Bacterial community and potential ecological risk from heavy metals in soils of coal mines

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ABSTRACT

Coal consumption increases consistently, and heavy metal contamination in soils surrounding coal mines has been a severe environmental issue. An increasing number of evaluation studies are being conducted on the effects of heavy metals on the soil environment based on various organisms that inhabit soil. Among these organisms, bacteria are the smallest species but comprising the largest population in soil environment. Bacteria possess large specific surface areas and are rich in both extracellular and intracellular substances, making bacteria crucial as pioneer species in ecological succession. It is important to conduct in-depth investigations on the effects of soil contamination based on bacterial community analysis and to establish a versatile estimation method.

The objective of this study was to establish a method for quantitatively evaluating the relations between bacterial communities and potential ecological risks posed by heavy metals in soils surrounding coal mines. Targeting on six coal mines with different production scales (annual production: $0.6 \sim 8.0 \times 10^6$ tons), we analyzed the bacterial community structure with the combination of quantitative PCR and high-throughput sequencing, and estimated the potential ecological risk from heavy metals, based on these analyzed the relations between bacterial communities and the potential ecological risks. The main results of this research are as follows:

Regarding the bacterial community, differences in the abundance of total bacteria based on 16S rDNA were observed in the soils surrounding different coal mines, with the mine having the smallest production scale being about an order of magnitude higher than that having the largest production scale. Bacterial diversity was evaluated based on the Shannon and Simpson indexes, and no obvious differences were observed between the coal mines. Furthermore, according to the analysis using high-throughput sequencing, the bacteria inhabiting the soils surrounding each coal mine were mainly Proteobacteria, Acidobacteriota, Actinobacteria, Firmicutes, and Bacteroideta, accounting for approximately 70% of the total bacteria; yet slight differences relating to their presence levels among the six mines were revealed. The potential ecological risk from heavy metals was estimated using the Potential Ecological Risk Index model, and it was

suggested that among the six coal mines, the one with the largest coal production scale had the lowest ecological risk value. Among the seven heavy metals tested, Cd was the most significant contributor, accounting for nearly 50% of the overall ecological risk. Analysis based on Geographical Detector (GeoDetector) model revealed elevation and total phosphorus (TP) as two key influencing factors influencing the distribution of heavy metals, and elevation showed a positive correlation, while TP exhibited a negative correlation with metal concentrations. Additionally, analysis based on Positive Matrix Factorization (PMF) model identified three potential pollution sources, with overall contributions ranging of coal transportation (39.4%) > coal mining activities (32.2%) > geology (28.4%).

Regarding the relations between bacterial community and potential ecological risk, it was suggested that the bacterial abundance was negatively correlated with the overall ecological risk from heavy metals, and the bacterial diversity was negatively correlated with Zn. Furthermore, 12 species were identified as bacteria those sensitive to the overall risk and the individual risk from Cr, Cd, and Zn, and relations between their density and ecological risk were determined. Corresponding linear models at $p < 0.05$ level were established based on their absolute abundance and the potential ecological risk from heavy metals.

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Chapter 1

INTRODUCTION

1.1 BACKGROUND

1.1.1 Coal mining industry

Since the onset of the Industrial Revolution in the mid-19th century, coal has assumed a paramount position as the primary source of energy. With the growth of industry and advancements in technology, other sources of energy, such as hydroelectric power, nuclear energy, and renewable energy, have been progressively developed and utilized. However, presently the global energy system is still reliant on oil, coal, and natural gas (Haszeldine, 2009; Arratia-Solar, 2019; Jakob *et al.*, 2020).

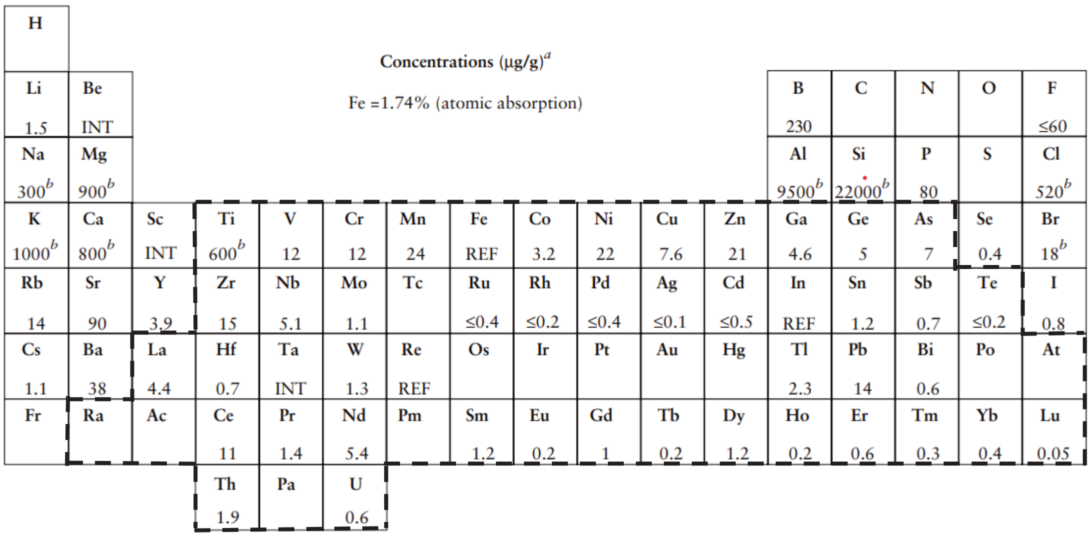
As Covid-19 restrictions ease and economic activity recovers, there has been a sharp increase in energy consumption, leading to greater demands on coal supplies (BP, 2023). Global coal consumption increased by 6% in 2021, mainly contributed by China, India, Europe, and North America, as reported by the IPCC (2022) and BP (2023). The global coal production matched consumption, and reached a historic high in 2022. China recorded an 11% increase in its output as compared to 2021, while India's production escalated by 16%, additionally, the United States experienced a 3% rise in production in 2022 (BP, 2023).

There is an increasing need for interdisciplinary scientific assessment of the environmental impacts of coal production to help promote regional sustainable development of the coal production industry (Ma et al., 2021).

