



Original Research

Soil microbiomes divergently respond to heavy metals and polycyclic aromatic hydrocarbons in contaminated industrial sites



Zhen-Ni Yang^{a,1}, Ze-Shen Liu^{a,1}, Ke-Huan Wang^{a,b}, Zong-Lin Liang^{a,b}, Rashidin Abdugheni^{a,b}, Ye Huang^{a,b}, Run-Hua Wang^{a,b}, Hong-Lin Ma^{a,b}, Xiao-Kang Wang^{a,b}, Mei-Ling Yang^c, Bing-Ge Zhang^d, De-Feng Li^a, Cheng-Ying Jiang^{a,*}, Philippe F.-X. Corvini^e, Shuang-Jiang Liu^{a,f,**}

^a State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China

^b University of Chinese Academy of Sciences, Beijing, 100049, China

^c School of Life Sciences, Hebei University, Baoding, 071002, Hebei Province, China

^d School of Public Health, Xuzhou Medical University, Xuzhou, 221004, Jiangsu Province, China

^e School of Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland, Muttenz, 4132, Switzerland

^f State Key Laboratory of Microbial Technology, Microbial Technology Institute, Shandong University, Qingdao, 226237, Shandong Province, China

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ABSTRACT

Contaminated sites from electronic waste (e-waste) dismantling and coking plants feature high concentrations of heavy metals (HMs) and/or polycyclic aromatic hydrocarbons (PAHs) in soil. Mixed contamination (HMs + PAHs) hinders land reclamation and affects the microbial diversity and function of soil microbiomes. In this study, we analyzed HM and PAH contamination from an e-waste dismantling plant and a coking plant and evaluated the influences of HM and PAH contamination on soil microbiomes. It was noticed that HMs and PAHs were found in all sites, although the major contaminants of the e-waste dismantling plant site were HMs (such as Cu at $5,947.58 \pm 433.44 \text{ mg kg}^{-1}$, Zn at $4,961.38 \pm 436.51 \text{ mg kg}^{-1}$, and Mn at $2,379.07 \pm 227.46 \text{ mg kg}^{-1}$), and the major contaminants of the coking plant site were PAHs (such as fluorene at $11,740.06 \pm 620.1 \text{ mg kg}^{-1}$, acenaphthylene at $211.69 \pm 7.04 \text{ mg kg}^{-1}$, and pyrene at $183.14 \pm 18.89 \text{ mg kg}^{-1}$). The microbiomes (diversity and abundance) of all sites were determined via high-throughput sequencing of 16S rRNA genes, and redundancy analysis was conducted to investigate the relations between soil microbiomes and contaminants. The results showed that the microbiomes of the contaminated sites divergently responded to HMs and PAHs. The abundances of the bacterial genera *Sulfuritalea*, *Pseudomonas*, and *Sphingobium* were positively related to PAHs, while the abundances of the bacterial genera *Bryobacter*, *Nitrospira*, and *Steroidobacter* were positively related to HMs. This study promotes an understanding of how soil microbiomes respond to single and mixed contamination with HMs and PAHs.

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1. Introduction

Soil ecosystems are important parts of terrestrial ecosystems upon which humans rely [1]. Anthropogenic activities such as mining, coking, and electronic waste (e-waste) dismantling

processes have led to widespread pollution of soil with heavy metals (HMs) and refractory organics such as polycyclic aromatic hydrocarbons (PAHs) [2–7]. The contamination by HMs and PAHs poses a huge threat to soil microbiomes, human health, and natural ecosystems [8–13]. Soil microorganisms are sensitive to pollutants and can be used as ecological indicators to evaluate soil contamination [14]. The mixed contamination of HMs and PAHs shows different influences on soil microorganisms compared to the single contamination [15]. An intensive understanding of how soil microbiomes respond to single and mixed contamination with HMs and PAHs is essential for evaluating soil health and developing

* Corresponding author.

** Corresponding author. State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China

E-mail addresses: jiangcy@im.ac.cn (C.-Y. Jiang), liusj@im.ac.cn (S.-J. Liu).

¹ The two authors contributed equally to this work.

Abbreviations			
E-waste	electronic waste	CHR	chrysene
HMs	heavy metals	BbF	benzo[b]fluoranthene
PAHs	polycyclic aromatic hydrocarbons	BkF	benzo[k]fluoranthene
OC	organic carbon	BaP	benzo[a]pyrene
NAP	naphthalene	DhA	dibenzo[a,h]anthracene
ACY	acenaphthylene	BgP	benzo[ghi]perylene
FLO	fluorene	IcP	indeno[1,2,3-cd]pyrene
PHE	phenanthrene	16S rRNA	16S ribosomal RNA
ANT	anthracene	ASVs	amplicon sequence variants
FLA	fluoranthene	PCoA	principal coordinate analysis
PYR	pyrene	LEfSe	linear discriminant analysis (LDA) coupled with effect size measurements
BaA	benzo[A]anthracene	RDA	redundancy analysis

effective bioremediation processes [16].

HMs such as Cd, Cr, and Pb are widely present in soil, which are toxic to organisms [17–20]. Studies have reported changes in microbial metabolic activity, population diversity, and abundance of soil microbiomes in response to HMs [21–23]. Bacteria such as *Proteobacteria*, *Acidobacteria*, and *Bacteroidetes* are found in soil that is contaminated with HMs [9,24,25]. Bacteria may be able to eliminate the toxicity of HMs via adsorption, oxidation, and reduction mechanisms [26,27]. In addition to HMs, PAHs are also common soil pollutants produced from industrial processes and human activities, e.g., incomplete combustion and pyrolysis of organic substances, waste transportation, and incineration [28–30]. PAHs cause great concerns due to their high hydrophobicity, toxicity, and low bioavailability, especially those with more than four aromatic rings [31]. Soil contamination with PAHs has been reported to result in decreases in soil microbial diversity, abundance, and metabolic function [32]. Bacterial genera such as *Rhizobacter*, *Sphingobium*, *Mycobacterium*, *Bacillus*, and *Pseudarthrobacter* have been found to increase in abundance in response to PAHs [8,33,34].

Besides, many contaminated sites are affected by both HMs and PAHs [35,36]. Bourceret et al. [37] found that *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were the dominant phyla in soil that was contaminated with HMs and PAHs. Gran-Scheuch et al. [38] reported that genera such as *Sphingomonas*, *Ferruginibacter*, *Pseudomonas*, *Rhodanobacter*, and *Sphingobium* were abundant in the soil of King George Island (Antarctica), where HMs and PAHs were detected. Although the soil microbiomes in soil contaminated by HMs and PAHs have been reported, the differences of the soil microbiomes with single and mixed contamination of HMs and PAHs are still confusing.

In this study, we aimed to explicate the characteristics of soil microbiomes from an e-waste dismantling plant and a coking plant with single and mixed contamination with HMs and PAHs. Microbial communities of four soil samples, including one with higher concentrations of HMs, one with higher concentrations of PAHs, and two with higher concentrations of HMs and PAHs, were compared to a control sample from a paddy field by the Illumina MiSeq sequencing and redundancy analysis. We found that the microbial community structure of soil microbiomes with high concentrations of HMs was different from that with high concentrations of PAHs. This study promotes an understanding of how soil microbiomes respond to single and mixed contamination with HMs and PAHs and provides insight into soil health evaluation.

2. Materials and methods

2.1. Materials

Chemical reagents, including hydrochloric acid, nitric acid, acetone, *n*-hexane, and acetonitrile of analytical grade, were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Decafluorobiphenyl was purchased from Shanghai Eon Chemical Technology Co., Ltd. (China), and dichloromethane was purchased from Shanghai Macklin Regent Co., Ltd. (China). A standard solution of metals (National Institute of Metrology, China) was used to measure the concentrations of K, Ca, Na, Mg, Fe, Al, and Ti; multi-element calibration standard 3 (PerkinElmer, USA) was used to measure those of Mn, As, Cr, Cd, Pb, Cu, Zn, Ni, Ba, Sr, V, Li, and Co; and Wave Cal solution (PerkinElmer, USA) was used to measure those of La, Mo, and Sc. Acetonitrile of HPLC grade was obtained from Concord Technology (Tianjin) Co., Ltd. (China). Certificated Reference Materials for the Chemical Composition of Soils (GBW07556, China) from the Institute of Geophysical and Geochemical Exploration was used to measure the recoveries of metals. A standard solution of 16 PAHs was obtained from TMstandard Co., Ltd. (China). Standard fluorene from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (China), fluoranthene from sigma-aldrich Co., Ltd. (USA), pyrene from Shanghai Meryer Chemical Technology Co., Ltd. (China), and benzo[a]anthracene from Shanghai Macklin Regent Co., Ltd. (China) were used to evaluate the recoveries of PAHs. A DNeasy® PowerSoil® Kit (QIAGEN, Germany) was used to extract DNA from soil samples.

