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Original Research

Utilization and Characterization of Microbes for Heavy Metal Remediation

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Abstract

Heavy metals in forest soils have substantial ecological implications, affecting the soil and the surrounding ecosystem. These naturally occurring elements, with high atomic weights, become toxic at elevated concentrations. Notable heavy metals in forest soils include lead, cadmium, mercury, and arsenic. The profound toxicity of heavy metal pollution poses a significant risk to modern agriculture, with the potential for accumulation in crops and soils, threatening food security. A crucial aspect of addressing this challenge involves promptly restoring disrupted agricultural land. This study contributes to agricultural soil restoration by employing a combination of microorganisms, which have proven effective in alleviating heavy metal pollution. When paired with Sunflower (*Helianthus annus*), the combination ensures soil restoration and enhances food security. The study investigated microorganisms from contaminated soil, revealing Gram-negative bacilli and cocci arrangements. The colony characteristics, including hues and diameters, were assessed, with notable findings in samples 1, 2, and 5. The microbial ability to remove heavy metals (Pb, As, Hg, Ni, and Cd) was quantified, highlighting the diverse capacities among isolates. Selected isolates (1, 3, 6, 7, and 10) exhibited 25% higher biomass accumulation than the control, extracting at least 40 mg/L of each metal. Bacterial identification using a Vitek 2 Compact analyzer revealed *Pantoea sp*., *Achromobacter denitrificans*, *Klebsiella oxytoca*, *Rhizobium radiobacter*, and *Pseudomonas fluorescens*. Biocompatibility testing led to the formation of consortia for soil remediation, with Coalition D (*Achromobacter denitrificans*, *Klebsiella oxytoca*, and *Rhizobium radiobacter* in a 1:1:2 ratio) confirming the effective removal of Ni and Pb. Various consortia showed differing performances in removing composite contaminants, with Coalition D being promising, indicating a potential for phytoremediation. Optimal cultivation conditions were identified, with Coalition D excelling at metal removal and biomass accumulation. The temperature, pH, and soil conditions were crucial for Coalition D efficiency, and combining them with phytoremediation techniques showed promise. Laboratory experiments with sunflower seedlings confirmed the efficacy of Coalition D in enhancing soil phytoremediation, improving plant survival, and removing mixed heavy metals.

Keywords: contamination, heavy metal, soil and ecosystem health, bioremediation, microbes

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Introduction

Heavy metals in forest soil can originate from natural geological processes such as the weathering of rocks and volcanic activity. Human activities, including mining, industrial emissions, agricultural practices, and the use of certain pesticides and fertilizers, introduce heavy metals into forest ecosystems [1]. Over time, these heavy metals accumulate in forest soils through deposition from the atmosphere or runoff from nearby contaminated areas. The accumulation depends on factors such as soil pH, organic matter content, and the specific characteristics of the heavy metals involved [2].

High concentrations of heavy metals can alter the physical, chemical, and biological properties of forest soils, affecting nutrient cycling, microbial activity, and overall soil fertility [3, 4]. Soil pH crucially determines the availability and mobility of heavy metals [5]. Plants in forest ecosystems absorb heavy metals from the soil. The extent of uptake depends on the plant species and the specific metal. Once absorbed, heavy metals accumulate in various plant tissues, potentially affecting vegetation health and the entire food web [6, 7].

Forest-dwelling organisms, including insects, fungi, and larger fauna, may be exposed to heavy metals through soil and plant pathways. Bioaccumulation in wildlife can adversely affect reproduction, growth, and overall health. Heavy metals can leach from forest soils into groundwater or surface water, impacting water quality [8] and posing downstream risks to aquatic ecosystems and human water supplies.

Sustainable forest management practices can minimize the introduction of heavy metals [9, 10]. Remediation techniques, such as phytoremediation (using plants to extract or stabilize contaminants) and soil amendments, can mitigate the impact of heavy metals [11].

Monitoring and comprehending the dynamics of heavy metals in forest soils are essential for sustainable ecosystem management, conservation, and the protection of human and wildlife health [12]. Heavy metal toxicity in soil and plants has significant implications for human health because these elements can accumulate in consumable crops, entering the human food chain [13]. This contamination directly threatens human well-being through various pathways, such as the consumption of tainted food and water and the inhalation of polluted air [14]. This study examined the adverse effects of heavy metal toxicity in soil and plants on human health.

The challenges posed by rapid population growth and industrialization necessitate a comprehensive strategy considering social justice, technological innovation, environmental sustainability, and international cooperation [15]. Sustainable farming practices must align with prudent land use and resource management to ensure long-term global food security [16]. On the other hand, the escalating human load, which diminishes soil fertility by depleting nutrients and organic matter while introducing contaminants such as pesticides, heavy metals, and hydrocarbons, threatens these endeavors [17].

When plants absorb heavy metals from contaminated soil, these toxic elements concentrate in the edible parts, such as fruits, vegetables, and grains [18], leading to chronic exposure and potential adverse health effects. Common toxic heavy metals include lead, cadmium, mercury, and arsenic, which can cause various health issues, including neurological disorders, kidney damage, cardiovascular problems, and cancer [19, 20]. Children, pregnant women, and the elderly are particularly vulnerable because of their developing bodies or weakened immune systems [21].

The bioaccumulation of heavy metals in the human body over time suggests that even low levels of exposure can lead to long-term health problems [22, 23]. In addition, heavy metals can undergo biomagnification in the food chain, posing higher exposure risks for individuals consuming animal products from contaminated areas [24].

