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ORIGINAL RESEARCH ARTICLE

Testing the ability of *Vetiveria zizanioides* plants to bind cadmium and its influence on soil microbial diversity

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Abstract: Phytoremediation is an environmentally friendly and cost-effective approach for the remediation of heavy metals from contaminated environments. However, its effectiveness can be influenced by various factors, particularly the structure and diversity of soil microbial communities, which play a crucial role in enhancing or hindering the phytoremediation process. In this study, the remediation of cadmium-contaminated soil was investigated through the cultivation of *Vetiveria zizanioides*, examining its effects on the diversity of soil microbial communities. The concentration of Cd in roots and leaves reached more than 600 mg/kg DW and 400 mg/kg DW, respectively, at 60 mg/kg Cd treatment. Next-generation sequencing was used to characterize the soil microbial community. It was shown that the increased Cd contaminant from 20 mg/kg to 60 mg/kg of soil noticeably reduced the microbial count. A significant increase in species numbers was observed in the clean soil containing the *V. zizanioides* plants. In addition, soil samples from Cd-contaminated soil showed a considerable change in microbial structure at the genus level with the *Sphingomonas* bacteria becoming the most dominant genus against Cd-contamination.

Keywords: Phytoremediation; Cadmium remediation; Soil microbial composition; Soil contamination; *Vetiveria zizanioides*; *Sphingomonas*

1. Introduction

Soil is a vital component of ecosystems and significantly contributes to the sustainability of human populations. Over the years, researchers have been investigating methods for the prevention and treatment of contaminated soils, with significant progress made since 1985. Soil contamination with various inorganic and organic pollutants has led to the degradation of vast areas of urban and agricultural land worldwide.[1] Industrial activities, particularly from sectors such as mining and petroleum, have been identified as major contributors to the release of toxic heavy metals (HMs) into the soil, negatively affecting soil fertility, microbial activity, and agricultural productivity.[2] As this contamination becomes a pervasive global issue, it is crucial that we address the challenges in soil degradation to safeguard food security and human health.[3,4] It is important to note that contamination of the lithosphere has been aggravated in the past decade due to rapid industrialization, with petroleum and mining industries being the primary contributors.[5]

Among various contaminants, HMs such as zinc, cadmium, arsenic (As), and lead are of particular concern due to their carcinogenic and mutagenic properties, and their removal from ecosystems is imperative.[6] Consequently, the urgency for environmental protection and the development of effective remediation techniques has increased.[7,8] In recent years, several remediation technologies—including physical,[9,10] chemical,[11] and biological[12,13] approaches – have been considerably applied to treat HM contamination. Traditional remediation methods, such as chemical techniques involving immobilization, soil washing, and vitrification, are often expensive and not feasible for large-scale applications.[1] As such, there is an urgent need for cost-effective, eco-friendly, and sustainable alternatives.[14] Biological methods, particularly phytoremediation, offer a promising solution to this challenge.[15] Phytoremediation involves the adsorption of contaminants, such as HMs, in plant roots and leaves.[16] This process can be further divided into various mechanisms such as phytostabilization, phytoextraction, phytovolatilization, rhizofiltration, and others, which enable plants to remove, stabilize, or degrade pollutants.[17] Among the various plant species used in phytoremediation, *vetiver* stands out due to its remarkable characteristics. Vetiver, previously identified as *Vetiveria zizanioides* Nash, has been reclassified as *Chrysopogon zizanioides* (L.) Roberty, known for its tall, fast-growing perennial nature and deep root system that can extend 3 – 4 m into the soil, which is particularly effective at remediating contaminated sites. This grass can tolerate a wide range of harsh environmental conditions, including high concentrations of HMs, making it an ideal candidate for phytoremediation in various contaminated environments.[18,19]

In addition, microbes play a key role in most of the Earth's biogeochemical cycles and are crucial to the functioning of virtually all ecosystems. Certain microorganisms in the rhizosphere form symbiotic relationships with plants, suggesting that phytoextraction, supported by plant growth-promoting bacteria, could be an effective approach to improve phytoremediation in heavily contaminated soil. For instance, *Serratia* spp., isolated from cadmium-contaminated soils, has been shown to improve the efficiency of phytoremediation by altering the physicochemical properties of the soil. Therefore, a comprehensive investigation of the structure and function of rhizosphere microbial communities is crucial for evaluating the quality of contaminated agricultural soils. Under HM stress, microbes either die due to the toxicity caused by the metals or survive and thrive by employing various resistance mechanisms against them. HM ions help protect the internal bacterial cells, and the formation of a biofilm on *Pseudomonas aeruginosa* increases its resistance to Cu, Pb, and Zn.[20,21]