2.2. Soil sampling

An initial investigation of soil contamination with HMs and PAHs was carried out at an e-waste dismantling plant and a coking plant. The two plants were located 48.8 km from each other and showed similar climatic features. Five samples (annotated samples A-E) were selected for further investigation according to their concentrations of HMs and PAHs. Sample A was collected from a paddy field near the e-waste dismantling plant and served as the control in this study. Sample B was obtained from the e-waste dismantling plant, and samples C-E were obtained from the coking plant. Sample C was collected from the field where coal and iron were stacked, sample D was obtained near the coking workshop, and sample E was significantly polluted with tar oil. Compared to sample A, which had relatively low concentrations of HMs and

PAHs, samples B and E were characterized by high concentrations of either HMs or PAHs, and samples C and D featured high concentrations of both HMs and PAHs.

Sampling was conducted on December 12th, 2020. The local temperature was 5–9 °C. The upper 30 cm of the soil from each site was collected within a 50 × 50 cm square area after removing the root mat. Five soil subsamples were mixed to obtain one composite soil sample. The soil samples were stored in sterile sampling bags at 4 °C and transported to the laboratory. After drying and sieving (0.15 mm), triplicate soil samples of 100 g for each sampling site were used for soil characterization. The analysis methods of soil properties, including moisture, organic carbon (OC), ammonia-nitrogen, and nitrate-nitrogen, are specified in the Supporting Information.

2.3. Chemical analysis

2.3.1. Analyses of HMs

The concentrations of HMs were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES; Optima 5300 DV, PerkinElmer, USA) after acidic digestion [39]. Triplicate soil samples (approximately 1.0 g each) in 7.0 mL of aqua regia (HCl:HNO₃, 3:1, v/v) were digested at 185 °C for 40 min using a microwave digestion system (Topwave, analytikjena, Germany). After digestion, the acid solution was boiled gently on a hot plate in a fuming cupboard until a volume of 2–3 mL remained. The residual acid solution was dissolved in ultrapure Milli-Q water to a final volume of 50 mL. The concentrations of metals (Al, Fe, Ca, Mg, Na, K, Ti, Cd, Mn, Cu, Ni, Pb, Zn, Ba, Sr, Co, Mo, Cr, As, V, La, Li, and Sc) in the solutions were measured using ICP-OES. The certified soil GBW07556 (GSS-65) in triplicate was digested and measured at the same condition [34,40]. Recoveries of the metals in the certified soil were 85.02–87.39% (Al), 86–104.65% (Fe), 85.3–91.37% (Ca), 89.71–97.37% (Mg), 88.78–92.78% (Na), 83.58–93.41% (K), 86.06–94.83% (Ti), 93.39–98.3% (Cu), 89.47–94.07% (Zn), 88.8–90.37% (Mn), 101.64–102.41% (Pb), 89.91–102.06% (Ba), 91.97–93.64% (Ni), 90.8–93.11% (Co), 94.72–98.11% (Sr), 86.21–97.37% (Cd), 92.53–100.41% (Mo), 94.67–100.68% (As), 92.78–101.36% (Cr), 94.58–97.06% (La), 99.13–105.02% (Li), 90.5–93.15% (V), and 94.56–98.93% (Sc).

2.3.2. Analyses of PAHs

PAHs in the soil were extracted using the Soxhlet method and analyzed by high-performance liquid chromatography (HPLC; 1260 Infinity, Agilent Technologies, USA) [41]. Triplicate soil samples of 10.0 g each were extracted with 100 mL of acetone and *n*-hexane (1:1, v/v) using a Soxhlet extractor and spiked with 200 ng of decafluorobiphenyl. Then, the extract was filtered through fiberglass meshes and concentrated and cleaned up using a rotary evaporator and Si SPE cartridge (CNW, ANPEL Laboratory Technologies (Shanghai) Inc., China), respectively. A mixture of *n*-hexane and dichloromethane (1:1, v/v) was used to dissolve the PAHs. The extract was concentrated by a rotary evaporator and dissolved in acetonitrile to a final volume of 5.0 mL. The concentrations of PAHs in the concentrated acetonitrile solution were analyzed using HPLC with an Eclipse plus C18 column (Agilent, USA) and acetonitrile-water as the mobile phase according to the following gradient: 6:4 acetonitrile:water from the start to 18 min; only acetonitrile from 18 to 28.5 min; and 6:4 acetonitrile:water from 28.5 min to the end. The analyzed PAHs were naphthalene (NAP), acenaphthylene (ACY), fluorene (FLO), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DhA), benzo[ghi]perylene (BgP), and indeno [1,2,3-cd]

pyrene (IcP). The concentrations of the 15 PAHs in the soil samples were determined, and the total concentration (Σ PAHs) was calculated. Soil samples A and E with the lowest and highest concentrations of PAHs in duplicates, respectively, were used for quality control [40,42]. The recoveries of the 15 PAHs in sample A were 68.5–72.95% (NAP), 48.72–53.56% (ACY), 46.44–49.55% (FLO), 84.34–85.34% (PHE), 79.04–80.75% (ANT), 110.05–132.29% (FLA), 77.08–77.08% (PYR), 80.65–82.59% (BaA), 82.56–85.37% (CHR), 75.25–75.25% (BbF), 82.61–82.76% (BkF), 80.43–82.74% (BaP), 85.57–85.63% (DhA), 75.87–77.94% (BgP), and 86.51–87.01% (IcP), respectively. The recoveries of FLO, FLA, PYR, and BaA in sample E were 70.51–108.91%, 97.10–115.30%, 75.65–78.08%, and 110.62–119.34%, respectively. The methods are specified in the Supporting Information.

2.4. Characteristics of microbial communities

Soil DNA was extracted from triplicate samples using a DNeasy® PowerSoil® Kit. The primer set 515F (5'-GTGCCAGCMGCCGCGG-3')/907R (5'-CCGTCGAATTCMTTTRAGTTT-3') was used to amplify the V4–V5 region of bacterial 16S ribosomal RNA (16S rRNA) genes [43–45]. The PCR products were sequenced by Shanghai Majorbio Biopharm Technology Co., Ltd. (China) using the Illumina MiSeq Platform (San Diego, USA). The microbial diversity and composition were analyzed using QIIME2 (v2020.2). The Shannon index was calculated using MOTHUR software (v1.30). One-way analysis of variance (ANOVA) was used to establish significant differences between the groups with $p < 0.05$. Beta diversity at the level of amplicon sequence variants (ASVs) was evaluated by principal coordinate analysis (PCoA). Linear discriminant analysis (LDA) coupled with effect size measurement (LefSe) analysis was performed to screen for various bacterial species. Redundancy analysis (RDA) was conducted with R software (v.3.3.1) using the Vegan package to determine the correlation between the soil microbiome diversity and the level of PAHs/HMs.

3. Results

3.1. The e-waste dismantling plant and the coking plant sites feature HMs and PAHs

Soil samples are collected from the coking plant (samples C, D, and E), the e-waste dismantling plant (sample B), and the nearby paddy field (sample A). The soil properties (moisture, pH, organic carbon, ammonia nitrogen, and nitrate nitrogen) of the five soil samples are presented in Table S1. The moisture of the soil samples was approximately 8.31–26.49%. The soil pH was in the range from 5.96 ± 0.08 (control) to 8.56 ± 0.28 (sample C). The concentration of organic carbon in sample D (51.93 ± 0.88 mg kg⁻¹) was significantly higher than those in the other samples (13.28–31.59 mg kg⁻¹). The concentrations of ammonia nitrogen and nitrate nitrogen in samples A and E (5.77–10.38 mg kg⁻¹ and 14.16–24.12 mg kg⁻¹, respectively) were higher than those in samples B, C and D (<2.21 mg kg⁻¹ and <1.06 mg kg⁻¹, respectively).