Mitigating the adverse health effects of heavy metal toxicity involves improving soil and water quality through remediation techniques [25], implementing strict industrial and agricultural regulations [26], and promoting awareness and safe practices among vulnerable communities [27]. Addressing heavy metal contamination in soil and plants is vital for safeguarding human health and ensuring food safety [28]. Global concerns regarding heavy metal poisoning in agricultural areas are escalating [29, 30]. As and Hg, often originating from industrial facilities, along with metals such as Cd, Pb, Zn, and Cu from fertilizers, including organic ones [31], contribute to soil contamination. These persistent metals alter the biochemical processes crucial for crop production, posing risks through bioaccumulation and biomagnification in the food chain [32]. Agricultural and industrial practices, including transportation, garbage burning, mining, and oil spills, intensify heavy metal pollution in soils [33].

Agricultural practices introduce heavy metals through excessive fertilizer and pesticide use, harming the environment by affecting plant growth [34]. Removing heavy metals from soil involves physicochemical and biological techniques. Although physicochemical methods can significantly alter soil quality, they are expensive and labor-intensive. Alternatively, biological techniques, such as bioremediation, utilizing microorganisms and plants (phytoremediation), provide a more economical and environmentally friendly alternative [35, 36]. Bioremediation, emphasizing microbial activity, shows promise in removing heavy metals through various methods [37].

Microorganisms are crucial in soil restoration, and selecting resilient strains is vital. Proteobacteria show resilience to lead and zinc, while Bacteroidetes and Firmicutes are suitable for arsenic-contaminated environments [38, 39]. The oxidation states of heavy metals, such as Cu, Se, Pb, Cr, As, and Ni, affect their mobility and toxicity [40, 41]. Microorganisms can alter these states through enzymatic transformations, with certain bacteria displaying high biosorption capacities [42, 43]. Hence, using microbes and plants together is a practical strategy for influencing metal phosphates [44],

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producing chelating agents, and causing redox shifts that affect heavy metal mobility [45, 46]. Sunflower (*Helianthus annuus*) is a viable option for improving agricultural soils, demonstrating resistance to heavy metal toxicity and effective detoxification of contaminated sites through its concentration of heavy metals in the root system [47-52].

Material and Methods

Microorganisms were cultured in a liquid medium enriched with heavy metals (0.4 g KH_2PO_4, 3.0 g $(NH4)_2SO_4$, 3.0 g $Na_3C_6H_5O_7$, 0.01 g $CuSO_4$, 0.005 g $ZnSO_4$, 0.001 g $FeSO_4$, 0.2 g $CdCl_2$, 0.5 g $MgSO_4$, 0.1 g $MnSO_4$, 8.0 g peptone, 5.0 g maltose, 20.0 g agar-agar, and 1 L water). Subsequently, microorganisms were retrieved after being cultured. One gram of soil was added to 100 mL of sterile medium, and the mixture was agitated for 24 hours at 25 °C and 100 rpm in an LSI-3016A shaker–incubator [2, 3]. Pure isolates were produced by seeding 1 mL of the liquid culture onto a solid medium of the same composition and incubating it for 24 hours at 25 °C in a TSO–1/80 SPU thermostat. The morphological traits were determined using single colonies produced on ordinary agar via the streak plate method. The Gramme method describes the biochemical profile of the cell wall, and an Axio Scope A1 microscope (Zeiss, Jena, Germany) was used to observe the samples. The samples were tested for their capacity to take up heavy metals from the nutrient medium while retaining biomass growth compared to the control (no heavy metals) to identify promising strains. The consortia were formed based on biocompatibility testing, and their capacity to absorb heavy metals and combinations was evaluated [2, 4]. After selecting a consortium for soil purification, its effect on plant growth and development was examined, particularly through the synthesis of phytohormones, including indoleacetic acid.

Isolated microorganism inoculums were added to beef extract broth to assess potential metal uptake (Pb, Ni, As, Cd, and Hg). Flasks were incubated in an LSI-3016A shaker at 100 rpm and 25 °C for five days after adding metal salts (Na_3AsO_4, $Pb(NO_3)_2$, $Ni(NO_3)$, $Cd(NO_3)_2$, and $Hg(NO_3)_2$) at 1 mg/mL. The residual heavy metals were analyzed using an ICPE-9820, Shimadzu, Kyoto, Japan, atomic emission spectrometer with standard metal ion reductions as reference samples. After centrifuging the culture medium, the supernatant was hydrolyzed and treated with concentrated HNO_3. The microbial suspension was added to the beef extract broth, and working solutions of Pb, Ni, As, Cd, and Hg were added to assess the absorption capability. The conditions were consistent in both substance analysis and cultivation. After centrifuging the culture liquid for 15 minutes at 8000 rpm to assess biomass accumulation, the biomass was dried in an oven at 60°C until a consistent weight was reached. The isolates were grown at 25°C for 18–72 hours and identified using a Vitek 2 Compact automatic microbiological analyzer. The microbiological suspension was prepared according to the McFarland density scale, and a Densi CHEK plus densimeter quantified the optical density. The co-cultivation approach evaluated biocompatibility by growing microbial cultures on beef extract broth and applying the test culture to plain agar [6, 7]. The total indole-3-acetic acid content in the consortia was calculated, and HPLC registered the amount of indole-3-butyric acid. Sunflower was used to examine the capacity of Coalition D to improve phytoremediation efficiency. Heavy metal salts were added to the soil, which was then spread over Petri plates. Two methods of treating seeds were tested: soaking them in water or Coalition D, seeding them on Petri dishes, and providing them with water [27]. An atomic emission spectrometer measured the remaining metals in the soil after ten days of germination. Every experiment was run in triplicate, and statistical analysis was conducted using Microsoft Office 2007 (excel sheet analysis) with a t-test.

