Bioremediation of toxic metals and *Limnocharis flava* (L.) Buchenau growth in acidic contaminated soil supported by microbial inoculants

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Abstract. An in situ pot experiment was carried out in a net house to study the combined effect of a mixture of microbial inoculants at different levels of 0, 1, 1.5, 2, 2.5, and 3 g/kg in dry soil with *Limnocharis flava* (L.) Buchenau plants grown on agricultural land contaminated with lead (Pb) and cadmium (Cd). The results showed that plant growth, fresh inflorescence yields and accumulation of Pb and Cd in *L. flava* plant parts gradually increased with increasing levels of the applied microbial inoculants in the contaminated soil. The highest total content of Pb and Cd was determined in roots (6.63–32.46 and 0.12–0.72 mg/kg dw), followed by stems and leaves (1.20–3.08 and 0.04–0.09 mg/kg dw) and inflorescence parts (0.012–0.120 and 0.011–0.065 mg/kg fw). The application of microbial inoculants in the range of 2–2.5 g/kg dry soil stimulated the growth and fresh inflorescence yields of *L. flava*, increased the accumulation of Pb and Cd in inedible parts, ensuring safety for edible parts of *L. flava* plants in accordance with QCVN8-2:2011/BYT and FAO/WHO regulations. The results of the present work indicate that the flexibility of Pb and Cd can be increased when soil is mixed with microbial inoculants. Long term ex situ experiments are needed to investigate the impact of microbial inoculants mixed with biochar, minerals and nanomaterial for phytomanagement of soil contaminated with multiple metals.

Keywords: soil pollution, heavy metals, Pb, Cd, contamination reduction, microbial remediation, vascular plant growth.

1. Introduction

Soil pollution has worldwide turned into a dangerous problem due to its great harm to food security and ecosystem health (Bolan et al., 2004; Xiang et al., 2022; Münzel et al., 2023). Toxic metals (TMs) are known to be non-biodegradable and can cause toxicity to humanbeing, animals, birds and plants due to their long-term persistence in soil system (Zhao et al., 2022). There has been made much determination to solve the subject of soil contamination, which has ended up to a great number of policies and methods against contamination reduction and control (McLaughlin et al., 2000). For example, Pb and Cd are clearly exhibit toxicity due to continuous accumulation in the environment. Cumulative tendency in the biomass of the food chain poses a serious threat to plants, animals and humans even when exposed to low concentrations (Bolan et al., 2014; Sarwar et al., 2017). In-situ restoration approaches of pollutants are mostly ecologically friendly and cost-effective (Akcil et al., 2015). Removing these TMs from agricultural soil to limit their effect on the quality of farming products is therefore essential to maintaining a safe food chain and a healthy agricultural ecosystem. Bioremediation is performed through the use of microorganisms, plants or combination of both to restore the polluted environment to the initial state (Mani & Kumar, 2014; Dixit et al., 2015). In particular, the combination of plants and microorganisms is not only a means of promoting the TMs removal (Ma et al., 2017), but also a way to increasing activity and diversity of microorganisms in the soil, keeping the ecosystem healthy (Chen, 2000; Atuchin et al., 2023). It is a necessary solution for sustainable development (Zayed & Terry, 2003). Many species of fungi and bacteria have been used effectively in combination with plants to promote plant growth and control TMs stress in the soil, such as the application of two indigenous fungal species *Mucor circinelloides* and *Trichoderma asperellum* in combination with *Arabidopsis thaliana* (L.) Heynh for phytoremediation of Cd-and Pb-contaminated soil (Zhang et al., 2018). Fungus *Aspergillus aculeatus* in combination with *Cynodon dactylon* (L.) Pers. may cause the accumulation of different metabolites related to Cd tolerance in *Cynodon dactylon* plants (Li et al., 2017). The injection of *Ralstonia eutropha* and *Chryseobacterium humi* in *Zea mays* L. showed that plant biomass increased in shoot levels, and increased Cd accumulation in the root (Moreira et al., 2014). *Micrococcus* sp. TISTR2221 also promoted the root length, shoot length, and dry biomass and increased Cd accumulation in the hearts and shoots of *Zea mays* plants (Sangthong et al., 2016). AMF mycorrhizal bio-product had increased total dry biomass of *Pteris vittata* L., and Pb content was uptaken by ferns plants up 129 times compared to the control without bio-product (Nguyen, 2016). *Enterobacter* sp. (S2) promoted plant growth and enhanced Cd accumulating rice plants

(Mitra et al., 2018). Bacteria *Bacillus licheniformis* and fungus *Penicillium chrysogenum* have also been studied in combination with many different plants to promoted plant growth under stressed soil conditions (García et al., 2004; Jamil et al., 2013; Park et al., 2017; Won et al., 2019). However, these studies mainly focused on bacterial, or fungal strains combined with plants. The use of a mixture of different microorganisms strains to take advantage of each species and increase synergistic effects in supporting plant growth, accumulating, and eliminating heavy metal pollution in the soil is still limited.