The contamination of the soil samples was evaluated based on the concentrations of metals (Al, Fe, Ca, Mg, Na, K, Ti, Cd, Mn, Cu, Ni, Pb, Zn, Ba, Sr, Co, Mo, Cr, As, V, La, Li, and Sc) and PAHs (NAP, ACY, FLO, PHE, ANT, FLA, PYR, BaA, CHR, BbF, BkF, BaP, DhA, BgP, and IcP), which are presented in Fig. 1 and Table S1. Metals Al, Fe, and Ca were present in all the soil samples at high concentrations (1,000–10,000 mg kg⁻¹); metals Mg, K, Ti, Mn, Zn, and Ba at moderate concentrations (100–1,000 mg kg⁻¹); and most of the remaining metals, such as Cd, Mo, La, Li, and Sc, at concentrations of less than 100 mg kg⁻¹. HMs such as Mn, Zn, and Cu showed significant influences on the microbial community structures, in

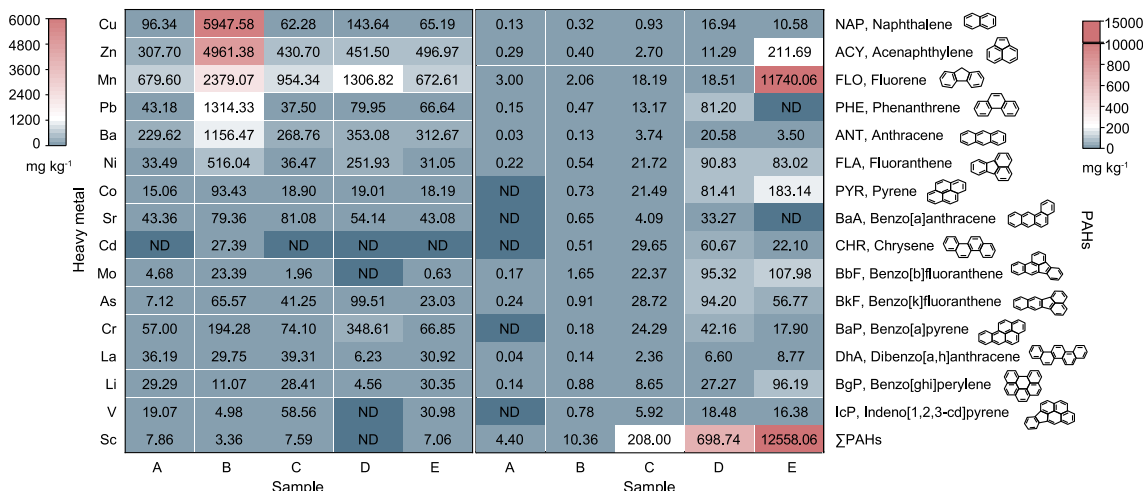


Fig. 1. Concentrations of HMs (left) and PAHs (right) in the soil samples. The number in each cell is the average concentration. ND denotes not detected.

contrast to common metals such as Al, Fe, and Ca, according to RDA results (Fig. 4), and were therefore mainly considered in this study. In addition, most kinds of PAHs were present at a concentration of 100 mg kg⁻¹. Various kinds of PAHs, such as ACY, FLO, and PYR, in Sample E were present in concentrations that exceeded 100 mg kg⁻¹. Pearson correlation analysis (Fig. S1) showed that HMs such as Mn, Zn, Cu, and Mo were highly related to each other (*p* < 0.5) and that PAHs such as ACY, PYR, and FLO were significantly related to each other (*p* < 0.5).

The concentrations of HMs and PAHs in sample A were relatively low compared to those in the remaining soil samples. For example, the concentrations of Zn (307.7 ± 21.4 mg kg⁻¹), Ba (229.62 ± 25.95 mg kg⁻¹), Co (15.06 ± 1.37 mg kg⁻¹), and As (7.12 ± 0.27 mg kg⁻¹) in sample A were much lower than those in other samples. The total concentration of PAHs (ΣPAHs) was 4.40 ± 0.84 mg kg⁻¹, which was the lowest among these samples. In addition to FLO (3.00 ± 0.68 mg kg⁻¹), the other PAHs were present in concentrations of below 0.30 mg kg⁻¹.

Sample B showed the highest concentrations of HMs among the five samples but low concentrations of PAHs. The concentrations of HMs, including Cu (5,947.58 ± 433.44 mg kg⁻¹), Zn (4,961.38 ± 436.51 mg kg⁻¹), Mn (2,379.07 ± 227.46 mg kg⁻¹), Pb (1,314.33 ± 55.92 mg kg⁻¹), and Ba (1,156.47 ± 103.39 mg kg⁻¹), were approximately tenfold higher than those in the other groups. Cd was only detected in sample B (27.39 ± 3.94 mg kg⁻¹). The ΣPAHs in sample B (10.36 ± 0.74 mg kg⁻¹) was slightly higher than that in sample A. In contrast, sample E showed a low concentration of HMs but a high concentration of PAHs. The concentrations of HMs such as Mn (672.61 ± 7.13 mg kg⁻¹), Ni (31.05 ± 1.95 mg kg⁻¹), and Sr (43.08 ± 4.09 mg kg⁻¹) were even lower than those in sample A. The concentration of FLO was 11,740.06 ± 620.1 mg kg⁻¹, followed by those of ACY (211.69 ± 7.04 mg kg⁻¹), PYR (183.14 ± 18.89 mg kg⁻¹), and BbF (107.98 ± 4.44 mg kg⁻¹), which resulted in ΣPAHs of as high as 12,558.06 ± 611.19 mg kg⁻¹.

Most of the concentrations of HMs and PAHs in samples C and D were between those in samples B and E. The concentrations of HMs such as Mn, Zn, and Ba in sample C (954.34 ± 93.97 mg kg⁻¹, 430.7 ± 15.79 mg kg⁻¹, and 268.76 ± 11.99 mg kg⁻¹, respectively) were lower than those in sample D (1,306.82 ± 48.57 mg kg⁻¹, 451.5 ± 7.66 mg kg⁻¹, and 353.08 ± 41.14 mg kg⁻¹, respectively). The ΣPAHs in sample C (208.00 ± 52.21 mg kg⁻¹) was also lower than that in sample D (698.74 ± 176.62 mg kg⁻¹). The main PAHs in sample C were CHR (29.65 ± 9.71 mg kg⁻¹), BkF

(28.72 ± 0.28 mg kg⁻¹), and BaP (24.29 ± 7.35 mg kg⁻¹), and the main PAHs in sample D were BbF (95.32 ± 20.40 mg kg⁻¹), BkF (94.20 ± 20.76 mg kg⁻¹), and FLA (90.83 ± 29.15 mg kg⁻¹).

Compared to sample A, samples B and E featured single contamination with HMs and PAHs, respectively, and samples C and D were characterized by mixed contamination with HMs and PAHs. The high concentrations of HMs in sample B could be related to the e-waste dismantling process, whereas the high concentrations of PAHs in sample E should be related to the oil tar [46]. The coking and steeling processes may have led to increased concentrations of both HMs and PAHs in samples C and D [47].

3.2. α- and β-diversities of the soil microbiomes

The 16S rRNA genes of bacteria were sequenced to analyze the soil microbiomes. The rarefaction curves (Fig. S2) indicated that the measurement of the sequences was sufficient. After quality control, 831,803 sequences of 16S rRNA genes were obtained. These sequences were clustered into 28,746 ASVs and assigned to 1,271 genera of 50 bacterial phyla. The α-diversities of the soil microbiomes (the Shannon, Simpson, Ace, and Chao1 indices) were evaluated (Table S2). The Shannon indices of samples A, C, and D reached as high as 7.10–7.33 and were close to one another (*p* > 0.05). Compared to that of sample A, the Shannon indices of samples B (6.31 ± 0.15) and E (5.01 ± 0.20) decreased significantly (*p* < 0.05), thereby showing that the richness of soil microbiomes in samples B and E was apparently lower than that in samples A, C, and D. This result showed that the soil microbiomes could survive at certain contamination levels of HMs and PAHs (samples C and D), but that high concentrations of either HMs or PAHs (samples B and E) decreased the α-diversities of the soil microbiomes. The β-diversity of the soil microbiomes was evaluated by PCA (Fig. S3). The samples were distant from one another, which suggested that the community structures of the soil microbiomes were different from one another.

