and chemical properties of soil (Kanwar et al. 2023).

The contamination factors (CF) of heavy metal were calculated from the average total concentration of each heavy metal from 16 sampling sites. It was found that Cd had the highest CF at 49.0 (Table 1), indicating that Cd had the highest contamination level in the soil. According to the classification levels of CF by Thongyuan et al. (2021), Cu had a moderate degree, while Cd and Pb had a very high degree of contamination. The background concentration ranges of Cd, Cu, Pb, and Zn in Thai soil have been reported to be 0.01–0.17, 0.16–43.6, 0.10–54.6, and 0.10–71.0 mg kg^{-1} , respectively (Zarcinas et al. 2004). The average soil concentrations of Cd (0.49 mg kg^{-1}) and Pb (59.36 mg kg^{-1}) in the CMWC landfill exhibited greater values than the reported background concentration ranges. Cd, Cu, Pb, and Zn in landfill soil had higher concentrations than that in the soils in the Brazilian Amazon which were reported as follows (mg kg^{-1}): Cd (0.1), Cu (2.8), Pb (4.4), and Zn (5.7) (do Nascimento et al. 2018). The concentration of heavy metals in the soil is influenced by the geological characteristics of the area (Daulta et al. 2023). Importantly, this landfill site is located adjacent to residential and agricultural areas. This suggests that heavy metals present in leachate and landfill soil can disperse into surrounding areas, potentially posing a risk to human health. Thongyuan et al. (2021) reported that prolonged exposure to landfill soil, which was severely polluted with Cd and moderately polluted with Cu, would possibly pose non-carcinogenic threats to the health of affected individuals of all ages. It is recommended that preventive measures to limit the distribution of heavy metals from leachate and landfill soil be urgently implemented.

Accumulation of heavy metals within plants growing naturally at the landfill site

Table 2 presents the Cd, Cu, Pb, and Zn concentrations present in the shoots and roots of local plants. The findings

Table 1 Ranges of heavy metal concentrations in landfill soils and contamination factors of heavy metals

Heavy metal form	Heavy metal concentration (mg kg^{-1})			
	Cd	Cu	Pb	Zn
Bioavailable form				
Minimum	< 0.001 [#]	0.06 ± 0.01	0.29 ± 0.06	0.16 ± 0.01
Maximum	0.08 ± 0.01	0.33 ± 0.02	5.06 ± 0.23	2.75 ± 0.02
Total (acid-digested) form				
Minimum	0.13 ± 0.02	0.77 ± 0.04	28.17 ± 9.62	3.57 ± 0.06
Maximum	0.80 ± 0.10	14.37 ± 2.05	100.77 ± 13.57	38.07 ± 0.25
Average	0.49	3.70	59.36	19.24
Contamination factor	49.0	1.6	12.1	5.3

[#]Detection limits: Cd < 0.001 mg kg^{-1}



Table 2 Heavy metal concentrations accumulated in local plants grown at landfill site and their translocation factors