Currently, there also are not many in-depth studies on multi-purpose plants species which are capable of accumulating TMs and using as foodstuffs under food safety regulations. Water cabbage *Limnocharis flava* (L.) Buchenau, is a such wild aquatic plant, tuberous roots stick to the soil and survive every year, furthermore this plant was introduced in Asia as an ornamental species due to its attractive foliage and colorful blossoms (Nishan & George 2018; WFO 2024). It has been known as a metal hyper-accumulator species, which proved in many studies to treat TMs in wetlands, water and wastewater (Kamarudzaman et al., 2011; Chandran, 2009; Vijayaraghavan & Yeoung, 2008; Abbas et al., 2014; Malik & Jaiswal, 2000). These studies only used *Limnocharis flava* as a wild species to remove highly effective TMs without regard to the safety of the edible parts of the plant. In Vietnam, *L. flava* is grown in wetlands, sold submerged in some southern provinces for food. The edible part of inflorescence is used to be processed into daily vegetable dishes of the Southern people with an average daily intake of over 50 g / person to provide the content of nutrients viz. Ca and Fe for the body (Pandey et al., 2014). Research combining both bacteria and fungi with *L. flava* is a new direction.

Towards safe and sustainable agriculture, this study aims to assess the potential of the combination of microorganism with *L. flava* in acidic agriculture polluted soil aiming to enahance the phytomanagement of Pb and Cd by plant. The suitable level of inoculants at 0, 1, 1.5, 2, 2.5, and 3 g/kg were selected to enhance plant growth, fresh inflorescence yields, and accumulation of Pb and Cd in parts of *L. flava* plants grown on contaminated agricultural soil for the field scale in the future. It was hypothesized that the combination of microorganism with *L.flava* plants in acidic agriculture polluted soil might be highly effective for bioremadition of Pb and Cd in-situ framework.

2. Materials and methods

2.1. Soil collection

The experimental soil was collected from an abandoned vegetable field at a depth of 0-20 cm in Thoi An, 12 District, Ho Chi Minh City, Vietnam (10°53'40.0"N 106°39'04.5"E; Fig. 1). The studied soils was Gleyic Acrsoils type in nature as per some physical and chemical properties. In this present work *Limnocharis flava* seeds were purchased from Southern Seed Company (SSC) in Vietnam and sown on clean mud soil for one month in the net house of branch of Hanoi University of Natural Resources and Environment in Thanh Hoa, Vietnam (20°05'04.1"N 105°51'34.6" E), until seedlings are mature and healthy at an average height of 28 cm with a number of leaves of 4-5 leaves used as plants for experimental purpose. The selected physico-chemical properties of studied soils were indicated in (Table 1).

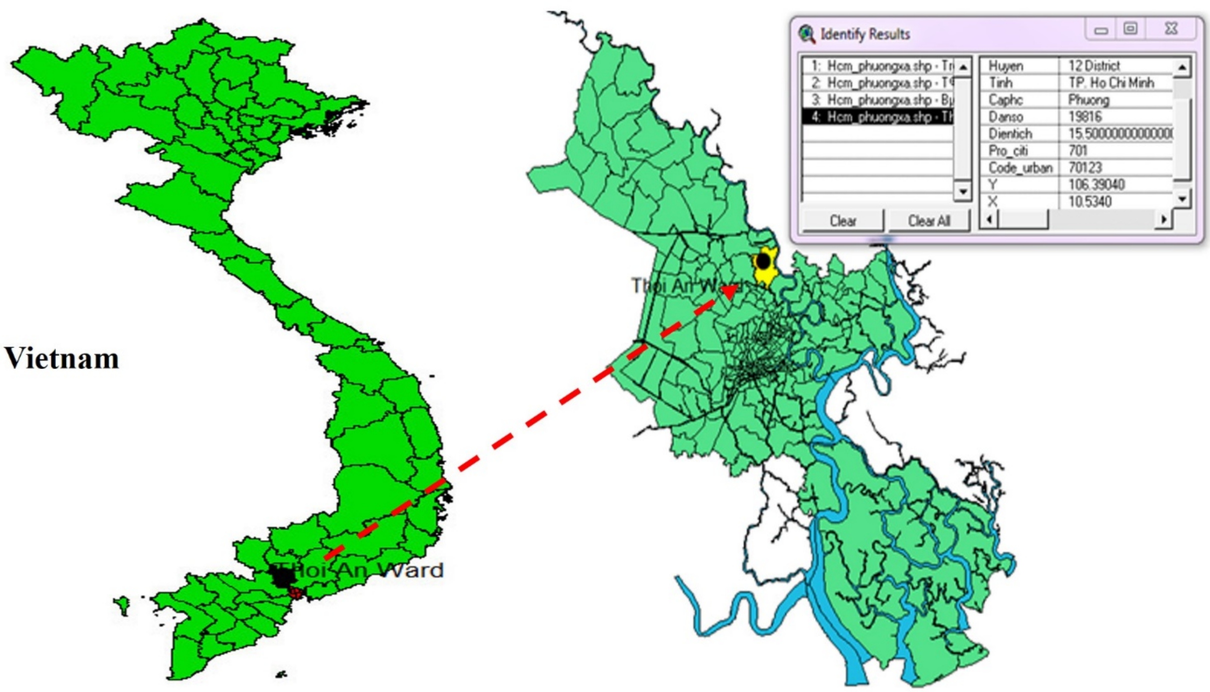

Figure 1. The map location of study area

Table 1. Some physical and chemical properties of the experimental soil.