Statistical Analysis

Data were statistically analyzed using the method reported elsewhere [31], and means were compared with the LSD test ($P < 0.05$). The significance of the main effects and treatments in a control vs. rest comparison was determined through ANOVA. A two-way ANOVA compared the treated conditions (Factors A, B, and C) with the non-treated plots (control vs. rest) and assessed their significance relative to the control. The ANOVA output commonly presents the main effect of the "control vs. rest" factor.

Results and Discussion

Microorganism Isolation and Characteristics

Experimental findings revealed that all identified microorganisms were Gram-negative bacilli, typically occurring singly or in pairs, except for sample 8, where cocci were found arranged in chains resembling streptococci. The colonies exhibited a variety of colors, including yellow, orange, light brown, or light beige, with samples 1, 2, and 5 appearing predominantly yellow [28, 29]. Samples 4 and 6 displayed the largest colony diameters, ranging from 2.0 to 3.5 mm and 2.1 to 2.8 mm, respectively [30, 31]. Notably, samples 1, 2, and 5 were consistently distinguished by their yellow hue. The ability of the microorganisms to acquire biomass and eliminate specific heavy metals, including Pb, As, Hg, Ni, and Cd, varied among isolates (Table 1). The selected isolates (1, 3, 6, 7, and 10) showed 25% higher biomass accumulation than the control, extracting at least 40 mg/L of each metal. Bacterial identification using a Vitek 2 Compact analyzer revealed *Pantoea sp.*, *Achromobacter denitrificans*, *Klebsiella oxytoca*, *Rhizobium radiobacter*, and *Pseudomonas fluorescens*. These isolates were further tested for biocompatibility to form consortia for soil remediation. Coalition D, with *Achromobacter denitrificans*, *Klebsiella oxytoca*, and

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Table 1. Deposition of various heavy metals and biomass in an environment of each metal.

	Deposition of various heavy metals concentrations, g/L					
	Cd	Ni	Hg	As	Pb	Control (No Metal)
1	0.88 ± 0.06	0.82 ± 0.05	0.68 ± 0.05	0.75 ± 0.04	0.86 ± 0.07	0.89 ± 0.07
2	0.52 ± 0.04	0.86 ± 0.05	0.20 ± 0.04	0.24 ± 0.05	0.85 ± 0.07	0.81 ± 0.05
3	0.85 ± 0.07	0.91 ± 0.06	0.75 ± 0.05	0.75 ± 0.06	0.84 ± 0.07	0.91 ± 0.05
4	0.56 ± 0.04	0.56 ± 0.03	0.53 ± 0.04	0.65 ± 0.04	0.64 ± 0.05	0.91 ± 0.05
5	0.27 ± 0.03	0.43 ± 0.02	0.42 ± 0.04	0.35 ± 0.04	0.54 ± 0.04	0.93 ± 0.05
6	0.84 ± 0.05	0.76 ± 0.05	0.83 ± 0.03	0.94 ± 0.04	0.64 ± 0.06	0.90 ± 0.05
7	0.75 ± 0.05	0.91 ± 0.06	0.64 ± 0.05	0.74 ± 0.05	0.83 ± 0.07	0.88 ± 0.05
8	0.38 ± 0.03	0.58 ± 0.03	0.43 ± 0.06	0.24 ± 0.04	0.34 ± 0.03	0.88 ± 0.05
9	0.20 ± 0.02	0.57 ± 0.03	0.74 ± 0.07	0.15 ± 0.04	0.34 ± 0.03	0.84 ± 0.05
10	0.80± 0.05	0.71± 0.08	0.65± 0.06	0.95± 0.06	0.75± 0.07	0.97± 0.06

Rhizobium radiobacter at a (1:1:2) ratio, demonstrated effective removal of Ni and Pb. The study explored various Coalition D alternatives based on biocompatibility tests, with Coalition D showing promising properties for further investigation and consideration in soil remediation scenarios. The study highlights the potential use of Achromobacter sp. for the bioremediation of heavy metal-contaminated soils, highlighting its ability to immobilize and remove divalent cadmium [53, 54]. *Achromobacter denitrificans* was reported to produce phytohormones, organic acids, siderophores, and ammonia, highlighting its multifunctional capabilities [55]. Klebsiella bacteria, including *Klebsiella oxytoca isolates*, showed resistance against various heavy metals [56]. Certain Rhizobium species, in addition to having heavy metal resistance, can act as biofertilizers because of their nitrogen-fixing capacity [39, 57].

Heavy Metal Removal Efficiency

Due to the predominant presence of Gram-negative bacilli arrangements among the isolated microorganisms, the colonies presented a diverse array of hues, encompassing tones of yellow, orange, light brown, or light beige, with samples 1, 2, and 5 manifesting a predominant yellow coloration. Samples 4 and 6 were distinguished by their conspicuous colony diameters. Conversely, sample 8 deviated from this pattern, exhibiting cocci akin to those resembling streptococci. The microbial ability to remove heavy metals, as detailed in Table 2, varied among isolates. Samples 1, 3, and 7 showed the highest Pb removal, ranging from 22.04 mg/L to 74.26 mg/L. Selected isolates (1, 3, 6, 7, and 10) showed 25% higher biomass accumulation than the control, extracting a minimum of 40 mg/L of each metal. Bacterial identification using a Vitek 2 Compact analyzer revealed *Pantoea sp*., *Achromobacter denitrificans*, *Klebsiella oxytoca*, *Rhizobium radiobacter*, and *Pseudomonas fluorescens*.