While numerous studies have investigated the effectiveness of *V. zizanioides* in removing contaminants from soils and groundwater,[22,23] there is a notable lack of research on the biological interactions between plants and microorganisms in the context of HM phytoremediation. Recent studies have begun to explore these interactions, demonstrating that combining plants with microorganisms can enhance phytoremediation efficiency. Specifically, *Serratia* spp. has been shown to significantly improve cadmium accumulation in *V. zizanioides*.[24] Furthermore, research into microbial diversity under HM stress has shown that microbes either survive through resistance mechanisms or perish due to metal toxicity.[25] Ng *et al.*[26] modified the HM phytoremediation using *V. zizanioides* by ethylenediaminetetraacetic acid addition into the soil and ranked metal accumulation as follows: Zinc >>> Copper > Lead >> Cadmium. Since microorganisms promote most biogeochemical cycles on the Earth, their role is not negligible to the ecosystems.[27]

In addition to plant leaves or roots, soil microorganisms are crucial in phytoremediation.[25] The existing studies focus on investigating the phytoremediation process rather than biological interactions.[28-30] By investigating the impact of two grasses, *V. zizanioides* and *Juncus effusus* L., on the wetland bacterial and archaeal diversity, Long *et al.*[31] revealed that archaea faced decreasing abundance with increasing plant bacterial diversity. In another study, ecotoxicological impacts of Cd on the soil microorganisms were studied through polymerase chain reaction (PCR) denaturing gradient gel technique, showing that bacteria, fungi, and actinomycete are sensitive to Cd.[32]

The research to date has tended to focus on phytoremediation process rather than biological interactions.[28] Overall, there is a lack of comprehensive research thoroughly investigating soil biodiversity in the context of HM phytoremediation. To address this gap, this study primarily aims to examine the impact of Cd phytoremediation on microbial variation in soils assessed using next-generation sequencing (NGS) to identify the key bacteria that are crucial for the remediation process.

2. Materials and methods

2.1. Soil preparation and plant material

Soil samples were taken from Chitgar Forest Park, Tehran, Iran, and were transferred to the laboratory. According to the soil taxonomy, the samples were classified as typic haplocambids. The samples were air-dried at room temperature; a 2 mm mesh screen was used to sieve the samples. In addition, the soil texture type was defined using a hydrometer test, and soil electrical conductivity, cation-exchange capacity, pH, total organic carbon, CaCO3, and elements[33] were measured according to standard methods[34] (Table 1).

$Cd(NO_3)_2$ solution was applied as a single dose, at concentrations of 20 and 60 mg/kg soil, with the control group (0 mg/kg) consisting of soil without metal addition. Table 2 presents the treatment plan for various soil samples. All experiments involving these treatments were replicated 3 times. All soil samples were maintained under field capacity moisture conditions for 3 weeks. Each pot contained 2 kg of soil. Eight seeds were planted in each pot. After 10 days, five unhealthy seedlings were eliminated. The pots were weighed and irrigated at intervals of 24 – 48 h. The plants were harvested once the growing season was completed.

Table 1. Chemical and physical characteristics of substrates used in the study

Sand	Silt	N	EC	pH	P	K	Zn	Mn	Fe
(%)					(Ds/m)			(mg/kg)	
27	53	0.09	62.3	7.8	18.9	940	0.63	5.43	3.53

Table 2. Experimental treatments

Sample abbreviation	Treatment name
B	Clean soil, no Cd, and no *V. zizanioides* plants (negative control)
V	Clean soil in the presence of *V. zizanioides* plants (positive control)
Cd20	Soil with 20 mg/kg Cd+ *V. zizanioides* plants
Cd60	Soil with 60 mg/kg Cd+ *V. zizanioides* plants

Abbreviation: *V. zizanioides*: *Vetiveria zizanioides*; B: Clean soil without *Vetiveria zizanioides*; V: Clean soil in the presence of *V. zizanioides*; Cd20: Cd-contaminated soil (20 mg/kg); Cd60: Cd-contaminated soil (60 mg/kg).

2.2. Plant sampling and analysis

2.2.1. Leaves and roots dry weight measurement

Leaf and root samples were dried at 70°C for 48 h and subsequently weighed.

