

Site Remediation of Multiple Heavy Metals Contaminated Iron Tailings by Compound Bacterial Flora Combined with Peganum harmala

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Abstract

Many researchers have paid attention to solving the problem of multiple heavy metals pollution in the tailings area. However, the studies only stay at the laboratory level, which cannot completely demonstrate an efficient approach to remediate polluted environment due to iron tailings. This study aimed at investigating the effects and mechanisms of compound bacterial flora combined with *Peganum harmala* to remediate the tailings area of Linze County, Zhangye City, Gansu Province due to the accumulation of numerous heavy metals such as Pb, Cd and Mn. The preliminary experiment was conducted in the laboratory to obtain data that should be compared with the tailings data. Compared with the control, after 84 days of restoration, parameters of the tailings soil inoculated with the compound bacterial flora were improved, pH was decreased from 7.74 to 7.18, the electrical conductivity was decreased by 21.15%~70.49%, and activities of alkaline phosphatase, sucrase and urease were increased by 95.94%~99.64%, 88.42%~98.43%, 83.28%~86.95% respectively. The improvement of soil parameters was more evident in the treatment groups inoculated with compound bacterial flora and *Peganum harmala*. The results of high-throughput sequencing at different remediation stages showed that the structure of the soil microbial community changed after inoculation with compound bacterial flora, but the dominant species remained unchanged, only their relative abundance changed. At the phylum level, the dominant species in T (total tailings soil treatment groups) were Proteobacteria, Bacteroidota and Actinobacteriota, while the dominant species in 3:1 (H: add yellow cinnamon soil treatment groups) were more than Chloroflexi and Acidobacteriota. According to the analysis of Heatmap, the environmental factors such as pH, WHC, EC, Pb, Cd and Mn have evinced a significant impact on the bacterial community structure. This study provides a reference basis for the remediation approach to the soil polluted by different heavy metals.

1 Introduction

The increasing demand for mineral resources has led to large-scale mining, beneficiation and smelting of mine resources all over the country. The development of mineral resources not only brings economic benefits, but also causes serious environmental pollution. High concentrations of heavy metals in water or soil environments, affect human health and safety (Mengbo Liu et al., 2021). Tailings are substances that can be excavated again after the extraction and beneficiation of ores in the mining industry (Hai-Hong Gu et al., 2017), but tailings often contain a variety of high concentrations of heavy metals (Hanno Matthaei et al., 2020; Sha Li et al., 2021). With the circulation of the biological chain, a large number of tailings are accumulated in the iron ore reservoir of Linze County, Zhangye City, Gansu Province, in which the contents of Pb, Cd and Mn seriously exceed the local soil background value, and these heavy metals are very easy to migrate and cause pollution of the surrounding environment and harm to human health. Studies have shown that long-term exposure to lead and cadmium will cause acute or chronic damage to the human nervous system, renal insufficiency and respiratory system (Paul B. Tchounwou et al., 2012). For years, people have been looking for ways to get rid of heavy metal pollution in the environment that are both safe and long-lasting. The research showed that heavy metal pollution is hard to get rid of

because it stays in the environment and is toxic to organisms (Siallelli et al., 2011; Fan Du et al., 2018; Liu et al., 2018).

Many researchers have favored bioremediation technology in the past few years because of its eco-friendliness and cost-effectiveness. To remediate heavy metal-contaminated soil, bioremediation uses bacteria and/or plants (Biao Song et al., 2017). Many studies have shown that the combined repair technology of compound flora and plants can shorten the repair time and improve the repair effect, which is significantly better than that of inoculating only single strain or compound flora or plants (Hassan Etesami, 2018; Akhtar et al., 2018; Arora et al., 2016; Utobo, E.B. et al., 2015). After the complex flora is connected to the soil polluted by heavy metals, it can not only interact with heavy metals, but also stimulate the adjustment of soil bacterial community structure, promote plant growth and development, further absorb heavy metals in the soil and repair the soil polluted by heavy metals more effectively. However, so far, most studies have stayed at the laboratory level, and tailings are large-area open-air areas. The impact of environmental factors on their remediation technology cannot be solved in laboratory research, and more practical remediation data cannot be obtained.

Since the relevant personnel of our laboratory has screened out the strains with high tolerance to heavy metals and the hyperaccumulation plants suitable for the growth of this region (*Peganum harmala*) in the preliminary study, the purpose of this test is to (a) construct the best ratio of compound bacterial flora and apply it to the outdoor site remediation test to evaluate its impact on the parameters of tailings soil; (b) explore the changes of the microbial diversity and community structure of tailings soil in different periods after inoculation with compound bacterial flora. In this research period, the experimental data on tailings remediation in Northwest China will be closer to the level of real-world use of the lab.

2 Materials And Method

2.1 Reagents and metal standard solutions

NaCl, yeast extract, tryptone, and agar used for cell culture were purchased from Sinopharm Chemical Reagent Co., Ltd and Guangdong Huankai Microbial Technology Co., Ltd, respectively. The following chemicals were obtained from Tianjin Damao Chemical Reagent Factory: urea, methylbenzene, phenol, methanol, acetone, citric acid, ammonium sulphate, disodium phenyl phosphate, borax, 4-aminoantipyrine, K [Fe (CN)], glucose, 3,5-Dinitrosalicylic acid, and potassium sodium tartrate. Cd (NO₃)₂·4H₂O, Pb (NO₃)₂, and MnCl₂·4H₂O used for metal standard solutions were separately dissolved in sterile water supplied by Sinopharm Chemical Reagent Co., Ltd. All solutions were reserved in the dark at 4°C. The chemical reagents used in this experiment were analytical grade.

2.2 Selection and characterization of metal-tolerant strains

According to the types of excessive heavy metals in the tail mining area, the strains used in this experiment were based on our previous studies which were C1-h, C4-h, C8-h, P2-h, P3-h, M1-h, M3-h (Lu Zhang et al., 2022). Cadmium-tolerant strains were C1-h, C4-h, and C8-h; lead-tolerant strains were P2-h

and P3-h; and manganese-tolerant strains were M1-h and M3-h. Relevant personnel in the laboratory had identified the seven strains in the previous study. Each strain belongs to the following genus: C1-h (*Klebsiella pneumoniae*), C4-h (*Bacillus*), C8-h (*Pseudomonas*), M1-h (*Bacillus*), M3-h (*Bacillus pumilus*), P2-h (*Klebsiella pneumoniae*) and P3-h (*Enterobacter Xiangfang*). The Agar Diffusion Method (Parisot H J., 2008) was used to observe whether there was mutual antagonism between them. The growth curve was determined using a UV-vis Spectrophotometer (UV-4802, Unico, USA) at 600 nm.

2.3 Selection of ratios and action conditions by growing cells

The strains were cultured in LB liquid medium at 150 rpm and 30°C for 12 h and harvested. After centrifugation at 6000 r/min for 5 minutes, the supernatant was discarded, the bacteria were collected, washed with sterile water for 2 ~ 3 times, and finally resuspended with sterile water and the optical density (OD) at 600 nm was adjusted to 1.0. The prepared cells with eight different ratios were inoculated into 100mL of liquid sterile growth medium containing 15 mg/L Cd (II), 2500 mg/L Pb (II) and 1000 mg/L Mn (II), and an inoculum size of 2% (v/v) was used. Then the removal efficiency of heavy metals at 12 h, 24 h, and 48 h were measured to determine the best ratio. Eight different ratios were determined according to the growth curve of each strain, which were 1) 1:1:1:1:1:1:1; 2) 2:1:1:1:1:1:1; 3) 1:2:1:1:1:1:1; 4) 1:1:2:1:1:1:1; 5) 1:1:1:2:1:1:1; 6) 1:1:1:1:2:1:1; 7) 1:1:1:1:1:2:1 and 8) 1:1:1:1:1:1:2. The content of heavy metals in the supernatant was determined by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies Co. Ltd., ICP-MS 7900). Set three parallel and the medium without inoculating cells and only adding metal was used as the blank control.

The prepared cells were inoculated into the medium containing 15 mg/L Cd (II), 2500 mg/L Pb (II) and 1000 mg/L Mn (II) under different conditions in a ratio of 1:1:1:1:1:1:1, including different gradients of pH, temperature, salt concentration and inoculation amount (Table S1), cultured for 12 h, and the supernatant was taken after centrifugation to determine the heavy metals, so as to explore the optimal action conditions of mixed culture. Similarly, set three parallel and the medium in which the prepared cells were not inoculated but only the metals were added was used as the blank control.

2.4 Outfield site test

The tailings soil was taken from the iron ore reservoir in Pingchuan Town, Linze County, Zhangye City, Gansu Province, China (100°27'21"E, 38°56'11"N). The soil samples were obtained after screening, and their main characteristics were as follows: pH 7.73, water holding capacity 6.28%, conductivity 772.449 us/cm, porosity 28.91%, Pb 2194 mg/kg, Cd 11.8 mg/kg, Mn 989 mg/kg (heavy metal content was higher than in Gansu Province soil (Pb 20.83 mg/kg, Cd 0.13 mg/kg, Mn 624 mg/kg)). Tailings soil samples were sub packed in each cloth bag (diameter×height: 30cm×25cm), and the soil weight in each cloth bag was 14 kg. The 7 strains were activated respectively and mix-cultured to form a compound flora. After the liquid compound bacterial agent was cultured for 24 h, the sterilized bran was added according to the proportion of 15% (Liqun Sun. 2017), and the moisture content was maintained at 60%~70%. The solid compound bacterial flora was vibrated and cultured for 24 h at 28°C to make it mixed evenly to prepare

the solid compound bacteria group and inoculate it into different groups. According to the previous research of relevant laboratory personnel (Lu Zhang. 2021), the groups of outfield tests of the improved site were set as follows: (a) T (CK, total tailings soil, no-inoculated compound flora and no-inoculated seeds); (b) 3:1 (CK, add yellow cinnamon soil, no-inoculated compound flora and no-inoculated seeds); (c) T + M (total tailings soil, inoculated compound flora but no-inoculated seeds); (d) T + P (total tailings soil, no-inoculated compound flora but inoculated seeds); (e) T + M + P (total tailings soil, inoculated compound flora and inoculated seeds); (f) 3:1 + M (add yellow cinnamon soil, inoculated compound flora but no-inoculated seeds); (g) 3:1 + P (add yellow cinnamon soil, no-inoculated compound flora but inoculated seeds); (h) 3:1 + M + P (add yellow cinnamon soil, inoculated compound flora and inoculated seeds). Each group was set up with three parallels. For the above groups added with yellow cinnamon soil, the proportion between total tailings soil and yellow cinnamon soil was 3:1. The CM was inoculated into the corresponding group of cloth bags at 20 g/m² (Hao Xu et al., 2020) and balanced indoors for 2 days. Then choose a wider outfield to place all the cloth bags. The plump and healthy seeds of *Peganum harmala* were disinfected, washed repeatedly with sterile water for 4h, and then inoculated in the cloth bag of the above-mentioned corresponding group (50 seeds/bag). After the plants grow, 20 plants with similar growth would be left for a follow-up study.