Plant species	Heavy metal concentration in roots (mg kg ⁻¹)				Heavy metal concentration in shoots (mg kg ⁻¹)			
	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
<i>Acacia auriculi-</i> <i>formis</i> Benth	1.00 ± 0.42 ^c TF	2.90 ± 1.20 ^b	2.00 ± 0.80 ^a	14.20 ± 5.60 ^a	0.90 ± 0.22 ^c 0.90	4.00 ± 1.25 ^a 1.38	2.00 ± 1.10 ^{ab} 1.00	34.48 ± 12.12 ^{ab} 2.43
<i>Amaranthus viridis</i> L	< 0.001 TF	1.30 ± 0.32 ^a	4.10 ± 1.05 ^{ab}	29.27 ± 7.41 ^b	< 0.001 -	1.90 ± 1.00 ^a 1.46	3.20 ± 0.70 ^{bc} 0.78	43.47 ± 8.90 ^b 1.49
<i>Brachiaria mutica</i> Forssk	0.03 ± 0.01 ^a TF	31.40 ± 5.80 ^c	10.50 ± 2.08 ^c	39.42 ± 10.10 ^{bc}	0.20 ± 0.10 ^b 6.67	7.50 ± 2.42 ^c 0.24	4.20 ± 1.62 ^c 0.40	47.75 ± 16.07 ^b 1.21
<i>Celosia argentea</i> L	2.20 ± 1.12 ^d TF	6.20 ± 1.27 ^c	10.00 ± 3.53 ^c	81.56 ± 22.00 ^e	2.10 ± 0.63 ^d 0.95	6.40 ± 1.20 ^b 1.03	1.10 ± 0.00 ^a 0.11	89.80 ± 14.50 ^d 1.10
<i>Coccinia grandis</i> L	0.70 ± 0.10 ^c TF	5.40 ± 2.40 ^c	2.00 ± 0.82 ^a	41.17 ± 9.67 ^c	0.20 ± 0.05 ^b 0.29	7.20 ± 0.33 ^{bc} 1.33	1.00 ± 0.20 ^a 0.50	68.37 ± 9.80 ^c 1.66
<i>Ipomoea aquatica</i> L	0.40 ± 0.02 ^b TF	8.60 ± 3.00 ^d	2.30 ± 1.00 ^a	35.22 ± 5.40 ^{bc}	0.20 ± 0.00 ^b 0.50	5.80 ± 1.07 ^b 0.67	< 0.015 -	23.32 ± 7.62 ^a 0.66
<i>Mimosa pudica</i> L	32.00 ± 3.53 ^e TF	6.30 ± 1.30 ^c	13.40 ± 2.57 ^c	29.67 ± 11.50 ^b	1.40 ± 0.45 0.04	3.40 ± 0.85 ^a 0.54	< 0.015 -	36.71 ± 11.50 ^{ab} 1.24
<i>Ricinus communis</i> L	0.01 ± 0.00 ^a TF	6.00 ± 0.52 ^c	28.20 ± 6.20 ^d	34.81 ± 7.95 ^{bc}	0.02 ± 0.00 ^a 2.00	2.20 ± 0.82 ^a 0.37	1.00 ± 0.10 ^a 0.04	35.07 ± 6.93 ^{ab} 1.01
<i>Sida cordifolia</i> L	0.80 ± 0.27 ^c TF	7.20 ± 2.10 ^{cd}	3.10 ± 0.93 ^a	57.81 ± 10.90 ^{cd}	0.60 ± 0.27 ^c 0.75	4.10 ± 1.33 ^a 0.57	6.20 ± 2.58 ^{cd} 2.00	36.88 ± 7.37 ^{ab} 0.64
<i>Urena lobata</i> L	1.60 ± 0.72 ^{cd} TF	2.70 ± 0.83 ^b	6.50 ± 1.20 ^b	31.93 ± 7.70 ^b	3.00 ± 0.93 ^{de} 1.88	5.50 ± 2.12 ^{ab} 2.04	7.10 ± 1.37 ^d 1.09	87.77 ± 21.80 ^d 2.75

Different letters denote significant differences in each heavy metal accumulation in roots and shoots among plant species ($p < 0.05$), as determined by Duncan's multiple range test

revealed the greatest Cd, Cu, Pb, and Zn concentrations in the roots could be found in *Mimosa pudica* L., *Brachiaria mutica* Forssk., *Ricinus communis* L., and *Celosia argentea* L., respectively. *Urena lobata* L. accumulated the greatest Cd and Pb concentrations in its shoot tissues. *B. mutica* showed an excellent potential for the accumulation of Cu within the shoots and roots, while the highest concentration of Zn within the roots and shoots was found with *C. argentea*. Hussain et al. (2024) concurred, noting that that *C. argentea* exhibited the highest efficiency in accumulating Zn in its shoots, with a bioconcentration factor for Zn exceeding 1.

However, undetectable amounts of Cd (< 0.001 mg kg⁻¹) in *Amaranthus viridis* L. shoots and roots were observed, indicating a cadmium excluder trait of *A. viridis*. In addition, lead was also undetected (< 0.015 mg kg⁻¹) in *Ipomoea aquatica* L. and *M. pudica* shoots. The highest TF values of Cd, Cu, Pb, and Zn could be observed in *B. mutica*, *U. lobata*, *Sida cordifolia* L., and *U. lobata*, respectively (Table 2). Our findings suggest that *U. lobata* had a high potential for heavy metals phytoextraction. These results match the work conducted by Tripathi and Misra (2013), who claimed that among 12 plant species, *U. lobata* is the most proficient plant in accumulating Pb grown on a municipal waste dump site, demonstrating a TF of 20.2.

