Application of plant growth-promoting rhizobacteria (PGPR) associated with energy plant, Pennisetum purpurenum, in cadmium and zinc contaminated soil

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Abstract

Heavy metal contamination of soil and water causes environmental problems by inhibiting plant growth and harming microbial communities. Cooperative interactions between plants and bacteria can promote the growth and survival of the two taxa in harsh environments. Plants have been shown to secrete nutrients for enhancing growth of certain bacteria. In return, these bacteria chelate toxic metals, dissolve minerals, produce auxin, or even fix nitrogen for plants. Our work explored the interactions between economically important plants and a bacterial community with potential to be a biofertilizer for cadmium contaminated areas. Experiments were performed in a greenhouse under controlled conditions. Three Napier grass (*Pennisetum purpurenum*) variants (Pak Chong 1, King Grass, and Emperor) were planted with the addition of selected microbial species, *Klebsiella huaxiensis* and *Pantoea cypripedii*, in contaminated soil from an agricultural area in Mae Sot District, Tak Province, Thailand. Each plant variant was separated into three groups; control, plants with *P. cypripedii* (Pc), and plants with *K. huaxiensis* (Kh). Solutions containing cadmium and zinc were added to each pot daily to simulate conditions at a contaminated site. After 60 days, Pc and Kh groups of all Napeir grass variants showed significantly higher plant growths than the controls. Total Cd uptakes of PCPc, PCKh, and KGPc were significantly higher than those in control groups, while Zn uptakes were significantly higher in KG and EM plants with the addition of Pc and Kh than other treatments. Growing Napier grass with the addition of Pc or Kh as biofertilizer in the cadmium and zinc contaminated agricultural area in Mae Sot District, Tak Province could be considered for high biomass production. Future field experiments for greater understanding of the heavy metal uptake and development of sustainable solutions are recommended.

Introduction

Mining industries have been one of the important activities driving Thailand's economy for the past few decades. However, many adverse effects occur through mining practices. Padaeng Zinc Mine, located in Phra That Pha Daeng Sub-District, Mae Sot District, Tak Province, Thailand, has been reported as a source of high cadmium and zinc contamination along Mae Tao River Basin and the agricultural land nearby (Simmons et al. 2005; Swaddiwudhipong et al. 2007; Limpatanachote et al. 2009; Chayapan et al. 2015a,b; Prasad et al. 2015; Putwattana et al. 2015). Cadmium is a threat to both a sustainable environment and human health (Duruibe et al. 2007; Wiangkham and Prapagdee 2018). Cadmium (Cd) and zinc (Zn) concentrations in this area keep rising through accumulation of contaminated sediments from the basin (Chayapan et al. 2015a,b). Even though, living organisms generally cannot survive in heavy metal contaminated areas, some species can develop defense or detoxification mechanisms in order to adapt to high heavy metal contaminations (Pan and Yu 2011). Moreover, many organisms in such areas reportedly have positive interactions with one another. For example, soil microorganisms supply absorptive forms of nutrients to plants, while plants secrete exudates which microorganisms can use. Thus, the rhizosphere plays an important role in plant growth and survival, especially in contaminated environments (Gresshoff et al. 1984; Ramos et al. 2002; Vessey 2003; Handelsman 2004; Uren 2007; Sachdev et al. 2009; Gilbert and Dupont 2011; Kirchman DL 2012; Sharma et al. 2013; Henao and Ghneim-Herrera 2021).

One promising technology to solve heavy metal contamination problems is phytoremediation (Petruzzelli et al. 1987; Kumar et al. 1995; Keller et al. 2005; He et al. 2009; Tangahu et al. 2011). This technology uses green plants to reduce or remove the pollutants from the soil and claims to be a low cost and environmentally friendly technology (Meeinkuit et al. 2012; Ali et al. 2013; DalCorso et al. 2019). Although low levels of cadmium are not toxic to plants, high levels cause phytotoxicity by inhibiting root growth and cell division, resulting in retarded plant growth and cadmium uptake (Wang et al., 2007). Several plants have been used for cadmium phytoremediation (Keller et al. 2005; Ping et al. 2008; He et al. 2009; Phaenark et al. 2009; Sun et al. 2009; Chayapan et al. 2015a,b; Liu et al. 2017; Wiangkham and Prapagdee 2018). Napier grass has been reported to have a high potential in remediation high heavy metal contaminated environment (Takara and Khanal 2015; Wiangkham and Prapagdee 2018). Moreover, Napier grass is also considered to be an energy plant. Plant biomass can be combusted to produce hot gas (at 800–1000°C) to use in the boiler system of steam-turbine power generators, and all plants have similar energy contents (McKendry 2002). Therefore, phytoremediation using Napier grass has been of interest for both benefits of remediation of Cd contaminated soil and bioenergy production from the harvestable parts of the plant (Wiangkham and Prapagdee 2018).

This research aimed to investigate the ability of plant growth-promoting bacteria to improve Napier growth and accumulation of cadmium and zinc in cadmium contaminated soil collected near a mining area. In addition, total dry biomass was calculated to evaluate the possibility of using harvested Napier grass as a biomass fuel.

Materials And Methods

Soil Collection

Soil samples were collected at 20–40 cm depth from soil surface in an agricultural area (16°40'35"N, 98°37'36") located in the watershed area near Mae Tao Basin, Ban Phadae, Mae Sot District, Tak Province, Thailand (Fig. 1). Relative humidity was measured, and then the soil was air-dried in a greenhouse located in Mahidol University Nakhonsawan Campus, Thailand. Dried samples were analyzed for their characteristics and stored in closed containers.