2.5 Analysis of soil properties and metal content

The whole outfield site test cycle was 84 days. Soil samples were taken every two weeks to analyze their physical and chemical properties, including enzyme activity (sucrase, urease, alkaline phosphatase), soil temperature, pH, water holding capacity, porosity and electrical conductivity. The activities of urease, alkaline phosphatase and sucrase were determined according to the methods of R.P. Dick et al., 1996, Xuedan Li et al., 2019 and Arsalan A. Quresh et al., 2020. The porosity was measured by the bulk density specific gravity method, and the conductivity was measured by conductivity meter (Shanghai Leici dds-307, DDS-307A). The determination of the content of heavy metals in soil includes the total concentration of heavy metals before and after remediation. Pretreatment of soil samples: weighed 0.1g of soil samples, added 5ml aqua regia, and conducted microwave digestion. After digestion, drove the acid to viscous state at 140°C, fixed the volume to 25ml, and tested on the machine (ICP-MS).

2.6 Analysis of plant indexes and metals translocation

Plant indexes include plant height, root length, biomass and chlorophyll content (Zhengshu Su et al., 1989). After drying the plant samples at 85°C, grinded and screened them, determined the content of heavy metals in the plants, and calculated the enrichment coefficient of heavy metals. Sample pretreatment method: weighed 0.5 g plant samples, added 5ml nitric acid and 1ml hydrogen peroxide, placed it overnight for microwave digestion. After digestion, drove the acid to 1 ml at 140°C, fixed the volume to 25 ml, and tested on the machine (ICP-MS).

Heavy metal enrichment factor = Heavy metal content in plants/Heavy metal content in soil*100%

2.7 Analysis of microbial diversity and community structure

For the soil samples on the 28th, 42nd, 56th and 84th days of the repair process and the control, another part of the soil samples need to be taken and stored in the refrigerator at -80°C for subsequent delivery to the company (Shanghai Majorbio Bio-pharm Technology Co., Ltd) for high-throughput sequencing. The obtained data was used to analyze the changes in microbial diversity and community structure during the restoration process.

2.8 Data analysis

All experiments were performed in triplicate. The experimental data were statistically analyzed in Microsoft Excel 2010 and SPSS 25.0, using Duncan's multiple comparative analysis method, and visualized in Origin 2019. Statistical significance was defined as $p < 0.05$.

3 Results

3.1 Characteristics of the seven strains

3.1.1 Antagonistic experiment

The antagonistic experiment was carried out on 7 strains in pairs (Fig. S1B). If there is an antagonistic effect between strains, it will affect the effect of the compound bacteria group. It can be seen from the figure that there was no bacteriostatic circle between the seven selected heavy metal tolerant strains, so there was no antagonism. Therefore, follow-up experiments can be continued.

3.1.2 Growth characteristics

The growth curves of the 7 strains (Fig. 1A) and the growth curves of microbial compound agents with different ratios (Fig. 1B) were measured by a spectrophotometer. As shown in figure A, except for M1-h, the growth trend of the other six heavy metal tolerant strains is basically the same. 2 ~ 6 hours is the delayed period, 6 ~ 24 hours is the logarithmic growth period (the logarithmic growth period of M1-h is relatively long, 6 ~ 42 hours), and after 24 hours, it enters the stable period (after M1-h is 42 hours). It can be seen from Fig. B that the growth trend of the compound flora with different ratios is basically the same. The growth of the compound flora directly enters the logarithmic period (0 ~ 20 h). Compared with the single bacterium, the adaptability of the flora is stronger and enters the stable period after 20h, but the OD value of the compound flora with the ratio of 1:1:2:1:1:1:1 (C1-h: C4-h: C8-h: M1-h: M3-h: P2-h: P3-h) increases significantly at 32h after entering the stable period, it decreases to synchronization with other proportions at 52h.

3.2 Construction of compound flora

3.2.1 Determination of optimum ratio

To replicate the environment polluted by heavy metals in tailings, 15 mg/L Cd, 2500 mg/L Pb, and 1000 mg/L Mn were added to LB medium. Compound bacteria were added at the above 8 different ratios,

cultured at 30 °C and 120 r/min, and then the heavy metal removal rates of 12 h, 24 h and 48 h were measured. The results showed that the removal rate of heavy metals in 12 h was higher than that in 24 h and 48 h. At 12 h, the removal rate of heavy metals in the compound bacterial agent with a ratio of 1:1:1:1:1:1:1 was higher than that in the other seven ratios (Fig. 2ABC). The removal rates of Mn^{2+} , Cd^{2+} and Pb^{2+} were 55.3%, 65.1% and 78.4% respectively. Therefore, the best ratio of compound bacterial agent is 1:1:1:1:1:1:1, and the removal rate of heavy metals under 12 h is selected in the subsequent optimization experiment of compound bacterial agent action conditions.

3.3 Evaluation of outfield site test repair effect

3.3.1 Change of tailing soil pH during remediation

The pH of tailings soil at the sampling point is alkaline, the pH of control group t is 7.74, and the pH of control group 3:1 added with yellow cinnamon soil is 7.24. In addition, compared with the control group, during the repair process, the pH of different treatment groups gradually decreased and tended to be neutral (Fig. 3A), especially in the treatment group of 3:1 + M + P. The pH of all treatment groups decreased from 7.74 to 7.18 (T + M), 7.15 (T + P), and 7.11 (T + M + P) after 84 days, and the pH of all 3:1 treatment groups decreased from 7.24 to 7.08 (3:1 + M), 7.15 (3:1 + P), and 7.02 (3:1 + M + P). The ability of plant microbial combination technology to improve soil pH is higher than that of a single microorganism or plant.

3.3.2 Change of tailings soil water holding capacity during remediation

Compared with the control group, the soil water holding capacity of tailings in different treatment groups increased (Fig. 3B). Whether inoculated with flora, plants or combined technology, there was little difference in the change in water holding capacity between them, but in general, the water holding capacity of all 3:1 treatment groups was higher than that of all T treatment groups.

3.3.3 Changes of porosity and conductivity of tailings soil during remediation

During the repair process, the porosity of the treatment group and the control group inoculated with only compound bacteria was measured. The results showed that with the increase of repair time, the porosity of tailings soil added with compound bacteria increased, and the treatment effect of 3:1 + M was particularly obvious (Fig. 3C). At 84 days, the porosity of 3:1 + M and T + M were 49.54% and 41.91% respectively, which increased by 13.69% and 8.53% respectively compared with the control.

In the process of remediation, the changes of soil conductivity in different treatment groups fluctuated, but on the whole, the soil conductivity increased first and then decreased with the increase of remediation time (Fig. 3D), and the conductivity of the treatment group inoculated with both compound flora and

plants fluctuated significantly compared with the treatment group inoculated with only flora or plants, with an overall decrease of 21.15% ~ 70.49%. Compared with the 0-day control, at 84 days of repair, the conductivity of 3:1 + M, T + M, 3:1 + P, T + P, 3:1 + M + P and T + M + P were 307 us/cm, 611 us/cm, 284 us/cm, 245 us/cm, 228 us/cm and 265 us/cm respectively. The decrease of conductivity in the control group is only related to temperature (linear correlation), while the decrease of conductivity in other treatment groups is related to the temperature changes and available state of heavy metals.

3.3.4 Changes of enzyme activities in tailings soil during remediation

During the restoration process, tailing soil samples were taken every 14 days to determine the enzyme activities of alkaline phosphatase, sucrase and urease (Fig. 4ABC). The three enzyme activities of T and 3:1 in the control group were almost unchanged during the sampling process, while the enzyme activities of all 3:1 treatment groups (3:1 + M, 3:1 + P, 3:1 + M + P) were higher than those of the corresponding T treatment groups (T + M, T + P, T + M + P). Compared with the initial control, the activities of alkaline phosphatase, sucrase and urease in the six experimental groups increased significantly on the 14th day. From 14 to 42 days, the activity of alkaline phosphatase changed little and began to increase gradually after 42 days. Compared with the control, the alkaline phosphatase activity of all treatment groups at 84 days were 3:1 + M + P (75.6 $\mu\text{g/g}$) > 3:1 + M (44.6 $\mu\text{g/g}$) > T + M + P (43.8 $\mu\text{g/g}$) > 3:1 + P (33.9 $\mu\text{g/g}$) > T + M (33.4 $\mu\text{g/g}$) > T + P (16 $\mu\text{g/g}$).

Compared with the control group, the activity of sucrase increased at 14 days, except for the T + P treatment group. After 42 days, the enzyme activity of all T treatment groups increased slowly, and the enzyme activity of the corresponding 3:1 treatment group increased rapidly. Although the enzyme activity of 3:1 + P treatment group fluctuated, at 84 days, the sucrase activity of all treatment groups was 3:1 + M + P (35.6 mg/g) > 3:1 + M (34.6 mg/g) > 3:1 + P (32.2 mg/g) > T + M + P (26.7 mg/g) > T + M (25.5 mg/g) > T + P (19.7 mg/g).

Compared with alkaline phosphatase and sucrase, urease activity fluctuated greatly in the repair process. The enzyme activity of different treatment groups had a trend of “first rising, then falling and then rising again”, but the repair time points corresponding to their decline and rise were different. Compared with the control group, the urease activity of all treatment groups showed an upward trend as a whole. At 84 days, the urease activity of all treatment groups were 3:1 + P (0.39 mg/g) > T + P (0.38 mg/g) > 3:1 + M (0.35 mg/g) > 3:1 + M + P (0.34 mg/g) > T + M + P (0.32 mg/g) > T + M (0.3 mg/g).

3.3.5 Plant index

At the end of the whole restoration process (84 days), the plants of different treatment groups were taken to measure their plant height, root length, aboveground and underground biomass and the chlorophyll content of their leaves (Fig. S2). It can be seen from figures A and B that the plant height, root length and biomass of the treatment group with compound bacterial agent are greater than those of the treatment

group without compound bacterial agent, and the plant height, root length and biomass of all 3:1 treatment groups (3:1 + P, 3:1 + M + P) are greater than those of all T treatment groups (T + P, T + M + P).