3.3. Features of the soil microbial community at the phylum and genus levels

The microbial community structures of the soil microbiomes were analyzed at the phylum and genus levels (Fig. 2). There were twelve bacterial phyla with relative abundances of greater than 1% in the soil samples (Fig. 2a and Fig. S4). The top ten phyla that were

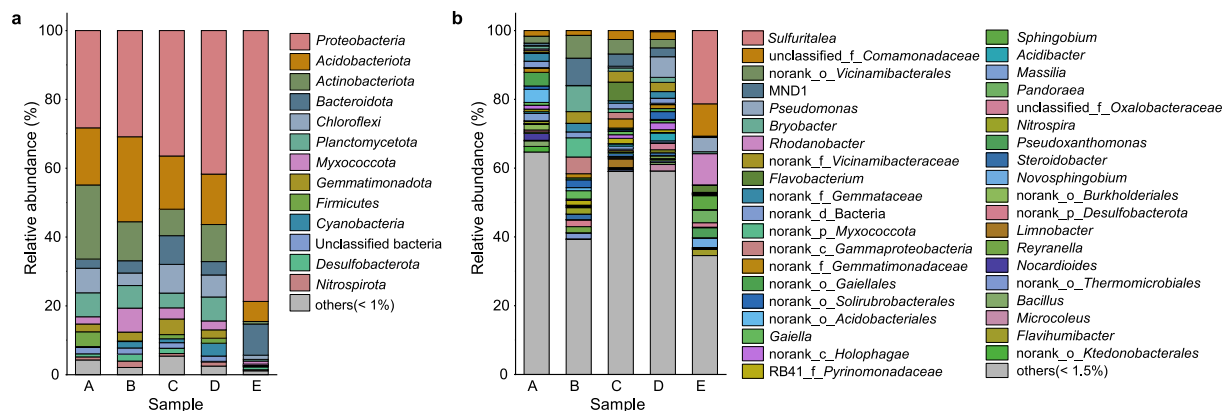


Fig. 2. Bacterial phyla (a) and genera (b) in the soil samples. Unclassified genera in various domains (d), phyla (p), classes (c), orders (o), and families (f) are specified.

found in the soil samples were *Proteobacteria*, *Acidobacteriota*, *Actinobacteriota*, *Bacteroidota*, *Chloroflexi*, *Planctomycetota*, *Myxococcota*, *Gemmatimonadota*, *Firmicutes*, and *Cyanobacteria*. *Actinobacteriota* (21.56%) and *Firmicutes* (4.31%) were relatively abundant in sample A but less abundant in the other samples. *Acidobacteriota* (24.71%) and *Myxococcota* (6.90%) showed the highest relative abundances in sample B. The relative abundance of *Proteobacteria* increased significantly from 28.24% to 78.69% from sample A to sample E. These results were similar to those of previous studies in which *Acidobacteriota* and *Myxococcota* were more frequently detected in HM-contaminated environments [48] and *Proteobacteria* were found to be more abundant when the concentration of PAHs was higher [49].

Thirty-nine genera had relative abundances of above 1.5%, including twenty-two classified genera (56.41%) and seventeen unclassified genera (43.59%) (Fig. 2b and Fig. S5). This high proportion of unclassified genera showed that many bacteria still need to be characterized in co-contaminated sites. The genera in sample A were quite diverse, and each of them was present with a similar relative abundance. In sample B, MND1 (7.95%), *Bryobacter* (7.54%), and an unclassified genus of *Vicinamibacteriales* (6.66%) showed high relative abundances. *Flavobacterium* showed the highest abundance in sample C (5.39%), and *Pseudomonas* was well represented in sample D (6.01%). *Sulfuritalea* was detected in samples D (0.36%) and E (21.34%) but barely detected in samples A, B, and C. In addition, *Rhodanobacter* (9.06%), an unclassified genus of *Comamonadaceae* (9.35%), *Sphingobium* (4.03%), *Pandoraea* (3.70%), and *Pseudoxanthomonas* (2.92%) showed higher relative abundances in sample E.

LEfSe with an LDA score of >4.0 was used to identify the main genera that were differently represented in the microbiomes of the soil samples (Fig. 3a). There were eight distinctive genera with LDA scores of >4.0 in sample B, six in sample E and three in samples A, C, and D. The three most distinctive genera of each sample are presented in Fig. 3b, and the others are presented in Fig. S5.

Massilia (2.18%) and unclassified genera of *Gaiellales* (3.93%) and *Acidobacteriales* (3.85%) were present mainly in sample A, and all of them showed relative abundances of <1% in the other samples, which implied that these genera were not capable of resisting HMs or PAHs. Genera such as MND1, *Bryobacter*, and an unclassified genus of *Vicinamibacteriales* showed the highest relative abundances in sample B (6.66–7.95%). Genera including *Sulfuritalea*, *Rhodanobacter*, and an unclassified genus of *Comamonadaceae* were the most abundant in sample E (9.06–21.34%), whereas the three genera showed relatively lower abundances <2.26% in samples A, B, C, and D. *Flavobacterium* in sample C (5.39%) and *Pseudomonas* in

sample D (6.01%) showed higher abundances than other genera in the soil with high concentrations of HMs and PAHs [37,38]. The results implied that the bacteria which were tolerant of high concentrations of HMs were also found in soil with mixed contamination with HMs and PAHs but that the bacteria which were dominated by high concentrations of PAHs hardly survived in soil with mixed contamination with HMs and PAHs, which has rarely been reported.

3.4. HMs and PAHs divergently impact the soil microbiomes

The impacts of HMs and PAHs on the soil microbiomes were evaluated with RDA (Fig. 4, Table S3, and Table S4). The results showed that the microbial community structures of these soil samples were influenced mainly by PAHs (such as ACY, FLO, and BbF) and HMs (such as Mn, Pb, and Ni) and slightly by soil properties (such as pH, moisture, and ammonia). Sample A was far from the others and less influenced by HMs and PAHs. The microbiomes of sample B were influenced mainly by HMs, and the microbiome of sample E was highly influenced by PAHs. In contrast, the microbial community structure of sample D was influenced by both HMs and PAHs, and the influences were more significant than those of sample C. In addition, the community structure of the soil bacteria in sample D was also significantly influenced by soil organic carbon. The RDA results were in accordance with soil properties and the concentrations of HMs and PAHs.

The correlations between HMs and PAHs and bacterial phyla and genera are presented in Fig. 5 and Fig. S6. Impressively, the bacteria that showed positive relations with HMs or PAHs were obviously different. At the phylum level (Fig. 5a), *Acidobacteriota*, *Myxococcota*, and *Nitrospirota* were positively related to HMs but negatively related to PAHs, whereas *Proteobacteria* and *Bacteroidota* were positively related to PAHs. At the genus level (Fig. 5b), MND1, *Gaiella*, *Nitrospira*, and *Steroidobacter* were positively related to HMs and negatively related to PAHs [9], whereas *Rhodanobacter*, *Sphingobium*, and *Pseudoxanthomonas* were positively related to PAHs and negatively related to HMs [34]. The results were in accordance with the LEfSe results.

4. Discussions

The results demonstrated that the microbiomes divergently responded to single contamination with HMs or PAHs and mixed contamination with HMs and PAHs in soil. The dominant bacteria in this study were in accordance with previous reports (Table S5). For example, Rogiers et al. and Qin et al. reported that bacterial phyla

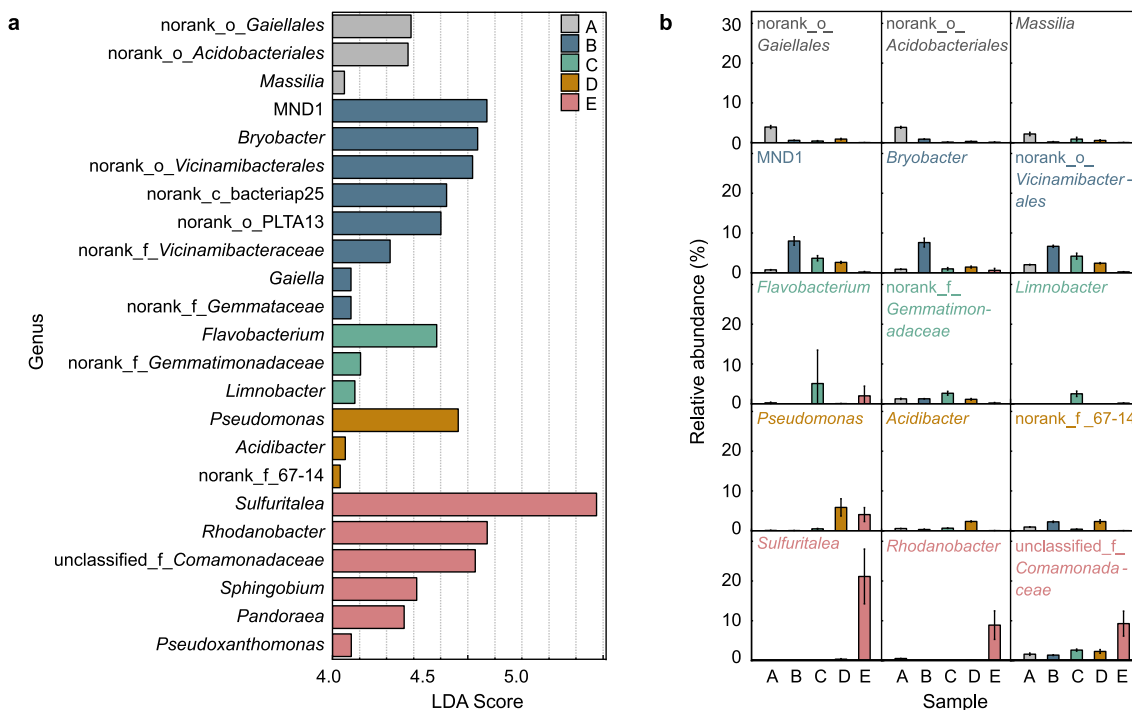


Fig. 3. LEfSe results of bacterial genera (a) and relative abundances of the distinctive genera (b).