Selection and preparation of soil bacteria as biofertilizers

Bacterial strains which did not show adverse effects to one another were isolated from the collected soil and chosen under criteria of (1) cadmium and zinc tolerance, (2) production of plant growth promoter, (3) nitrogen fixation.

Bacterial isolation

Dried soil (10 g) was mixed with 90 ml minimal salt and 2.5 ppm $Cd(NO_3)_2$ solution using an orbital shaker at 200 rpm for 24 hr, and diluted to factors of 10^0 to10^{-4}. Each solution was spread on 20-ppm-Cd King's B agar, and all the plates were incubated at 30°C for seven days. Single-colony isolation method was performed. Then, isolated bacteria were preserved in glycerol at -80°C.

Plant growth promoting rhizobacteria selection

- Indole acetic acid (IAA) producing bacteria

Isolated bacteria were grown in 3 mL King's B broth containing 2.5 mM L-tryptophan. The solution was then shaken at 200 rpm at 30°C for 1–2 days until the OD_{600} reached 0.05–0.1. Then, 1.5 mL aliquots were centrifuged at 5,400x g for 10 min at room temperature. The supernatants (500 µL) were mixed with 500 µL Salkowski's reagent and kept in the dark for 30 min. The solutions containing IAA-producing bacteria turned pink and were analyzed for IAA content by spectrophotometric comparison with a standard curve. (The method was applied from Gang et al. (2019).)

- Tricalcium phosphate compound solubilizing bacteria

Isolated bacterial solutions were streaked on GY/Tricalcium phosphate agar and incubated at 30°C for seven days. Bacterial strains with tricalcium phosphate solubilizing ability exhibited translucent circles around streaked bacteria (Ambrosini and Passaglia 2017).

- Siderophore producing bacteria

Isolated bacterial solutions were streaked on chrome azurol S (CAS) agar and incubated at 30°C for three days. Agar color around bacterial strains with siderophore producing ability changed from blue to yellow or orange (Louden et al. 2011).

Soil bacterial strains selection for biofertilization purposes

Selected bacterial strains (*Pantoea cypripedii* P8S2 (Ec) and *Klebsiella huaxiensis* F2S4 (Kh)) were inoculated in 10 mL LB broth and shaken at 200 rpm at 30°C for 18–20 hr. 1 mL of the mixture was then transferred to LB broth at 37°C and shaken at 200 rpm until the OD_{600} reached to 0.6. After 5 min of 3,000 rpm centrifugation, LB broth was removed, and phosphate buffer solution was added. The final mixture was ready for experiment.

Growing energy plants in association with selected bacterial strains

Three Napier grass (*Pennisetum purpureum*) variants (Pak Chong 1 (PC), King Grass (KG), and Emperor (EM)) collected from Uthai Thani Agricultural Research and Development Center were planted under controlled conditions in a greenhouse located on the Mahidol University Nakhonsawan Campus. Experiments were performed for the total of 60 days with a Completely Randomized Design (CRD). Napier grass stems were put in each pot containing 5 kg dry contaminated soil, 7 g Osmocote fertilizer, and 20 g chicken manure fertilizer. There were five replicates for each Napier variant and each bacterial strain, one plant in each pot represented one replicate. Each pot received 125 mL of 0.064 mg L^{-1} $Cd(NO_3)_2$ and 0.128 mg L^{-1} $Zn(NO_3)_2$ solutions two times per day. The experiment was designed to be as similar as possible to the conditions at the contaminated site in Mae Sot Province where the soil is exposed through contamination in Mae Tao River Basin. Each pot received 25 mL of Ec or Kh solution every 30 days for 60 days; only one strain was added in each pot. Control (Ct) pots received 25 mL phosphate buffer solution.

Plants and soil samples analysis

Plant samples

Plant samples were rinsed with tap water and oven dried at 70°C for five days. After grinding, 0.5 g of dry plant samples were digested with nitric acid and hydrochloric acid (TraceMetal® grade) using microwave digestion method (ETHOS One, Milestone Inc., Italy) and analyzed for Cd and Zn contents using flame atomic absorption spectrophotometry (FAAS; AAnalyst 200, PerkinElmer®) (Phusantisampan et al. 2016).

Soil samples

After rhizospheric soil samples were oven-dried at 70°C for seven days and sieved through a 2 mm mesh sieve, 0.5 g samples were digested with nitric acid and hydrogen peroxide (TraceMetal® grade) using microwave digestion method (ETHOS One, Milestone Inc., Italy). Cd and Zn contents were determined using flame atomic absorption spectrophotometry (FAAS; AAnalyst 200, PerkinElmer®) (Phusantisampan et al. 2016).

Soil pH was determined with pH meter (Accumet®, USA). Soil organic matter was analyzed using the Walkley-Black titration method (Walkley and Black 1934), while soil texture was analyzed by the hydrometer method (Allen et al. 1974). Electrical conductivity was calculated with an EC meter

(Hanna instruments HI 993310, USA). Cation exchange capacity was examined with the method described by Sparks (1996). Kjeldahl method (Black 1965), and the Bray II (Bray and Kurtz 1945) method were used to determine total nitrogen and exractable phosphorus, respectively. Extractable potassium was extracted with NH_4OAC and determined with atomic absorption spectrophotometry (Sparks 1996).

Growth and metal uptake indices

Plant relative growth rate (RGR) is used to indicate growth rate of the plant. It was calculated according to Hunt'sequation (Hunt 1978):

$$RGR = \frac{lnw2 - lnw1}{T2 - T1}$$

where RGR is the relative growth rate (g g^{-1} d^{-1}); w_1, T_1 and w_2, T_2 are initial and final dry weight and times for each treatment, respectively.