It can be seen from figure C that the chlorophyll content of each treatment group is significantly different. The plant chlorophyll content of the treatment group with compound flora was higher than that of the treatment group without compound flora. The chlorophyll content was 3:1 + M + P (1.94 mg/g) > T + M + P (1.86 mg/g) > 3:1 + P (1.75 mg/g) > T + P (1.61 mg/g).

3.3.6 Removal rate of heavy metals in soil and enrichment coefficient of heavy metals in plants

The heavy metal content of TCK before remediation and each treatment group’s soil heavy metal content after remediation were measured to evaluate the heavy metal removal rate of different treatment groups in the whole remediation process (Table 1 and Fig S3). Among them, 3:1 yellow cinnamon soil is added, which is equivalent to diluting the heavy metals in the whole tailings soil, so it also has a certain remediation effect. It can be seen from the figure that the repair effect of all 3:1 treatment groups are better than that of all T treatment groups, and the repair effect of only inoculating compound flora was better than that of only inoculating plants, while the microbial plant joint repair effect was the best, and the removal rates of Pb, Cd and Mn were 54.8%, 57.5% and 25.1% respectively.

The plant heavy metal enrichment coefficients (Table S3) of T + P and T + M + P treatment groups were measured. From the data in the table, it can be seen that *Peganum harmala* has the best enrichment effect on Mn, while the enrichment effect of the treatment group inoculated with both flora and plants are significant compared with the treatment group inoculated with only plants.

Table 1
Content and removal rate of Pb, Cd and Mn in tailings soil after remediation.

HMs(mg/kg)/Groups	Pb	Cd	Mn	Pb removal rate	Cd removal rate	Mn removal rate
TCK	2194	11.8	989	/	/	/
HCK	1568	8.87	849	28.53%	24.83%	14.16%
TM	1692	10.6	791	22.88%	10.17%	20.02%
HM	1439	7.44	766	34.41%	36.95%	22.55%
TP	1792	11.0	760	18.32%	6.78%	23.15%
HP	1450	8.12	750	33.91%	31.19%	24.17%
TMP	1662	9.8	748	24.25%	16.95%	24.37%
HMP	993	5.02	742	54.74%	57.46%	24.97%

3.4 Analysis of microbial diversity and community structure

3.4.1 Diversity of soil microbial community

During the remediation test, samples of different treatment groups were taken for 0 days (control T and 3:1), 28 days, 42 days, 56 days and 84 days respectively to determine the diversity of the soil microbial community. A total of 2620238 bacterial 16SrRNA gene sequences were obtained from soil samples collected from all treatment groups. After data processing, 2360069 effective sequences were obtained, with an average length of 417bp. A total of 5507 OTUs were obtained by cluster analysis at a 97% similarity level.

The α diversity index of different treatment groups (3:1 treatment group and T treatment group) was used for statistics (Table 2), including the richness index (Chao index and ACE index) and diversity index (Shannon index and Simpson index). The ACE index of all T treatment groups was between 368.39 ~ 633.18, Shannon index was between 0.95 ~ 4.28, and Simpson index was between 0.028 ~ 0.627. Compared with the control TCK, the richness index and diversity index of the treatment groups with flora and plants were improved. Compared with all T treatment groups, the bacterial richness index and diversity index of all 3:1 (H) treatment groups added with yellow cinnamon soil were improved, and their ACE index was between 2811.34 ~ 3577.25, Shannon index was between 5.74 ~ 6.76, and Simpson index was between 0.003 ~ 0.013. Similarly, compared with the control HCK, the richness index and diversity index of the treatment group connected with flora and plants were improved. The Chao index, Shannon index, Simpson index and ACE index showed that the richness and diversity of the treatment group added with compound bacterial flora and the treatment group with combined microbial plant repair were significantly higher than those of the control group ($p>0.05$), and their richness index and diversity index were also increasing with the increase in repair time.

Table 2

The Chao index, Shannon index, Simpson index and ACE index of different treatment groups, H group is the 3:1 treatment group added with yellow cinnamon soil, and T group is the treatment group of whole tailings soil.

Group H	Chao	Shannon	Simpson	ACE
HCK	2806.68 ± 212.98d	5.74 ± 0.12e	0.013 ± 0.005a	2942.58 ± 438.56bc
HM1	2845.32 ± 71.08d	6.03 ± 0.02d	0.008 ± 0.001b	2811.34 ± 75.34c
HM2	3029.69 ± 45.06bcd	6.21 ± 0.03bcd	0.007 ± 0.001b	3016.42 ± 33.37bc
HM3	2908.67 ± 81.17cd	6.14 ± 0.27cd	0.007 ± 0.002b	2897.05 ± 115.14bc
HM4	3102.09 ± 157.33bc	6.31 ± 0.09bc	0.007 ± 0.001b	3062.57 ± 118.80bc
HMP1	3189.47 ± 38.43b	6.24 ± 0.05bc	0.007 ± 0.0001b	3133.89 ± 47.81bc
HMP2	3177.37 ± 168.47b	6.3 ± 0.07bc	0.006 ± 0.0004b	3170.27 ± 196.29b
HMP3	3238.94 ± 128.07b	6.36 ± 0.05b	0.006 ± 0.0006bc	3216.63 ± 59.18b
HMP4	3571.99 ± 77.31a	6.76 ± 0.02a	0.003 ± 0.0001c	3577.25 ± 53.06a
Group T				
TCK	444.32	0.95	0.627	475.84
TM1	400.84 ± 36.75b	3.85 ± 0.06de	0.055 ± 0.002a	409.75 ± 21.22b
TM2	398.91 ± 19.80b	3.91 ± 0.03cde	0.045 ± 0.005ab	397.73 ± 28.88b
TM3	476.53 ± 54.82ab	4.19 ± 0.04ab	0.034 ± 0.001bc	479.65 ± 44.04ab
TM4	505.65 ± 98.75ab	4.28 ± 0.05a	0.028 ± 0.003c	522.33 ± 137.83ab
TMP1	363.88 ± 39.53b	3.69 ± 0.17e	0.054 ± 0.005a	368.39 ± 38.92b
TMP2	418.17 ± 62.24b	3.97 ± 0.11bcd	0.04 ± 0.005bc	441.94 ± 86.12ab
TMP3	593.48 ± 77.93a	4.06 ± 0.24abcd	0.047 ± 0.016ab	633.18 ± 92.10a
TMP4	616.23 ± 193.4a	4.13 ± 0.16abc	0.039 ± 0.006bc	612.22 ± 213.83a
Data are shown as means ± standard deviations (n = 3). The different lowercase letters in the same column indicate significant differences ($p < 0.05$) among samples.				

3.4.2 Composition and structure of soil microbial OTUs

The Venn diagram of T treatment group shows that after access to the compound bacterial flora, the common OTUs of TM and TCK in different repair periods is 55 (Fig. 5A), and the unique OTUs are 170 (TCK), 40 (TM1), 71 (TM2), 97 (TM3) and 106 (TM4) respectively. The common OTUs of TMP and TCK in different repair periods is 58 (Fig. 5B), and the number of unique OTUs are 155 (TCK), 23 (TMP1), 47 (TMP2), 109 (TMP3) and 228 (TMP4) respectively. The results show that, the number of original unique

OTUs in tailings soil decreased after the bacteria were connected, and the number of unique OTUs were slowly restored and increased with the increase of repair time, and the number of unique OTUs in TMP treatment group was more than that in TM treatment group. The common OTUs of TM and TMP in different repair periods is 231 (Fig. 5C). Common bacterial OTU may be the crystallization of survival in the tailings' specific environment, and it is the key to repairing heavy metal pollution in the tailings soil.

The Venn diagram of 3:1 treatment group (H) (Fig. 5D) shows that after access to the flora, the number of common OTUs of HM and HCK in different repair periods is 1772, and the number of unique OTUs are 170 (HCK), 193 (HM1), 169 (HM2), 167 (HM3) and 259 (HM4) respectively. The number of common OTUs of HMP and HCK in different repair periods is 1726 (Fig. 5E), and the number of unique OTUs are 85 (HCK), 115 (HMP1), 153 (HMP2), 159 (HMP3) and 501 (HMP4) respectively. Compared with the T treatment group, the 3:1 (H) treatment group had more common bacterial OTUs than the T treatment group, and the inoculation of compound flora had less impact on the 3:1 control group (HCK), and the number of endemic species did not suddenly decrease, but decreased slightly. However, with the increase of repair time, the number of endemic species increased all the time, and the number of endemic species in the treatment group inoculated with both compound flora and plants increased the most. The number of common OTUs of HM and HMP in different repair time periods is 1589 (Fig. 5F), which is more than that of the whole tailings soil after adding compound bacterial flora. It may be due to the dilution effect of yellow cinnamon soil on the content of heavy metals, so that more bacteria can survive in the environment of the 3:1 treatment group. The common bacterial OTUs of the two groups are also the key to repairing the soil polluted by heavy metals.

The number of cores OTUs of HM HMP TM and TMP in different repair periods is 124 (Fig. 5G). These common OTUs belong to Proteobacteria (45.05%), Actinobacteriota (20.74%) and Bacteroidota (18.29%). These common bacteria are formed after connecting with the composite flora and coexist in the whole tailings and tailings soil added with yellow cinnamon soil, which may be the dominant flora for repairing the soil polluted by multiple heavy metals.

3.4.3 Changes of soil microbial community structure

Analysis of Circos diagram (Fig. 6), before adding the compound bacterial flora, the primary microflora in the whole tailings soil (TCK) were Firmicutes (80%) and Actinobacterita (17%), and the main microflora in the control group (HCK) added with yellow cinnamon soil were Proteobacteria (41%), Actinobacterita (31%) and Bacteroidota (11%), at the Phylum level. After adding the compound bacterial flora, whether plants are planted or not, the prominent flora of all T treatment groups are Proteobacteria, Bacteroidota and Actinobacterita. In contrast, at the phylum level the prominent flora of all 3:1 (H) treatment groups are Proteobacteria, Bacteroidota, Actinobacteriota, Chloroflexi and Acidobacteriota, but the relative abundance of the main flora microorganisms of the two treatment groups changes with the increase of repair time. It can be seen that the access of compound bacterial flora changes the microbial community structure in the soil samples of two different treatment groups, and whether the plants are inoculated or not has little effect on the microbial community structure.