The biocompatibility testing led to the formation of consortia, with Coalition D exhibiting impressive results in removing Ni (98.22 mg/L) and Pb (91.13 mg/L). On the other hand, mercury adversely affected biomass accumulation. Various consortia of alternatives were explored based on biocompatibility tests (Fig. 1). Coalition D, with a ratio of *Achromobacter denitrificans*, *Klebsiella*

Table 2. Various Heavy metals Absorption (mg/L) absorption by the individual microbe.

Microorganism Isolates	Various Heavy metals Absorption, mg/L				
	Hg	Ni	Cd	As	Pb
1	45.44±2.23	48.10±2.36	57.51±2.65	37.44±1.65	62.15±3.17
2	13.32±0.96	47.41±2.04	19.14±1.14	25.76±1.03	46.02±2.25
3	73.08±3.82	53.32±2.29	62.41±2.99	40.03±1.60	73.10±2.55
4	41.44±1.70	36.08±1.62	38.50±1.85	33.21±1.43	34.76±1.77
5	21.46±1.01	39.74±1.22	16.72±0.87	28.47±1.31	36.02±1.66
6	51.33±2.31	47.32±2.13	78.02±3.67	44.37±1.86	51.14±2.35
7	61.57±3.16	56.96±2.90	41.65±2.04	59.52±2.86	74.26±3.42
8	32.34±1.68	48.19±1.97	29.97±1.44	24.57±1.03	25.11±1.16
9	64.38±2.64	43.50±2.11	11.84±0.50	10.36±0.41	22.04±1.06
10	56.16±2.75	43.32±2.21	53.46±2.19	43.45±2.16	46.36±2.04

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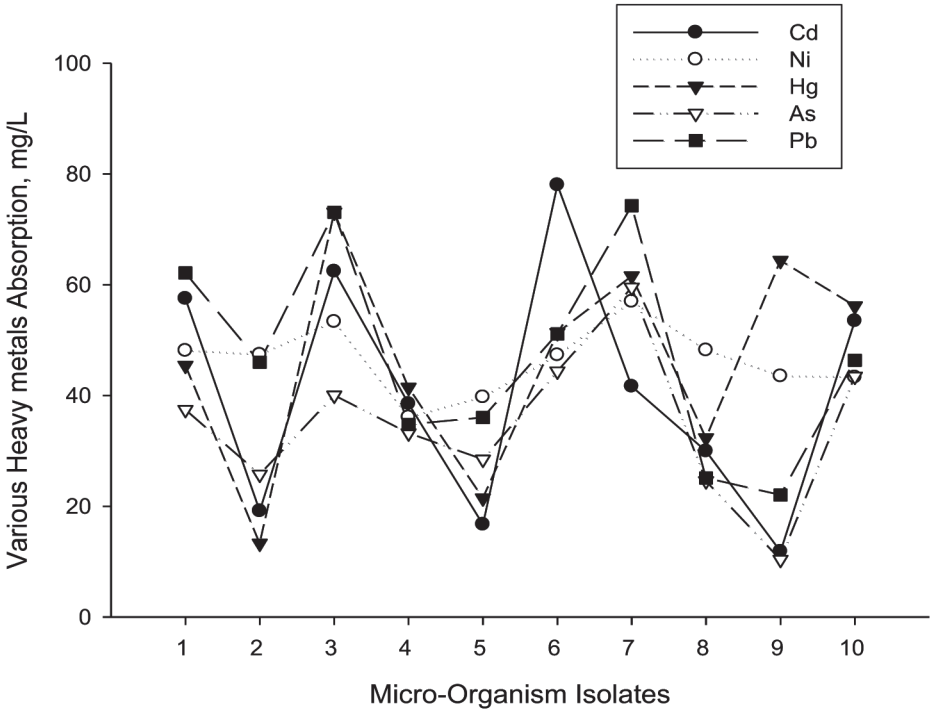

Fig. 1. Various heavy metals (Cd, Ni, Hg, As, and Pb) trapped by different microorganisms.

oxytoca, and *Rhizobium radiobacter* (1:1:2), demonstrated effectiveness in removing heavy metals and showed promise in mixed heavy-metal contamination scenarios. The ability of Coalition D to synthesize phytohormones was highlighted, making Coalition D a promising choice (Table 3). Optimal cultivation conditions were identified, with temperature, pH, and soil conditions crucial for the efficiency of Coalition D. Coalition D, when combined with phytoremediation techniques, showed promise in enhancing plant survival and removing heavy metals from contaminated soil. Laboratory experiments with Sunflower seedlings confirmed the efficacy of Coalition D in enhancing soil phytoremediation**.** The study emphasizes the significant threat of heavy metal pollution to modern agriculture, jeopardizing food security [58]. The assembly of Coalition D microorganisms isolated from technogenic areas, particularly Coalition D, showed promise in addressing heavy metal pollution in agriculture and ensuring soil restoration and food security [59]. The effectiveness of Coalition D in removing heavy metals from contaminated soils, its ability to synthesize phytohormones, and its application in phytoremediation techniques make it a valuable solution for sustainable soil restoration in agriculture. *Achromobacter denitrificans*, *Klebsiella oxytoca*, and *Rhizobium radiobacter* efficiently remove heavy metals, such as Cd, Hg, As, Pb, and Ni [60]. Coalition D was designed to combat Pb, As, Hg, Ni, and Cd simultaneously, highlighting its effectiveness in mixed heavy-metal contamination scenarios. The collaborative approach involving *Pseudomonas fluorescens*, *Pantoea sp*., *Achromobacter denitrificans*, *Klebsiella oxytoca*, *Rhizobium radiobacter*, and *Rhizobium radiobacter* is a comprehensive method for heavy metal removal [14].