Selection of heavy metal resistant rhizosphere bacteria and the ability on IAA production

Viable bacteria numbers in rhizosphere soils and plant roots collected from the landfill site ranged from 1.8×10^5 to 1.4×10^8 CFU g⁻¹. The soil texture was sandy loam soil with slightly acidic pH (6.0). The results of isolation of heavy metal resistant rhizobacteria found that a total of 48 isolates were able to grow on SLP supplemented with either Cd, Cu, or Pb. Among forty-eight isolates of heavy metal resistant rhizobacteria, twenty-eight isolates resisted at least two heavy metals were found (Table S2). Most of them (25.0%) resisted Cd and Pb. Only nine isolates (18.7%) were triple-heavy metal resistance called multi-heavy metal resistant-rhizobacteria. The genes involving the mechanisms of heavy metal resistance in bacteria are located on plasmids and some are found in chromosomes (Nanda et al. 2019). Bacteria have developed various heavy metal resistant mechanisms that support them to survive in heavy metal-polluted soils (Fakhar et al. 2022). Examples of heavy metal resistant mechanisms are transportation across cell membrane and intracellular bioaccumulation, metal binding or surface biosorption, enzymatic detoxification, and metal precipitation (Banerjee et al. 2015; Nanda et al. 2019).



The range of IAA levels produced from the 48 isolates of heavy metal resistant rhizobacteria is presented in Table 3. Fifteen isolates produced IAA levels at the range of 0–10 $\mu\text{g mL}^{-1}$. The isolate Cd3A7 demonstrated the highest IAA production among the tested isolates, with a level of $232.08 \pm 9.27 \mu\text{g mL}^{-1}$, followed by Cd2ES7, which produced $164.06 \pm 12.32 \mu\text{g mL}^{-1}$ of IAA. Cd3A7 and Cd2ES7 exhibited resistance to Cd, Cu, and Pb, categorizing them as multi-heavy metal-resistant PGPR. The research substantiates that rhizobacteria isolated from native plants in land-fill sites possess the capability to withstand heavy metals and synthesize significant quantities of IAA. Isolation of rhizobacteria which are resistant to heavy metals and produce IAA from various plants growing in soil polluted by heavy metals have been reported, e.g. *H. annuus* (Siripan et al. 2018), *Odontarrhena obovata* C.A. Mey. (Kumar et al. 2021).

Bacterial characterization and time-course of IAA production from multi-heavy metal-resistant PGPR

The results of selected multi-heavy metal-resistant PGPR identification using 16S rDNA sequencing found that Cd3A7 and Cd2ES7 showed a close relationship with *Enterobacter cloacae* subsp. *dissolvens* [99.79% similarity] (Accession number Z96079) and *Klebsiella pneumoniae* subsp. *rhinoscleromatis* [100% similarity] (Accession number ACZD01000038), respectively. Both *E. cloacae* and *K. pneumoniae* are Gram-negative, rod-shaped bacteria within the family Enterobacteriaceae. These bacteria are commonly found in natural environments, including soil, water, and the gastrointestinal tracts of humans and animals (González-Gómez et al. 2024). Although both *E. cloacae* and *K. pneumoniae* are considered opportunistic pathogens, several studies have highlighted their potential in promoting plant growth, particularly through the production of IAA (Pattnaik et al. 2020; Khanthom et al. 2021). Notably, certain strains of *K. pneumoniae* have been identified as non-pathogenic and exhibit significant plant growth-promoting capabilities

(Pramanik et al. 2017). Furthermore, numerous investigations have demonstrated the effectiveness of *E. cloacae* and *K. pneumoniae* in enhancing the phytoremediation of heavy metal-contaminated soils (Pramanik et al. 2018; Xu et al. 2020; Chakraborty et al. 2021).