3.4.4 Effects of environmental factors on microbial communities

The effects of different environmental factors on the horizontal community composition of soil bacterial phyla in different treatment groups are analyzed through the correlation heatmap diagram (Fig. 7AB). The results show that the soil bacterial communities in different treatment groups are affected by water holding capacity, soil temperature, soil pH and soil conductivity at the same time. Different bacterial phyla are affected differently by these environmental factors, among which soil temperature has little effect on most bacterial phyla, and water holding capacity is positively correlated with most bacterial categories, while soil pH and soil conductivity are negatively correlated with most categories, and the correlation is significant, especially for soil pH. The results show that the content of heavy metals in the soil has an impact on the bacterial community. The impact of Mn on the soil bacterial community is not very significant. It may be because the toxicity of Mn to tailings soil is smaller than that of Pb and Cd. Pb and Cd are negatively correlated with most bacterial categories, and the correlation is significant.

4 Discussion

Bioremediation of waste tailings is a green and environmentally friendly remediation method with the concept of sustainable development, which mainly includes microbial remediation, phytoremediation and microbial plant combined remediation (Zhijia Lu et al., 2018; Bin Xuan et al., 2017). Current studies have found that some functional groups on the surface of microbial cells can adsorb or precipitate heavy metals (Ying Wang et al., 2020), and microorganisms can also secrete some secondary metabolites, that will interact with heavy metals, such as mineralization (C.-H. Kang et al., 2015), dissolution of heavy metals by organic acids (N. Papassiopi et al., 2014), etc. However, the process of remediation after adding microorganisms to the soil is very complex, and the growth of microorganisms also has a certain period of time, so the fate of microorganisms in the later stage is also difficult to solve. Phytoremediation can remove heavy metals from soil, and plants are easy to collect in the later stage, so they can deal with heavy metals intensively. However, the efficiency of phytoremediation technology is often limited by the high concentration of heavy metals, coexistence of multiple heavy metals and low-level nutrition (Benidir L et al., 2020). Therefore, before planting plants in heavy metal contaminated soil, inoculate microorganisms to reduce the soil toxicity of tailings, promote plant growth, reduce stress and chelate heavy metals (Nida Zainab et al., 2020), and give full play to the effect of combined microbial and plant remediation. In fact, the resistance of soil bacteria to heavy metals and their ability to promote plant growth make them the first choice for phytoremediation of heavy metal contaminated soil in previous studies (Tirry et al., 2018). It has been found that the inoculated compound bacterial flora resistant to heavy metals can affect the mobility and availability of heavy metals in plants through rhizosphere chelation, acidification and oxidation-reduction reactions, which is conducive to the absorption of heavy metals by plants, thus directly affecting the remediation effect (Ma et al., 2011), which further illustrates the important role of microorganisms in heavy metal contaminated soil. Therefore, in this study, a compound bacterial flora resistant to heavy metals was constructed, and the three bioremediation

methods were set up in different groups for tailings remediation test, so as to obtain more practical remediation schemes and reference data according to the characteristics and properties of the collected soil samples.

Seven strains of Pb, Cd and Mn resistant strains with no antagonistic effect between two were selected in the test (Fig. S1B). After demonstrating the growth characteristics of strains (Fig. 1. AB), it was built as the best ratio of compound bacterial agents (Fig. 2. ABC). The antagonistic effect between strains is that the life activities of one microorganism or its metabolites inhibit or interfere with the life activities of another microorganism, which will make the constructed complex flora unable to give full play to its efficacy and repair the tailings soil to the greatest extent. In this study, the antagonistic experiment results of the seven strains showed that they had no antagonistic effect and could coexist under the same cultural conditions, so they could be used for the construction of subsequent compound bacterial flora. In addition, the ratio among the strains will also affect the effectiveness of the compound bacterial flora, this study also explored the best ratio among the seven strains. It can be seen from the follow-up outdoor site remediation test results that the appropriate ratio of compound flora indeed played an important role in the remediation process of iron tailings soil.

The existence and migration of heavy metals in soil are determined by many factors, among which soil pH and conductivity (EC) play an important role. The change in pH will change the solubility of heavy metals in soil (Teng Xu et al., 2020; Alicja Kinska et al., 2022). In particular, the bacteria combined plant technology is adopted in this study, and the change of soil pH will affect the bioavailability of nutrients or heavy metals absorbed by plants in the rhizosphere (E. Oburger et al., 2020). In the process of remediation, the pH of the tailings soil changes from alkaline to neutral. At the end of remediation, the pH of tailings soil of different treatment groups is stable at about 7.1. The neutral environment is conducive to the survival of microorganisms, and relevant studies have found that an appropriate pH can promote microbial activity (Myoung-Jin Kim et al., 2004). At the same time, soil pH value is also considered to be the main driving force behind changes in the bacterial community (Xiaoxu Sun et al., 2020). The correlation heatmap diagram of environmental factors shown in Fig. 7A. At the phylum level, pH is negatively correlated with most phyla and positively correlated with a few phyla. Therefore, pH affects the distribution of the bacterial community in different treatment groups.

Soil conductivity (EC) can indirectly reflect the concentration of metal ions in soil, while in soil polluted by multiple heavy metals such as tailings, EC can indirectly determine the concentration of heavy metal ions (Kasemodel et al., 2019). The test results show that although EC fluctuates with the increase of remediation time, it may be due to the dynamic transformation of different forms of heavy metal ions during the remediation process, which still shows a downward trend as a whole (Fig. 3D), indicating that the addition of flora or planting plants will reduce the concentration of heavy metal ions in tailings soil, it may be due to the adsorption heavy metal ions on the cell surface of the flora and the interaction of the secondary metabolites produced by the flora with heavy metal ions. Therefore, the overall reduction of EC also indicates that the combined microbial phytoremediation technology can reduce the content of heavy metals in the soil and reduce soil toxicity. This guess is also verified by the results of the removal rate of

heavy metals in the soil at the end of the experiment. The changing trend of soil porosity is just the opposite to that of electrical conductivity. It is well known that the size of soil porosity depends on soil quality. During the experimental remediation process, the soil porosity of different treatment groups after adding flora gradually was increased (Fig. 3C). This indicates that the quality of tailings soil has improved after adding flora, which is consistent with the previous results of electrical conductivity.

Soil enzyme activity is a good indicator of soil quality improvement, and its change can provide characteristics and relevant information about microbial community succession (Adewele T. Adetunji et al., 2017). The test results show that the addition of compound bacterial flora will improve the enzyme activities of sucrase, urease and alkaline phosphatase in tailings soil (Fig. 4ABC), which is consistent with the results reported by Souhir Abdelkrim (2020) and others. When the soil inoculated with PGPR was compared with the soil without inoculation, the activities of β -glucosidase, urease and alkaline phosphatase increased significantly. In addition, other literatures had also reported the increase of soil enzyme activity after bacterial inoculation (Upadhyay et al., 2016; Utobo-Tewari, 2015; Wenliang Ju et al., 2019). The increase of soil enzyme activity is conducive to the growth and development of plant roots. For example, the increase of urease activity will promote the absorption of nitrogen by plant roots (Nosheen, A. and Bano, A., 2014), which is very friendly to the microbial plant joint remediation technology adopted in this experiment, so as to achieve the improvement of soil quality by microorganisms, and the improvement of soil quality promotes the growth and development of plants, so as to achieve a mutually beneficial and win-win relationship.

The test results show that, the bacterial Chao index, Shannon index, Simpson index and ACE index, it is found that the richness index and diversity index of the treatment group added with yellow cinnamon soil are higher than those of the whole tailings treatment group, which may be due to the dilution of the content of heavy metals in the whole tailings soil by adding exogenous yellow cinnamon soil, resulting in the decrease of soil toxicity. At the same time, the richness index and diversity index of the treatment group with complex flora and plants are higher than those of the control, and with the growth in repair time, the richness index and diversity index of different treatment groups are also increasing, indicating that the addition of flora will stimulate the change of local community structure to achieve the effect of biological enhancement. This result is consistent with the report of RAÚl Zornoza (2017) and others. From the number of cores OTUs in different treatment groups, after access to the flora, the number of cores OTUs in the 3:1 (H) treatment group in different repair time periods is greater than that in the T treatment group, which also proves the results of the previous diversity. The number of original unique OTUs in TCK decreases after being connected to the flora. With the increase of repair time, the number of unique OTUs is slowly recovering and increasing. This may be due to the high toxicity of the whole tailings soil, the need for adaptation of the complex flora and the interaction with the original flora in TCK soil. Compared with the T treatment group, the inoculation of compound bacterial flora had less impact on the 3:1 control group (HCK), and the number of unique OTU did not suddenly decrease, but decreased slightly. With the increase of repair time, the number of unique species has increased. This may be because the addition of cinnamon soil diluted the heavy metal toxicity of the whole tailings soil, and the adaptation of compound bacterial flora in the soil of the 3:1 (H) treatment group is relatively easy, and

quickly coexist with other flora in HCK soil. The number of common OTUs of HM, HMP, TM and TMP in different repair periods is 124. At the phylum level, the bacteria belonging to these common OTUs are mainly Proteobacteria, Actinobacteriota and Bacteroidota. These common bacteria are formed after inoculating the compound bacterial flora, which may be the dominant bacterial flora for repairing the soil polluted by multiple heavy metals under the stimulation of the compound bacterial flora. At the phylum level, the main phyla in TCK soil are Firmicutes and Actinobacteriota before inoculating the compound flora, and the main phyla of HCK are Proteobacteria, Actinobacteriota and Bacteroidota. Previous studies have reported similar findings (Lin-xing Chen et al., 2013; Yuanqing Chao et al., 2016; Jianwei Xing et al., 2017; Lur Epelde et al., 2015). These phyla should be the resident phyla in the tailings area. After access to the compound flora, the main phyla of all T treatment groups in different repair time periods are Proteobacteria, Bacteroidota and Actinobacteriota, and the main phyla of all 3:1 (H) treatment groups in different repair time periods are Proteobacteria, Bacteroidota, Actinobacteriota, Chloroflexi and Acidobacteriota, but the relative abundance of several main phyla changes with the increase of repair time. These results show that the bacterial community structure in the tailings area has changed after the compound bacterial flora was inoculated, which is consistent with the research results of Wood (2016) et al, Cd changes the competitive dynamics in the soil bacterial community, causing changes in the bacterial community. The change of the soil bacterial community structure shows that it is not only the inoculated compound bacterial flora. This result has been reported by M Shuaib (2018), which may be because the access of compound bacterial flora stimulates the reproduction of some native microorganisms and forms a unique dominant bacterial flora with the inoculated compound bacterial flora. Moreover, the bacterial community was significantly affected by environmental factors such as pH, water holding capacity, EC and heavy metal content. A. Markowicz (2016) and others have studied the impact of heavy metal variables on the bacterial community, and the results were consistent with this study.