Table 3. Synthesis of phytohormones by consortia.

	Indole-3-Acetic Acid, µg/mL of Nutrient Medium	Indole-3-Butyric Acid, µg/mL of Nutrient Medium
A	IA13.63±0.64	IA1.92±0.05
B	IA12.32±0.54	IA0.62±0.05
C	IA12.12±0.45	IA0.94±0.04
D	IA16.03±0.94	IA2.02±0.06

Biomass Accumulation and Coalition D Formation

The chosen isolates had a 25% higher biomass accumulation rate than the control and could extract at least 40 mg/L of each specific metal. Samples 1, 3, 6, 7, and 10 contained these bacteria. Fig. 2 presents various consortia of alternatives depending on the results of biocompatibility tests. A comprehensive and collaborative method for removing heavy metals from contaminated soils is shown (Table 4), using a variety of microbes, including *Pseudomonas fluorescens*, *Pantoea sp*., *Achromobacter denitrificans*, *Klebsiella oxytoca*, *Rhizobium radiobacter*, and *Rhizobium radiobacter*. The biomass acquisition and heavy metal removal abilities of the microbes varied. Samples 1, 3, and 7 showed the highest Pb removal. Selected isolates showed 25% higher biomass accumulation than the control, extracting at least 40 mg/L of each metal. Bacterial identification

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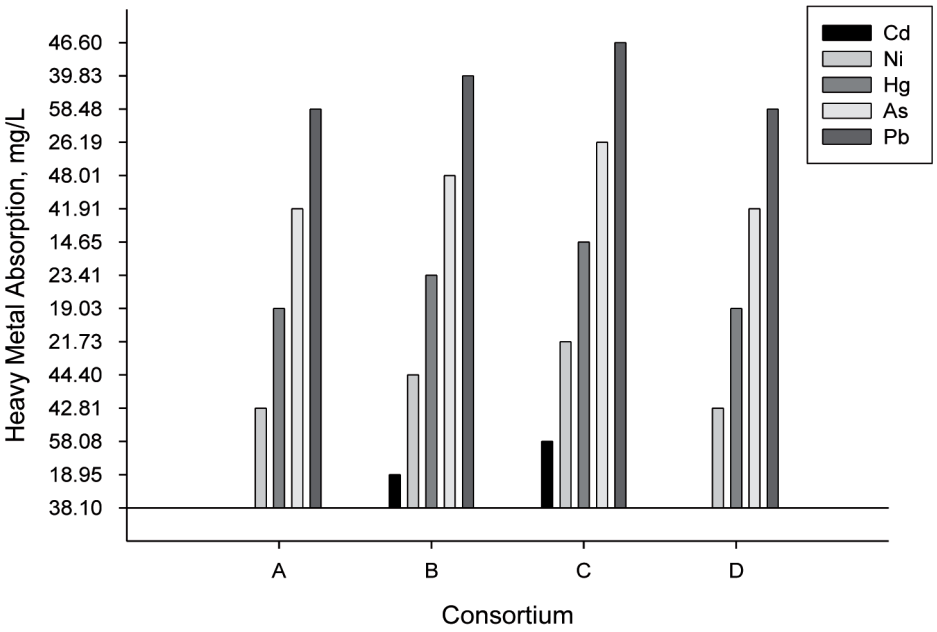

Fig. 2. Coalition D absorption efficiency to different heavy metals.

Table 4. Biomass accumulation and total absorption of mixture metals by various coalition. D.

Coalition D	Heavy Metal Absorption, mg/L					Biomass Accumulation, g/L	
	Cd	Ni	Hg	As	Pb	Composite pollutants	Control
A	38.10±1.87	42.81±2.10	19.03±0.93	41.91±2.06	58.48±2.87	0.86±0.06	0.89±0.07
B	18.95±4.12	44.40±4.04	23.41±2.33	48.01±2.60	39.83±3.72	0.82±0.07	0.90±0.07
C	58.08±2.85	21.73±1.07	14.65±0.72	26.19±1.28	46.60±2.29	0.84±0.06	0.87±0.07
D	38.10±1.87	42.81±2.10	19.03±0.93	41.91±2.06	58.48±2.87	0.86±0.06	0.89±0.07

revealed *Pantoea sp*., *Achromobacter denitrificans*, *Klebsiella oxytoca*, *Rhizobium radiobacter*, and *Pseudomonas fluorescens*. The selected isolates, *Pantoea sp*., *Achromobacter denitrificans*, *Klebsiella oxytoca*, *Rhizobium radiobacter*, and *Pseudomonas fluorescens*, underwent biocompatibility testing to form consortia for soil remediation. Coalition D, containing *Achromobacter denitrificans*, *Klebsiella oxytoca*, and *Rhizobium radiobacter* at a 1:1:2 ratio, effectively removed Ni and Pb. Various consortia performed differently, with Coalition D showing promise. The ability of a consortium to synthesize phytohormones has the potential for phytoremediation. Optimal cultivation conditions were identified, with Coalition D excelling at metal removal and biomass accumulation. The temperature, pH, and soil conditions are crucial for the efficiency of Coalition D. The study explored various Coalition Ds for heavy metal removal, with Coalition D being effective. Laboratory experiments with Sunflower seedlings confirmed the efficacy of Coalition D in enhancing soil phytoremediation, improving plant survival, and removing mixed heavy metals. The combination of Achromobacter, Rhizobium, and Klebsiella is proposed for agricultural use to remove heavy metals and promote plant development in contaminated areas [21, 61]. The Coalition D formed within the parameters of the project, particularly Coalition D, shows promise for heavy metal removal (Fig. 3) and plant development in mixed pollution settings [13, 43]. Plant inoculation with PGPR Variovorax NB24 increased nickel accumulation in the roots and aerial parts, reducing heavy metal-induced oxidative stress and increasing biomass [15, 47].