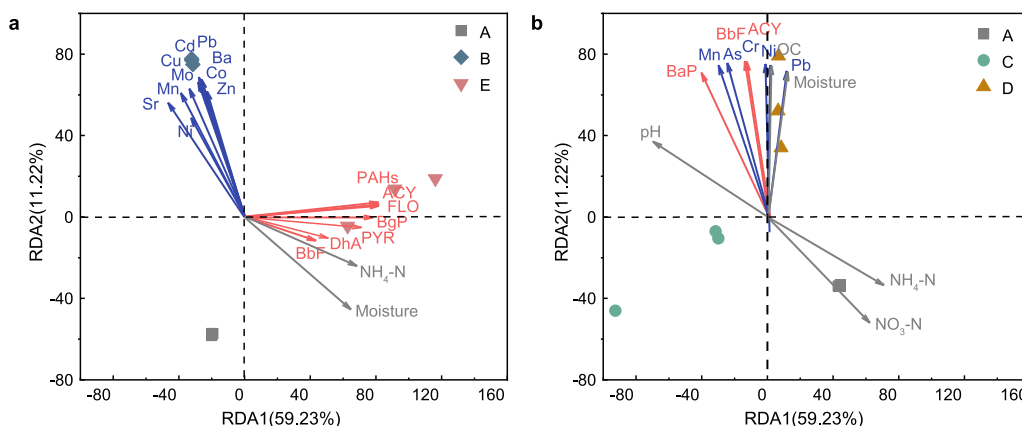


Fig. 4. Redundancy analysis (RDA) of environmental factors and the community structure of bacteria in soil with single contamination (a) and mixed contamination (b) of HMs and PAHs.

such as *Proteobacteria* (23.8–43.0%) and *Acidobacteria* (14.6–34.5%) and bacterial genera such as *Nitrosospora* (0.27–3.48%) were abundant in soil that was contaminated with HMs such as Pb (19–225 mg kg⁻¹), Cr (180–410 mg kg⁻¹), and Cd (2.4–110 mg kg⁻¹) [9,24]. Geng et al., Liu et al., and Miao et al. reported that bacterial phyla such as *Proteobacteria* (20.86–81.37%) and bacterial genera such as *Pseudomonas*, *Sphingomonas*, *Flavobacterium*, and *Pseudoxanthomonas* were abundant in soil that was contaminated with PAHs (0.11–56.11 mg kg⁻¹ in total) [8,34,50]. In addition, bacterial phyla such as *Proteobacteria* (35.3–98.45%), *Bacteroidetes* (0.3–18.6%), and *Actinobacteria* (0.6–13.4%) and bacterial genera such as *Pseudomonas* (0.7–25.0%) and *Flavobacterium* (5.2%) were found in soil that was contaminated with HMs (such as Pb at a concentration of 19–1,133 mg kg⁻¹, Mn at 64–435 mg kg⁻¹, and Cr at 0.04–1,033 mg kg⁻¹) and PAHs (15–2,002 mg kg⁻¹ in total) [37,38,51]. Compared to previous

reports, the concentrations of HMs and PAHs in soil samples in this study were much higher, and the divergent responses of microbiomes to such high concentrations of HMs and PAHs were rarely reported. Our study may facilitate understanding of how microbiomes respond to HMs and PAHs.

Bacteria may interact with HMs and PAHs via multiple strategies. For example, bacteria could use HMs as electron donors or acceptors for energy production [52] or use PAHs as both carbon and energy sources for growth [53]. Bacterial genera such as MND1 and *Bryobacter* decreased in abundance with decreasing concentrations of HMs but remained abundant in the presence of PAHs. In contrast, bacterial genera such as *Sulfuritalea* and *Sphingobium* failed to survive when the concentrations of PAHs were reduced. Therefore, it is inferred that PAH degraders are more sensitive to the concentrations of HMs and PAHs than HM-resistant bacteria.

In addition to HMs and PAHs, microbiomes can also be

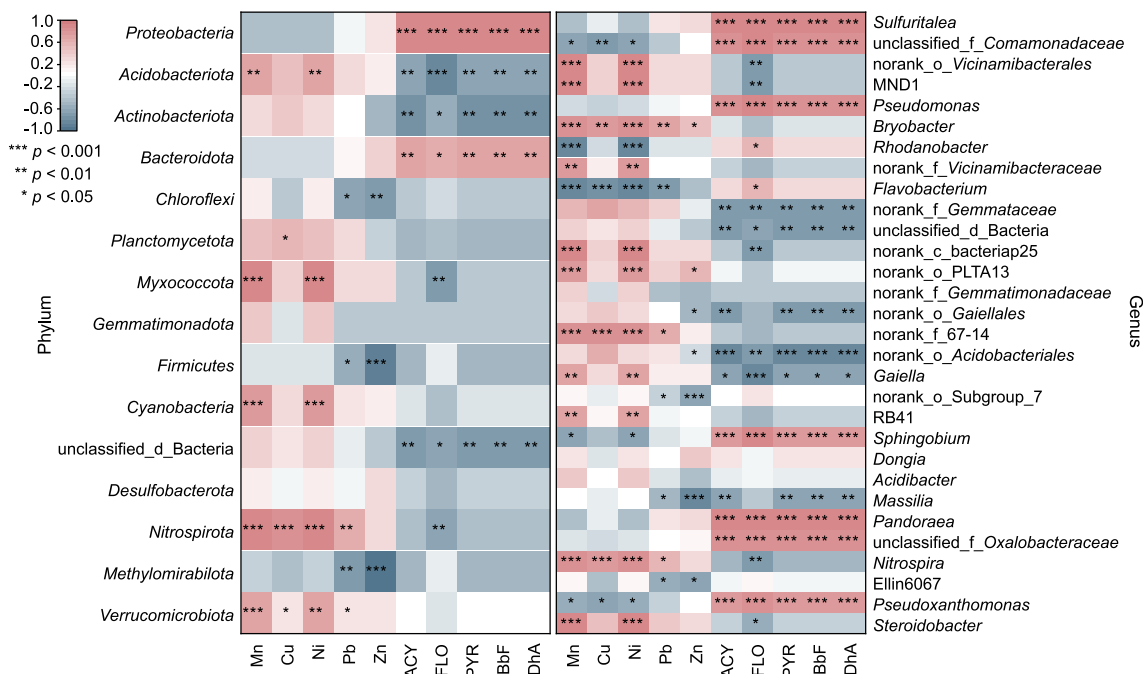


Fig. 5. Correlations between the contaminants and bacterial phyla (left) and genera (right).

influenced by soil physicochemical properties [36]. Eutrophic *Pseudomonas*, *Sulfuritalea*, and *Pandoraea* were abundant in sample D. In contrast, the relative abundance of a putative oligotrophic and unclassified genus of *Oxalobacteraceae* increased in sample C.

Bacteria surviving with HMs, PAHs or HM and PAH mixtures may serve as potential resources for bioremediation and for investigation of bacterial evolution. Various studies reported that *Bryobacter*, *Gaiella*, and *Nitrospira* were able to resist HMs [54–56], and *Pseudomonas*, *Sphingobium*, and *Pseudoxanthomonas* were able to degrade PAHs [57–59]. There were also reports that *Pseudomonas* and *Sphingobium* strains were able to resist HMs and degrade PAHs [58,60]. For example, *Pseudomonas putida* UW4 could degrade FLA in the presence of Pd [60], and the Cu-tolerant *Sphingobium* sp. PHE-1 could degrade PHE [61]. However, the knowledge on *Sulfuritalea*, *Bryobacter*, and *Pseudoxanthomonas* is still limited to the bacterial community level [38,62,63], and their adaptation mechanism to HMs/PAHs and application potential for bioremediation need to be explored.