2.2.2. HM leaching and analytical process

After harvesting, the leaves and roots of *V. zizanioides* were carefully washed with deionized water to remove any surface contaminants. The washed plant material was then dried at 70°C for 48 h to ensure complete moisture removal. Once dried, the plant tissues were finely ground to a homogeneous powder to facilitate efficient digestion and analysis. For metal analysis, the samples were digested in a mixture of concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) in order to break down the plant matrix and release the metals into solution. This step was crucial to ensure that the metals of interest were fully extracted from the plant material. The digestates were then filtered and diluted with deionized water as needed. Finally, metal concentrations in the resulting solutions were determined using (furnace atomic absorption spectrometry; model 603 Perkin-Elmer, PerkinElmer, Inc., USA). This technique was chosen for its sensitivity and precision in measuring trace metal concentrations.

2.3. Soil microbial community analysis

2.3.1. DNA extraction

Microbial DNA from the total soil samples (Table 1) was extracted using the NucleoSpin® Microbial DNA Isolation Kit (Macherey-Nagel, Germany) following the manufacturer's instructions. The quality of the extracted DNA was assessed using Nano-drop ultraviolet-Vis's spectrophotometer (Thermo Fisher Scientific, USA) as previously reported by Yang *et al.*[9] Then, agarose gel electrophoresis (1%) was applied to study the structural integrity of the DNA molecules. DNA from the soil samples was utilized to analyze bacterial diversity through 16S rRNA amplicon sequencing.

2.3.2. Illumina sequencing data analysis

In the present study, the impact of Cd-phytoremediation by *V. zizanioides* grass on the soil microbial diversity was investigated using NGS. The 16S protocol was designed to amplify both bacteria and archaea using paired-end 16S community sequencing on the Illumina platform. PCR amplification was performed following standard procedures, using primers with barcodes: 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT), which target the V4 region of the 16S rRNA. Subsequently, sequencing libraries were prepared using the TruSeq DNA PCR-Free Sample Prep Kit (Illumina, USA) following the manufacturer's guidelines. The quality of the library was assessed using the Qubit 2.0 Fluorometer (Thermo Scientific) and the Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on the Illumina HiSeq 2500 platform, generating 250 bp paired-end reads (Beijing Genomics Institute, China).

2.3.3. Statistical and bioinformatics analysis

In addition, the sequences were aligned and compared with those in the gene database using the basic local alignment search tool program (National Center for Biotechnology Information) for identification. Strains with 97% similarity or more in sequence were identified as identical. To calculate alpha diversity, species diversity complexity was analyzed using several indices, including Chao 1, ACE, Shannon, and Simpson, with QIIME (V. 1.7.0). The results were visualized using R software (V. 2.15.3). Furthermore, beta diversity was evaluated using both weighted and unweighted UniFrac distances, analyzed with QIIME (V. 1.7.0). Principal coordinate analysis (PCoA) was also employed to visualize and identify differences between the samples based on the beta diversity distance matrix.

3. Results

3.1. Responses and plant growth

Empirical studies have indicated that Cd exerts a substantial influence on plant growth. As illustrated in Figures 1 and 2, *V. zizanioides* dry weight was dramatically reduced by increasing soil contaminant concentration. The leaves' weight was lower than 19 g for the contaminated soil, whereas it was measured up to 27 g for the clean samples. In addition, the same trend was observed for the roots weight, reaching 2.7, 1.92, 1.56, and 1.41 g for the B, V, Cd20, and Cd60 samples, respectively.

3.2. Phytoremediation potential of *Vetiveria*

Leaf necrosis and discoloration are common indicators of HM toxicity (Figure 3). Cadmium accumulation in *V. zizanioides* was measured by extracting HM elements from the leaves and roots. The results indicated that this plant demonstrates promising potential for Cd phytoremediation, with Cd metal concentrations exceeding 600 mg/kg DW in the leaves and more than 400 mg/kg DW in the roots (Figures 4 and 5).

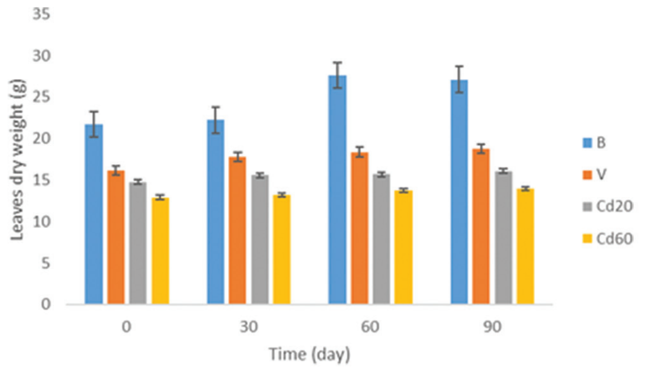

Figure 1. Dry weight of leaves of *Vetiveria zizanioides* grass during exposure time. Data points and error bars represent mean±SD of three replicates (*n*=3). Soil treatment definitions: B=Clean soil without *V. zizanioides*; V=Clean soil in the presence of *V. zizanioides*; Cd20=Cd-contaminated soil (20 mg/kg); Cd60: Cd-contaminated soil (60 mg/kg)