1.1.2 Heavy metals in coal

Coal is a type of sedimentary rock and organo-clastic in occurrence, that forms from the remains of plant material that were deposited in a swampy environment and later buried by other sedimentary layers. It consists mainly of organic matter, mineral substances, and water (Adaikpoh et al., 2006; Lett et al., 2004). The mineral elements in coal are derived from multiple sources, including sedimentary materials deposited alongside the coal, minerals transported by water, and inherent materials present in the plant precursors (Lett et al., 2004). **Table 1** illustrates the typical concentration levels of mineral elements in a West Virginia bituminous coal sample. Heavy metals comprise most of the mineral elements present in coal. The concentration levels of heavy metals differed significantly among various types of coal, as illustrated in **Table 2**. The heavy metal elements including Co, Ni, Cu, Zn, As, Mo, Cd, Hg, and Pb are of potential environmental concern (Lett et al., 2004).

Table 1 Semi-Quantitative Mineral Element Survey of West Virginia (Ireland Mine) hvAb Coal (Lett et al., 2004)



Concentrations (μg/g)[a]

Fe =1.74% (atomic absorption)

H																
Li 1.5	Be INT											B 230	C	N	O	F ≤60
Na 300[b]	Mg 900[b]											Al 9500[b]	Si 22000[b]	P 80	S	Cl 520[b]
K 1000[b]	Ca 800[b]	Sc INT	Ti 600[b]	V 12	Cr 12	Mn 24	Fe REF	Co 3.2	Ni 22	Cu 7.6	Zn 21	Ga 4.6	Ge 5	As 7	Se 0.4	Br 18[b]
Rb 14	Sr 90	Y 3.9	Zr 15	Nb 5.1	Mo 1.1	Tc	Ru ≤0.4	Rh ≤0.2	Pd ≤0.4	Ag ≤0.1	Cd ≤0.5	In REF	Sn 1.2	Sb 0.7	Te ≤0.2	I 0.8
Cs 1.1	Ba 38	La 4.4	Hf 0.7	Ta INT	W 1.3	Re REF	Os	Ir	Pt	Au	Hg	Tl 2.3	Pb 14	Bi 0.6	Po	At
Fr	Ra	Ac	Ce 11	Pr 1.4	Nd 5.4	Pm	Sm 1.2	Eu 0.2	Gd 1	Tb 0.2	Dy 1.2	Ho 0.2	Er 0.6	Tm 0.3	Yb 0.4	Lu 0.05
			Th 1.9	Pa	U 0.6											

- - - : Heavy metal elements

[a] Determined by spark source mass spectrometry on low-temperature ash renormalized to whole coal basis unless otherwise indicated. Volatile elements such as halogens, S, and Sb are largely lost in ashing procedure.

[b] Determined by X-ray fluorescence on whole coal.

Note. REF, reference; Fe is used as an internal reference; In and Re are added as additional low-concentration references; INT, intereference.

Source: National Energy Technology Laboratory unpublished data

Table 2 Quantitative analysis of mineral elements in coal samples collected from coal mining sites across Pakistan and one Chinese coal sample (Chilikwazi et al., 2023)

The concentration of minor elements in coal samples (ppm)					
Elements	Chinese	Lakhra	Duki	Makerwal	Thar
Ba	63 ± 6	37 ± 6	-	54 ± 8	53 ± 7
Ce	-	-	-	-	20 ± 7
Cr	-	70 ± 8	-	153 ± 12	14 ± 6
Fe	-	647 ± 21	-	3190 ± 123	1147 ± 37
K	316 ± 11	594 ± 20	833 ± 24	649 ± 20	749 ± 24
Li	574 ± 18	338 ± 17	291 ± 11	620 ± 33	458 ± 27
Mg	701 ± 27	207 ± 24	255 ± 31	3680 ± 106	379 ± 43
Mn	147 ± 21	137 ± 14	137 ± 17	-	166 ± 16
Na	1861 ± 58	736 ± 18	93 ± 11	1020 ± 32	978 ± 30
Pb	-	-	252 ± 27	210 ± 29	-
Sr	-	777 ± 31	-	405 ± 13	566 ± 16
Ti	-	1391 ± 41	-	1154 ± 43	906 ± 29
Zn	33 ± 8	-	-	-	70 ± 13

1.1.3 Heavy metals in coal dust, coal gangue, and mine drainage

The heavy metal in coal poses significant environmental safety concerns. Coal production results in the release of heavy metals, making it a major source of environmental metal pollution (Liu et al., 2019; Liang et al., 2017; Zhang et al., 2020). These toxic metals can be discharged into the environment via coal dust, coal gangue, and acid mine drainage, resulting in long-term environmental damage such as the destruction of ecosystems and biodiversity, land disruption, and environmental contamination (Pandey & Agrawal, 2014; Niu et al., 2017).

Dust acts as a carrier for heavy metals, as illustrated in **Fig. 1**. Sultana et al. (2022) assessed the concentration of heavy metals in coal dust from mines in Pakistan and observed that the mean concentration decreased in the following order:

Cr>Mn>Zn>Ni>Pb>Co>Cu>Cd, with heavy metals predominantly found in fine dust particles. The size of dust particles is a critical determinant of the risk posed to human health, as indicated by Csavina et al. (2012), Shen et al. (2021), and Sultana et al. (2022). Fine dust particles can penetrate deeply into the lungs, carrying contaminants directly into the bloodstream, whereas larger particles tend to settle in the upper respiratory tract (Valiulis et al., 2008; Sultana et al., 2022).

Coal production generates large amounts of coal gangue, which is typically enriched with heavy metals. Chilikwazi et al. (2023) investigated the heavy metal concentrations in coal gangue (mudstone and sandstone) from Zambia and presented their findings in **Table 3**. Overall, mudstone exhibited higher levels of heavy metals than sandstone. The descending order of mean heavy metal concentrations in mudstone and sandstone were as follows: Fe, Pb, Zn, Cu, Ni, and Cd, and Fe, Pb, Zn, Ni, Cu, and Cd, respectively. Cd poses a medium risk in both mudstone and sandstone.