Properties	Values
Silt %	43.0
Clay %	40.8
Sand %	16.2

Textural class	Silty clay
pH_{KCl}	4.5
Total MO %	2.5
N %	0.14
P_2O_5 %	0.04
K_2O %	0.24
CEC (meq/100g soil)	3.53
Total Pb	70.40
Total Cd	2.05

In addition, indigenous fungi MHCM15-VN1 and bacteria MHCM7-VK2 were selected from agricultural wetland in the Vegetable Growing Areas of Ho Chi Minh City, Vietnam. MHCM15-VN1 strain has a 100% similar sequence to the fungus *Penicillium chrysogenum*, and MHCM7-VK2 strain has a 99.73% similar sequence to *Bacillus licheniformis* bacteria. Microbial inoculants produced and tested in SFRI laboratory with peat carrier and microorganism density >$4x10^8$ CFU/g for use in combination with *Limnocharis flava* plants.

2.2. Eexperimental set-up

The experiment was designed in the net house at the Department of Environment, Branch of Hanoi University of Natural Resources and Environment in Thanh Hoa, Vietnam. A randomized complete block (RCB) was applied four times for each treatment. It was carried out with ceramic pots of 45 cm x 30 cm x 30 cm, containing 10 kg Pb and Cd-contaminated dry mud soil. This soil was collected from an abandoned vegetable field, dried naturally, smashed and then passed through a 2 mm sieve and was sterilized by autoclave at 121ºC (1 atm) for 30 minutes before placing in the experimental pots. The mixture of microbial inoculant at different levels are weighed and marked the name corresponding to 6 treatments such as 0, 1, 1.5, 2, 2.5, and 3 g/kg are indicated in (Table 2).

Table 2. Treaments used in the polluted soil.

Code	**Treatment description**

T1	Ssubstrate (Control)
T2	Substrate + 1 microbial inoculants g/kg dry soil
T3	Substrate + 1.5 g microbial inoculants/kg dry soil
T4	Substrate + 2 g microbial inoculants/kg dry soil
T5	Substrate + 2.5 microbial inoculants/kg dry soil
T6	Substrate + 3 g microbial inoculants/kg dry soil

Note: The amount of manure was used 50g / pot for all pots experimental.

The experimental soil was restored bioactivity at average 30°C and 60-70% humidity for two weeks. Three *Limnocharis flava* seedlings were planted in each pot for plants to clinging to the soil stable and grow their roots. After three days of planting, these plants are flooded with 10 cm of clean tap water (from the ground to the water surface) during the experiment.

The indicators were observed including height, dry biomass, the number and fresh inflorescence yields of the plant, TMs accumulated in the inedible part (roots, stems, leaves) and the edible inflorescence part (flower, flower-peduncle, flower buds).

2.3. Harvest and analyze TMs content in parts of *Limnocharis flava* plants

After 60 days, the *L. flava* samples were harvested, washed with tap water and rinsed with distilled water. The root, stems-leaves and edible parts (flowers, peduncle, and flower buds) were weighed, recorded, and shredded into labeled silver paper bags, respectively before drying at 75°C (until the weight remains constant). The dried samples were transferred to a desiccator for cooling prior to weighing the dry mass. Then the dried samples were crushed into fine powder and stored in a sealed plastic bag. 1 g crushed sample was combusted at 550^0C for 4-8 hours (until the sample completely turned white). The cooled sample was mixed with a mixture of HNO_3: HCl (ratio 1: 3) to a volume of 50 ml and filtered before analyzing using the atomic absorption spectrometer machine (AA280FS, Varian, Australia) in Lab of National Institute of Agriculture Planning and Projection.).

The safety limit of Pb and Cd in the edible inflorescence part was assessed according to National technical standards on the pollution limit of heavy metals in food (QCVN8-2: 2011/BYT) and World Food Organization/World Health Organization: General standard for contaminants and toxins in food and feed (FAO/WHO) (FAO, 2009).

2.4. Statistical analysis

All data were analyzed for one-way ANOVA for all variables of height, dry biomass, inflorescence, roots, stems-leaves and edible part with four times repetition. Treatments (T) have significantly different effects on different parts *Limnocharis flava* at probability level $p < 0.05$. Then group Tukey^{SHD} with alphabetic characters for modal mean values to compare differences with the 95% confidence interval to select the most effective treatment to meet the research purpose. Coefficient of variations, which represents the ratio of Standard deviation to the mean calculated, as an index of reproducibility and precision of the measurements. Minimum Significant Difference (MSD) was calculated at 5% probability for treatments (T1-T6). Using SAS 9.1 on Windows 7 Ultimate computer operating system. Pearson correlation coefficient between studied parameters was analyzed by usisng IBM SPSS21 version. Data calculated using SAS 9.1 software, and the results are displayed in a graphical format using sigma plot 14.0 software.