Therefore, for the tailings soil polluted by multiple heavy metals, the access of compound bacterial flora may stimulates the reproduction of original species in the tailings soil, and forms a dominant flora with the access of compound bacterial flora to repair the tailings soil and reduce the toxicity of heavy metals in the tailings soil, so that plants can grow and develop on the tailings soil, further absorb heavy metals in the tailings soil, and achieve the dual effect of microbial plant joint remediation.

5 Conclusions

Our results emphasized that the soil physical and chemical properties and plant indexes of the treatment groups inoculated with the compound bacterial flora were significantly improved with the growth of remediation time, and the results of soil microbial diversity and community structure analysis showed that the compound bacterial flora increased the soil microbial diversity of iron tailings and changed the original community structure of iron tailings. The results showed that the compound bacterial flora we constructed had good remediation effect on the soil polluted by iron tailings. We removed most of the existing laboratory level research to the outfield for site remediation test, and obtained some valuable data, which is a great leap forward for further exploring the remediation of complex polluted environment

in tailings area. These data will provide a reference for researchers who conduct subsequent outfield tests. However, we still need to further explore the specific causes and laws of the changes of soil bacterial community structure after inoculation with compound bacterial flora, so as to master a more specific remediation scheme of soil polluted by multiple heavy metals.

Declarations

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Ethical Approval and Consent to Participate

The manuscript does not involve human or animal experiments.

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Authors Contributions Statement

Huan Wang: Investigation, Formal analysis, Data curation, Writing-original draft. **Lingui Xue:** Conceptualization, Supervision, Funding acquisition, Writing - review & editing. **Yanli Huo:** Formal analysis, Data curation. **Yecheng Ma:** Investigation, Data curation. **Jiahui Li:** Methodology, Validation.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the author on reasonable request.

References

1. Arsalan A. Quresh, Tasneem G. Kazi, Jameel A. Baig, et al (2020) Exposure of heavy metals in coal gangue soil, in and outside the mining area using BCR conventional and vortex assisted and single step extraction methods. Impact on orchard grass. *Chemosphere* 255:126960. <https://doi.org/10.1016/j.chemosphere.2020.126960>.
2. Adewole T. Adetunji, Francis B. Lewu, Reckson Mulidzi, Bongani Ncube (2017) The biological activities of β -glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of Soil Science and Plant Nutrition* 17 (3):794-807. <https://doi.org/10.4067/S0718-95162017000300018>.
3. Biao Song, Guangming Zeng, Jilai Gong et al (2017) Evaluation methods for assessing effectiveness of in situ remediation of soil and sediment contaminated with organic pollutants and heavy metals. *Environment International* 105:43-55. <http://dx.doi.org/10.1016/j.envint.2017.05.001>.
4. Akhtar, M.J., Ullah et al (2018) Nickel phytoextraction through bacterial inoculation in *Raphanus sativus*. *Chemosphere* 190:234–242. <https://doi.org/10.1016/j.chemosphere.2017.09.136>.
5. Arora, Naveen Kumar, Mehnaz et al (2016) Bioformulations: for Sustainable Agriculture. Effect of Bioinoculants on the Quality of Crops. Springer India, Chapter 5:93–124. https://doi.org/10.1007/978-81-322-2779-3_5.
6. Bin Xuan, Ji Wang, et al (2017) Review on Contamination and Remediation Technology of Heavy Metal in Agricultural Soil *Advances in Environmental Protection* 7(1): 26-34. <https://doi.org/10.12677/aep.2017.71004>.

7. Benidire L, Madline A, S.I.A. Pereira et al (2020) Synergistic effect of organo-mineral amendments and plant growth promoting rhizobacteria (PGPR) on the establishment of vegetation cover and amelioration of mine tailings Chemosphere. <https://doi.org/10.1016/j.chemosphere.2020.127803>.
8. Chang-Ho Kang, Soo Ji Oh, YuJin Shin, et al (2015) Bioremediation of lead by ureolytic bacteria isolated from soil at abandoned metal mines in South Korea Ecological Engineering 74:402-407. <http://dx.doi.org/10.1016/j.ecoleng.2014.10.009>.
9. E. Oburger, C.V. Cid, D. Schwertberger, et al (2020) Response of tungsten (W) solubility and chemical fractionation to changes in soil pH and soil aging. Science of the Total Environment 3(5). <https://doi.org/10.1016/j.scitotenv.2020.139224>.
10. Fan Du, Zhaoguang Yang, Peng Liu et al (2018) Accumulation, translocation, and assessment of heavy metals in the soil-rice systems near a mine-impacted region. Environmental Science and Pollution Research 25:32221-32230. <https://doi.org/10.1007/s11356-018-3184-7>.
11. Hao Xu, Jian Sun, Liansheng Xu, Hong Zhao, Xiaohan Chen, Zhaojie Cui, Rui Zhao (2020) The invention relates to a microbial agent and a method for planting plants to improve tailings sand. P. China, CN 112410265 A.
12. Hai-Hong Gu, Zheng Zhou, Yu-Qian Gao et al (2017) The Influences of Arbuscular Mycorrhizal Fungus on Phytostabilization of Lead/Zinc Tailings Using of Four Plant species International Journal of Phytoremediation 19:739-745. <https://doi.org/10.1080/15226514.2017.1284751>.
13. Hassan Etesami (2018) Bacterial mediated alleviation of heavy metal stress and decreased accumulation of metals in plant tissues: Mechanisms and future prospects. Ecotoxicology and Environmental Safety 147:175-191. <http://dx.doi.org/10.1016/j.ecoenv.2017.08.032>.
14. Jianwei Xing, Jinming Song, Huamao Yuan et al (2017) Atmospheric wet deposition of dissolved trace elements to Jiaozhou Bay, North China: Fluxes, sources and potential effects on aquatic environments. Chemosphere 174:428-436. <https://doi.org/10.1016/j.chemosphere.2017.02.004>.
15. Julien Sialelli, Christine M. Davidson et al (2011) Human bioaccessibility of Cr, Cu, Ni, Pb and Zn in urban soils from the city of Torino, Italy. Environ Chem Lett 9:197-202. <https://doi.org/10.1007/s10311-009-0263-5>.
16. J.L. Wood, W. Liu, C. Tang, A.E. Franks (2016) Microorganisms in heavy metal bioremediation: strategies for applying microbial-community engineering to remediate soils. AIMS Bioengineering 3 (2) 211. <https://doi.org/10.3934/bioeng.2016.2.211>.
17. Kicinska, A., Pomykała, R., & Izquierdo-Diaz, M (2022) Changes in soil pH and mobility of heavy metals in contaminated soils. European Journal of Soil Science 73(1)-e13203. <https://doi.org/10.1111/ejss.13203>.
18. Lu Zhang, Lingui Xue, Huan Wang, Sijing Chang, Y-yuan He, Yingtong Liu, Yajie Xu (2022) Immobilization of Pb and Cd by two strains and their bioremediation effect to an iron tailings soil. Process Biochemistry 113:194-202. <https://doi.org/10.1016/j.procbio.2021.12.026>.
19. Lu Zhang (2021) Study on the construction of composite microbial agent and its remediation effect on Pb and Cd contaminated soil from iron tailings. Lanzhou Jiaotong University.

<https://doi.org/10.27205/d.cnki.gltec.2021.000761>.

20. Liqun Sun (2017) Soil utilization of iron tailings and microbial remediation of heavy metal pollution. Shandong University.
21. Lin-xing Chen, Jin-tian Li, Ya-ting Chen et al (2013) Shifts in microbial community composition and function in the acidification of a lead/zinc mine tailings. *Environmental Microbiology* 15(9):2431-2444. <https://doi.org/10.1111/1462-2920.12114>.
22. Lur Epelde, Anders Lanzen, Fernando Blanco et al (2015) Adaptation of soil microbial community structure and function to chronic metal contamination at an abandoned Pb-Zn mine. *FEMS Microbiology Ecology* 91(1):1-12. <https://doi.org/10.1093/femsec/fiu007>.
23. Lianwen Liu, Wei Li, Weiping Song et al (2018) Remediation techniques for heavy metal-contaminated soils: Principles and applicability. *Science of the Total Environment* 633:206-219. <https://doi.org/10.1016/j.scitotenv.2018.03.161>.
24. Myoung-Jin Kim, Yejin Jung (2004) Vertical Distribution and Mobility of Arsenic and Heavy Metals in and around Mine Tailings of an Abandoned Mine. *Journal of Environmental Science and Health Part A* 39(1):203-222. <https://doi.org/10.1081/ESE-120027379>.
25. M.C. Kasemodel, I.K. Sakamoto, M.B.A. Varesche, V.G.S. Rodrigues (2019) Potentially toxic metal contamination and microbial community analysis in an abandoned Pb and Zn mining waste deposit. *Science of the Total Environment* 675:367-379. <https://doi.org/10.1016/j.scitotenv.2019.04.223>.
26. Mengbo Liu, Shengli Wang, Meng Yang et al (2021) Experimental study on treatment of heavy metal-contaminated soil by manganese-oxidizing bacteria. *Environmental Science and Pollution Research*. <https://doi.org/10.1007/s11356-021-15475-0>.
27. Martha Ndeapo, Uugwanga, Kgabi, et al (2020) Assessment of metals pollution in sediments and tailings of Klein Aub and Oamites mine sites, Namibia. *Environ. Adv.* 2:100006. <https://doi.org/10.1016/j.envadv.2020.100006>.
28. Ma, Y., Prasad, M.N.V., Rajkumar, M., Freitas, H. (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. *Biotechnology Advances*. 29:248–258. <https://doi.org/10.1016/j.biotechadv.2010.12.001>.
29. M. Shuaib, K. Ali, S. Ahmed, F. Hussain, M. Ilyas, N. Hassan, I. Khan, F. Hussain (2018) Impact of rapid urbanization on the floral diversity and agriculture land of district Dir, Pakistan. *Acta Ecol. Sin.* 38 (6): 394–400. <https://doi.org/10.1016/j.chnaes.2018.04.002>.
30. N. Papassiopi, K. Vaxevanidou, C. Christou, et al (2014) Synthesis, characterization and stability of Cr(III) and Fe(III) hydroxides. *Journal of Hazardous Materials* (264):490-497. <http://dx.doi.org/10.1016/j.jhazmat.2013.09.058>.
31. Nida Zainab, Amna, Bashir Ud Din et al (2020) Deciphering metal toxicity responses of flax (*Linum usitatissimum* L.) with exopolysaccharide and ACC-deaminase producing bacteria in industrially contaminated soils. *Plant Physiology and Biochemistry* (152):90-99. <https://doi.org/10.1016/j.plaphy.2020.04.039>.