Coal production produces acid mine drainage (AMD) that contains heavy metals. Improper disposal of AMD can result in the release of harmful metal elements into the environment. Heavy metals from mine drainage can become mobilized and contaminate soil and river sediments. Shylla et al. (2021) investigated the heavy metal concentrations in drainage from coal mines in India and reported their findings in **Table 4**. The concentration of individual metals in the samples was in the following order: Fe>Mn>Zn>Cr>Pb>Cu>Cd. Fe concentration exceeded that of other metals by more than 80% in all the tested samples. The major metal contaminants in the drainage were Mn, Pb, Fe, and Cr.

The release of heavy metals through coal dust, gangue, and drainage can cause serious consequences for the environment surrounding coal mines, contaminating natural ecosystems, and negatively impacting biodiversity. Heavy metals such as As, Cd, Cr, Cu, Ni, Pb, Zn, and Hg are considered environmentally hazardous elements and the most covert, persistent, and irreversible pollutants in the ecosystem (Wang et al., 2001; Belkin et al., 2008; Saikia et al., 2014). Heavy metal pollution poses a threat to the health and well-being of animals and humans, since they can accumulate in vegetation, aquatic species like fish, and livestock (Cardwell et al., 2002; Pruvot et al., 2006; Nabulo et al., 2010). Consumption of contaminated pastures by livestock and contaminated vegetables and dairy products by humans may cause adverse health effects (Liu et al., 2019).

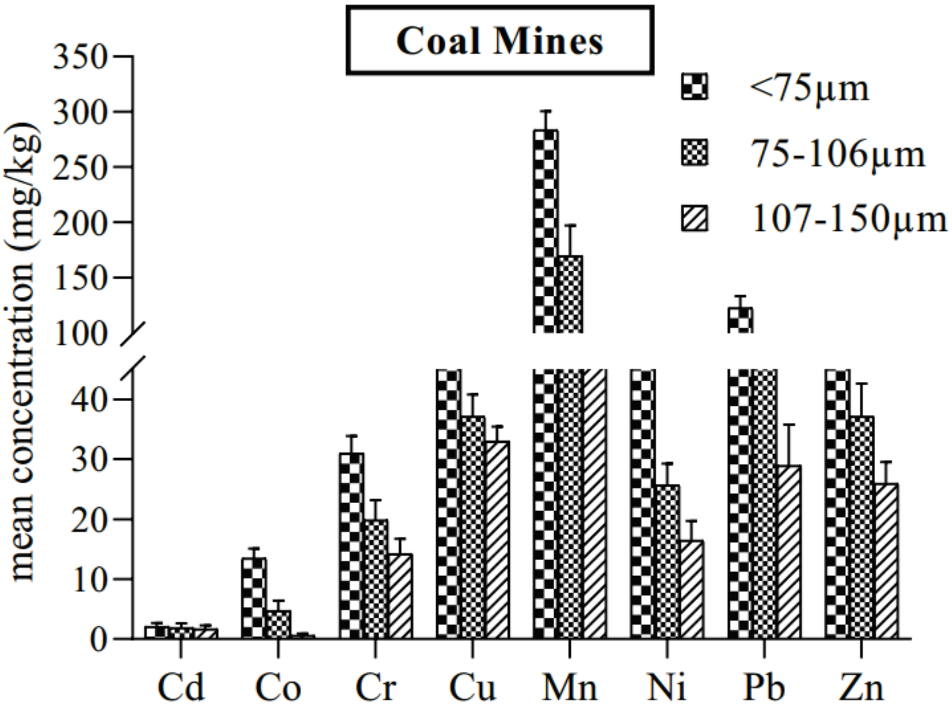


Fig. 1 Mean concentration of the heavy metals in three size fractions of dust in coal mines (Sultana *et al.*, 2022)

Table 3 Mean concentrations (mg/kg) in coal gangues (Chilikwazi *et al.*, 2023)

Heavy metals	Mudstone	Sandstone	Control sample	F value	*P* value
Zn	63.5a	36.62b	38.34b	4.811	0.038
Cu	44.28c	1.51d	17.17e	1613.145	0
Fe	11,619.86a	12,193.46a	12,991.74a	0.537	0.602
Ni	7.89b	6.48b	7.25b	2.309	0.155
Cd	0.97c	1.17c	0.62b	9.777	0.006
Pb	70.18a	58.67b	23.42c	205.231	0

In each row, means with the same letters are not significantly different, while those with different letters are significantly different ($p<0.05$) according to the Duncan's multiple range test.

Table 4 Heavy metal concentration (mg/L) in acid mine drainage (Shylla et al., 2021)

Heavy metals	**WS1**				**WS2**				**WS3**				**WS4**				**WC**			
	PRE	MON	POS	MCM	PRE	MON	POS	MCM	PRE	MON	POS	MCM	PRE	MON	POS	MCM	PRE	MON	POS	MCM
Fe	207.9	54.092	136.8	132.93	178.1	42.036	78.9	99.678	143.6	22.8	40.5	68.967	259.4	96.7	195.3	183.8	0.765	0.529	0.46	0.585
Mn	3.19	0.279	1.65	1.706	7.16	0.2	4.32	3.893	5.538	0.144	2.34	2.674	5.34	0.201	2.741	2.761	0.055	0.007	0.019	0.027
Cu	0.004	0.005	0.002	0.004	0.002	0.003	0.004	0.002	0.004	0.002	0.003	0.003	0.007	0.005	0.003	0.005	0.001	BDL	0.002	0.001
Pb	0.031	0.006	0.026	0.021	0.044	0.008	0.04	0.031	0.098	0.009	0.0119	0.04	0.027	0.006	0.016	0.016	0.002	0.001	0.002	0.001
Zn	6.75	0.489	3.95	3.73	4.69	0.42	2.77	2.627	4.03	0.055	2.34	2.142	5.34	0.922	0.575	2.279	0.26	0.025	0.012	0.099
Cd	0.002	0.001	0.001	0.001	0.001	BDL	BDL	0.001	BDL	BDL	0.001	0.001	0.001	0.001	0.007	0.003	BDL	BDL	BDL	0
Cr	2.8	0.216	0.089	1.035	3.4	0.124	0.713	1.412	1.146	0.082	0.146	0.458	3.7	0.194	0.98	1.625	0.022	0.002	0.001	0.009

PRE pre-monsoon, MON monsoon, POS post-monsoon, MCM mean concentration of metal, BDL below detection level.