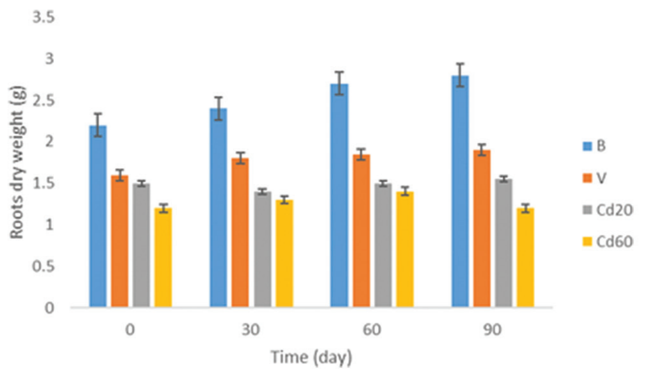

Figure 2. Dry weight of roots of *Vetiveria zizanioides* grass during the exposure time. Data points and error bars represent mean±SD of three replicates (*n*=3). Soil treatment definitions: B: Clean soil without *V. zizanioides*; V: Clean soil in the presence of *V. zizanioides*; Cd20: Cd-contaminated soil (20 mg/kg); Cd60: Cd-contaminated soil (60 mg/kg)

3.3. Impacts of HMs on the soil microorganisms

To investigate the relationships between soil microorganisms and plant roots, NGS was utilized to identify the bacteria species involved in phytoremediation. All effective reads were clustered into operational taxonomic units (OTUs) based on 97% DNA sequence similarity to facilitate analysis of soil microbial diversity. By comparing the number of OTUs, we revealed that the number of microorganisms were enriched in the presence of *V. zizanioides* grass. Increasing the contaminant concentration from 20 to

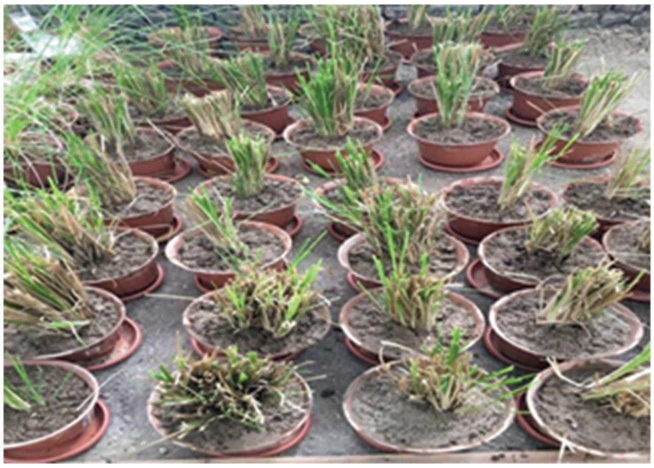

Figure 3. Morphological symptoms in *Vetiveria zizanioides* plants exposed to Cd contamination

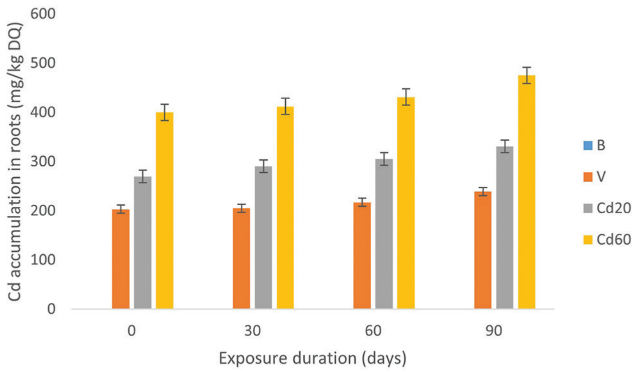

Figure 4. Heavy metal (Cd) accumulation in the roots of *Vetiveria zizanioides* grass across different metal exposure duration. Data points and error bars represent mean±SD of three replicates (*n*=3). Soil treatment definitions: B: Clean soil without *V. zizanioides*; V: Clean soil in the presence of *V. zizanioides*; Cd20: Cd-contaminated soil (20 mg/kg); Cd60: Cd-contaminated soil (60 mg/kg)

60 mg/kg of soil noticeably reduced the microbial count (Figure 6).