The bioconcentration factor (BCF) is used to indicate to potential for heavy metal translocation within different plant parts (Dodgen et al. 2015):

$$BCF = \frac{Cpp}{Cs}$$

where BCF is the bioconcentration factor for the plant part (pp) (i.e., shoot, stem, or root), C_{pp} is the metal concentration in plant part, and C_s is the metal concentration in soil.

Statistical analysis

The data were analyzed statistically using analysis of variances (ANOVA) with SPSS® Software for MacOS Ver.27 (SPSS, Inc., USA), and results with a $p < 0.05$ were considered statistically significant.

Results

Soil characteristics

Soils collected from the agricultural area in Ban Phadae, Mae Sot District, Tak Province were characterized as sandy-loam with pH 6.89, electrical conductivity (EC) of 0.5 dS m^{-1}, cation exchange capacity (CEC) of 12%, and 2.8% organic matter. Soil contained 0.3 mg kg^{-1} nitrogen, 13 mg kg^{-1} phosphorus, 2,700 mg kg^{-1} calcium, 183 mg kg^{-1} magnesium and 65 mg kg^{-1} potassium. Cadmium and zinc contents were 0.4 mg kg^{-1} and 11 mg kg^{-1}, respectively (Table 1).

Table 1
Physical and chemical properties of Cd/Zn contaminated soil collected from the study area

Parameters	
pH	6.89
EC (dS m−1)	0.5
CEC (%)	12.0
Organic matter (%)	2.8
Soil texture	Sandy loam
N (%)	0.3
P (mg kg−1)	13
Ca (mg kg−1)	2,700
Mg (mg kg−1)	183
K (mg kg−1)	65
Cd (mg kg−1)	0.4
Zn (mg kg−1)	11

Selection and preparation of soil bacteria as biofertilizers

After screening, 218 isolates (out of 262 isolates in total) were able to grow in 20 ppm Cd agar. Eighty three isolates out of 218 isolates (32%) could produce siderophores, 19 isolates (7%) were phosphate-solubilizing bacteria, while only seven isolates could both produce siderophores and solubilize phosphate. The 16s rDNA sequences were analyzed and compared to the information provided in NCBI's genebank. After screening the isolates for their ability to promote plant growth, produce siderophores, and solubilize tricalcium phosphate, bacterial strains *Klebsiella huaxiensis* F2S4 (Kh) and *Pantoea cypripedii* P8S2 (Pc) were selected for further study.

Plant growth

Plant dry biomass

The average total dry biomass (g) of all Napier grass variants is represented in Table 2. The biomass values shown in the table indicate the increase in the dry biomass of Napier stems since the start of the experiment (Day 0). Thus, the values on Day 0 represent zero increase in mass. The Ct group of PC showed an increase in dry biomass of 5.17 ± 1.09 g on Day 15, and reached 44.50 ± 3.64 g on Day 60. Plant dry biomass of the Pc group, which was added with *P. cypripedii*, increased to 6.10 ± 0.86 g on Day 15, 15.53 ± 1.74 g on Day 30, 26.68 ± 2.25 g on Day 45, and 54.95 ± 2.70 g on Day 60. A similar trend was also observed in the Kh group, with *K. huaxiensis* added, when the plants had gained 5.89 ± 3.53 g on Day 15, 18.05 ± 2.28 g on Day 30, 8.41 ± 1.61 g on Day 45, and 54.83 ± 4.45 g on Day 60. KG Napier grasses exhibited similar trends to PC plants. The Ct group of KG Napier showed an increase in dry biomass from 0 g to 47.23 ± 2.76 g on Day 60. KG dry biomass of the Pc group increased to 55.92 ± 3.49 g on Day 60. Plants of the Kh group added 57.25 ± 2.80 g on Day 60. EM Napier grasses also exhibited similar trends to EC and KG plants. The Ct group showed an increase in dry biomass of 48.79 ± 2.95 g on Day 60. The dry biomass of the Pc group increased by 55.85 ± 3.02 g on Day 60. Plants of Kh group had grown 55.64 ± 2.21 g on Day 60. The final plant dry biomasses of Pc and Kh groups of all variants were significantly higher than those of the control groups ($p < 0.05$).

Table 2
Total dry biomass

Napier variety	Bacteria	Dry biomass (g)				
		Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 60**
PC	Ct	0	5.17 ± 1.09	13.71 ± 2.10	22.09 ± 1.03	44.50 ± 3.64
	Pc	0	6.10 ± 0.86	15.53 ± 1.74	26.68 ± 2.25	54.95 ± 2.70*
	Kh	0	5.89 ± 3.53	18.05 ± 2.28	28.41 ± 1.61	54.83 ± 4.45*
KG	Ct	0	6.70 ± 1.46	17.16 ± 2.21	24.87 ± 3.01	47.23 ± 2.76
	Pc	0	8.63 ± 2.89	20.93 ± 2.34	30.00 ± 2.08	55.92 ± 3.49*
	Kh	0	7.59 ± 0.78	20.54 ± 1.83	27.12 ± 2.28	57.25 ± 2.80*
EM	Ct	0	5.62 ± 0.85	14.08 ± 1.57	25.36 ± 2.45	48.79 ± 2.95
	Pc	0	4.69 ± 0.84	17.17 ± 1.19	30.30 ± 1.17	55.85 ± 3.02*
	Kh	0	5.39 ± 1.10	17.58 ± 1.98	30.36 ± 1.11	55.64 ± 2.21*

Data are given as means ± SD. Asterisk marks illustrated differences of values among different bacterial treatments in a Napier variety on day 60 ($P < 0.05$, LSD).