1.2 REVIEW OF LITERATURE

1.2.1 Soil bacterial community

In the soils of coal mines in previous studies, Proteobacteria, Acidobacteriota, and Actinobacteria were found most dominant (**Table 5**). The sum of their relative abundance averagely accounted for about 67% of the total bacteria. The review studies based on soils from 30 coal mines worldwide and 50 coal mines in China demonstrated that heavy metal contamination was one common and long-term environmental issue for soils surrounding coal mines (Liu et al., 2019; Sahoo et al., 2016). The similarity of bacterial community structure in the soils of coal mines in previous studies may be due to the long-term disturbance from heavy metals, even though the contamination level was low. Small but frequent imputes of toxic pollutants over a long time were found to be more important in changing the microbial properties, including their biomass, diversity, and structure, compared to much larger short-term inputs (Frossard et al., 2017; Song et al., 2018). Long-term coal mining activities offered origins for frequent inputs of heavy metals into the soils and may cause the changes of bacterial community. This assumption can be supported by the limited shift of soil bacterial community after revegetation and fertilization in the soils surrounding coal mines (Ngugi et al., 2017; Wang et al., 2020; Du et al., 2021). However, comprehensive studies regarding the relations between the soil bacterial community and heavy metals are needed to get direct evidence.

Moreover, in the soils contaminated with heavy metals studied in the reviewed literature, Proteobacteria was the most abundant taxa, and its relative abundance accounted for 42% of the total bacteria on average. The dominance of Proteobacteria in the soils contaminated with heavy metals indicated their strong tolerance to heavy metals. Johnson et al. (2019) reported that Proteobacteria had 46% of the 170,000 heavy metal-resistant genes published in the National Center for Biotechnology Information (NCBI) database. These genes play vital roles in molecular transport, energy production, and macromolecular biosynthesis and can promote the survival of Proteobacteria in environments with high concentrations of heavy metals (Jaiswal et al., 2011; Johnson et al., 2019). For Actinobacteria, its tolerance to Cr was found in the sediment contaminated with heavy metals (Rajeev et al., 2021), and Cr and Pb were reported as necessary trace elements for the growth of Actinobacteria (Liu et al., 2019).

Table 5 Bacterial community in the soils of coal mines of this study and previous studies, and the soils contaminated by heavy metals evaluated through high-throughput sequencing.

Soils	**Dominant taxa in phylum level and the corresponding relative abundance to the total bacteria**	**References**
Coal mine soil (Low risk from heavy metals)	Proteobacteria (42%), Acidobacteriota (10%), Actinobacteria (7%), Firmicutes (5%), Bacteroidota (4%), Crenarchaeota (3%), Myxococcota (2%), Chloroflexi (2%), Verrucomicrobiota (2%), Gemmatimonadota (2%)	This study
Coal mine soil (Risk not mentioned)	Actinobacteria (44%), Proteobacteria (27%), Acidobacteria (10%), Chlorofexi (8%), Gemmatimonadetes (3%), Firmicutes (2%), Cyanobacteria (1%), Bacteroidota, Verrucomicrobia, Rokubacteria	Du et al., 2021
Coal mine soil (Risk not mentioned)	Proteobacteria (32%), Actinobacteria (21%), Acidobacteria. (18%), Gemmatimonadetes (8%), Chloroflexi (5%), Bacteroidota (4%), candidate division WPS-1 (5%), Planctomycetes (3%), Firmicutes (3%), Verrucomicrobia (2%)	Guo et al., 2021
Coal mine soil (Risk not mentioned)	Proteobacteria (20%), Actinobacteria (17%), Acidobacteria (10%), Chloroflexi (7%), Bacteroidota (2%), Gemmatimonadetes (6%), Cyanobacteria (0.7%), Firmicutes (4%), Planctomycetes (1%), TM7 (0.4%)	Wang et al., 2020
Coal mine soil (Risk not mentioned)	Actinobacteria (51%), Acidobacteria (12%), Proteobacteria (11%), Chloroflexi (8%), Gemmatimonadetes (6%), Thaumarchaeota (5%), Planctomycetes (2%), Verrucomicrobia (1%), and Firmicutes (0.70%)	Chen et al., 2020a
Coal mine soil (Risk not mentioned)	Proteobacteria (28%), Acidobacteria (22%), Actinobacteria (19%), Chloroflexi (8%), Gemmatimonadetes (7%), Bacteroidota (5%), Firmicutes (3%), Patescibacteria (2%), Verrucomicrobia (2%), and Nitrospirae (2%)	Guo et al., 2022
Coal mine soil (Risk not mentioned)	Actinobacteria (-), Proteobacteria (-), Acidobacteria (-), and Chloroflexi (-)	Ngugi et al., 2017
Soil (High risk from Pb, Zn, and Cd)	Actinobacteria (32%), Proteobacteria (27%), Chloroflexi (14%), Acidobacteria (13%), Bacteroidota (6%), Firmicutes (4%), Cyanobacteria (3%), Nitrospirae (2%)	Pan et al., 2020
Soil (High risk from Cu, Zn, Pb, Ni)	Proteobacteria (42%), Firmicutes (20%), Acidobacteria (10%) and Bacteroidota (8%)	Zhao et al., 2019
Soil (High risk from Cd, Cu, and Zn)	Proteobacteria (35%), Actinobacteria (40%), Acidobacteria (20%), Chloroflex (10%), Firmicutes (5%), Gemmatimonadetes (1%) Planctomycetes (0.2%), Verrucomicrobia (0.1%), Bacteroidota (0.05%)	Song et al., 2018
Soil (High risk from Zn and Cu)	Proteobacteria (50%), Actinobacteria (32%), Chloroflexi (24%), Acidobacteria (11%), Gemmatimonadetes, Firmicutes, Grenarchaeota, Bacteroidota, Nitrospirae	Tseng et al., 2021