3.4. Soil microbial community structure

Community analysis was applied to estimate the impacts of HM contamination on the soil microbial community structure during the phytoremediation process through *V. zizanioides* grass. The results, presented in Figure 7A, demonstrated that exposing the soil samples to Cd results in microbial destruction at the genus level. *Sphingomonas* was identified as the strongest genus against Cd. Furthermore, it was revealed that planting *V. zizanioides* grass remarkably changes the soil microbiome. Considerable results were

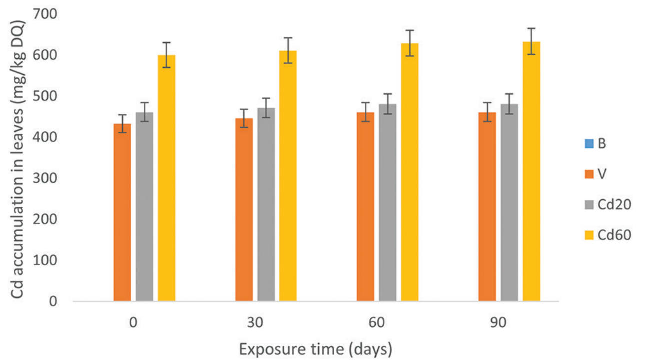

Figure 5. Heavy metal (Cd) accumulation in leaves of *Vetiveria zizanioides* grass across different metal exposure duration. Data points and error bars represent mean±SD of three replicates (*n*=3). Different letters in the same color indicate significant difference determined by analysis of variance followed by the least significant difference test (*p*<0.05). Soil treatment definitions: B: Clean soil without *V. zizanioides*; V: Clean soil in the presence of *V. zizanioides*; Cd20: Cd-contaminated soil (20 mg/kg); Cd60: Cd-contaminated soil (60 mg/kg)

additionally observed at the phylum level. Relative abundance of *Actinobacteria* was dramatically reduced from 0.39 in sample B to 0.03, 0.07, and 0.05 in samples V, Cd20, and Cd60, respectively. As illustrated in Figure 7B, Cd relatively removed *Firmicutes* from the soil; meanwhile, it had no significant impact on *Acidobacteria*, *Bacteriodetes*, *Gammatimonadete*, *Ghloroflexi,* and *Verrucomicrobia*.

3.4.1. Alpha diversity analysis

Alpha diversity analysis was used to measure species richness, which refers to the number of different species present. The results showed significantly increasing observed species in soil by Vetiveri culture, which raised from 1223 to 1864 (Table 3). The addition of contaminant (20 mg/kg) into soil resulted in slight species reduction, whereas it dramatically reduced to 1620 at 60 mg/kg Cd contamination.

Studying the species evenness by Shannon, Chao1, and ACE indexes, the Cd20 sample showed a relatively higher index, while the Simpson index did not show any considerable changes. It can be concluded that the species were present in all four samples, with the highest abundance of microorganisms observed at the same level in the Cd20 sample. Taken together, the results indicate that all the studied indices are more responsive