Enterobacter cloacae Cd3A7 had respective MIC levels against Cd, Cu, and Pb toxicity of 2000, 1000, and 3500 $\mu\text{g mL}^{-1}$. Meanwhile, the respective MIC levels of *E. cloacae* Cd3A7 and *K. pneumoniae* Cd2ES7 against Cd, Cu, and Pb toxicity were 1000, 1000, and 3000 $\mu\text{g mL}^{-1}$. Our results indicated that *E. cloacae* Cd3A7 had more resistance to Cd and Pb than *K. pneumoniae* Cd2ES7. The findings reported here concurred with those in the work conducted by Mitra et al. (2018) and Khanthom et al. (2021) who reported higher MIC values in the context of *E. cloacae* upon Cd at 3500 and 1500 $\mu\text{g mL}^{-1}$, respectively. *Enterobacter* spp. and *K. pneumoniae* emerged as the genera displaying the greatest resistance to Cd and Pb, supported by evident molecular and biochemical mechanisms (Henao and Ghneim-Herrera 2021). It is of importance to bear in mind that *E. cloacae* and *K. pneumoniae*, Gram-negative bacteria, demonstrate superior heavy metal resistance in comparison to Gram-positive bacteria (Paredes-Páliz et al. 2016).

The IAA production outcomes by *E. cloacae* and *K. pneumoniae* at different growth periods are presented in Fig. 2. *E. cloacae* exhibited maximal growth at 24 h and produced a maximum of 280.51 $\mu\text{g mL}^{-1}$ of IAA at 24 h of growth. For *K. pneumoniae*, maximal growth was observed at 36 h and the production of IAA peaked at 161.74 $\mu\text{g mL}^{-1}$ after 24 h of incubation. Afterward, the growth and IAA production in both bacterial strains declined. This suggests that *E. cloacae* produced IAA more rapidly than *K. pneumoniae* and secreted IAA greater than *K. pneumoniae* by 1.73-fold. Among the various genera of bacterial endophytes, *E. cloacae* has been demonstrated to possess the ability to stimulate plant growth and development in various plant types (Mitra et al. 2018; Khanthom et al. 2021). Additionally, findings reported by Biswas et al. (2023) revealed that *K. pneumoniae* exhibited a high level of IAA ($195.66 \pm 2.51 \mu\text{g mL}^{-1}$) and significantly enhanced the lengths of the roots and shoots of *Vigna unguiculata*.

Table 3 The ranges of IAA levels produced from isolated heavy metal-resistant rhizobacteria

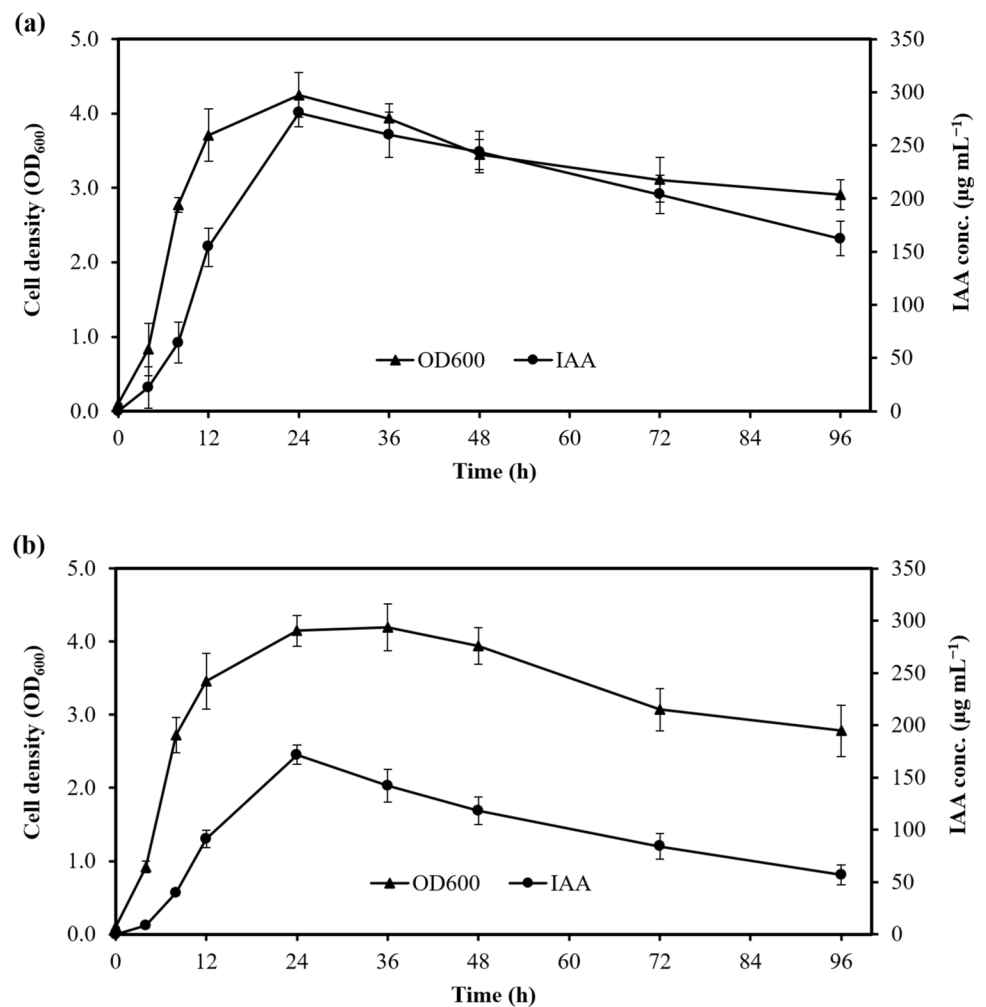
Level of IAA ($\mu\text{g mL}^{-1}$)	Number of isolates	Percentage
> 200.0	1	2.1
150.1–200.0	1	2.1
100.1–150.0	12	25.0
50.1–100.0	9	18.7
25.1–50.0	1	2.1
10.1–25.0	9	18.7
0–10.0	15	31.3
Total	48	100.0