1.2.2 Heavy metal contamination

Soil heavy metal contamination is a significant global concern associated with coal mining activities. Numerous studies have been conducted to assess the concentrations of heavy metals in soils of coal mines worldwide. For instance, Li and Ji (2017) reported high concentrations of Cd, Cu, and Pb in surface soils of coal-mine brownfields in Beijing, China. Similarly, Agrawal et al. (2016) found elevated levels of Cu and Zn in surface soils near coal mining areas of the Jharia coalfields, India, and Tozsin (2014) reported high levels of Cr in soil of Oltu coal mines, Turkey. A review study by Liu et al. (2019) compared heavy metal concentrations in soils from 50 typical coal mines in China and revealed that soil heavy metal contamination is a prevalent environmental issue associated with coal production. Statistical analysis of heavy metal concentrations in these soils showed that mean concentrations of As (29.283 mg/kg), Cd (0.518 mg/kg), Cr (92.890 mg/kg), Ni (47.101 mg/kg), and Pb (41.012 mg/kg) exceeded the corresponding Chinese soil guidelines (grade I). The concentrations of As and Cd were particularly high, being about 1.95 and 2.59 times greater than their corresponding Chinese soil guidelines (grade I), respectively. Elevated heavy metal concentrations in mining-affected soils have also been reported in other regions, such as China (Gu, 2011), Bangladesh (Bhuiyan et al., 2010), and India (Mishra et al., 2008), compared to natural soils. The average concentrations of Cu and Pb were higher than global average values of 30 mg/kg, whereas the concentrations of Zn and Ni were lower than global baselines for uncontaminated soils of 80-120 mg/kg and 20 mg/kg, respectively (Adriano, 2001; Kabata-Pendias and Pendias, 1992; Ross, 1994).

Evaluating the ecological risk indexes of heavy metals was a common approach to determine their toxicity to soil organisms surviving in the areas surrounding coal mines (Sahoo et al., 2016; Liu et al., 2019). The review study by Liu et al. (2019) demonstrated that based on the concentrations of heavy metals in 50 typical coal mines in China, the heavy metals had median risk indexes in the following order: Cd (21.30 mg/kg) > Hg (6.84 mg/kg) > As (3.96 mg/kg) > Pb (3.61 mg/kg) > Ni (3.31 mg/kg) > Cu (1.59 mg/kg) > Cr (0.58 mg/kg) > Zn (0.27 mg/kg) (**Table 6**). Cd was found to be the element causing the highest ecological risk, contributing about 50% of the overall potential ecological risk from heavy metals (Cd, Hg, As, Pb, Ni, Cu, Cr, and Zn). This finding is consistent with the results of studies conducted in six coal mines in India and three coal mines in Europe, which showed that Cd contributed a significant proportion of the potential ecological risk in the soil, averaging 71% and 30%, respectively (Sahoo et al., 2016) (**Fig. 2**). These results suggest that Cd is a common contaminant in coal mine soils and should be given great attention and are most toxic to the soil ecology.

Table 6 Basic statistical information about average heavy metal concentration in Chinese coal mine soils (heavy metal concentration unit: mg/kg-dry) (Liu et al., 2019)

	As	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**	**Hg**
10th	4.785	0.111	29.455	11.363	8.895	16.078	46.848	0.029
25th	7.834	0.144	39.255	18.824	21.841	22.34	54.865	0.036
50th	10.466	0.268	66.37	28.618	34.51	26.7	67.16	0.056
75th	22.032	0.443	83.476	37.054	48.243	39.181	84.577	0.096
90th	25.821	1.473	105.849	66.369	134.05	58.673	119.056	0.289
Mean	29.283	0.518	92.89	33.18	47.101	41.012	79.404	0.09
Skewness	4.415	2.676	4.399	2.245	2.733	4.05	2.423	2.428
Kurtosis	19.637	7.674	19.602	6.55	8.235	16.619	6.582	5.689
Chinese soil guidelines (grade I)	15	0.2	90	35	40	35	100	0.15
Chinese soil guidelines (grade II)								
pH<6.5	40	0.3	150	50	40	250	200	0.3
6.5<pH<7.5	30	0.6	200	100	50	300	250	0.5
pH >7.5	25	1	250	100	60	350	300	1

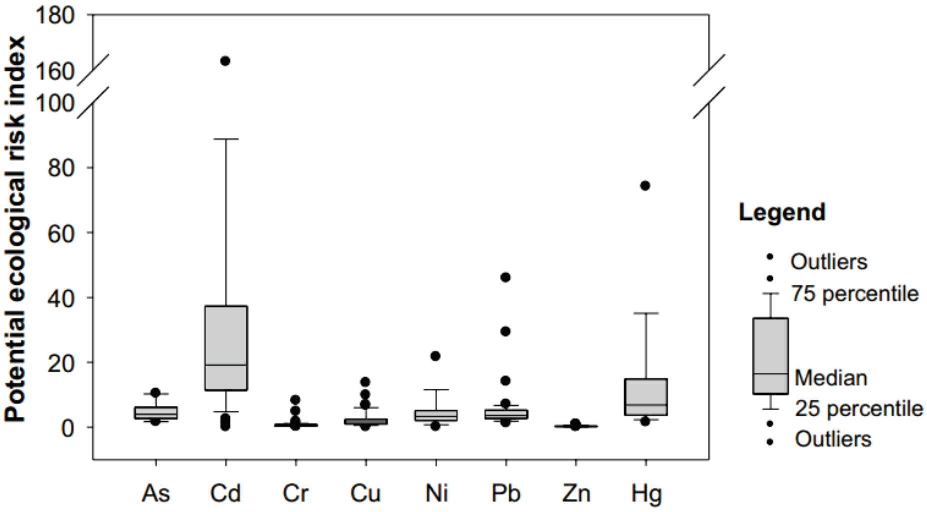


Fig. 2 Potential ecological risk index calculated with Chinese soil guidelines (grade II) (Liu et al., 2019)