Even though the final dry biomasses (Day 60) of the plants in pots with the same bacterial strains were not significantly different, the trends of increasing dry biomass in all Napier variants from Day 0 to Day 60 were different in details (Fig. 2). Starting from Day 0, all plant variants in control groups showed lower growth potential compared to those in Pc and Kh groups. The highest dry biomass values on Day 15 (8.63 ± 2.89 g), 30 (20.93 ± 2.34 g), and 45 (30.00 ± 2.08 g) were observed in KG Napier plants of Pc groups. However, KG plants of Kh group exhibited the highest dry biomass value (57.25 ± 2.80 g) on Day 60.

Relative growth rate (RGR)

Plants of all control groups had lower RGRs compared to Pc and Kh groups (Fig. 3). The lowest value (0.21637 ± 0.00136 g g^{-1} day^{-1}) was indicated in control group of PC, while the highest RGR value in a control group was observed in EM plants (0.21827 ± 0.00100 g g^{-1} day^{-1}). In the Pc group, RGR of PC (0.22026 ± 0.00080 g g^{-1} day^{-1}) was the lowest compared to KG (0.22055 ± 0.00105 g g^{-1} day^{-1}) and EM (0.22053 ±

0.00088 g g^{-1} day^{-1}). KG showed the highest RGR (0.22095 ± 0.00082 g g^{-1} day^{-1}) among other variants (0.22020 ± 0.00135 g g^{-1} day^{-1} in PC, and 0.22048 ± 0.00067 g g^{-1} day^{-1} in EM) in Kh group.

Heavy metal contents

Soil Cd and Zn

Soils in all treatments of PC contained significantly higher Zn contents 15 to 60 days after planting compared to Day 0, except for the PCPc on Day 15 (15.68 ± 2.42 mg kg^{-1}) and PCKh on Day 30 (15.94 ± 1.62 mg kg^{-1}). KG exhibited higher Zn concentrations on Day 30 (Ct: 18.51 ± 2.74 mg kg^{-1}, Pc: 16.80 ± 4.92 mg kg^{-1}, Kh: 18.78 ± 2.60 mg kg^{-1}), Day 45 (Ct: 17.91 ± 1.32 mg kg^{-1}, Pc: 16.88 ± 1.68 mg kg^{-1}, Kh: 20.21 ± 2.17 mg kg^{-1}), and Day 60 (Ct: 25.86 ± 2.27 mg kg^{-1}, Pc: 28.39 ± 2.26 mg kg^{-1}, Kh: 25.81 ± 2.73 mg kg^{-1}) than those on Day 0 and 15. Similar trends were also observed in all treatments of EM, when Zn concentrations of Day 15 to 60 were significantly greater than Day 0 (11.21 ± 1.85 mg kg^{-1}). However, Cd concentrations in soil did not change from Day 0 to the last day of the experiment (Table 3).

Table 3
Rhizospheric soil Cd and Zn contents after experiment

Napier variety	Bacteria	Heavy metal content (mg kg^{-1})	
		Cd	**Zn**
PC	Ct	0.44 ± 0.05	22.34 ± 1.34
	Pc	0.46 ± 0.02	22.92 ± 2.49
	Kh	0.43 ± 0.07	24.17 ± 4.22
KG	Ct	0.46 ± 0.02	25.86 ± 2.27
	Pc	0.50 ± 0.04	28.39 ± 2.26
	Kh	0.48 ± 0.06	25.81 ± 2.73
EM	Ct	0.47 ± 0.04	25.11 ± 2.00
	Pc	0.48 ± 0.06	20.90 ± 3.06
	Kh	0.43 ± 0.05	23.70 ± 1.49

Cd and Zn concentrations and total uptake in plants

Concentrations of Cd and Zn in each plant part (leaf, stem, and root) and total uptakes increased over time from Day 0 to Day 60 in all treatments (Table 4). Only the data on Day 60 were statistically analyzed. On Day 60, Cd concentrations in leaves of PCCt (0.76 ± 0.03 mg kg^{-1}), PCPc (0.72 ± 0.04 mg kg^{-1}), PCKh (0.81 ± 0.07 mg kg^{-1}), KGCt (0.93 ± 0.08 mg kg^{-1}), KGPc (0.77 ± 0.10 mg kg^{-1}), and EMCt (0.73 ± 0.08 mg kg^{-1}) were significantly more than those of KGKh, EMPc, and EMKh. No differences of Cd concentrations in stems were determined in all treatments. Ct groups of all PC and KG roots exhibited higher Cd concentrations (PCCt: 0.64 ± 0.06 mg kg^{-1}, and KGCt: 0.66 ± 0.07 mg kg^{-1}) than other treatments. However, Zn concentrations in plants were quite different from Cd concentrations. Greater Zn concentrations in leaves on Day 60 were observed in PCCt and PCPc at the values of 25.37 ± 2.62 mg kg^{-1} and 28.86 ± 1.34 mg kg^{-1}, respectively, compared to other treatments on the same day. Addition of Pc and Kh enhanced Zn concentrations in PC plants over the control ones, while Zn concentration in roots of Pc group (23.51 ± 2.71 mg kg^{-1}) was as high as it was determined in Ct group (24.12 ± 3.28 mg kg^{-1}) of KG, with significantly lower Zn concentration in KGKh (15.69 ± 3.33 mg kg^{-1}). No difference in Zn concentrations was observed among stems of all treatments of KG. Moreover, Pc and Kh additions did not affect Zn concentrations in leaves, stems, and roots of EM plants as compared to the Ct plants.