3. Result and Discussion

3.1. Soil properties of polluted soil

The physical and chemical properties of the contaminated soil used in the experiment had a certain influence on the capacity of *Limnocharis flava* to remove Pb and Cd when combined with the microbial inoculants properties are indicated in (Table 1). Soil experiment was taken in the Thoi An Ward, District 12, Ho Chi Minh City, Vietnam contained 40.8% clay, up to 43.0% silt, and 16.2% sand (Table 1). According to the United States Department of Agriculture's classification of soil textures, the soil was silty clay, and it belongs to Gleyic Acrsoils. pHKCl-H20 of soil was as low as 4.5 and 5.8, indicating acidic soil (FAO, 2009). Low soil pH stimulates the flexibility of cations in the soil, leading to high risk of TMs contamination (Nguyen et al., 2006). According to the Vietnam soil classification, parameters of N, P_2O_5, K_2O reached the average group, and organic content of 3.53 meq/100g soil was low (Nguyen et al., 2006). The soil bulk density of compacted soil (ρ) is 1.35 g/cm^3. This property helped reduce TMs leaching to the deep layers, reduce the spread of pollutants around, and lower the possibility of aquifer pollution.

The total Cd concentration of soil was recorded at 2.05 mg/kg higher than the permissible limits for Cd in agricultural land according to the Vietnam standards of QCVN 03-MT: 2015/BTNMT. Pi value of Cd was 1.37 in the range of $1 < Pi < 2$, which were classified as the low pollution degree (Hui et al., 2017). Pi value of Pb of 1.06 was also long to the low pollution group of $1 < Pi \leq 2$ (Hui et al., 2017). Polluted agricultural land by Pb and Cd in this area might have undergone weathering process of parent rock to form soils containing natural Pb and Cd content (substrate). According to Le Duc and Tran Thi Tuyet Thu, different parent rocks contain various elements of TMs. For example, in the soil, Pb content: 2-300 ppm (average 19 ppm); Cd: 0.01-2 ppm (mean 0.35 ppm) (Le et al., 2000). Cd pollution in soil might occur by the influx of urban wastewater, and industrial wastewater from Ho Chi Minh City via Tham Luong - Ben Cat, Tran Quang Co into this wetland during the rainy seasons every year. An earlier report noted that water from the canals of Ho Chi Minh City was used for irrigating agricultural lands, causing Cd accumulation a concentration of 9.9-10.3 mg/kg in the soil. This concentration was five times higher than the permitted threshold (Nguyen & Le, 2003). In an other report also showed that the Pb content was 14 - 85 ppm in paddy soil in the south of the Ho Chi Minh City (Quynh & Ba, 2003). In addition, another possible cause of Cd pollution in soil was due to the fact that farmers used various kinds of Pb and Cd-containing fertilizers and crop protection chemicals for increasing crop yields. In most fertilizer samples used in the southern provinces of Vietnam, Cd was presented at different concentrations raning from 0.59 to 186.2 mg/kg. As a consequence, this metal gradually accumulated in the soils (Cao, 2007).

As located in a low terrain, the experiment soil is likely to receive water from nearby areas, and less drain so it is suitable for growing aquatic plants, which are able to grow well on acidic soils which are poor in nutrients, and contaminated with heavy metals.

3.2. Effect of different levels of microbial inoculants on the growth of *Limnocharis flava* plants

The height and biomass of the plant are the most important indicators for assessing the growth potential of *L. flava* plants. These results demonstrated that the use of different levels of microbial inoculants affected plant height and dry biomass significantly different at $p < 0.05$. The height and total dry biomass of *L. flava* plants increased in treatments (T2-T6) by 48.27 - 68.50 cm and 24.95 - 35.11.46 g/plant, higher than the control - without microbial inoculants (46.83 cm and 23.93 g/plant). In which, the height and total dry biomass of the plants reached the highest values at microbial inoculants level of 3 g/kg dry soil with 1.46 and 1.47 times higher than the control. The height and dry biomass of plants reached the lowest values in treatment of T2, and these results were not significant differences compared to the control of T1 at P <0.05 (Fig. 2 a,b).