5. Conclusions

In our study, soil microbiomes divergently responded to single and mixed contamination with HMs and PAHs. The bacterial genera *Sulfuritalea*, *Pseudomonas*, and *Sphingobium* were positively related to PAHs, and the bacterial genera *Bryobacter*, *Nitrospira*, and *Steroidobacter* were positively related to HMs. This study showed how hazardous HMs/PAHs changed the soil microbiome.

Declaration of 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.

Author contributions

Methodology, data analysis and original draft preparation, Z. N.

Yang and Z. S. Liu; measurements of PAHs and HMs, K. H. Wang and Z. L. Liang; sample preparation and DNA extraction, R. H. Wang, H. L. Ma, X. K. Wang, M. L. Yang, and B. G. Zhang; bacterial analysis, R. Abdugheni and Y. Huang; review and editing, C. Y. Jiang, D. F. Li, P. F.-X. Corvini, and S. J. Liu; and funding acquisition, S. J. Liu and C. Y. Jiang. All authors have read and agreed to the published version of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ese.2022.100169>.

References

- [1] N. Fierer, Embracing the unknown: disentangling the complexities of the soil microbiome, *Nat. Rev. Microbiol.* 15 (10) (2017) 579–590, <https://doi.org/10.1038/nrmicro.2017.87>.
- [2] S.Y. Sun, Y.N. Hou, W. Wei, H.M.A. Sharif, C. Huang, B.J. Ni, H.B. Li, Y.Y. Song, C.C. Lu, J.B. Guo, Perturbation of clopyralid on bio-denitrification and nitrite accumulation: long-term performance and biological mechanism, *Environ. Sci. Ecotech.* 9 (2022) 100144, <https://doi.org/10.1016/j.ese.2021.100144>.
- [3] D.G. Kong, Y.M. Du, M. Luo, G.W. Liu, X.T. Su, D.G. Luo, X.X. Huang, L.Z. Wei, Y. Liu, Q.H. Wu, Environmental Effects of Heavy Metals from the E-Waste Dismantling Site, South China, *Soil Sediment. Contam.*, 2021, pp. 1–16, <https://doi.org/10.1080/15320383.2021.1887810>.
- [4] M.K. Park, H.K. Cho, I.G. Cho, S.E. Lee, S.D. Choi, Contamination characteristics of polychlorinated naphthalenes in the agricultural soil of two industrial cities in South Korea, *Chemosphere* 273 (2021) 129721, <https://doi.org/10.1016/j.chemosphere.2021.129721>.
- [5] A. Azari, M.H. Mahmoudian, M.H. Niarie, I. Es, E. Dehganifard, A. Kiani,