Table 4
Cd and Zn concentrations and total uptakes in plant

Napier variety	Bacteria	Cd concentration (mg kg^{-1})			Total Cd uptake	Zn concentration (mg kg^{-1})			Total Zn uptake
		Leaf	**Stem**	**Root**	(ng $plant^{-1}$)	**Leaf**	**Stem**	**Root**	(mg $plant^{-1}$)
PC	Ct	$0.76 \pm 0.03^{a,A,Y}$	$0.54 \pm 0.07^{a,A,X}$	$0.64 \pm 0.06^{b,A,XY}$	$28.43 \pm 2.12^{a,A}$	$25.37 \pm 2.62^{b,B,Z}$	$8.74 \pm 2.10^{a,A,X}$	$14.44 \pm 2.42^{a,A,Y}$	$0.77 \pm 0.09^{a,A}$
	Pc	$0.72 \pm 0.04^{a,B,Y}$	$0.60 \pm 0.07^{a,A,X}$	$0.48 \pm 0.06^{a,AB,X}$	$35.48 \pm 1.65^{b,B}$	$28.86 \pm 1.34^{b,B,Z}$	$7.89 \pm 1.33^{a,A,X}$	$17.48 \pm 2.76^{a,A,Y}$	$0.98 \pm 0.31^{a,A}$
	Kh	$0.81 \pm 0.07^{a,B,Y}$	$0.58 \pm 0.05^{a,A,X}$	$0.51 \pm 0.09^{ab,A,X}$	$37.03 \pm 3.15^{b,B}$	$19.49 \pm 2.87^{a,A,Y}$	$9.63 \pm 1.70^{a,A,X}$	$18.14 \pm 3.53^{a,A,Y}$	$0.84 \pm 0.16^{a,A}$
KG	Ct	$0.93 \pm 0.08^{b,B,Y}$	$0.67 \pm 0.07^{b,A,X}$	$0.66 \pm 0.07^{b,A,X}$	$37.12 \pm 2.68^{b,B}$	$16.92 \pm 2.43^{a,A,Y}$	$11.11 \pm 2.91^{a,A,X}$	$24.12 \pm 3.28^{b,B,Z}$	$0.75 \pm 0.10^{a,A}$
	Pc	$0.77 \pm 0.10^{ab,B,Y}$	$0.61 \pm 0.04^{b,A,X}$	$0.53 \pm 0.04^{a,B,X}$	$38.07 \pm 1.47^{b,B}$	$19.58 \pm 3.57^{ab,A,Y}$	$10.07 \pm 1.20^{a,A,X}$	$23.51 \pm 2.71^{b,B,Y}$	$0.93 \pm 0.06^{b,A}$
	Kh	$0.69 \pm 0.06^{a,B,Y}$	$0.47 \pm 0.06^{a,A,X}$	$0.54 \pm 0.08^{ab,A,X}$	$32.41 \pm 0.79^{a,A}$	$22.06 \pm 1.94^{b,A,Z}$	$10.79 \pm 1.71^{a,A,X}$	$15.69 \pm 3.33^{a,A,Y}$	$0.95 \pm 0.02^{b,A}$
EM	Ct	$0.73 \pm 0.08^{a,A,Y}$	$0.54 \pm 0.05^{a,A,X}$	$0.55 \pm 0.08^{b,A,X}$	$30.55 \pm 3.64^{a,A}$	$21.47 \pm 2.55^{a,B,Y}$	$7.40 \pm 1.74^{a,A,X}$	$19.15 \pm 4.88^{a,AB,Y}$	$0.74 \pm 0.05^{a,A}$
	Pc	$0.60 \pm 0.08^{a,B,Y}$	$0.54 \pm 0.06^{a,A,Y}$	$0.39 \pm 0.06^{a,A,X}$	$31.44 \pm 3.18^{a,A}$	$20.79 \pm 1.02^{a,A,Y}$	$7.48 \pm 1.54^{a,A,X}$	$21.08 \pm 5.44^{a,AB,Y}$	$0.86 \pm 0.04^{b,A}$
	Kh	$0.60 \pm 0.07^{a,A,X}$	$0.54 \pm 0.07^{a,A,X}$	$0.47 \pm 0.07^{ab,A,X}$	$31.66 \pm 4.28^{a,A}$	$21.62 \pm 1.21^{a,A,Y}$	$9.37 \pm 2.30^{a,A,X}$	$21.60 \pm 8.39^{a,A,Y}$	$0.94 \pm 0.07^{b,A}$

Values followed by the same letter did not differ, first letters illustrated differences of values among different bacterial treatments in a Napier variety ($P < 0.05$, LSD), the second letters illustrated differences in values of all Napier varieties in the same bacterial treatments ($P < 0.05$, LSD), and the last letters illustrated differences in Cd or Zn concentrations in different plant parts of each Napier variety in the same bacterial treatment ($P < 0.05$, LSD).

In consideration of total metal uptake, Pc and Kh remarkably increased the uptake of Cd in PC plants (PCPc: 35.48 ± 1.65 ng $plant^{-1}$, PCKh: 37.03 ± 3.15 ng $plant^{-1}$) compared to Ct (28.43 ± 2.12 ng $plant^{-1}$). However, Pc and Kh addition did not raise the total uptake of Cd in KG and EM plants. While only Pc significantly enhanced Zn uptake in PC plants, the addition of Ec and Kh could increase Zn uptakes in both KG (KGPc: 0.93 ± 0.09 mg $plant^{-1}$, KGKh: 0.95 ± 0.02 mg $plant^{-1}$) and EM (EMPc: 0.86 ± 0.04 mg $plant^{-1}$, EMKh: 0.94 ± 0.07 mg $plant^{-1}$) when compared to the Ct plants of the same Napier variety.