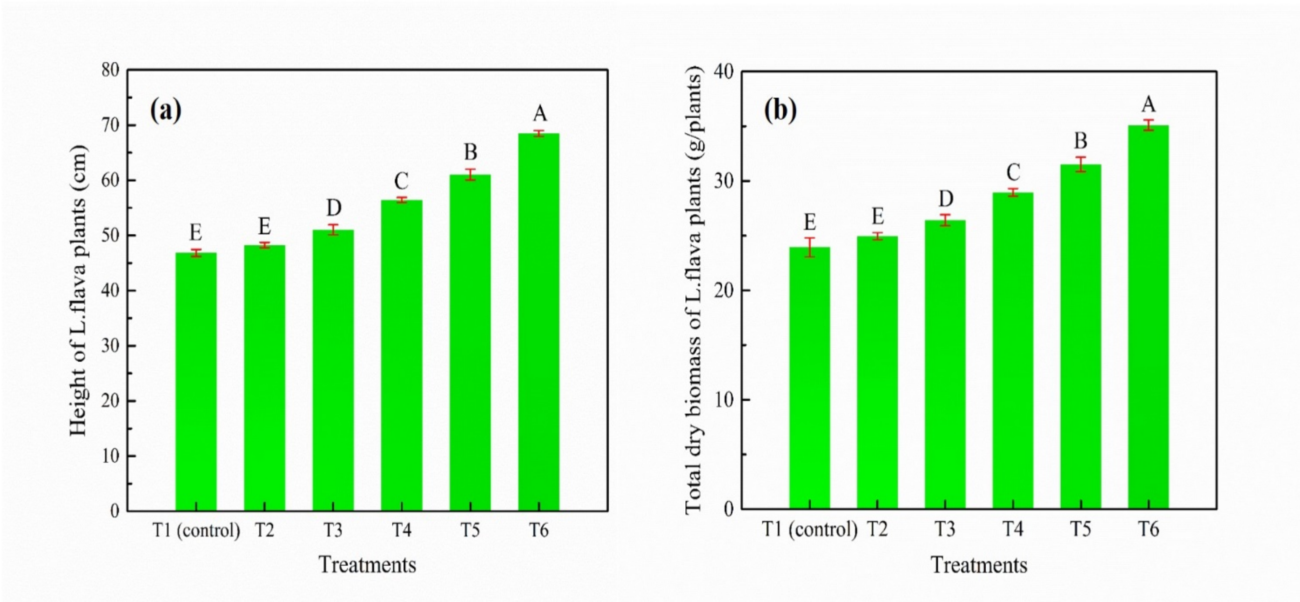

Figure 2. Effect of microbial inoculants levels on the height (a) and dry biomass (b) of *Limnocharis flava* plants. The bar chart displayed mean ± Std (n=4), and mean value with different letters indicate that values are significantly different at P<0.05. CV= 1.28 and MSD = 1.63; CV= 2.03 and MSD =1.33.

3.3. Effect of different levels of microbial inoculants on the inflorescence yields of *Limnocharis flava* plants

The inflorescence yields are also an important indicator to evaluate the growth potential of multi-purpose plants. In Vietnam, *L. flava* is grown as a vegetable in the edible part of inflorescence. Therefore, the number and fresh weight of inflorescence play an important role in showing the effect of a mixture of microbial inoculant in different levels on the crop yields. Experimental results showed that the combination of *L. flava* with probiotics in the six treatments was a small difference in the number of inflorescence with three inflorescences in treatments of T1, T2, and T3; four inflorescences in treatments of T4, T5, and T6.

The average fresh inflorescence yields of plants increased significantly different between treatments at level P <0.05. The mean fresh weight of inflorescence in treatment of T2 was not significant differences with T1 at P <0.05 (Fig. 3).