The use of this method for quantifying the toxicity of heavy metals to microorganisms is a subject of controversy, as the toxic factors were estimated based on the concentrations of heavy metals in igneous rocks, soils, freshwater, land plants, and animals (Hakanson, 1980), rather than on the concentrations accumulated in microorganisms. Although assessing metal accumulation in microorganisms poses challenges, existing limited research suggests a strong possibility of heavy metal accumulation in these microorganisms. Heavy metals have the potential to alter the abundance, diversity, and community structure of bacteria, as well as impact the genes involved in these cellular processes. Relying solely on ecological risk indexes to estimate the toxicity of heavy metals to microorganisms may not fully capture their true effects. Investigation of the bacterial communities in soils surrounding coal mines using approaches that enable a more comprehensive evaluation of the relations of bacterial abundance, diversity, and structure with heavy metals is very important for better understanding the impact of coal production on ecosystem health and for more effective and sustainable operation and management of the industry associated with coal production. Furthermore, in most cases, since soil pollution by heavy metals is complex pollution by many heavy metal elements, ecological risk evaluation in terms of an overall index that could take into account the impact from all heavy metals is also important in addition to the individual risk from each heavy metal element. Therefore, to obtain information that could benefit a more in-depth understanding of the impacts of heavy metals in the soil environment surrounding coal mines, a detailed investigation of relations between soil bacterial community and the potential ecological risk from heavy metals is desired.

1.3 OBJECTIVES OF THE STUDY

The main objective of this study was to investigate the impact of heavy metals on the bacterial community in coal mine soils, through a comprehensive assessment and modeling of the relations between bacterial abundance and the potential ecological risk posed by heavy metals. This involved three key aspects: evaluating the potential ecological risk from heavy metals, characterizing the properties of the bacterial community (abundance, structure, and diversity), and examining the relations between the bacterial community and the potential ecological risk from heavy metals.

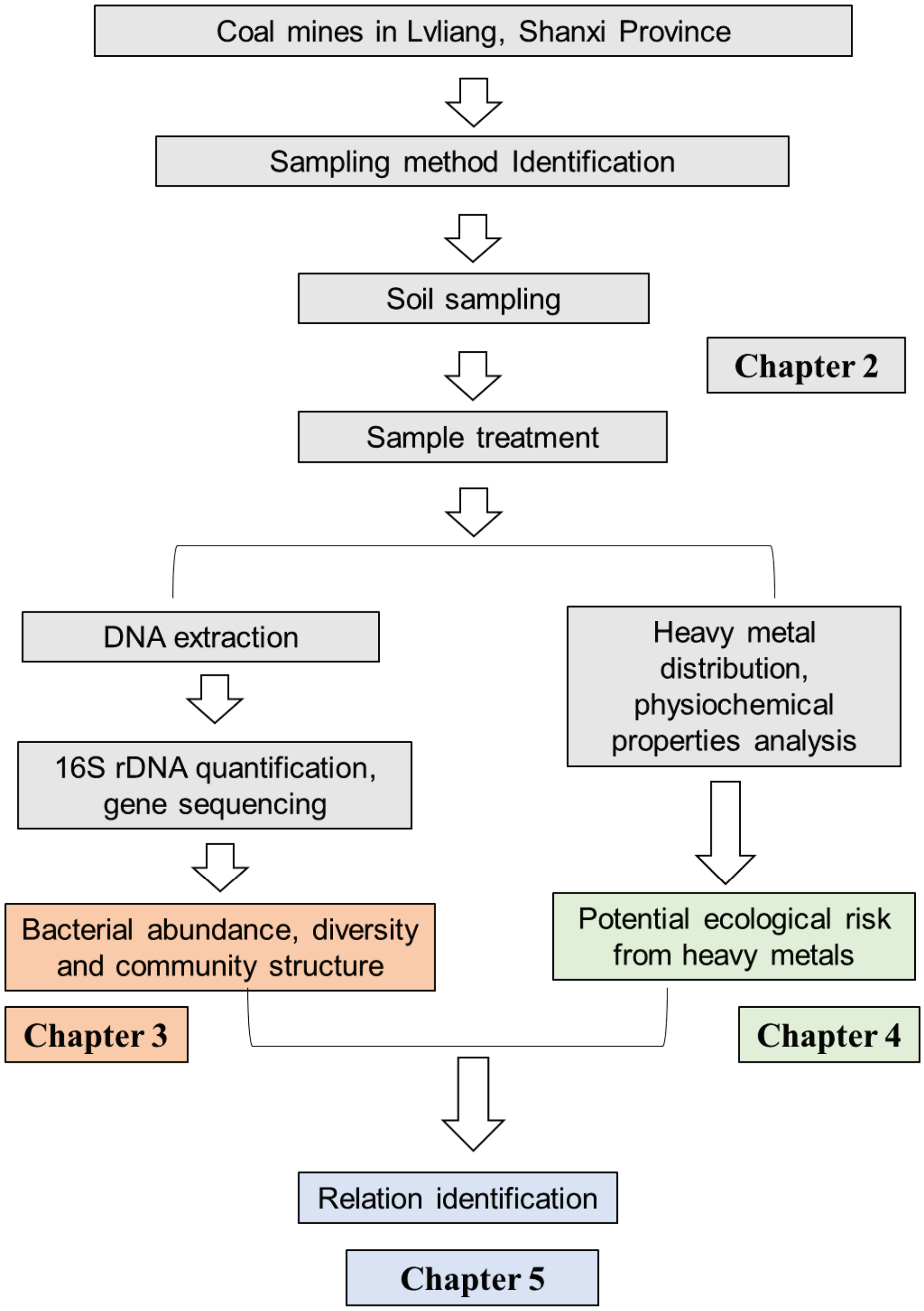
For achieving these, six coal mines in Luliang, Shanxi Province of China, were targeted and, from each coal mine, soil from three sites located 0, 1, and 3 km apart from its center was sampled for analysis of the concentrations of heavy metals (As, Cd, Cr, Cu, Ni, Pb, and Zn), based on which the associated potential ecological risk was computed. The bacterial community was evaluated based on bacterial abundance, diversity, and community structure through the combined application of quantitative PCR and high-throughput sequencing.

Their likely relations with the ecological risk from heavy metals, together with the possible involvement of soil water content, pH, EC, organic matter, total phosphorus, and total nitrogen were also evaluated through Redundancy analysis (RDA) and Spearman analysis.