Evaluation of Coalition D Performance

The ability of the microbes to accumulate biomass and remove specific heavy metals (Table 4) highlights a range of removal capacities among isolates. The selected isolates (1, 3, 6, 7, and 10) demonstrated 25% higher biomass accumulation than the control, extracting at least 40 mg/L of each metal. Bacterial identification using a Vitek 2 Compact analyzer revealed *Pantoea sp*., *Achromobacter denitrificans*, *Klebsiella oxytoca*, *Rhizobium radiobacter*, and *Pseudomonas fluorescens*.

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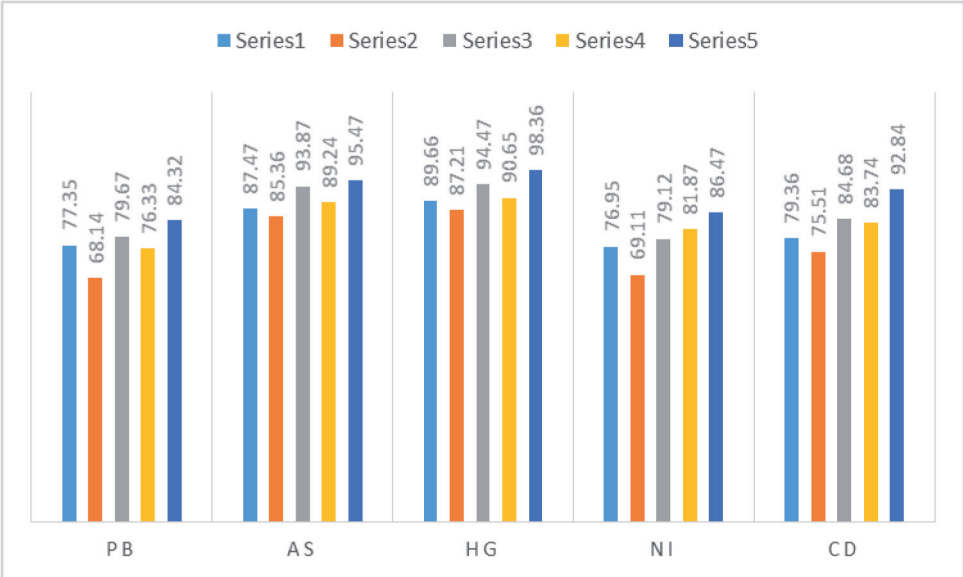

Fig. 3. Metal residues in soil and the effectiveness of soil phytoremediation when subjected to treatment with Coalition D.

Biocompatibility testing guided the formation of the consortium for soil remediation. Coalition D, with a 1:1:2 ratio of *Achromobacter denitrificans*, *Klebsiella oxytoca*, and *Rhizobium radiobacter*, effectively removed Ni and Pb (Fig. 3). Various consortia showed differential performance in removing composite contaminants, with Coalition D showing promise. The ability of the consortia to synthesize phytohormones indicated the potential for phytoremediation. Optimal cultivation conditions, which are crucial for the efficiency of Coalition D, were identified, with Coalition D excelling at specific metal removal and biomass accumulation. The temperature, pH, and soil conditions played vital roles, and combining them with phytoremediation techniques proved promising. Laboratory experiments with Sunflower seedlings confirmed the efficacy of Coalition D in enhancing soil phytoremediation, improving plant survival, and removing mixed heavy metals. The study explored various Coalition D alternatives based on biocompatibility test results. Coalition D, comprising *Achromobacter denitrificans*, *Klebsiella oxytoca*, and *Rhizobium radiobacter* in a 1:1:2 ratio, showed impressive results in removing Ni (98.22 mg/L) and Pb (91.13 mg/L). On the other hand, mercury adversely affected biomass accumulation. In composite heavy-metal contamination scenarios, Coalition D exhibited significant effectiveness, highlighting its promise for soil cleanup in agricultural areas. The production of indole-3-acetic and indole-3-butyric acids by the consortia can potentially enhance phytoremediation. The temperature and pH conditions crucial for bacterial growth were identified, with 25–30°C considered ideal for Coalition D cultivation and pH 7 optimal for growth. The application of Coalition D was limited in highly acidic and alkaline soils. Combining Coalition D with phytoremediation techniques showed promise for enhancing plant survival and removing heavy metals from contaminated soil. Seed treatment with Coalition D improved soil phytoremediation efficiency significantly, resulting in the removal of Pb (32%), As (15%), Hg (13%), Ni (31%), and Cd (25%). Despite the lower removal rates compared to the nutritional medium, the seed treatment showed higher phytoremediation effectiveness than the control techniques, offering potential benefits for plant survival under heavy metal-induced stress. Further research will be needed for comprehensive validation and practical applications in contaminated soil remediation [45]. The efficiency of Coalition D in removing heavy metals, such as Pb, As, Hg, Ni, and Cd, in mixed contamination scenarios is emphasized [17, 27]. This Coalition D, with specific ratios of *Achromobacter denitrificans*, *Klebsiella oxytoca*, and *Rhizobium radiobacter*, has significant promise in reducing heavy metal levels in the soil.