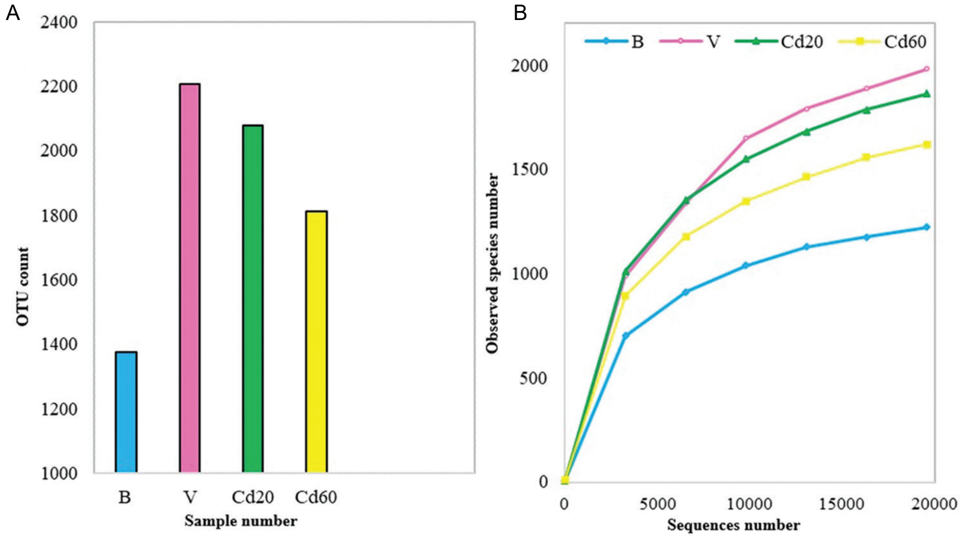

Figure 6. Soil microbial diversity under different treatment. (A) Over-the-counters count and (B) observed species number for the different soil sample types, The bacterial genus *Sphingomonas* is represented by purple bars in (A) belonging to the *Phylum Pseudomonatota*. This Phylum is synonym with *Phylum Proteobacteria* (B); (B: Clean soil without *Vetiveria zizanioides* planting; V: Clean soil in the presence of *V. zizanioides*; Cd20: Cadmium contaminated soil [20 mg/kg]; Cd60: Cadmium contaminated soil [60 mg/kg])

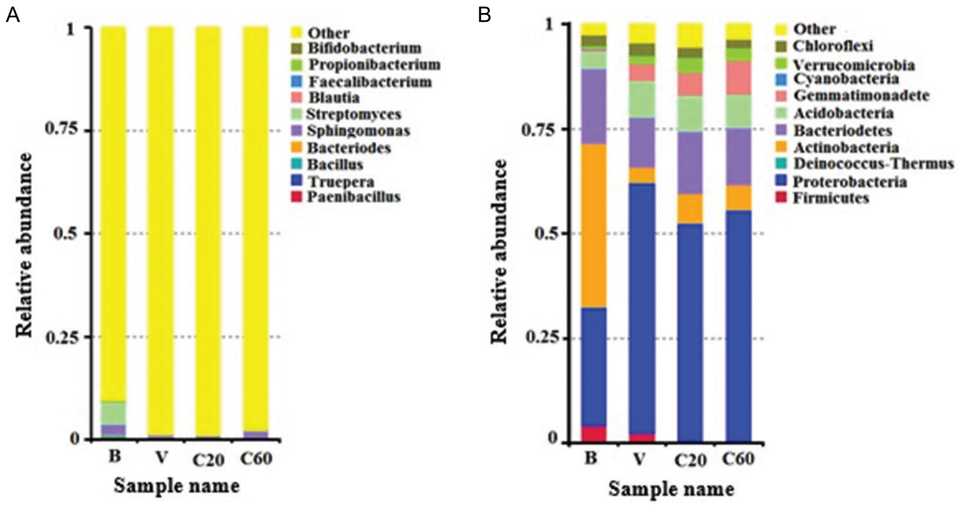

Figure 7. Species relative abundance at genus level (A) and phylum level (B). The bacterial genus *Sphingomonas* belongs to the *Pseudomonatota* phylum, which is synonymous with the *Proteobacteria* phylum. Soil treatment definitions: B: Clean soil without *Vetiveria zizanioides*; V: Clean soil in the presence of *V. zizanioides*; Cd20: Cd-contaminated soil (20 mg/kg); Cd60: Cd-contaminated soil (60 mg/kg)

Table 3. Indices of alpha diversity

Sample	Observed species	Shannon	Simpson	Chao1	ACE
B	1223	8.501	0.993	1410.789	1359.89
V	1864	9.395	0.996	2107.594	2130.127
Cd20	1853	9.473	0.997	2203.743	2164.503
Cd60	1620	9.231	0.996	1800.555	1865.044

Soil treatment definitions: B: Clean soil without *Vetiveria zizanioides*; V: Clean soil in the presence of *V. zizanioides*; Cd20: Cd-contaminated soil (20 mg/kg); Cd60: Cd-contaminated soil (60 mg/kg).

to microbial community evenness than to community richness.

3.4.2. Beta diversity analysis

PCoA was used to investigate differences in microbial community composition. Beta diversity analysis, accounting for 40.49% and 18.77% of the total variation in PC1 and PC2, respectively (Figure 8A), supported the results of the alpha diversity analysis. These findings showed that the microbial community structure of the blank sample was significantly different from that of the *V. zizanioides*-planted soils. The most exciting part of the results was the *V. zizanioides* grass controlling the negative impacts of HM on microbial diversity.