Enhanced root elongation and root colonization ability of PGPR with multi-heavy metal resistance

Figure 3 depicts the lengths of the *H. annuus* shoots and roots treated with multi-heavy metal-resistant PGPR under toxic heavy metal conditions, in comparison to untreated seedlings. Results demonstrated that *E. cloacae* and *K. pneumoniae* promoted the elongation of roots and shoots in *H. annuus* grown under conditions with and without Cd, Cu, or Pb. The *H. annuus* root lengths which were treated with *E. cloacae* and *K. pneumoniae* were 1.37- and 1.54-fold



Fig. 2 IAA levels time-courses generated by (a) *E. cloacae* Cd3A7 and (b) *K. pneumoniae* Cd2ES7



greater, respectively, than those of untreated *H. annuus* with no heavy metals present. Compared to untreated *H. annuus*, the lengths of *H. annuus* shoots treated with *E. cloacae* and *K. pneumoniae* increased by 1.41- and 1.96-fold, respectively. Untreated *H. annuus* plants exposed to heavy metals showed reduced root and shoot lengths compared to those grown without heavy metal exposure, reflecting heavy metal toxicity in this context. Notably, the root and shoot elongation of *H. annuus* seedlings was more significantly inhibited under Cd toxic conditions. The symptoms of Cd toxicity in *H. annuus* are associated with alterations in enzyme activity, carbohydrate metabolism, sub-cellular division, photosynthesis, and respiration (Hu et al. 2020). Additionally, exposure to Cd disrupts photosynthesis, directly impacts nutrient and water absorption, reduces root growth, and ultimately hampers the growth of the plants (Tan et al. 2023).

However, the growth of *H. annuus* seedling shoots and roots under Cu and Pb exposure were almost completely recovered in *H. annuus* treated with *E. cloacae* and *K. pneumoniae*. Owing to the high toxic effects of Cd, *H. annuus* treated with *E. cloacae* and *K. pneumoniae* had

higher root lengths than the untreated *H. annuus* by 1.27- and 1.40-fold, respectively. The results explicitly demonstrate that *E. cloacae* and *K. pneumoniae* possess a robust capability to enhance the elongation of *H. annuus* seedling shoots and roots under the presence or absence of heavy metals. Several strains of plant growth-promoting bacteria (PGPB), such as *Enterobacter* sp., *K. pneumoniae*, (Henao and Ghneim-Herrera 2021) and *Stutzerimonas stutzeri* WA4 (Saravanan et al. 2024), have been shown to mitigate the adverse effects of heavy metals and enhance plant growth. In addition, they were also capable of colonizing both the root surface and the internal root tissues of *H. annuus* seedlings. Several studies have highlighted the proficiency of effective PGPR in root colonization across a variety of plants, e.g., *Oryza sativa* L., *Arabidopsis thaliana*, and *Zea mays* L. (Cao et al. 2023). The utilization of bacterial endophytes has been advanced for the creation of biofertilizers, thereby supporting environmentally friendly and sustainable agricultural practices (Afzal et al. 2019). The implication of our findings can be extended to the utilization of PGPR with multi-heavy metal resistance to