Synergy with Phytoremediation Techniques

During cultivation in media contaminated with the composite, none of the Coalition D showed a discernible decrease in biomass accumulation. Many studies have highlighted the use of phyto- and bioremediation techniques in tandem. Some microbes can increase plant survival rates while eliminating heavy metals from the environment. The potential of Coalition D for phytoremediation has been studied because it can synthesize phytohormones. All the isolated microorganisms exhibited characteristics of Gram-negative bacilli, except for sample 8, which showed cocci in chains similar to streptococci. Colonies displayed various hues, such as yellow, orange, light brown, or light beige, with samples 1, 2, and 5 standing out as yellow. The highest colony diameters were observed in samples 4 and 6. Table 4 lists the ability of the microbes to accumulate biomass and remove specific heavy metals, with samples 1, 3, and 7 showing the highest Pb removal. Selected isolates (1, 3, 6, 7, and 10) exhibited 25% higher biomass accumulation than the control,

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extracting at least 40 mg/L of each metal. Bacterial identification using a Vitek 2 Compact analyzer revealed *Pantoea sp*., *Achromobacter denitrificans*, *Klebsiella oxytoca*, *Rhizobium radiobacter*, and *Pseudomonas fluorescens*. Biocompatibility testing led to the formation of consortiums for soil remediation. Coalition D, with a 1:1:2 ratio of *Achromobacter denitrificans*, *Klebsiella oxytoca*, and *Rhizobium radiobacter*, effectively removed Ni and Pb. Various consortia performed differently in removing the composite contaminants, with Coalition D showing promise. The ability of Coalition D to synthesise phytohormones indicated the potential for phytoremediation. The study assessed various Coalition Ds for heavy metal removal, with Coalition D being effective. Coalition D B exhibited efficient mercury removal, while Coalition D and C excelled in Cd extraction. The composite heavy-metal contamination removal rates were generally less successful than single-contaminant scenarios. The phytoremediation potential was highlighted, especially with Coalition D, which synthesized phytohormones. The optimal cultivation conditions were identified, with Coalition D excelling at specific metal removal and biomass accumulation. The temperature, pH, and soil conditions were crucial for the efficiency of Coalition D, and their combination with phytoremediation techniques showed promise. Laboratory experiments with Sunflower seedlings confirmed the efficacy of Coalition D in enhancing soil phytoremediation, improving plant survival, and removing mixed heavy metals. A seed treatment with Coalition D improved the soil phytoremediation efficiency, resulting in the removal of Pb (32%), As (15%), Hg (13%), Ni (31%), and Cd (25%). Despite lower removal rates than the nutritional medium, the seed treatment demonstrated higher phytoremediation effectiveness than the control techniques, offering potential benefits for plant survival under heavy metal-induced stress. Combining phyto- and bioremediation techniques is suggested as a practical and sustainable approach. The Coalition D of microbes, when used with Sunflower (Helianthus annuus), decreased the levels of Pb, As, Hg, Ni, and Cd significantly in the soil [11]. Phytoremediation involving the injection of Pseudomonas koreensis AGB-1 into Miscanthus sinensis A. was reported to enhance the solubilization and availability of heavy metals in the plant rhizosphere, leading to a decrease in heavy metal-induced oxidative stress and an increase in biomass [13, 24, 31].

Optimization of Cultivation Conditions

Temperature and pH are also important for the growth of bacteria. A good choice of these factors lowers industrial production costs while enhancing bioremediation. In particular, Coalition D, which was the best at eliminating specific Pb, Ni, As, and Cu pollutants, had excellent performance against mixed heavy-metal pollution under optimal cultivation conditions and is the leader in the production of indole-3-acetic and indole-3-butyric acids. The microbial ability to acquire biomass and eliminate specific heavy metals, detailed in Table 3, varied among isolates, with samples 1, 3, and 7 showing the highest Pb removal. Selected isolates (1, 3, 6, 7, and 10) exhibited 25% higher biomass accumulation than the control, extracting at least 40 mg/L of each metal.

The identities of these isolates determined using a Vitek 2 Compact analyzer were *Pantoea sp*., *Achromobacter denitrificans*, *Klebsiella oxytoca*, *Rhizobium radiobacter*, and *Pseudomonas fluorescens*. Biocompatibility testing formed consortia for soil remediation, with Coalition D showing the effective removal of Ni and Pb. The effectiveness of the Coalition D in removing a range of heavy metals, both individually and in mixed contamination scenarios, was notable. Using Coalition D in phytoremediation with Sunflower is a sustainable solution for agricultural areas. The Coalition D showed the ability to significantly lower the levels of Pb, As, Hg, Ni, and Cd in the soil, indicating potential benefits for soil restoration and food security [17, 21, 23]. Further research is needed for comprehensive validation and practical applications. Additional research will be needed to enhance the cultivation conditions, select suitable carriers, and understand the processes integral to heavy-metal bioremediation. This is crucial for efficiently utilizing the developed Coalition D in treating industrial water and wastewater contaminated with heavy metals [30]. Several consortia have exhibited promising characteristics, warranting thorough exploration and consideration, particularly in addressing heavy metal pollution in soil.

Enhancement of Phytoremediation Efficiency

In the laboratory, Sunflower seedlings were used to examine the efficacy of Coalition D in improving soil phytoremediation efficiency (Table 5). A pre-sowing seed treatment (soaking), particularly at a concentration of 2.5 in accordance with the McFarland standard, was the

Table 5. Efficiency of soil phytoremediation when treated with Coalition D.