1.4 CONTENTS OF THE DISSERTATION

This dissertation presents the experimental results and model analysis for the objectives of the study. Chapter 2 describes the study areas, sampling methods, and the analytical methods used for the bacterial community, relation analysis, and modeling. In Chapter 3 describes the properties of the bacterial community, including abundance, diversity, and structure. Chapter 4 shows the heavy metal distribution in soils surrounding coal mines and their potential ecological risks. Chapter 5 discusses the relations between the bacterial community and the potential ecological risk from heavy metals, along with model analyses using bacterial abundance and potential ecological risk. Additionally, models of sensitive bacteria and individual risk from each heavy metal are presented. Finally, Chapter 6 provides a summary of the research and considerations for future studies.

1.5 TECHNICAL ROUTE



Chapter 2

MATERIALS AND METHODS

2.1 SOIL SAMPLING

2.1.1 Sampling sites

Six coal mines, denoted as M1, M2, M3, M4, M5, and M6 at coordinates 37°08′53″-37°37′28″N and 110°39′45″-110°05′33″E, in Luliang, Shanxi Province, China were targeted, as illustrated in **Fig. 2.1**. These mines produce high-quality anthracite and coking coal, with annual productions of 0.6, 1.2, 1.5, 1.8, 3.0 and 8.0×10^6 t, respectively. Luliang has a semi-arid continental climate and is famous for its rich storage of high-quality anthracite and coking coal resource. The soil of the coal mines is mainly composed of cinnamon soil. The mean annual temperature, precipitation and evaporation of this region are 10.5 ºC, 472.3 mm and 1200 mm, respectively. The geochemical information, including DEM, slope, precipitation, and NDVI of the sampling sites of coal mines (M1-M6) are shown in **Table 2.1** and **Fig. 2.2**. The data of NDVI and precipitation were sourced from the database of the Institute of Geography at the Chinese Academy of Sciences and China Meteorological Network, respectively. The digital elevation model (DEM) data were acquired from the Geospatial Data Cloud, provided by the Computer Network Information Center, Chinese Academy of Sciences. The slope was computed using DEM.

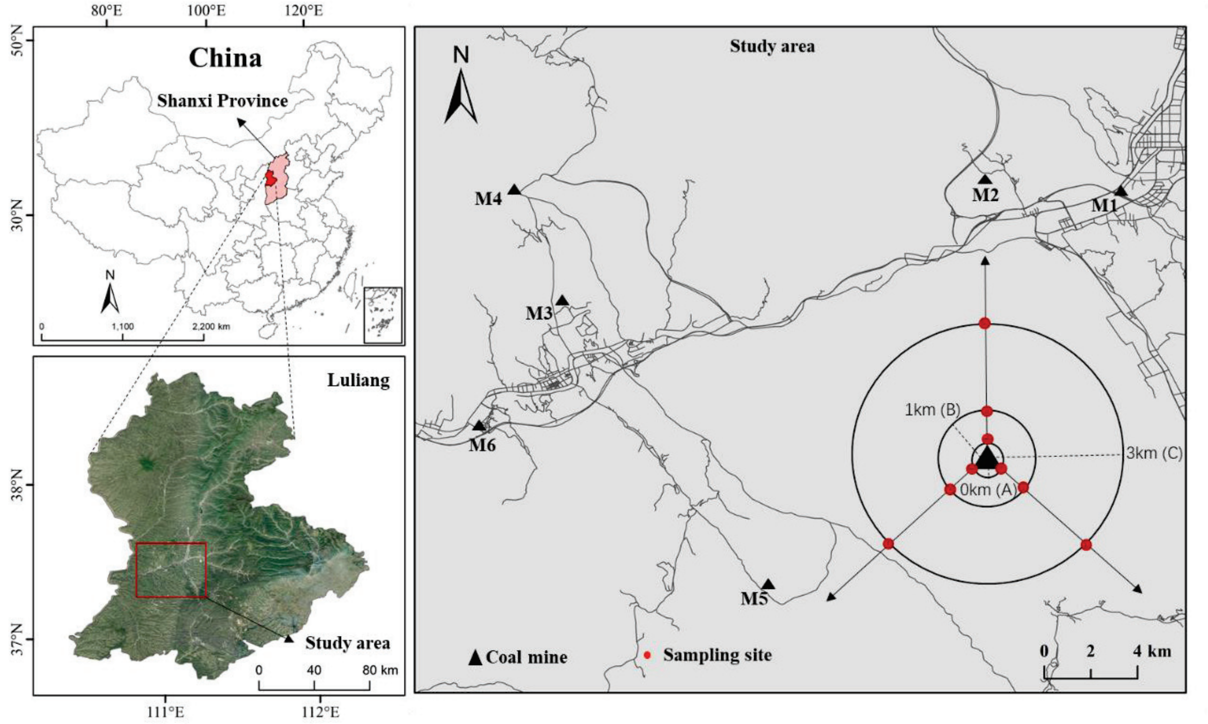


Fig. 2.1 Study area and locations of the six coal mines (M1-M6). A, B, and C indicate the soil sampling sites around each mine distanced 0, 1and 3km apart from the center of the mine, respectively. For each site, soil was taken at three spots distributed averagely on the circle of the corresponding distance and mixed for analysis.

Table 2.1 Geographical and meteorological information for the sampling sites of the coal mines (M1-M6).

Coal mine	**Index**	**DEM**	**Slope**	**NDVI**	**Precipitation**
	unit	m	degree	-	mm
M1	range	910-920	1.8-6.2	0.30-0.38	489.5-490.1
	mean	917^{c}	2.7^{a}	0.34^{b}	489.7^{d}
M2	range	932-1006	13.2-13.9	0.17-0.31	491.6-491.9
	mean	944^{d}	13.3^{c}	0.21^{a}	491.6^{f}
M3	range	886-886	9.7-9.7	0.2-0.2	487.5-487.5
	mean	886^{bc}	9.7^{b}	0.2^{a}	487.5^{a}
M4	range	856-856	3.6-3.8	0.34-0.34	489.3-489.3
	mean	856^{b}	3.7^{a}	0.34^{b}	489.3^{c}
M5	range	951-952	9.3-13.6	0.23-0.30	490.1-490.1
	mean	952^{d}	11.5^{b}	0.26^{ab}	490.1^{f}
M6	range	770-772	1.2-1.9	0.22-0.37	487.8-487.9
	mean	770^{a}	1.3^{a}	0.3^{ab}	487.9^{b}
	Average	889.40	7.10	0.30	489.40

Different letters (a, b, and c) show the differences between the groups in the same metal are statistically significant (p<0.05). The letter ‘ab’ in the group of coal mines means the difference of the value with the letters of ‘a’ and ‘b’ is not statistically significant. The letter ‘abc’ in the group of coal mines means the difference of the value with the letters of ‘a’, ‘b’ and ‘c’ is not statistically significant.