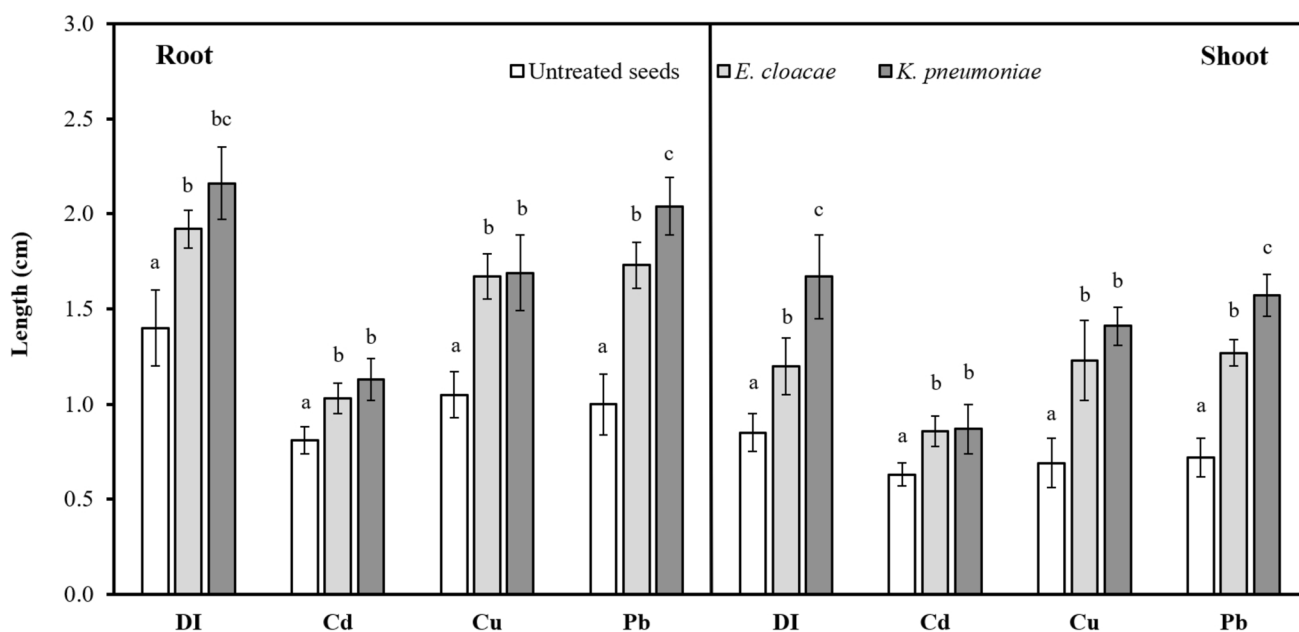


Fig. 3 Elongation of *H. annuus* seed shoots and roots treated with multi-heavy metal-resistant PGPR compared to the untreated seeds under the absence (DI water) and presence of heavy metals. Error

bars denote standard errors ($n=10$) pertaining to the mean values, while the letters shown above the bars are indicative of significant differences at $p < 0.05$ on the basis of DMRT

facilitate the growth of *H. annuus* in soil polluted with heavy metals.

Performance of multi-heavy metal-resistant PGPR on survival and mobilization of heavy metals in soil from landfills

It was found that landfill soil used for bacterial inoculation studies exhibited a number of attributes: sandy loam soil texture, 118 g kg^{-1} organic matter, pH 7.40, 68.45 g kg^{-1} organic carbon, 7.5 g kg^{-1} total nitrogen, 3.33 mg kg^{-1} of total Cd, 0.64 mg kg^{-1} of bioavailable Cd, $231.17 \text{ mg kg}^{-1}$ of total Cu, 15.57 mg kg^{-1} of bioavailable Cu, $115.67 \text{ mg kg}^{-1}$ of total Pb and 5.67 mg kg^{-1} of bioavailable Pb. Results of the viability of *E. cloacae* and *K. pneumoniae* in landfill soil polluted by heavy metals are presented in Fig. 4. Landfill soil provides an environment where *E. cloacae* and *K. pneumoniae* can survive, with viable bacterial cell numbers rising for both strains on the third day of the incubation period, remaining constant, and then declining after 21 days of incubation.