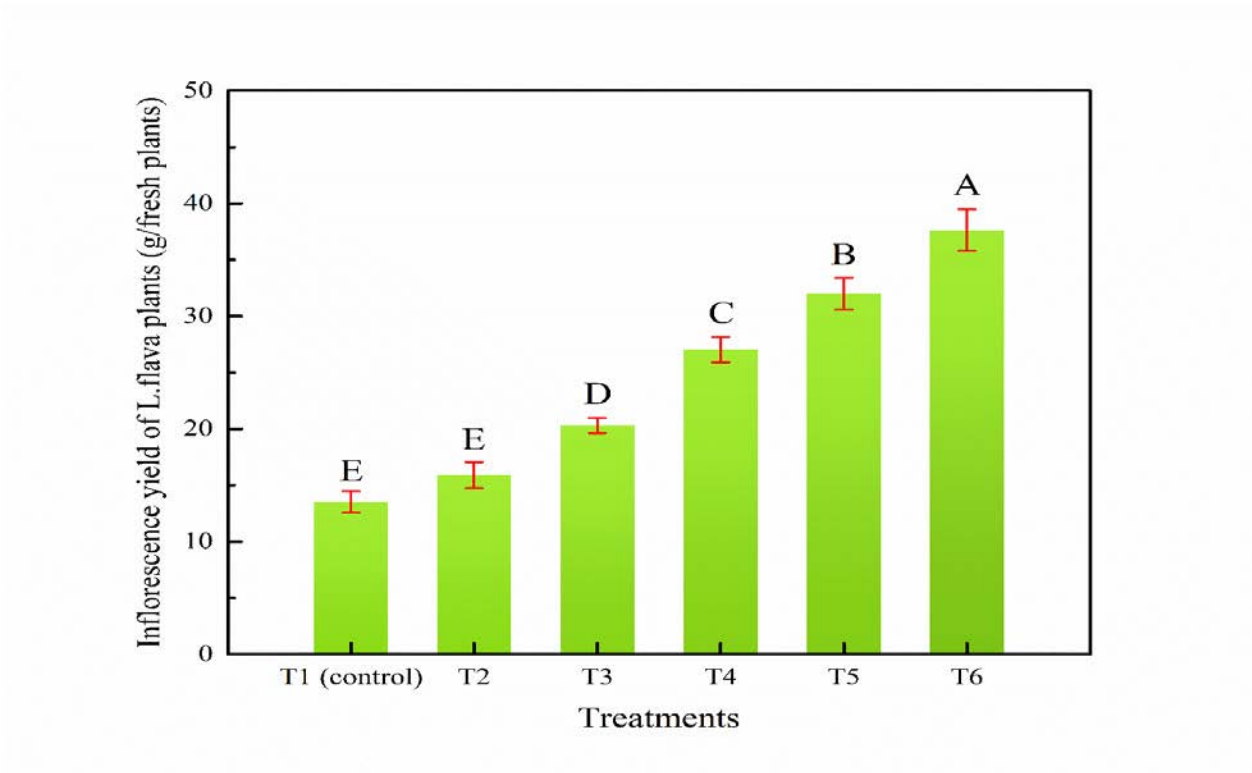

Figure 3. Effect of microbial inoculants levels on the inflorescence yields of *Limnocharis flava* plants. The bar chart displayed mean ± Std (n=4), and mean value with different letters indicate that values are significantly different at P<0.05. CV= 4.66 and MSD = 2.61.

Results showed that the yield of fresh inflorescences increased by 13.52, 15.91, 20.30, 27.04, 32.01, and 37.65 g/plant corresponding to the microbial inoculant levels of 0, 1, 1.5, 2, 2.5, and 3 g/kg dry soil. In treatments of T2 - T6, the yields of fresh inflorescence were higher than the control treatment - without microbial inoculants from 1.18 to 2.78 times.

3.4. Effect of microbial inoculants on the accumulation of Pb and Cd in the inedible part of *Limnocharis flava*

Results showed compared to the untreated control, in treatments are applied with the levels of microbial inoculants, which increased significantly different the accumulation of Pb and Cd in the inedible part (roots, stems - leaves) of *L. flava* plants at P <0.05. In which, Pb content increased the accumulation in roots and stems - leaves by 1.46 - 8.90 and 1.09 - 2.57 times, respectively; Cd content increased in roots and stems - leaves by 1.08 - 6.03 and 1.08 - 2.25 times, respectively, compared to the controls - without microbial inoculants. In the level of 3 microbial inoculants g / kg of dry soil, Pb and Cd content were accumulated highest in roots and stems - leaves. In the treatment of T2, the accumulation of Pb stems - leaves and Cd in both roots and stems - leaves of *L. flava* plants increased insignificantly compared to the control at P <0.05 (Table 3).

Table 3. Effect of inoculant levels to the accumulation of Pb and Cd in the inedible part of *Limnocharis flava.*

Treatments (T)	Pb		Cd	
	Roots (mg/kg dw)*	Stems-leaves (mg/kg dw)*	Roots (mg/kg dw)*	Stems-leaves (mg/kg dw)*
1 (Control)	6.63F	1.20E	0.120E	0.040E
2	9.70E	1.31E	0.129E	0.043ED
3	14.32D	1.73D	0. 253D	0.047D
4	19.25C	2.16C	0.322C	0.053C
5	25.39B	2.53B	0.509B	0.078B
6	32.46A	3.08A	0.724A	0.090A
$MSD_{0.05}$	0.63	0.11	0.01	0.005
CV%	1.53	2.50	1.23	3.91

Note: Means with the same letter(capital letter) in the column indicate that values are not significantly different at P<0.05 while different letters indicate the significant difference, *: Average of four times of repetition.

3.5. Effect of microbial inoculants on the accumulation of Pb, Cd in the edible part of *Limnocharis flava*

In T5 treatment, the TMs were accumulated second highest in the inflorescence with Pb content of 0.079 mg/kg fresh weight (Table 4).

Table 4. Effect of microbial inoculants levels on the accumulation of Pb and Cd in the edible part of *Limnocharis flava* plants.

Treatments (T)	Pb content	Cd content
	Inflorescence (mg/kg fw)*	Inflorescence (mg/kg fw)*

1 (Control)	0.012E	0.011E
2	0.015E	0.014E
3	0.027D	0.019D
4	0.049C	0.029C
5	0.079B	0.050B
6	0.120A	0.065A
$MSD_{0.05}$	0.006	0.003
CV%	4.85	4.76
QCVN8-2:2011/BYT	0.1	0.05
FAO/WHO	0.05	0.05

Note: Means with the same letter(capital letter) in the column indicate that values are not significantly different at P<0.05 while different letters indicate the significant difference, *: Average of four times of repetition.