- A. Javidi, H. Azari, Y. Fakhri, A.M. Khaneghah, Rapid and efficient ultrasonic assisted adsorption of diethyl phthalate onto $\text{Fe}^{\text{II}}\text{Fe}^{\text{III}}\text{O}_4\text{:GO}$: ANN-GA and RSM-DF modeling, isotherm, kinetic and mechanism study, *Microchem. J.* 150 (2019), <https://doi.org/10.1016/j.microc.2019.104144>.
- [6] E. Ahmadi, B. Kakavandi, A. Azari, H. Izanloo, H. Gharibi, A.H. Mahvi, A. Javid, S.Y. Hashemi, The performance of mesoporous magnetite zeolite nanocomposite in removing dimethyl phthalate from aquatic environments, *Desalination Water Treat.* 57 (57) (2016) 27768–27782, <https://doi.org/10.1080/19443994.2016.1178174>.
- [7] Y. Rashtbari, S. Hazrati, A. Azari, S. Afshin, M. Fazlzadeh, M. Vosoughi, A novel, eco-friendly and green synthesis of PPAC-ZnO and PPAC-nZVI nanocomposite using pomegranate peel: cephalene adsorption experiments, mechanisms, isotherms and kinetics, *Adv. Powder Technol.* 31 (4) (2020) 1612–1623, <https://doi.org/10.1016/j.apt.2020.02.001>.
- [8] R. Miao, M. Guo, X. Zhao, Z. Gong, C. Jia, X. Li, J. Zhuang, Response of soil bacterial communities to polycyclic aromatic hydrocarbons during the phyto-microbial remediation of a contaminated soil, *Chemosphere* 261 (2020) 127779, <https://doi.org/10.1016/j.chemosphere.2020.127779>.
- [9] T. Rogiers, J. Claesen, A. Van Gompel, N. Vanhoudt, M. Mysara, A. Williamson, N. Leys, R. Van Houdt, N. Boon, K. Mijnenonckx, Soil microbial community structure and functionality changes in response to long-term metal and radionuclide pollution, *Environ. Microbiol.* 23 (3) (2021) 1670–1683, <https://doi.org/10.1111/1462-2920.15394>.
- [10] L.J. Zhang, L. Qian, L.Y. Ding, L. Wang, M.H. Wong, H.C. Tao, Ecological and toxicological assessments of anthropogenic contaminants based on environmental metabolomics, *Environ. Sci. Ecotech.* 5 (2021) 100081, <https://doi.org/10.1016/j.ese.2021.100081>.
- [11] S.Y. Hashemi, M.Y. Badi, H. Pasalari, A. Azari, H. Arfaeina, A. Kiani, Degradation of ceftriaxone from aquatic solution using a heterogeneous and reusable $\text{O}_3/\text{UV}/\text{Fe}_3\text{O}_4/\text{TiO}_2$ systems: operational factors, kinetics and mineralisation, *Int. J. Environ. Anal. Chem.* (2020) 1–17, <https://doi.org/10.1080/03067319.2020.1817909>.
- [12] A. Azari, M. Yeganeh, M. Gholami, M. Salari, The superior adsorption capacity of 2,4-dinitrophenol under ultrasound-assisted magnetic adsorption system: modeling and process optimization by central composite design, *J. Hazard Mater.* 418 (2021) 126348, <https://doi.org/10.1016/j.jhazmat.2021.126348>.
- [13] M.Y. Badi, A. Esrafil, H. Pasalari, R.R. Kalantary, E. Ahmadi, M. Gholami, A. Azari, Degradation of dimethyl phthalate using persulfate activated by UV and ferrous ions: optimizing operational parameters mechanism and pathway, *J. Environ. Health Sci.* 17 (2) (2019) 685–700, <https://doi.org/10.1007/s40201-019-00384-9>.
- [14] J.C. Niemeyer, G.B. Lolata, G.M. de Carvalho, E.M. Da Silva, J.P. Sousa, M.A. Nogueira, Microbial indicators of soil health as tools for ecological risk assessment of a metal contaminated site in Brazil, *Appl. Soil Ecol.* 59 (2012) 96–105, <https://doi.org/10.1016/j.apsoil.2012.03.019>.
- [15] P.T. Gauthier, W.P. Norwood, E.E. Prepas, G.G. Pyle, Metal-PAH mixtures in the aquatic environment: a review of co-toxic mechanisms leading to more-than-additive outcomes, *Aquat. Toxicol.* 154 (2014) 253–269, <https://doi.org/10.1016/j.aquatox.2014.05.026>.
- [16] D.W. Lee, H. Lee, A.H. Lee, B.O. Kwon, J.S. Khim, U.H. Yim, B.S. Kim, J.J. Kim, Microbial community composition and PAHs removal potential of indigenous bacteria in oil contaminated sediment of Taean coast, Korea, *Environ. Pollut.* 234 (2018) 503–512, <https://doi.org/10.1016/j.envpol.2017.11.097>.
- [17] L. Li, X.Y. Yan, J. Li, Y.S. Tian, P. Ren, Advances in cotton tolerance to heavy metal stress and applications to remediate heavy metal-contaminated farmland soil, *Phyton-Int. J. Exp. Bot.* 90 (1) (2021) 35–50, <https://doi.org/10.32604/phyton.2021.012276>.
- [18] A.R.A. Usman, Y.A. Almaroai, M. Ahmad, M. Vithanage, Y.S. Ok, Toxicity of synthetic chelators and metal availability in poultry manure amended Cd, Pb and as contaminated agricultural soil, *J. Hazard Mater.* 262 (2013) 1022–1030, <https://doi.org/10.1016/j.jhazmat.2013.04.032>.
- [19] M.Z.U. Rehman, M. Rizwan, A. Hussain, M. Saqib, S. Ali, M.I. Sohail, M. Shafiq, F. Hafeez, Alleviation of cadmium (Cd) toxicity and minimizing its uptake in wheat (*Triticum aestivum*) by using organic carbon sources in Cd-spiked soil, *Environ. Pollut.* 241 (2018) 557–565, <https://doi.org/10.1016/j.envpol.2018.06.005>.
- [20] M.F. Seleiman, S. Ali, Y. Refay, M. Rizwan, B.A. Alhammad, S.E. El-Hendawy, Chromium resistant microbes and melatonin reduced Cr uptake and toxicity, improved physio-biochemical traits and yield of wheat in contaminated soil, *Chemosphere* 250 (2020) 126239, <https://doi.org/10.1016/j.chemosphere.2020.126239>.
- [21] R.E. Beattie, W. Henke, M.F. Campa, T.C. Hazen, L.R. McAilley, J.H. Campbell, Variation in microbial community structure correlates with heavy-metal contamination in soils decades after mining ceased, *Soil Biol. Biochem.* 126 (2018) 57–63, <https://doi.org/10.1016/j.soilbio.2018.08.011>.
- [22] Y.B. Lin, Y.M. Ye, Y.M. Hu, H.K. Shi, The variation in microbial community structure under different heavy metal contamination levels in paddy soils, *Ecotoxicol. Environ. Saf.* 180 (2019) 557–564, <https://doi.org/10.1016/j.ecoenv.2019.05.057>.
- [23] X.Q. Zhao, J. Huang, X.Y. Zhu, J.C. Chai, X.L. Ji, Ecological effects of heavy metal pollution on soil microbial community structure and diversity on both sides of a river around a mining Area, *Int. J. Environ. Res. Publ. Health* 17 (16) (2020) 5680, <https://doi.org/10.3390/ijerph17165680>.
- [24] C. Qin, X. Yuan, T. Xiong, Y.Z. Tan, H. Wang, Physicochemical properties, metal availability and bacterial community structure in heavy metal-polluted soil remediated by montmorillonite-based amendments, *Chemosphere* 261 (2020) 128010, <https://doi.org/10.1016/j.chemosphere.2020.128010>.
- [25] B. Li, R. Xu, X. Sun, F. Han, E. Xiao, L. Chen, L. Qiu, W. Sun, Microbiome-environment interactions in antimony-contaminated rice paddies and the correlation of core microbiome with arsenic and antimony contamination, *Chemosphere* 263 (2021) 128227, <https://doi.org/10.1016/j.chemosphere.2020.128227>.
- [26] S.P. Bachate, V.S. Nandre, N.S. Ghatpande, K.M. Kodam, Simultaneous reduction of Cr(VI) and oxidation of As(III) by *Bacillus firmus* TE7 isolated from tannery effluent, *Chemosphere* 90 (8) (2013) 2273–2278, <https://doi.org/10.1016/j.chemosphere.2012.10.081>.
- [27] Y.J. Yin, D. Li, Y.Q. Wang, Z.H. Xu, G.J. Xu, Z.Q. Zhao, S. Li, L.Y. Song, Concurrent removal of Mn(II) and Cr(VI) by *Achromobacter* sp. TY3-4, *Geomicrobiol. J.* 36 (4) (2019) 317–325, <https://doi.org/10.1080/01490451.2018.1544324>.
- [28] A.D. Wheatley, S. Sadhra, Polycyclic aromatic hydrocarbons in solid residues from waste incineration, *Chemosphere* 55 (5) (2004) 743–749, <https://doi.org/10.1016/j.chemosphere.2003.10.055>.
- [29] Y.J. Zhang, J. Wang, Distribution and source of polycyclic aromatic hydrocarbons (PAHs) in the surface soil along main transportation routes in Jiaying City, China, *Environ. Monit. Assess.* 182 (1–4) (2011) 535–543, <https://doi.org/10.1007/s10661-011-1895-z>.
- [30] W.H. Song, F. Cao, Y.C. Lin, M.M. Haque, X. Wu, Y.X. Zhang, C.Y. Zhang, F. Xie, Y.L. Zhang, Extremely high abundance of polycyclic aromatic hydrocarbons in aerosols from a typical coal-combustion rural site in China: size distribution, source identification and cancer risk assessment, *Atmos. Res* 248 (2021) 105192, <https://doi.org/10.1016/j.atmosres.2021.105192>.
- [31] B. Tomczyk, A. Siatecka, K. Jedruchiewicz, A. Sochacka, A. Bogusz, P. Oleszczuk, Polycyclic aromatic hydrocarbons (PAHs) persistence, bioavailability and toxicity in sewage sludge- or sewage sludge-derived biochar-amended soil, *Sci. Total Environ.* 747 (2020) 141123, <https://doi.org/10.1016/j.scitotenv.2020.141123>.
- [32] G. Gupta, V. Kumar, A.K. Pal, Microbial degradation of high molecular weight polycyclic aromatic hydrocarbons with emphasis on pyrene, *Polycycl. Aromat. Comp.* 39 (2) (2019) 124–138, <https://doi.org/10.1080/10406638.2017.1293696>.
- [33] X. Li, C. Qu, Y. Bian, C. Gu, X. Jiang, Y. Song, New insights into the responses of soil microorganisms to polycyclic aromatic hydrocarbon stress by combining enzyme activity and sequencing analysis with metabolomics, *Environ. Pollut.* 255 (Pt 2) (2019) 113312, <https://doi.org/10.1016/j.envpol.2019.113312>.
- [34] X. Liu, M. Liu, L. Zhou, L. Hou, Y. Yang, D. Wu, M.E. Meadows, Z. Li, C. Tong, J. Gu, Occurrence and distribution of PAHs and microbial communities in nearshore sediments of the Knysna Estuary, South Africa, *Environ. Pollut.* 270 (2021) 116083, <https://doi.org/10.1016/j.envpol.2020.116083>.
- [35] B. Biswas, B. Sarkar, A. Mandal, R. Naidu, Heavy metal-immobilizing organo-clay facilitates polycyclic aromatic hydrocarbon biodegradation in mixed-contaminated soil, *J. Hazard Mater.* 298 (2015) 129–137, <https://doi.org/10.1016/j.jhazmat.2015.05.009>.
- [36] S.H. Liu, G.M. Zeng, Q.Y. Niu, Y. Liu, L. Zhou, L.H. Jiang, X.F. Tan, P. Xu, C. Zhang, M. Cheng, Bioremediation mechanisms of combined pollution of PAHs and heavy metals by bacteria and fungi: a mini review, *Bioresour. Technol.* 224 (2017) 25–33, <https://doi.org/10.1016/j.biortech.2016.11.095>.
- [37] A. Bourceret, A. Cebron, E. Tisserant, P. Poupin, P. Bauda, T. Beguiristain, C. Leyval, The bacterial and fungal diversity of an aged PAH- and heavy metal-contaminated soil is affected by plant cover and edaphic parameters, *Microb. Ecol.* 71 (3) (2016) 711–724, <https://doi.org/10.1007/s00248-015-0682-8>.
- [38] A. Gran-Scheuch, J. Ramos-Zuniga, E. Fuentes, D. Bravo, J.M. Perez-Donoso, Effect of co-contamination by PAHs and heavy metals on bacterial communities of diesel contaminated soils of South Shetland Islands, Antarctica, *Microorganisms* 8 (11) (2020) 1749, <https://doi.org/10.3390/microorganisms8111749>.
- [39] F.B.V. da Silva, C.W.A. do Nascimento, P.R.M. Araujo, L.H.V. da Silva, R.F. da Silva, Assessing heavy metal sources in sugarcane Brazilian soils: an approach using multivariate analysis, *Environ. Monit. Assess.* 188 (8) (2016) 1–12, <https://doi.org/10.1007/s10661-016-5409-x>.
- [40] Y.Y. Zhao, F.A. Duan, Z.J. Cui, J.L. Hong, S.Q. Ni, Insights into the vertical distribution of the microbiota in steel plant soils with potentially toxic elements and PAHs contamination after 60 years operation: abundance, structure, co-occurrence network and functionality, *Sci. Total Environ.* 786 (2021) 147338, <https://doi.org/10.1016/j.scitotenv.2021.147338>.
- [41] S.X. Wu, X.R. Liu, M. Liu, X. Chen, S. Liu, L. Cheng, X.B. Lin, Y. Li, Sources, influencing factors and environmental indications of PAH pollution in urban soil columns of Shanghai, China, *Ecol. Indic.* 85 (2018) 1170–1180, <https://doi.org/10.1016/j.ecolind.2017.11.067>.
- [42] C. Wang, Y. Luo, H. Tan, H.K. Liu, F. Xu, H. Xu, Responsiveness change of biochemistry and micro-ecology in alkaline soil under PAHs contamination with or without heavy metal interaction, *Environ. Pollut.* 266 (2020) 115296, <https://doi.org/10.1016/j.envpol.2020.115296>.
- [43] M. Bill, L. Chidamba, J.K. Gokul, N. Labuschagne, L. Korsten, Bacterial community dynamics and functional profiling of soils from conventional and organic cropping systems, *Appl. Soil Ecol.* 157 (2021) 103734, <https://doi.org/10.1016/j.apsoil.2020.103734>.
- [44] J. Zhang, P.C. Wang, H.M. Tian, Q.Q. Xiao, H.K. Jiang, Pyrosequencing-based assessment of soil microbial community structure and analysis of soil properties with vegetable planted at different years under greenhouse conditions, *Soil Till. Res.* 187 (2019) 1–10, <https://doi.org/10.1016/j.still.2018.11.008>.