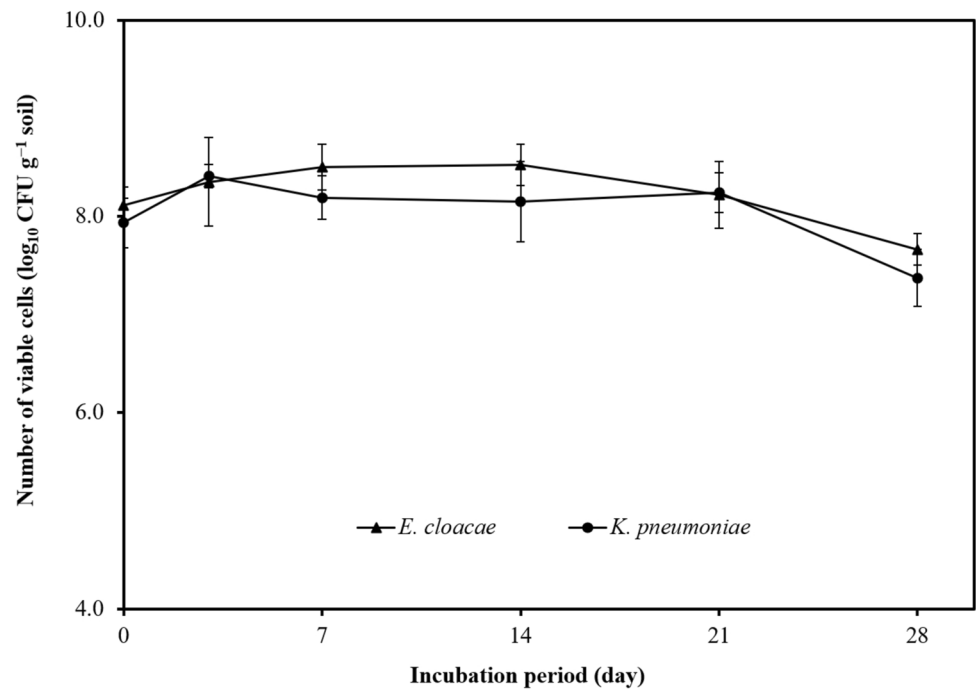
Bacterial survival in contaminated landfill soil was monitored over a one-month period, based on findings from our recent study, which recommended re-inoculation after one month to maintain consistent phytoremediation efficiency (Ketaubon et al. 2024). In heavy metal-contaminated soil, microorganisms are known to transform heavy metals to detoxify them (Zhang et al. 2024). In addition, heavy metal-resistant bacteria employ various mechanisms

to mitigate metal toxicity, including the use of efflux pumps via specific transport channels, enzymatic detoxification, intracellular sequestration, and biosorption through interactions with the bacterial cell wall and extracellular polymeric substances (EPS) (Prapagdee and Khonsue 2015; Pramanik et al. 2018; Singh et al. 2020; Chen et al. 2021). Intracellular sequestration refers to the binding of toxic metal ions by intracellular proteins, thereby reducing their bioavailability and toxicity. Additionally, biofilm formation offers a protective physical barrier that limits the penetration of heavy metals and shields bacterial cells from various environmental stressors (Flemming and Wuertz 2019). These mechanisms enable bacteria to tolerate and even thrive in environments heavily contaminated with heavy metals.

Generally, the bioavailability of heavy metals in contaminated soils is low because they have a binding affinity to soil particles, leading to reduced efficiency in heavy metal phytoextraction (Manoj et al. 2020; Montreemuk et al. 2024). Enhancing the bioavailability of heavy metals for plant uptake is a critical factor in the effectiveness of phytoextraction (Saravanan et al. 2024). Our results demonstrate the variations in the pH values of the soil and the concentrations of the bioavailable and non-bioavailable forms of Cd, Cu, Pb, and Zn in landfill soil with and without each multi-heavy metal-resistant PGPR inoculation (Table 4). The results found that soil pH values, ranging from 7.34 to 7.48 in all treatments, remained steady throughout the incubation period. There were no significant differences of soil



Fig. 4 Performance of multi-heavy metal resistant PGPR in terms of survival within landfill soil contaminated by heavy metals



pH across all treatments at $p < 0.05$. It is implied that these bacteria were unable to lower the soil pH to acidic levels.