Soil microorganisms such as bacteria and fungi are of great significance in the nutrition cycle of plants, improving soil texture, detoxifying plants, curbing harmful agents and stimulating plant growth (Nguyen et al., 2006; Le et al., 2000; Nguyen & Le, 2003; Cao, 2007). In particular, the microorganisms in the rhizosphere can enhance plant growth byways of directly, or indirectly through a variety of mechanisms, such as allowing plants to grow longer roots during periods of easy stages of growth by reducing ethylene production; nitrogen fixation; specific enzymatic activity; supply biological phosphorus and trace elements for plant uptake; production of phytohormone as auxins, cytokinins, and gibberellin; increase plant tolerance against stressful soil conditions (Abhilash et al., 2009; Deng et al., 2012; Bargaz et al., 2018; Jacoby et al., 2017; Souza et al., 2015), produce siderophores that played a role in solubilizing unavailable forms of heavy metals bearing minerals by complexation reaction (Rajkumar et al., 2012; Aafi et al., 2012). Results in this study showed that the *Limnocharis flava* plants were enhanced growth on the height, biomass, and inflorescence yields compared to the controls-without inoculants. These effects may be due to the role of two microbial species of bacteria *Bacillus licheniformis* and fungus *Penicillium chrysogenum* in the mutualism of symbiotic relationship in the soil and the root zone of *L. flava* plants promoted better plant growth. Similar results from previous studies on different plants, such as Pepper, Tomato, *Camellia japonica*, and Rice showed the plant growth promotion of *Bacillus licheniformis* (García et al., 2004; Jamil et al., 2013; Park et al., 2017), and the ability to promoted the yield and increased of Pak choi (*Brassica chinensis* L. = *B. rapa* L.; acc. WFO, 2024) shoot biomass and the plant growth-promoting effect of fungus *Penicillium chrysogenum* on crops (Won et al., 2019; Morsy et al., 2020; Galeano et al.,2023). Because in symbiotic mutualism relationship between microbial with the plant, plants provide microbial in the root zone with organic carbon sources that help microorganisms conduct metabolic activities as well as energy metabolism. In turn, these microbial also provide nutrients to plants and produce plant growth stimulants that assisting plants to grow and develop better. However, unlike previous studies, the use of both fungus *Penicillium chrysogenum* and bacteria *Bacillus licheniformis* in the form of a mixture of microbial inoculants induced positive and synergistic effects in increasing their capacity breaking down organic matter into nutrients for better *L. flava* plants growth. Effects of *Bacillus licheniformis* and *Penicillium chrysogenum* on the height, total dry biomass, and inflorescence yields were not only clearly compared to the controls but also expressed proportionally with the increasing levels of microbial inoculants. Because the higher levels of microbial inoculants increased the density of microbial and their activity in the soil and the root zone of *L. flava*, leading to increased interaction between soil - microorganisms - plants.

The combination of microbial and plants can enhance the biomass production of plants led to an increase in the efficiency of accumulation TMs in plant biomass (Ahmad et al., 2008). The TMs content accumulated by plants is an important indicator reflecting the ability of the plants to restore contaminated agricultural land. Each plant species can uptake various multi-elements, and in the different parts of plants also accumulated a certain amount of TMs depending on many factors as elemental nature and concentration of TMs, soil properties, microorganisms species can combine, etc. *L. flava* is a species of aquatic plants with a firm rooting in the mud that may without difficulty grow in severe environmental circumstance, and can produce excessive biomass, easy to harvest, and quickly accumulates heavy metals (Abhilash et al., 2009). This plant species was found with the advantage of developing on agricultural wetlands with the presence of TMs (Pb, Cd, As, and Hg) in the Southeast and Mekong Delta, Vietnam (Tran et al., 2014).

This study showed that the concentration of Pb and Cd was found to be highest in the roots of *L. flava*, which was followed by in the stems-leaves, and inflorescence compared with those in the controls - without microbial inoculants. These results are consistent with previous studies on Pb and Cd accumulation concentrated in roots compared to other remaining parts of *L. flava* plants in the range of different MTs concentration without using microbial inoculants (Hussein, 2008;

Vijayaraghavan & Yeoung, 2008; Abhilash et al., 2009; Kamarudzaman et al., 2011). However, in this study accumulation of TMs in the different parts of *L. flava* is directly proportional to the levels of microbial inoculants applied in treatments (T1-T6). These results showed the potential role of bacteria *Bacillus licheniformis* and fungus *Penicillium chrysogenum* that both promoted plant growth, and increased the accumulation of large amounts of TMs in the inedible part of roots, stems - leaves and only accumulated very little amounts of TMs in the inflorescence of *L. flava* plants. The reason may be that these microbials species can produce siderophore, organic acids, and amino acids that change the pH of soil that playes a major role in dissolving total TMs into minerals form which is easy for the uptake, accumulation of plants. In a previous study showed that the application high-density of *Bacillus licheniformis* in the rhizosphere of Indian mustard was enhanced the accumulation of Cd and Cr in the tissues of plants (Hussein, 2008). Increasing the density of *Bacillus licheniformis* in the root zone of Rice also reduced the amount of Ni available for the absorption plants; and increased the availability of other nutrients for Rice plants growth under Ni stress (Jamil et al., 2013).