32. N. Upadhyay, S. Verma, A. Pratap Singh et al (2016) Soil ecophysiological and microbiological indices of soil health: a study of coal mining site in Sonbhadra, Uttar Pradesh. *Journal of Soil Science and Plant Nutrition* 16 (3):778-800. <https://doi.org/10.4067/s0718-95162016005000056>.
33. Nosheen, A., Bano, A. (2014) Potential of plant growth promoting rhizobacteria and chemical fertilizers on soil enzymes and plant growth. *Pakistan J. Bot.* 46:1521–1530.
34. Parisot H J (2008) Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *Journal of Antimicrobial Chemotherapy* 61:1295–1301. <https://doi.org/10.1093/jac/dkn090>.
35. Paul B. Tchounwou, Clement G. Yedjou, Anita K. Patlolla et al (2012) Heavy Metal Toxicity and the Environment. *EXS* 101:133-164. https://doi.org/10.1007/978-3-7643-8340-4_6.
36. R.P. Dick, D.P. Breakwell, R.F. Turco (1996) Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. *Methods for Assessing Soil Quality So/7*. ISBN: 9780891188261.
37. Raúl Zornoza, Melisa Gómez-Garrido, Silvia Martínez-Martínez et al (2017) Bioaugmentation in Technosols created in abandoned pyritic tailings can contribute to enhance soil C sequestration and plant colonization. *Science of the Total Environment* 593-594, 357-367. <http://dx.doi.org/10.1016/j.scitotenv.2017.03.154>.
38. Souhir Abdelkrim, Salwa Harzalli Jebara, Omar Saadani et al (2020) In situ effects of *Lathyrus sativus*- PGPR to remediate and restore quality and fertility of Pb and Cd polluted soils. *Ecotoxicology and Environmental Safety* (192)110260. <https://doi.org/10.1016/j.ecoenv.2020.110260>.
39. Sha Li, Juanli Wu, Yanli Huo et al (2021) Profiling multiple heavy metal contamination and bacterial communities surrounding an iron tailing pond in Northwest China. *Science of the Total Environment* 752-141827. <https://doi.org/10.1016/j.scitotenv.2020.141827>.
40. Teng Xu, Feng Nan, Xiaofeng Jiang, et al (2020) Effect of soil pH on the transport, fractionation, and oxidation of chromium (III). *Ecotoxicology and Environmental Safety* 195-110459. <https://doi.org/10.1016/j.ecoenv.2020.110459>.
41. Tirry, N., Tahri Joutey, N., Sayel, H., Kouchou, A., Bahafid, W., Asri, M., El Ghachtouli, N. (2018) Screening of plant growth promoting traits in heavy metals resistant bacteria: prospects in phytoremediation. *Genet. Eng. Biotechnol.* 16 (2):613–619. <https://doi.org/10.1016/j.jgeb.2018.06.004>.
42. Utobo, E.B. – Tewari. (2015) Soil enzymes as bioindicators of soil ecosystem status. *Applied Ecology and Environmental Research* 13(1): 147-169. https://doi.org/10.15666/aeer/1301_147169.
43. Utobo, E.B., Tewari, L. (2015) Soil enzymes as bioindicators of soil ecosystem status. *Applied Ecology and Environment Research* 13(1): 147-169. https://doi.org/10.15666/aeer/1301_147169.
44. Wenliang Ju, Lei Liu, Linchuan Fang et al (2019) Impact of co-inoculation with plant-growth-promoting rhizobacteria and rhizobium on the biochemical responses of alfalfa-soil system in

- copper contaminated soil. *Ecotoxicology and Environmental Safety* (167)218-226.
<https://doi.org/10.1016/j.ecoenv.2018.10.016>.
45. Xuedan Li, Kemeng Xiao, Hang Ma, et al (2019) Mechanisms into the removal and translocation of cadmium by *Oudemansiella radicata* in soil. *Environmental Science and Pollution Research* 26 (7) 6388–6398. <https://dio.org/10.1007/s11356-018-4042-3>.
 46. Xiaoxu Sun, Tianle Kong, Rui Xu et al (2020) Comparative characterization of microbial communities that inhabit arsenic-rich and antimony-rich contaminated sites: Responses to two different contamination conditions. *Environmental Pollution* 260-114052.
<https://doi.org/10.1016/j.envpol.2020.114052>.
 47. Ying Wang, Yao Luo, Guoquan Zeng, et al (2020) Characteristics and in situ remediation effects of heavy metal immobilizing bacteria on cadmium and nickel co-contaminated soil. *Ecotoxicology and Environmental Safety* 192-110294. <https://doi.org/10.1016/j.ecoenv.2020.110294>.
 48. Yuanqing Chao, Wenshen Liu, Yanmei Chen et al (2016) Structure, Variation, and Co-occurrence of Soil Microbial Communities in Abandoned Sites of a Rare Earth Elements Mine. *Environmental Science and Technology* 50(21):11481-11490. <https://dio.org/10.1021/acs.est.6b02284>.
 49. Zhengshu Su, Xianzheng Zhang (1989) Comparison of several methods for determining plant chlorophyll content. *Plant Physiology Communication* (5): 77~78.
<http://www.cnki.com.cn/Article/CJFDTotal-ZWSL198905029.htm>.
 50. Zhijia Lu, Xiuhua Geng (2018) Remediation technology and application analysis of soil heavy metal pollution. *China resources comprehensive utilization* 036(011): 110-112.

Figures

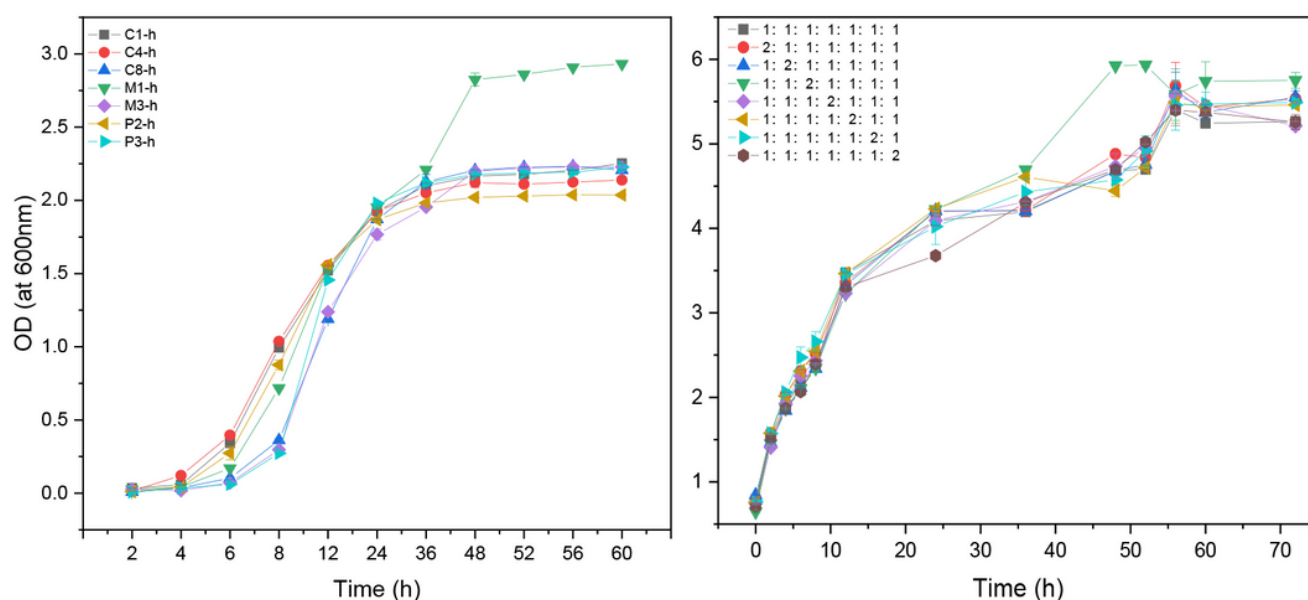


Figure 1

A: Growth curve of the 7 strains, **B:** Growth curves of compound microbial agents with different ratios (C1-h:C4-h:C8-h:M1-h:M3-h:P2-h:P3-h).