Bioconcentration factors (BCF)

BCF values of Cd were significantly greater in leaves than other parts of all variants with the highest value of 2.041 ± 0.288 in KGCt leaves. The lowest BCF of Cd was observed in the stem of EMPc at 0.826 ± 0.068. Only roots of Pc groups exhibited BCF values as high as those in leaves of all variants (**Table 5**). In contrast to BCF values of Cd, the BCFs of Zn in leaves and roots of all treatments, not only the Pc groups, were remarkably higher than those in stems. Each Napier variety revealed non-significant differences in BCF values of Zn in each plant part for all bacterial treatments. The highest BCFs of Zn was recorded at 1.292 ± 0.690 in PCPc, while the lowest value was found in EMCt at 0.295 ± 0.065.
Table 5 Bioconcentration factors (BCF) of Cd and Zn in plant parts on Day 60

Napier variety	Bacteria	Bioconcentration factors of Cd			Bioconcentration factors of Zn		
		Leaf	Stem	Root	Leaf	Stem	Root
PC	Ct	$1.715\pm0.196^{a,A,Y}$	$1.457\pm0.265^{b,A,XY}$	$1.226\pm0.238^{a,A,X}$	$1.136\pm0.106^{a,B,Y}$	$0.396\pm0.117^{a,A,X}$	$0.650\pm0.127^{a,A,Y}$
	Pc	$1.591\pm0.145^{a,A,Y}$	$1.059\pm0.134^{a,B,X}$	$1.307\pm0.144^{a,A,XY}$	$1.292\pm0.690^{a,A,Y}$	$0.352\pm0.089^{a,A,X}$	$0.770\pm0.157^{a,A,Y}$
	Kh	$1.940\pm0.308^{a,B,Y}$	$1.222\pm0.250^{a,A,X}$	$1.367\pm0.150^{a,A,X}$	$0.837\pm0.243^{a,A,Y}$	$0.402\pm0.052^{a,A,X}$	$0.768\pm0.182^{a,AB,Y}$
KG	Ct	$2.041\pm0.288^{b,B,Y}$	$1.452\pm0.235^{b,A,X}$	$1.457\pm0.195^{b,A,X}$	$0.661\pm0.129^{a,A,X}$	$0.436\pm0.129^{a,A,X}$	$0.944\pm0.193^{a,B,Y}$
	Pc	$1.529\pm0.203^{ab,A,Y}$	$1.061\pm0.053^{a,B,X}$	$1.228\pm0.152^{a,A,XY}$	$0.690\pm0.122^{a,A,Y}$	$0.358\pm0.062^{a,A,X}$	$0.834\pm0.134^{a,A,Y}$
	Kh	$1.454\pm0.134^{a,A,Y}$	$1.141\pm0.085^{ab,A,X}$	$1.010\pm0.219^{a,A,X}$	$0.866\pm0.144^{a,A,Y}$	$0.418\pm0.052^{a,A,X}$	$0.612\pm0.138^{a,A,XY}$
EM	Ct	$1.563\pm0.259^{a,A,X}$	$1.187\pm0.219^{a,A,X}$	$1.165\pm0.197^{a,A,X}$	$0.860\pm0.126^{a,AB,Y}$	$0.295\pm0.065^{a,A,X}$	$0.763\pm0.188^{a,AB,Y}$
	Pc	$1.265\pm0.210^{a,A,Y}$	$0.826\pm0.068^{a,A,X}$	$1.140\pm0.231^{a,A,Y}$	$1.012\pm0.158^{a,A,Y}$	$0.363\pm0.081^{a,A,X}$	$1.019\pm0.267^{a,A,Y}$
	Kh	$1.399\pm0.193^{a,A,X}$	$1.100\pm0.230^{a,A,X}$	$1.241\pm0.188^{b,A,X}$	$0.915\pm0.083^{a,A,Y}$	$0.394\pm0.084^{a,A,X}$	$0.905\pm0.333^{a,B,Y}$

Values followed by the same letter did not differ, first letters illustrated differences of BCF values among different bacterial treatments in each plant part of each Napier variety ($P < 0.05$, LSD), the second letters illustrated differences in BCF values of all Napier varieties in the same bacterial treatments ($P < 0.05$, LSD), and the last letters illustrated differences in BCF values of different plant parts in each Napier variety in the same bacterial treatment ($P < 0.05$, LSD).

Discussion

Using plants to absorb and accumulate heavy metals from polluted soil, followed by the recycling of explants containing heavy metals, can help achieve the goal of reverting contaminated soil to low heavy-metal content soil (Ko et al. 2017). The goal of using power plants in the application of phytoremediation purposes is not only to remediate contaminated environments, but also to gain high biomass plants as byproducts to further be converted to energy. The use of biomass as a power plant fuel is globally well accepted due to fossil oil depletion and environmental concerns (Henry 2010; Han et al. 2020). Biofuel production has been based upon the conversion of the storage carbohydrates (sugars and starch) in the plants into fuel (Schubert 2006; Graef et al. 2009). Napier grass (*P. purpureum*) is a well-recognized animal feed stock, due to its high growth rate and adaptability (Grant et al. 1974). From the results of this study, addition of bacteria as biofertilizer, Pc and Kh, seemed to increase plant total dry biomass of all Napier variants. Napier grass exhibited significantly higher RGRs when grown in the bacterial treatments, Pc and Kh. Generally, phytoremediation is the in situ application of plants and their associated microflora for environmental cleanup (Peters and Meeks 1989; Uren 2007; Rajkumar et al. 2010; Pan and Yu 2011; Antoniadis et al. 2017). Thus, the more association plants and local microflora have, the higher chance the plants will survive, even in highly contaminated areas.