- [45] X.J. Xiang, S.M. Gibbons, H. Li, H.H. Shen, J.Y. Fang, H.Y. Chu, Shrub encroachment is associated with changes in soil bacterial community composition in a temperate grassland ecosystem, *Plant Soil* 425 (1–2) (2018) 539–551, <https://doi.org/10.1007/s11104-018-3605-x>.
- [46] X.J. Tang, C.F. Shen, D.Z. Shi, S.A. Cheema, M.I. Khan, C.K. Zhang, Y.X. Chen, Heavy metal and persistent organic compound contamination in soil from Wenling: an emerging e-waste recycling city in Taizhou area, China, *J. Hazard Mater.* 173 (1–3) (2010) 653–660, <https://doi.org/10.1016/j.jhazmat.2009.08.134>.
- [47] M. Rachwal, T. Magieral, M. Wawer, Coke industry and steel metallurgy as the source of soil contamination by technogenic magnetic particles, heavy metals and polycyclic aromatic hydrocarbons, *Chemosphere* 138 (2015) 863–873, <https://doi.org/10.1016/j.chemosphere.2014.11.077>.
- [48] F. Dell'Anno, E. Rastelli, M. Tangherlini, C. Corinaldesi, C. Sansone, C. Brunet, S. Balzano, A. Ianora, L. Musco, M.R. Montoreali, A. Dell'Anno, Highly contaminated marine sediments can host rare bacterial taxa potentially useful for bioremediation, *Front. Microbiol.* 12 (2021) 326, <https://doi.org/10.3389/fmicb.2021.584850>.
- [49] X.R. Liu, M. Liu, X. Chen, Y. Yang, L.J. Hou, S.X. Wu, P.K. Zhu, Indigenous PAH degraders along the gradient of the Yangtze Estuary of China: relationships with pollutants and their bioremediation implications, *Mar. Pollut. Bull.* 142 (2019) 419–427, <https://doi.org/10.1016/j.marpolbul.2019.03.064>.
- [50] S. Geng, W. Cao, J. Yuan, Y. Wang, Y. Guo, A. Ding, Y. Zhu, J. Dou, Microbial diversity and co-occurrence patterns in deep soils contaminated by polycyclic aromatic hydrocarbons (PAHs), *Ecotox. Environ. Safe.* 203 (2020) 110931, <https://doi.org/10.1016/j.ecoenv.2020.110931>.
- [51] S. Kuppusamy, P. Thavamani, M. Megharaj, K. Venkateswarlu, Y.B. Lee, R. Naidu, Pyrosequencing analysis of bacterial diversity in soils contaminated long-term with PAHs and heavy metals: implications to bioremediation, *J. Hazard Mater.* 317 (2016) 169–179, <https://doi.org/10.1016/j.jhazmat.2016.05.066>.
- [52] C.A. Navarro, D. von Bernath, C.A. Jerez, Heavy metal resistance strategies of acidophilic bacteria and their acquisition: importance for biomining and bioremediation, *Biol. Res.* 46 (4) (2013) 363–371, <https://doi.org/10.4067/S0716-97602013000400008>.
- [53] P. Thavamani, M. Megharaj, R. Naidu, Bioremediation of high molecular weight polyaromatic hydrocarbons co-contaminated with metals in liquid and soil slurries by metal tolerant PAHs degrading bacterial consortium, *Biodegradation* 23 (6) (2012) 823–835, <https://doi.org/10.1007/s10532-012-9572-7>.
- [54] I.S. Kulichevskaya, N.E. Suzina, W.I.C. Rijpstra, J.S.S. Damste, S.N. Dedysh, *Paludibaculum fermentans* gen. nov., sp. nov., a facultative anaerobe capable of dissimilatory iron reduction from subdivision 3 of the *Acidobacteria*, *Int. J. Syst. Evol. Microbiol.* 64 (2014) 2857–2864, <https://doi.org/10.1099/ijs.0.066175-0>.
- [55] B.Q. Li, R. Xu, X.X. Sun, F. Han, E.Z. Xiao, L. Chen, L. Qiu, W.M. Sun, Microbiome-environment interactions in antimony-contaminated rice paddies and the correlation of core microbiome with arsenic and antimony contamination, *Chemosphere* 263 (2021), <https://doi.org/10.1016/j.chemosphere.2020.128227>.
- [56] L.T. Hao, B.G. Zhang, C.P. Feng, Z.Y. Zhang, Z.F. Lei, K. Shimizu, X.L. Cao, H. Liu, H.P. Liu, Microbial vanadium (V) reduction in groundwater with different soils from vanadium ore mining areas, *Chemosphere* 202 (2018) 272–279, <https://doi.org/10.1016/j.chemosphere.2018.03.075>.
- [57] J.Q. Liang, J.T. Xu, W.J. Zhao, J.F. Wang, K. Chen, Y.Q. Li, Y. Tian, Benzo[a]pyrene might be transported by a TonB-dependent transporter in *Novosphingobium pentaromativorans* US6-1, *J. Hazard Mater.* 404 (2021), <https://doi.org/10.1016/j.jhazmat.2020.124037>.
- [58] L. Madueno, B.M. Coppotelli, S. Festa, H.M. Alvarez, I.S. Morelli, Insights into the mechanisms of desiccation resistance of the Patagonian PAH-degrading strain *Sphingobium* sp. 22B, *J. Appl. Microbiol.* 124 (6) (2018) 1532–1543, <https://doi.org/10.1111/jam.13742>.
- [59] V.L. Shinde, V. Suneel, C. Rathore, B.D. Shenoy, Degradation of tarballs using associated bacterial consortia, *3 Biotech* 10 (3) (2020), <https://doi.org/10.1007/s13205-020-2095-8>.
- [60] S.L. Afegbua, L.C. Batty, Effect of plant growth promoting bacterium; *Pseudomonas putida* UW4 inoculation on phytoremediation efficacy of monoculture and mixed culture of selected plant species for PAH and lead spiked soils, *Int. J. Phytoremediation* 21 (3) (2019) 200–208, <https://doi.org/10.1080/15226514.2018.1501334>.
- [61] M.K. Song, Y. Yang, L.F. Jiang, Q. Hong, D.Y. Zhang, Z.G. Shen, H. Yin, C.L. Luo, Characterisation of the phenanthrene degradation-related genes and degrading ability of a newly isolated copper-tolerant bacterium, *Environ. Pollut.* 220 (2017) 1059–1067, <https://doi.org/10.1016/j.envpol.2016.11.037>.
- [62] X. Hao, J. Zhu, C. Rensing, Y. Liu, S. Gao, W. Chen, Q. Huang, Y.R. Liu, Recent advances in exploring the heavy metal(loid) resistant microbiome, *Comput. Struct. Biotechnol. J.* 19 (2021) 94–109, <https://doi.org/10.1016/j.csbj.2020.12.006>.
- [63] Q. Wang, J. Hou, J. Yuan, Y. Wu, W. Liu, Y. Luo, P. Christie, Evaluation of fatty acid derivatives in the remediation of aged PAH-contaminated soil and microbial community and degradation gene response, *Chemosphere* 248 (2020) 125983, <https://doi.org/10.1016/j.chemosphere.2020.125983>.