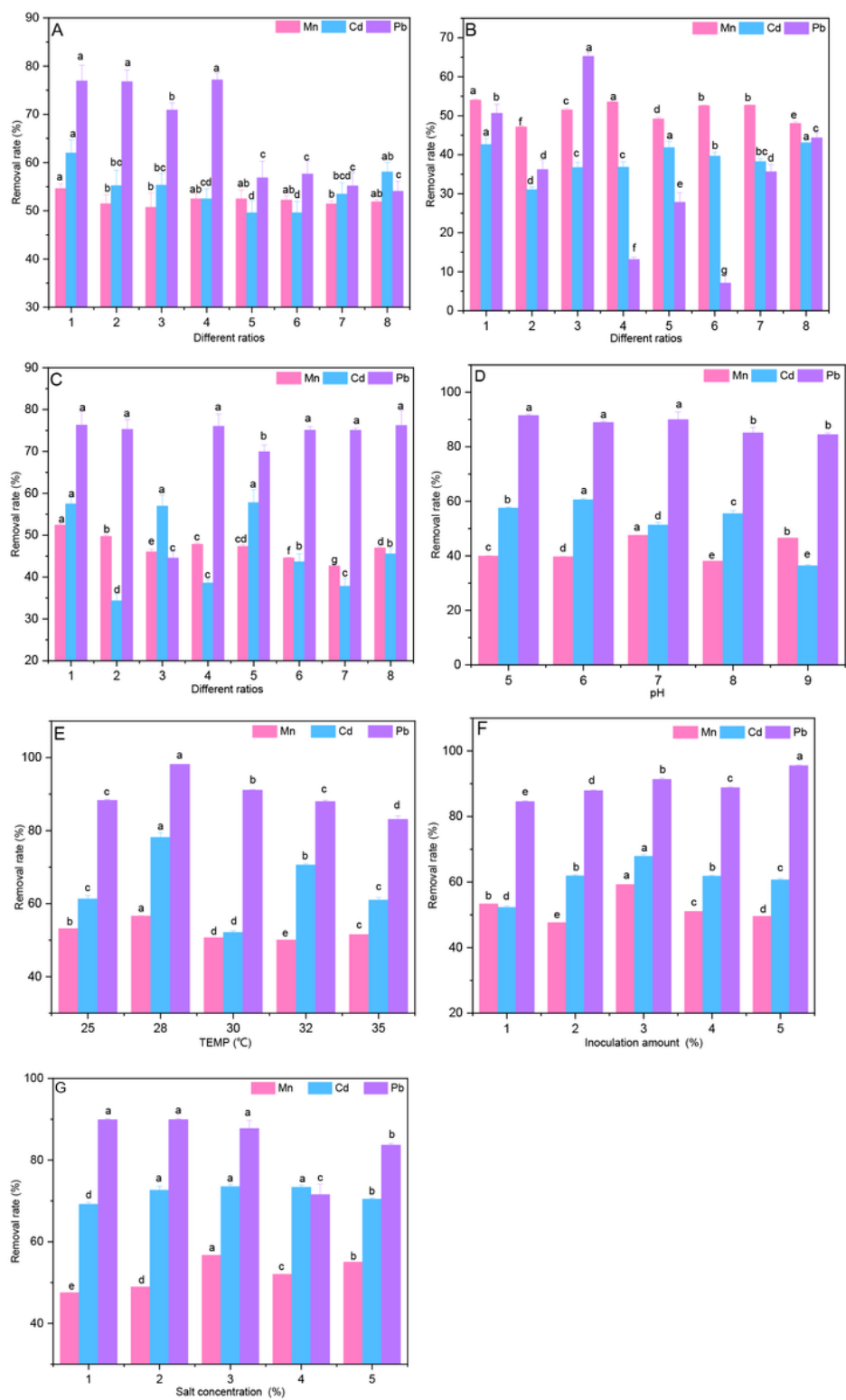


Figure 2

The heavy metal removal rates of 8 different ratios of compound bacteria under three periods (**A:** 12 h, **B:** 24 h, **C:** 48 h).

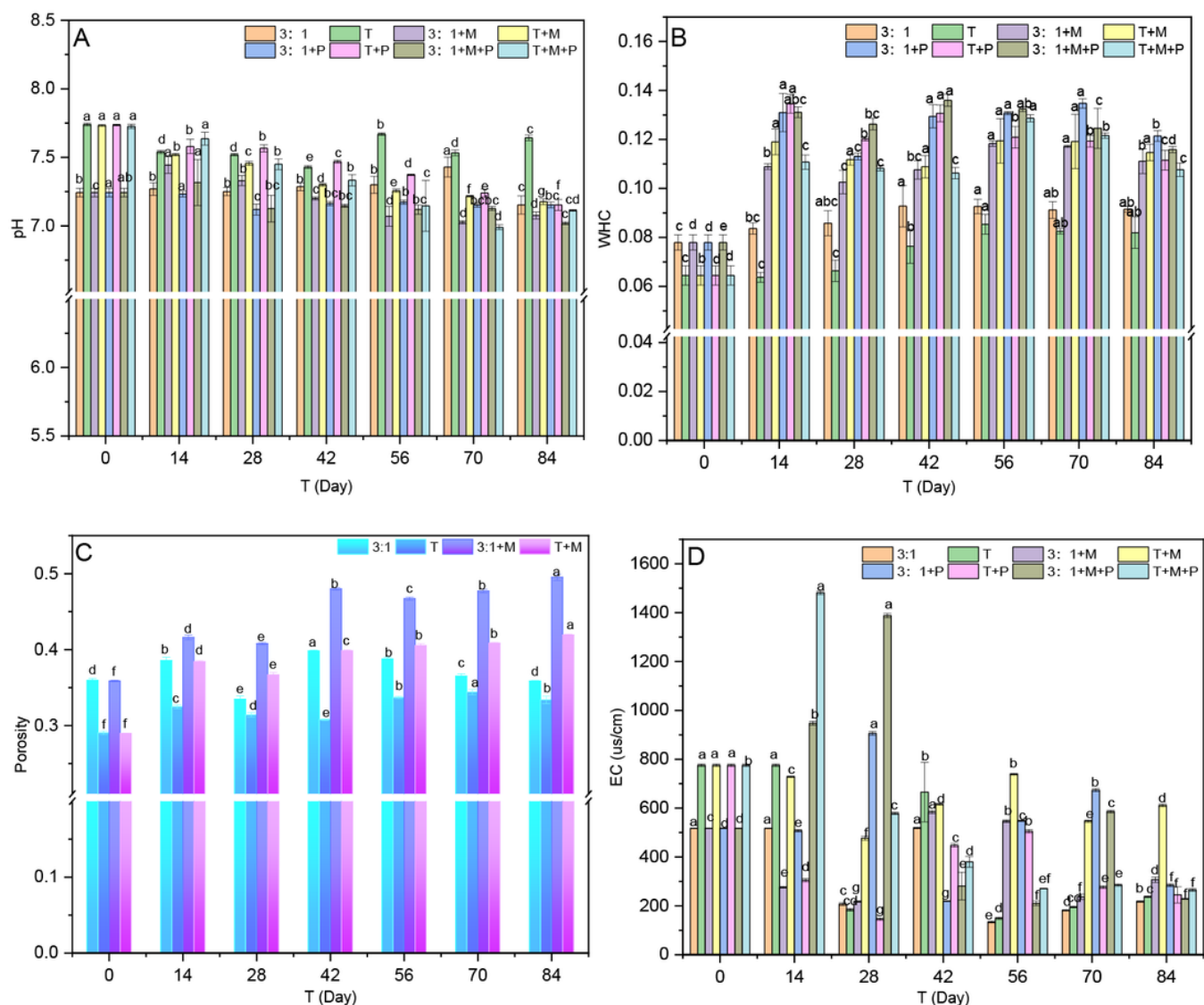


Figure 3

A: Changes of pH of tailings soil in different time periods, B: Changes of soil water holding capacity of tailings in different time periods, C: Changes of porosity in different time periods, D: Changes of soil conductivity of tailings in different time periods.

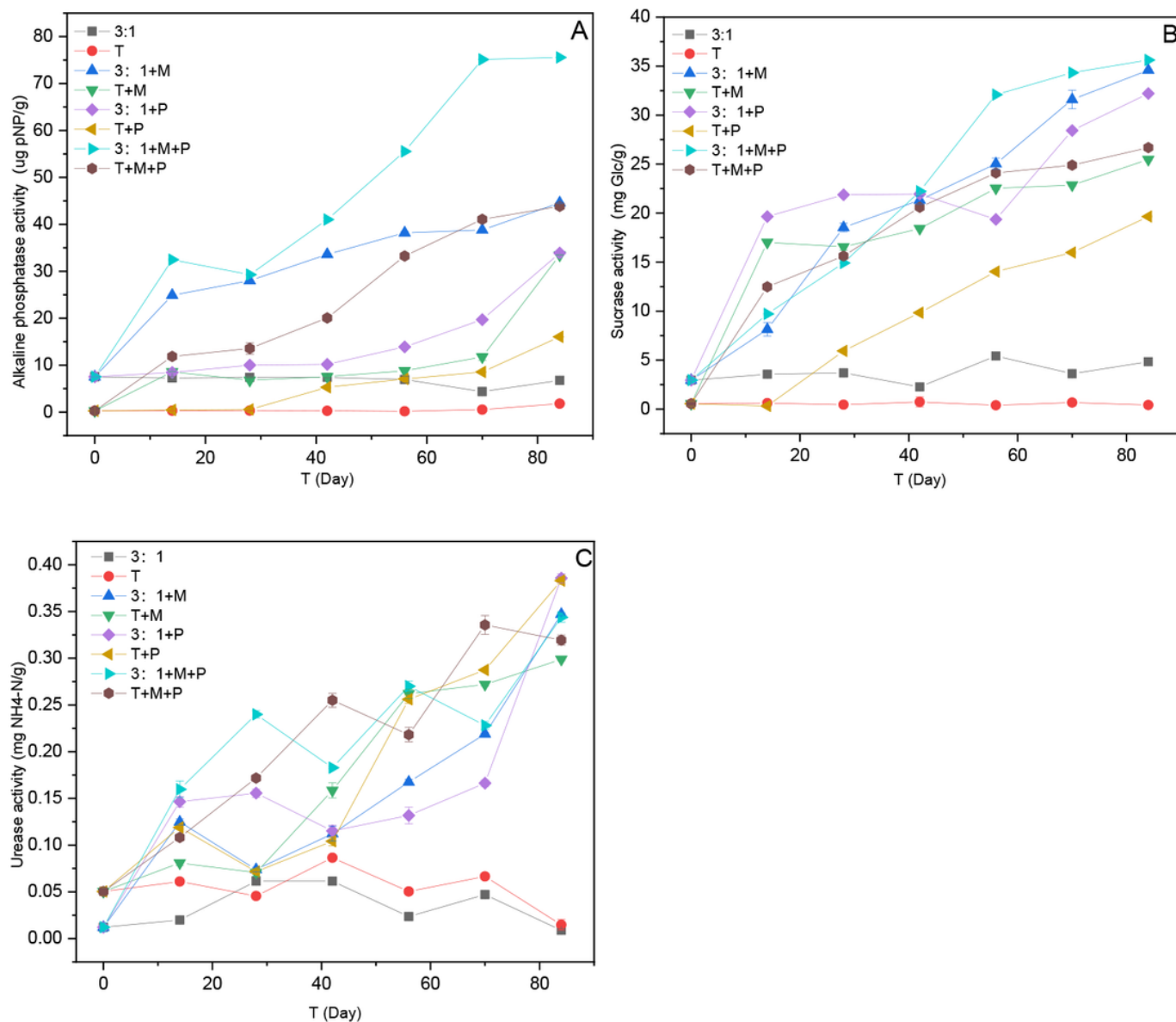


Figure 4

A: Changes of alkaline phosphatase during repair, **B:** Changes of sucrase during repair, **C:** Changes of urease during repair.