In this study, addition of high Cd- and Zn-tolerant bacteria, *K. huaxiensis* and *P. cypripedii*, improved Napier growth in contaminated soil. *Klebsiella* has been reported to be one bacterial genus having potential to promote plant growth under stress conditions (Singh et al., 2015; Liu et al. 2018; Sapre et al. 2018; Mohan et al. 2019; Kusale et al. 2021), including Cd stress (Pramanik et al. 2017; Mitra et al. 2018; Chakraborty et al. 2021). Among *Klebsiella* sp., most of the studies of plant growth-promoting ability of *Klebsiella* were on *K. pneumonia* (Iniguez et al. 2004; Singh et al. 2015; Pramanik et al. 2017; Mohan et al. 2019; Chakraborty et al. 2021; Rajkumari et al. 2021). However, *K. huaxiensis*, in this study, also showed high potential in improving plant biomass and relative growth rate when grown in Cd and Zn contaminated soil. In contrast to *K. huaxiensis*, *P. cypripedii*, previously named *Pectobacterium cypripedii* and *Erwinea cypripedii* (UniProt Taxonomy 2021; BacDrive 2021), reportedly produces orchid brown rod, a disease that affects many plant species such as *Carica papaya*, and types of orchids such as *Paphiopedilum*, *Phalaenopsis amabilis*, and others (Plantwise 2014; Ramírez-Rojas et al. 2016). The genus *Pantoea* comprises many versatile species that have been isolated from a multitude of environments such as soil, water, plants (e.g., epiphytes or endophytes), seeds, fruits (e.g., pineapple, mandarin oranges), human and animal gastrointestinal tracts, in dairy products, in blood, and in urine (Morin 2014; Walterson and Stavrinides 2015). Most *Pantoea* spp. are known to cause diseases in both plants and animals (Morin 2014). In spite of the fact that *P. cypripedii* enhanced Napier growth in this experiment, the ability of *Pantoea* to cause diseases to other crops should be taken into consideration before applying it in the field.

Even though Cd and Zn concentrations in soil did not significantly change from the first day to the final day of the experiment, there were significant uptakes in the plants. All Napier variants with the addition of Pc and Kh had higher Cd and Zn concentrations in leaves, while Zn contents in roots were also as high as they were in leaves. Pak Chong 1 in Pc and Kh groups and King grass in Ct and Pc groups showed higher Cd uptakes than other treatments. Pc and Kh additions also increased Zn uptake in King and Emperor grasses. The BCF values of Cd were very high in leaves, while BCFs for Zn were greater in both leaves and roots. Zn is one of the macronutrients plants need, while Cd is toxic to plants. Cd uptake might negatively affect plant growth and lead to high toxicity in plants grown in contaminated area (Pinto et al. 2004; Benavides et al.

2005; Kubier et al. 2019). Cd transferring to plant leaves could result in the loss of cell functions. According to Pena et al. (2012) and Yuan et al. (2013), Cd affected cell activities, e.g., lowering cyclin-dependent kinase activity by ROS reaction as a result of Cd toxicity. However, growing Napier grass in Cd contaminated areas could also result in high yields of biomass for ethanol production. According to the report by Ko et al. (2017), an increase in Cd concentration promoted the fermentation of alcohol from plants.

Conclusions

Klebsiella huaxiensis and *Pantoea cypripedii* showed an outstanding capability for Napier growth promotion. All Napier variants, Pak Chong 1, King Grass, and Emperor, were able to withstand cadmium and zinc contaminated conditions with 100% survival rate. However, the adverse effect of *P. cypripedii* on other plants should be taken into considerations before applying it in agriculture areas since many *Pantoea* spp. are known for their pathogenicity to plants. Growing Napier grass, *Pennisetum purpurenum*, with the addition of *K. huaxiensis* in the cadmium and zinc contaminated agricultural area in Mae Sot District, Tak Province could be considered (1) to remove Cd and Zn, and (2) to produce high biomass plant for bio-energy purposes. However, field trial studies are highly recommended since there are various factors involved in growing plants with biofertilizers in the field.

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Author contributions

Project conception and experimental design were conducted by Thitinun Sumranwanich, Weeradej Meeinkuirt, and Parichat Chayapan. Material preparation, data collection and analysis were performed by Thitinun Sumranwanich, Wasawat Leartsiwawinyu, Weeradej Meeinkuirt, and Parichat Chayapan. The first draft of the manuscript was written by Parichat Chayapan and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Agricultural area where soil was collected. The area was located in Mae Sot District, Tak Province, Thailand. (Google Earth®, Access date: Jan 23rd, 2022).