As shown in Figure 8B, the Cd20, Cd60, and V samples were distinct from the blank sample, indicating lower richness compared to the B sample. The results from unweighted UniFrac PCoA were consistent with those from the weighted UniFrac analysis, further supporting the findings from the alpha diversity analysis.

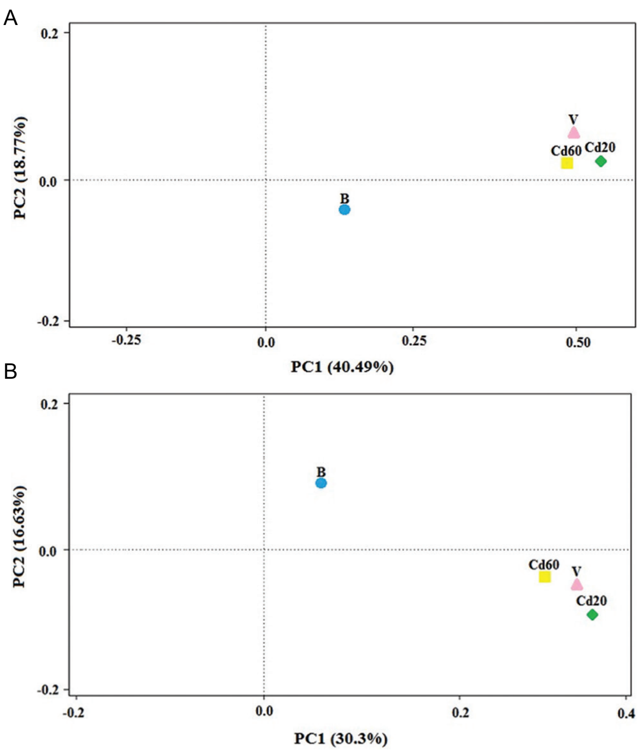

Figure 8. Two-dimensional principal coordinate analysis (PCoA) of the samples: (A) Weighted UniFrac and (B) Unweighted UniFrac PCoA. Soil treatment definitions: B: Clean soil without *Vetiveria zizanioides*; V: Clean soil in the presence of *V. zizanioides*; Cd20: Cd-contaminated soil (20 mg/kg); Cd60: Cd-contaminated soil (60 mg/kg)

4. Discussion

The results indicated that Cd in the solution had a negative impact on the dry weight of *V. Zizanioides* leaves and roots. It can be concluded that *V. zizanioides* is unable to grow effectively at high concentrations of Cd, as previously demonstrated by Liu *et al.*[35] They reported that when the Cd concentrations were >7.5 mg/L, the plant biomass decreased as Cd concentration in solution increased for both the roots and shoots. A similar pattern of reduced growth due to Cd toxicity has been observed in other species, such as *Brassica juncea* (L.) Czem, which showed a significant decrease in growth at Cd concentrations above 10 mg/L.[36] In contrast, Aibibu *et al.*[37] reported that a low concentration of Cd in the solution caused an increase in chlorophyll levels, root activity, and biomass accumulation in *V. zizanioides* after 15 days, with a 2.2% increase compared to the control group. Other studies on *Cicer arietinum* (chickpea) found no significant growth improvement in response to Cd exposure, highlighting species-specific responses to mental stress.[38] These contrasting findings highlight the varying effects of Cd at different concentrations on *V. zizanioides* growth.

Cadmium accumulation in the roots and leaves of plants can be more pronounced in those grown in contaminated soil compared to plants grown in non-contaminated soil. Yu *et al.*[39] indicated that at low cadmium concentrations (<3 mg/L), the stem tissue of *V. zizanioides* can utilize the osmosis of organic substances, such as carbohydrates and amino acids, to enhance cadmium tolerance. Interestingly, similar behavior was noted in *Helianthus annuus* (sunflower), which exhibited a stronger tolerance to Cd when root uptake was supported by osmotic adjustment mechanisms.[40] Cd accumulation in leaves did not show any considerable increase, while in roots, it was slightly increased over the experimental period. In further research, Phusantisampan *et al.*[41] stated that roots are more prone to Cd accumulation than leaves in *V. zizanioides* grass, a finding consistent with the observations for *Dittrichia viscosa*, which is also noted for efficient Cd uptake through root systems.[42]

This mechanism was observed not only in *V. zizanioides* but also in other plant-associated microbes, such as those in *C. arietinum* L., which showed increased glutathione synthesis in response to metal stress.[38] In line with this, our observation of microbial destruction at the genus level in *Actinobacteria* and *Sphingomonas* under cadmium exposure further highlights the detrimental impact of

Cd on soil microbial communities. Interestingly, the population of *Proteobacteria* was found to increase in the presence of *V. zizanioides*, indicating a potential synergy between these bacteria and the plant, similar to findings by Calcagnile *et al.*[43] for *Salix* species, which also demonstrated an increase in *Proteobacteria* under Cd stress. This finding is consistent with Long *et al.*[31] who reported that *Proteobacteria* was the dominant bacterial group in soil planted with *V. zizanioides*.