In addition, analysis revealed the bioavailable Cd and Pb concentrations in landfill soil inoculated with either *E. cloacae* or *K. pneumoniae* were higher than concentrations in the uninoculated landfill soil. However, inoculation with both multi-heavy metal-resistant PGPR did not increase Cu bioavailability. Additionally, bacterial cell walls contain functional groups, such as hydroxyl and carboxyl groups, which actively bind to positively charged metal ions (Iqbal et al. 2024). The increase in bioavailability of heavy metals in landfill soil could result from other bacterial mechanisms. Various bacterial mechanisms convert heavy metals from their insoluble to soluble forms, thereby enhancing the mobilization or bioavailability of heavy metals e.g., exopolymers, siderophores, and biosurfactants (Das et al. 2017; Xu et al. 2020; Roskova et al. 2022; Thooppeng et al. 2023). Therefore, the application of multi-heavy metal-resistant PGPR via root inoculation into soil holds significant potential, contributing to enhanced efficiency in heavy metal phytoremediation.

However, the field application of PGPR must account for several critical factors, including competition with indigenous microbial communities, variability in abiotic conditions (e.g., temperature, soil pH, and moisture), and the influence of site-specific soil properties (Ma et al. 2016; Iqbal et al. 2024). A comprehensive assessment of the environmental safety and long-term effectiveness of PGPR-assisted phytoremediation approaches requires the evaluation of prolonged changes in metal speciation and leaching behavior, particularly under field conditions. Future research should

incorporate sequential extraction techniques in conjunction with extended column leaching experiments to elucidate the influence of PGPR on transformation, mobility, and potential groundwater contamination risks associated with heavy metals over time (Gupta et al. 2024; Barreto et al. 2023). To establish the practical feasibility and ecological relevance of PGPR-assisted phytoremediation, field-scale studies are necessary to evaluate the bacterial colonization efficiency, functional persistence, and heavy metal remediation performance of selected PGPR strains under natural landfill conditions. Such investigations are essential for validating their long-term efficacy and operational potential in large-scale environmental remediation efforts (Ren et al. 2022; Modi et al. 2023).

Conclusion

MSW landfill soils had a higher contamination level of Cd than other heavy metals. *U. lobata*, a native plant, contained the highest Cd and Pb in its shoot tissues. Two new isolates from landfill soil, Cd3A7 and Cd2ES7, were resistant to Cd, Cu, and Pb. They produced high levels of IAA and were classified as multi-heavy metal-resistant PGPR. Cd3A7 and Cd2ES7 were identified to be *E. cloacae* and *K. pneumoniae*, respectively. Our findings highlight the effectiveness of multi-heavy metal-resistant PGPR in stimulating root elongation and colonizing on root surface and inside *H. annuus* root tissues. They also survived in landfill soil contaminated with heavy metals and increased the mobilization of Cd and Pb, but not Cu. Our results revealed several benefits of both



multi-heavy metal-resistant PGPR; therefore, these PGPR with multi-heavy metal resistance have the potential for enhancing heavy metal phytoremediation performance in soils from MSW landfills.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13762-025-06695-9>.

Acknowledgements The authors thank N. Ritthikasesm, T. Phalachit, and P. Phuiphan for their valuable technical assistance. Appreciation is expressed to Dr. T.N. Stewart for editing and proofreading.

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Funding Open access funding provided by Mahidol University. This research project has been funded by Mahidol University (Fundamental Fund: fiscal year 2024 by National Science Research and Innovation Fund) (Contract Number FF-141/2567) and Scholarships for Ph.D. Student (Contract Number PHD/0005/2565).

Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflicts of interest associated with this publication. The authors declare that they have no competing interests.

Ethical approval and consent to participate This article does not contain any studies with human participants or animals performed by any of the authors.

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