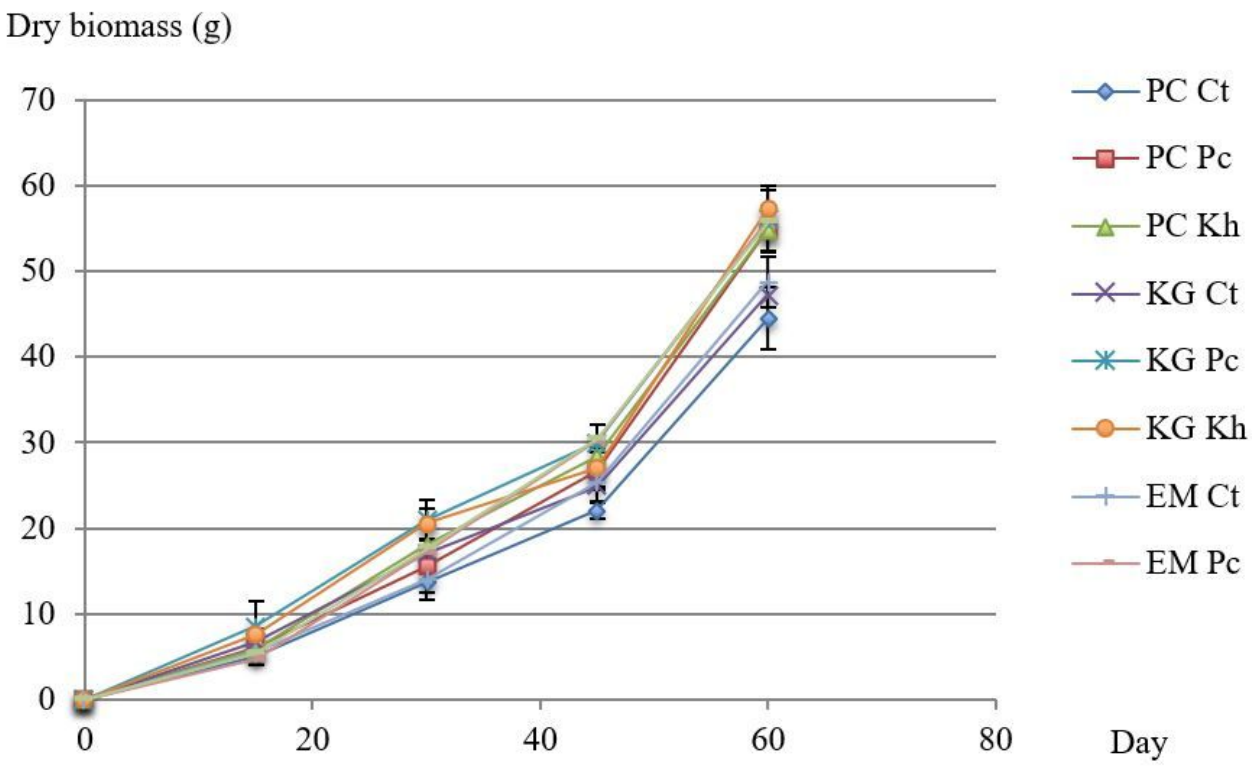

Figure 2

Total dry biomass. Data are given as means ± SD.

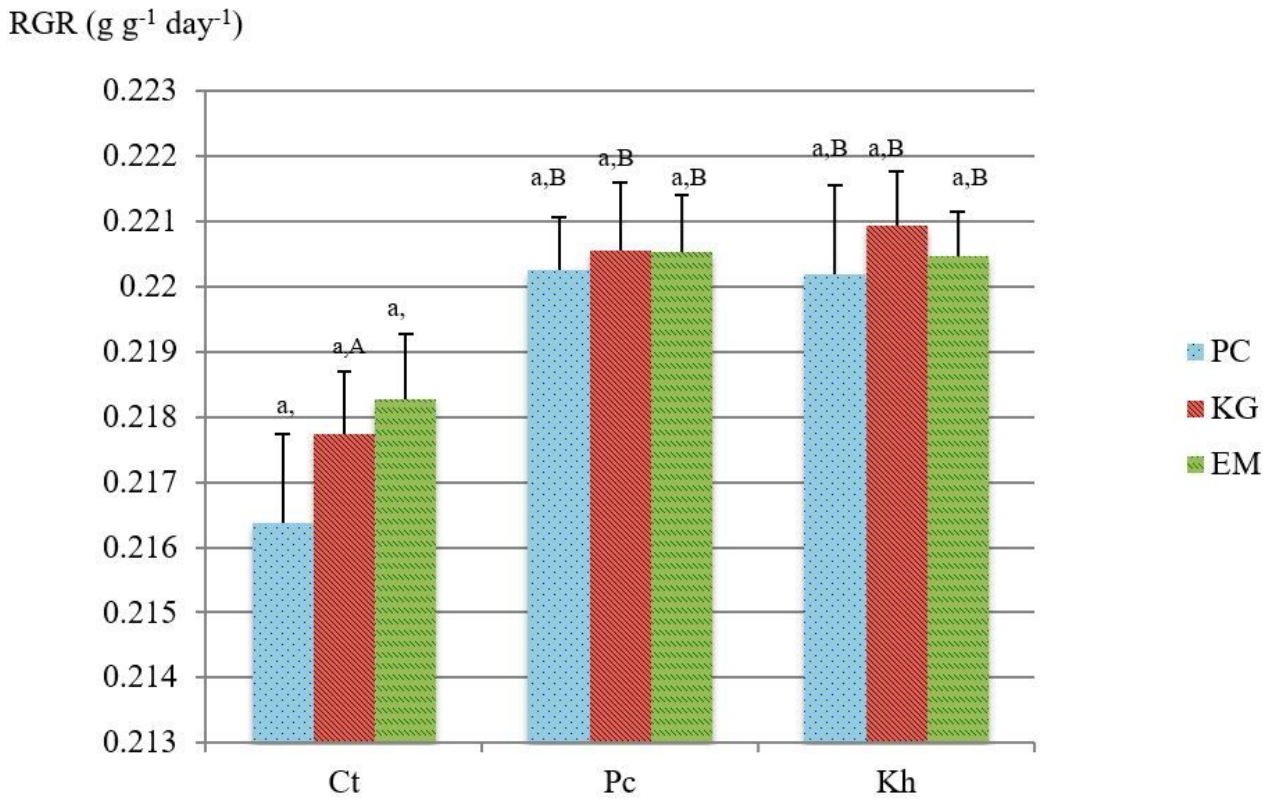

Figure 3

Relative growth rate (RGR). Data are given as means ± SD. Small letters indicated significant differences among plant varieties for the same bacterial treatments, and capital letters indicated significant differences among bacterial treatments for each plant variety ($P < 0.05$, ANOVA; LSD mean comparison test). Bars with the same letters were not significantly different.