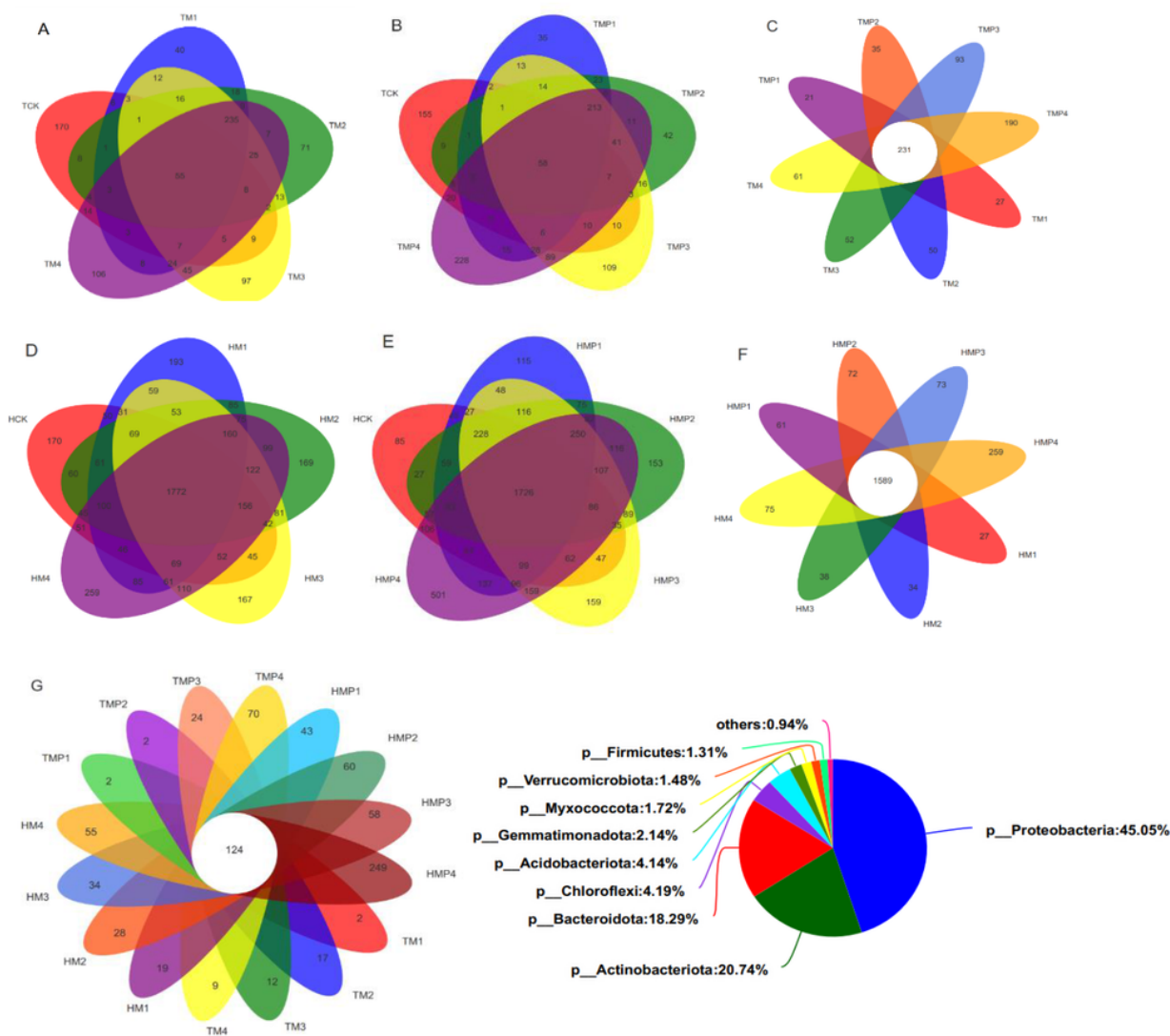


Figure 5

Common OTUs and unique OTUs of different treatment groups in different repair time periods. **A:** TCK and TM, **B:** TCK and TMP, **C:** TM and TMP, **D:** HCK and HM, **E:** HCK and HMP, **F:** HM and HMP, **G:** The common OTUs and unique OTUs of TM, TMP, HM and HMP treatment groups in different repair time periods, as well as the phylum to which the common OTUs belongs.

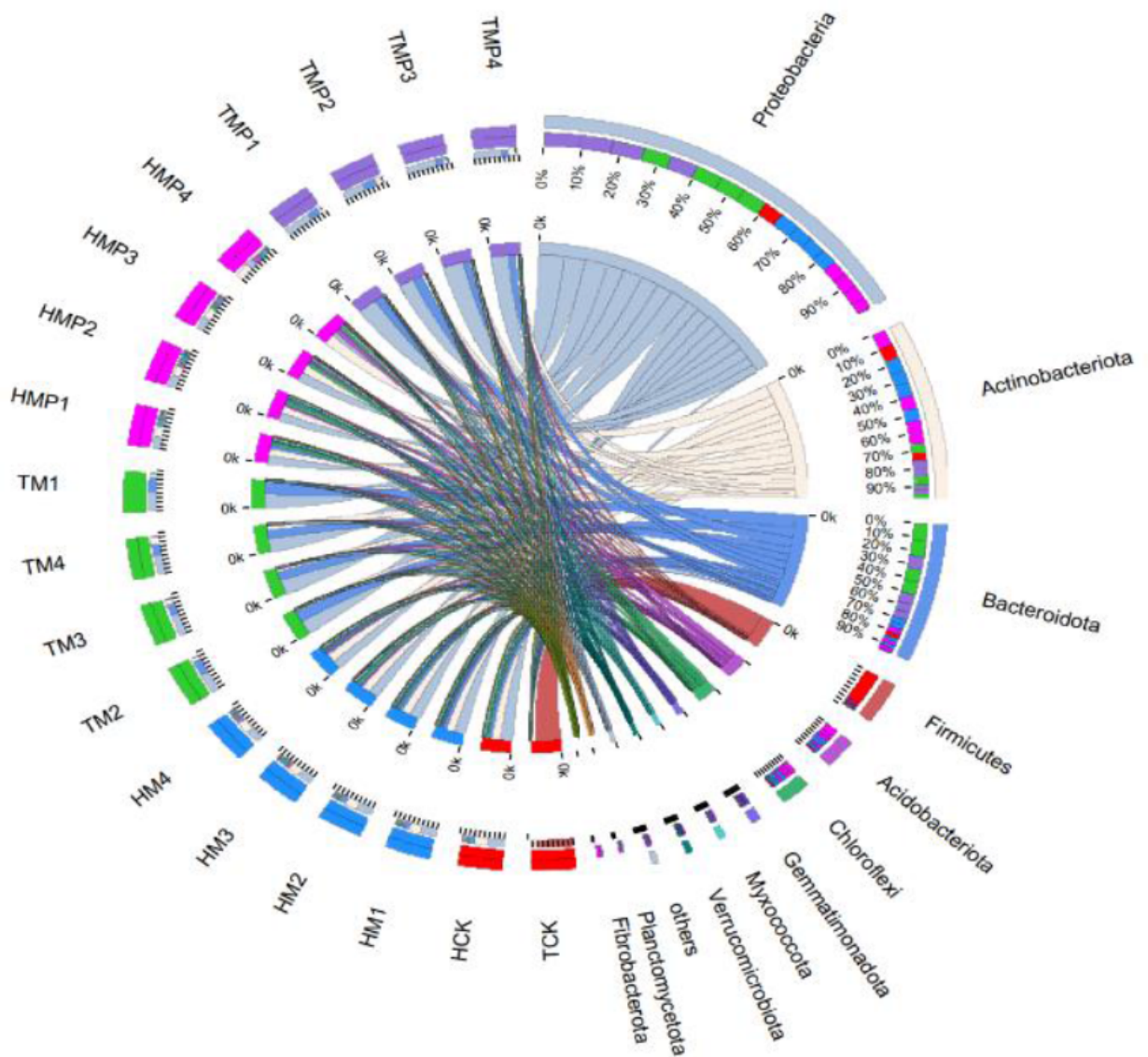


Figure 6

The Circos of soil bacterial community, from bottom to top, the treatment groups are TCK control HCK (control) HM1 HM2 HM3 HM4 TM2 TM3 TM4 TM1 HMP1 HMP2 HMP3 HMP4 TMP1 TMP2 TMP3 TMP4.

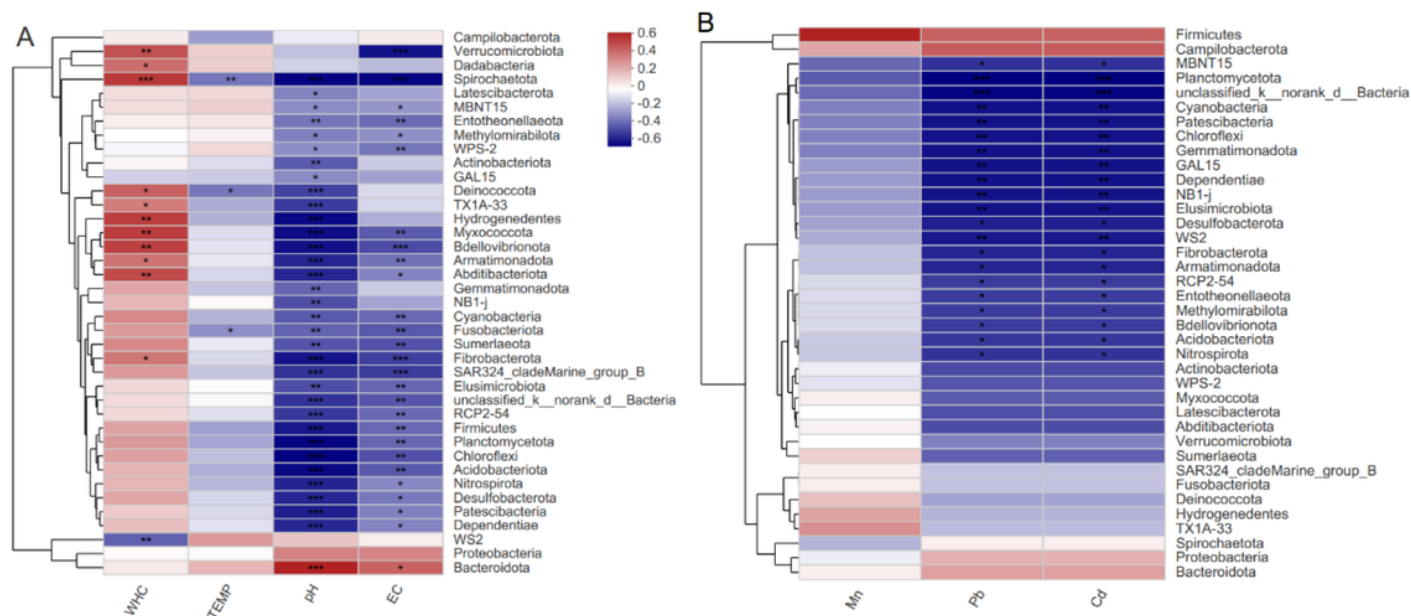


Figure 7

A: At the Phylum level, the effects of environmental factors (soil water holding capacity (WHC), soil temperature (TEMP), soil pH, soil conductivity (EC)) on bacterial community structure, **B:** At the Phylum level, effect of heavy metal content in soil on bacterial community.

Supplementary Files

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