It was observed that the Pb and Cd content in the edible part by *L. flava* increased gradually with an increase the concentarion of microbial inoculants amended in the polluted soil. In the control treatment T1, Pb and Cd contents were found lowest of 0.012 and 0.011 mg/kg fresh weight, respectively. The uptake of Pb and Cd in the highest edible part at the level of 3 microbial inoculants g/kg dry soil (T6) with the prportion of Pb was accumulated 0.120 mg/kg fresh weight, and the proportion of Cd absorbed upto 0.065 mg/kg fresh weight. These values have exceeded the safe limits for vegetables for both Pb and Cd according to QCVN8-2: 2011/BYT and FAO/WHO standards. The accumulation of Pb by edible part evidently exceeded 1.2 times as compared to the QCVN8-2:2011/BYT and exceeded 2.4 times the FAO/WHO standards for vegetable safety. The Cd content in vegetables also surpassed 1.3 folds as compared to the safety limit according to QCVN8-2: 2011/BYT and FAO/WHO standards. The results of present study are consistent with the conclusions made by Abhilash et al. (2009) revealed that the uptake of the Cd content in the roots by *L. flava* was higher than that of leaves, stalks, and flowers, with four Cd concentrations (0.5, 1, 2 and 4 mg/l) 30 days after the test. The hyphae of *Penicillium chrysogenum* growing on the roots create contact and wide spread during the soil (Abhilash et al., 2009; Deng et al., 2012). Consequently, a large quantity of Cd is kept in the mycelium structure in the roots and spores, dropping a large amount of Cd added to the soils, and enhancing possibility of accumulating Cd in the flower parts. Therefore, microbial products should be used in mixture with *L. flava* areas polluted with Cd <10 mg/kg for growing commercial vegetables, nonetheless should not be grown in extremely contaminated areas for of the high risk of toxicity.

3.6. Pearson correlation coefficient

The data in Table 5 indicate that Pb content in stems and leaves of *Limnocharis flava*, plant height, total dry biomass of *L. flava* plants and inflorescence yield of fresh *L. flava* plants were highly significantly positively correlated with Pb content in *L. flava* roots. On the other hand, Pb and Cd content in inflorescences was significantly negatively correlated with Pb content in *L. flava* roots. Pb content in stems and leaves of *L. flava* was significantly correlated with Pb content in stems and leaves of *L. flava*. Similarly, plant height, total dry biomass of *L. flava* plants and inflorescence yield of fresh *L. flava* plants were highly significantly correlated with Pb content in stems and leaves of *L. flava*. Pb and Cd content in inflorescences was negatively significantly correlated with Pb content in stems and leaves of *L. flava*. Plant height was significantly correlated with Cd content in *L. flava* roots, and the total dry biomass of *L. flava* plants and inflorescence yield of fresh *L. flava* plants were highly significantly correlated with Cd content in *L. flava* roots. Moreover, Cd content in inflorescences was highly significantly correlated with Pb content in inflorescences, while plant height, total dry biomass of *L. flava* plants and inflorescence yield of fresh *L. flava* plants were negatively correlated with Pb content in inflorescences. Similarly, plant height and total dry biomass of *L. flava* plants were significantly negatively correlated with Cd content in inflorescences, while inflorescence yield of fresh *L. flava* plants was highly significantly negatively correlated with Cd content in inflorescences. The total dry biomass of *L. flava* plants and inflorescence yield of fresh *L. flava* plants were highly significantly correlated with plant height. A positive, highly significant correlation was determined between the inflorescence yield of fresh *L. flava* plants and the total dry biomass of *L. flava* plants.

Table 5. Pearson correlation coefficient between studied parameters.

	Pb-R	**Pb-S**	**Cd-R**	**Cd-S**	**Pb-I**	**Cd-I**	**PH**	**TDB**	**IYF**
Pb-R	**1**								
Pb-S	.998**	**1**							

Cd-R	.917*	.915*	**1**						
Cd-S	-.437	-.478	-.603	**1**					
Pb-I	-.905*	-.901*	-.711	.165	**1**				
Cd-I	-.912*	-.914*	-.709	.225	.995**	**1**			
PH	.998**	.994**	.913*	-.407	-.898*	-.903*	**1**		
TDB	.997**	.996**	.926**	-.468	-.910*	-.916*	.991**	**1**	
IYF	.996**	.997**	.918**	-.466	-.917*	-.925**	.990**	.999**	**1**

Note: Pb-R= Pb in root by *Limnocharis flava*, **Pb- Note: S=** Pb in stem and leaves by *L. flava*, **Cd-R=** Cd in root by *L. flava*, **Cd-S=** Cd in stem and leaves by *L. flava*, **Pb-I=** Pb content in inflorescence, **Cd-I=** Cd content in inflorescence, **PH=** Plant height, **TDB=** Total dry biomass of *L. flava* plant, and **IYF=** Infloresecences yield of *L. flava* fresh plant.

4. Conclusions

The results of present study showed that the effects of *Bacillus licheniformis* and *Penicillium chrysogenum* were clearly on the height, total dry biomass, and inflorescence yields of *Limnocharis flava* plants compared to the controls-without inoculants. The application levels of microbial inoculants in the range of 2 - 2.5g /kg dry soil stimulated height and biomass of *L. flava*, as well as enhanced the accumulation of Pb and Cd in the inedible part, ensuring safety for the edible part of *L. flava* plants according to regulations of QCVN8-2: 2011 / BYT and FAO/WHO. However, in order to meet the requirement for Pb and Cd content in vegetables according to FAO/WHO regulations, the level of inoculant should be 2g/kg dry soil. The long-term impact of microbial inoculants on phytoremediation of multi-metal polluted soils should be verified in the field scale in the future.

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