Residual Content Metal in Soil, %					Average Survival Rate of Seedling
Pb	As	Hg	Ni	Cd	
77.35±3.01	87.47±2.31	89.66±2.27	76.95±2.21	79.36±2.08	8±1
68.14±2.96	85.36±1.84	87.21±2.73	69.11±2.85	75.51±2.16	9±2
79.67±2.71	93.87±2.98	94.47±1.32	79.12±3.03	84.68±2.48	7±2
76.33±1.99	89.24±2.04	91,36±2.62	81,87±2.28	83.74±1.74	9±1
84.32 ± 2.21	95.47 ± 1.72	98.36 ±2.14	86.47 ± 2.55	92.84 ± 1.89	5 ± 2

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most successful application of Coalition D. With 70% to 90% of seedlings surviving under heavy metal-induced stress, the use of Coalition D has promise for enhancing plant survival. The ability of microbes to acquire biomass and eliminate specific heavy metals was detailed, with samples 1, 3, and 7 demonstrating notable Pb removal [33]. Selected isolates (1, 3, 6, 7, 10) exhibited 25% higher biomass accumulation than the control, extracting at least 40 mg/L of each metal. Bacterial identification revealed *Pantoea sp*., *Achromobacter denitrificans* [17], *Klebsiella oxytoca* [29], *Rhizobium radiobacter* [13], and *Pseudomonas fluorescens* [15]. Biocompatibility testing led to the formation of consortia for soil remediation, with Coalition D (*Achromobacter denitrificans*, *Klebsiella oxytoca*, and *Rhizobium radiobacter* in a 1:1:2 ratio) demonstrating effective Ni and Pb removal. Various consortia alternatives were explored based on biocompatibility test results [35]. The effectiveness of Coalition D, especially in mixed heavy-metal contamination scenarios, was highlighted. Four different consortia were formed with *Achromobacter denitrificans*, *Klebsiella oxytoca*, and *Rhizobium radiobacter* ratios of 1:1:1, 2:1:1, 1:2:1, and 1:1:2. Coalition D exhibited impressive results in removing Ni (98.22 mg/L) and Pb (91.13 mg/L).

On the other hand, its biomass accumulation was retarded significantly in the presence of mercury. The efficiency of various consortia in eliminating specific heavy metals and building up biomass was evaluated [32, 36]. Coalition D showed promising results for composite pollution removal, making it a potential choice for addressing composite heavy-metal contamination. The ability of Coalition D to synthesize phytohormones, particularly indole-3-acetic and indole-3-butyric acids, was explored (Table 3). Combining Coalition D with phytoremediation techniques has great promise for removing heavy metals and enhancing plant survival (Table 5). The ideal cultivation conditions for Coalition D were identified, with temperature, pH, and soil conditions playing crucial roles. Coalition D demonstrated minimal biomass accumulation under highly acidic and highly alkaline conditions. The laboratory experiments with Sunflower seedlings confirmed that pre-sowing seed treatment (soaking) with Coalition D significantly improved the soil phytoremediation efficiency. This approach removed approximately 32% Pb, 15% As, 13% Hg, 31% Ni, and 25% Cd from the soil. Although removal rates were lower than the nutritional medium, seed treatment with Coalition D showed higher phytoremediation effectiveness than the control techniques. The survival rate of seedlings under heavy metal-induced stress ranged from 70% to 90%, indicating the potential of Coalition D for enhancing plant survival. Further research will be needed to validate these findings and explore practical applications in contaminated soil remediation. Efforts to explore and deepen the understanding of pollutant removal processes in heavy-metal bioremediation research are emphasized. This study suggests that a deeper comprehension of the procedures involved will increase the efficiency of cleaning contaminated environments [31]. The text provides a comprehensive overview of the negative impacts of heavy metal toxicity in soil and plants on human health. Elevated levels of lead, cadmium, mercury, and arsenic can lead to chronic exposure and cause various health problems, including neurological disorders, kidney damage, cardiovascular issues, and cancer. Children, pregnant women, and the elderly are particularly vulnerable populations. Specific negative impacts of common heavy metals are outlined. Elevated lead levels can impair plant growth and render soil unsuitable for agriculture. Cadmium readily accumulates in edible plant parts, posing health risks. High levels of mercury can have phytotoxic effects, and arsenic can interfere with nutrient uptake in plants. Excessive copper, zinc, nickel, and chromium levels can be toxic to plants and affect soil health.

Conclusions

The experiential findings revealed that *Achromobacter denitrificans* demonstrated efficient removal of heavy metals, utilizing 41.02 mg/L As and effectively removing Hg (72.07 mg/L), Pb (70.1 mg/L), and Cd (62.39 mg/L). *Klebsiella oxytoca* exhibited substantial Cd (78.03 mg/L), Hg (52.32 mg/L), and Pb (52.13 mg/L) removal, while *Rhizobium radiobacter* showed the highest Ni (56.94 mg/L), Hg (61.57 mg/L), and Pb (74.26 mg/L) removal. The microbial strains were biocompatible, forming consortia, particularly Coalition D and C, which exhibited significant phytohormone synthesis. Coalition D, characterized by a 1:1:2 ratio of *Rhizobium radiobacter*, *Klebsiella oxytoca*, and *Achromobacter denitrificans*, extracted heavy metals from individual and composite pollutants, demonstrating removal capacities at concentrations between 47.33 and 83.26 mg/L. In particular, Coalition D maintained biomass accumulation under diverse heavy-metal conditions, with an ideal range of pH 7 and 25–30°C. This underscores the potential of Coalition D in enhancing phytoremediation for mixed pollution scenarios, suggesting its applicability for promoting plant growth in disturbed regions.

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Conflict of Interest

The authors declare no conflict of interest.

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