In contrast to *V. zizanioides*, *Populus* species have been found to boost the microbial diversity of beneficial fungi and bacteria in Cd-contaminated soils, a mechanism that contributes to improved bioremediation efficiency.[44] Similarly, the cultivation of *Populus* species resulted in a significant increase in beneficial microbial activity, leading to improved phytoremediation potential.[45] While the findings for *V. zizanioides* indicate that Cd negatively impacts plant growth and microbial diversity, investigations on other species like *Populus*, *Salix*, and *C. arietinum* provide additional insights into the complex interactions between plants, microbes, and HMs. Future studies should continue exploring a broader range of species to better understand the full potential of phytoremediation and bioremediation processes.

On the other hand, extrapolating results from laboratory or greenhouse experiments to field conditions is a challenging task, especially in the context of HM remediation. Controlled environments, such as pot cultures, provide valuable insights but fail to replicate the complexities of natural ecosystems, where factors such as soil heterogeneity, microbial diversity, and environmental variability influence remediation processes. Studies have shown that while laboratory conditions can demonstrate the potential for phytoremediation, field studies are crucial to account for the unpredictable variables present in natural settings.[46,47] These factors include soil type, climatic conditions, and the interaction of plant species with the environment, which can significantly affect the efficiency of HM removal.[48] Furthermore, the presence in the soil of different species of the same HM, like in the case of As, might reduce the ability of the plants due to the toxicity of the different species.[49] Therefore, for a comprehensive understanding of phytoremediation potential, it is essential to conduct field trials that incorporate these natural variables.

The management of Cd-contaminated plants and soil, particularly regarding root disposal and the prevention of soil re-contamination, have been reviewed and explained by several scientific sources and studies, as follows:

(i) Root extraction and disposal. It is recommended that if feasible, the root system of the plant should be completely removed to prevent the re-release of Cd into the environment. The roots should then be disposed of properly, for instance, by incineration at high temperatures or burial in hazardous waste landfills to ensure that Cd does not leach into other areas (Phytoremediation Resource Guide, 1999).[50]

(ii) Soil remediation after root removal. After root extraction, the soil can be treated using methods like soil washing, bioremediation with microorganisms, or the addition of amendments (e.g., biochar, compost) that can immobilize the residual Cd and reduce its bioavailability. This ensures that the remaining Cd in the soil does not become accessible to plants.[51]

(iii) Long-term monitoring. Even after root extraction and soil treatment, continuous monitoring of Cd levels in the soil is essential. Periodic soil testing will help determine if any Cd remains or if additional remediation is required to ensure soil health and prevent re-contamination. Completely removing the root system is ideal to prevent re-contamination, but if this is not possible, treating the soil and monitoring it over time for cadmium levels should be prioritized to ensure long-term soil safety.

5. Conclusion

In the present study, the effect of *V. zizanioides* on the Cd-contaminated soil microbial structure was investigated. First, the plant growth on the different Cd concentration was studied by measuring the leaves and roots dry weight. Then, *V. zizanioides* grass potential in HM removal from soil was analyzed. Finally, the impact of *V. zizanioides* grass and Cd-contamination on the microbial structure of soil was studied by NGS. The results proved that HM contamination destroys the soil biodiversity. Planting *V. zizanioides* enriches the soil microbiome and helps to reduce the negative impacts of contaminants on the microorganisms. In conclusion, the combination uses of phytoremediation and bioremediation (stimulation of the native microorganisms) provides a more efficient approach to addressing soil contamination. Although analyzing the physicochemical properties and elemental content of the soil after the experiment would have provided valuable insights, we recommend that future studies include this aspect to gain a more comprehensive understanding of the phytoremediation process.

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Conflict of interest

The authors declare that there is no conflict of interest.

Author contributions

Conceptualization: Ebrahim Alaei
Formal analysis: Ebrahim Alaei
Methodology: Aida Abdali Dehdezi, Ebrahim Alaei
Writing – original draft: All authors
Writing – review & editing: All authors

Availability of data

Data is available from the corresponding author upon reasonable request.

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