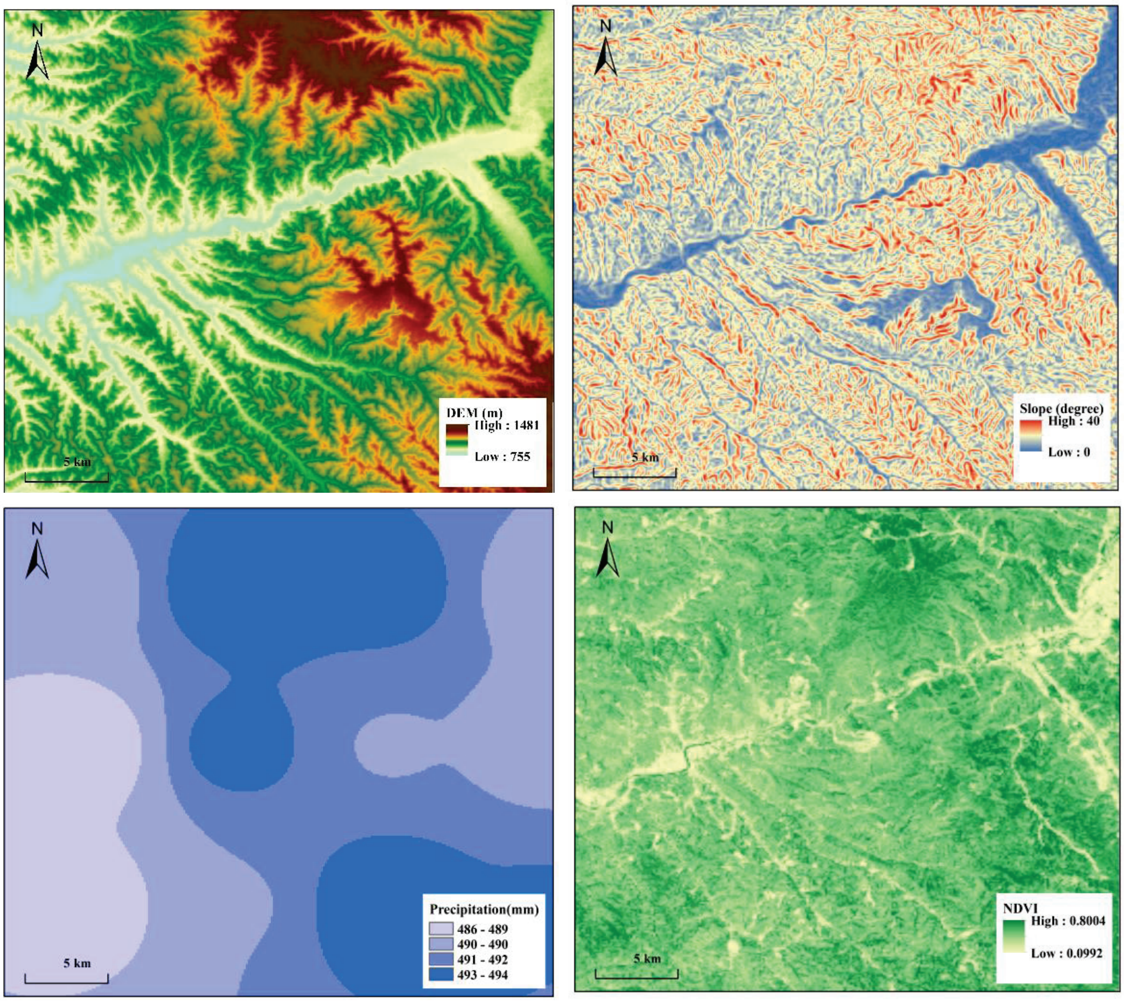


Fig. 2.2 Spatial distribution of natural factors including DEM, slope, precipitation, and NDVI of coal mines (M1-M6)

2.1.2 Sampling procedures

For each coal mine, three sampling sites for soil, labeled as A, B, and C with a distance about 0, 1, and 3 km apart from its center, respectively, were designated. The inclusion of site C 3 km away from the center of each mine for study was made by taking into consideration of the likely involvement of multiple influential factors, particularly the factors relating to wind, rainfall, coal transportation that may promote diffusion and transfer of heavy metals. For each site, surface soil samples (1-5 cm) were collected from three spots and were mixed for analysis. The mixed samples were transported to the laboratory in ice-cooled boxes. Each sample was separated into two parts, with one being used for the analysis of its basic physicochemical properties and the other one for analysis of heavy metals and bacterial community-related indexes. Before being subjected to analysis, the latter one was dried using a freeze-vacuum dryer, pulverized after removing stones and twigs larger than 2mm, and then stored at -25 ^{o}C in a deep freezer.

2.2 BACTERIAL COMMUNITY

Total genomic DNA was extracted for each soil sample using the Power Soil DNA Extraction Kit (MOBIO, USA) according to the manufacturer’s protocol. The integrity of the extracted DNA was evaluated by 1% agarose gel electrophoresis, and the purity of the DNA was checked on a microvolume spectrophotometer (NanoDrop, ND2000, ThermoScientific, USA) (Li et al., 2021a).

The abundance of total bacteria based on 16S rDNA gene was measured through quantitative PCR (qPCR) with SYBR® Premix Ex Taq™ (TaKaRa, Japan) according to the manufacturers' protocol. The 16S rDNA gene was amplified with the primers set Com1(5′-CAGCAGCCGCGGTAATAC-3′) and Com2 (5′-CCGTCAATTCCTTTGAGTTT-3′). The qPCR program consisted of initial denaturation at 95 ºC for 5 min followed by 40 three-step cycles of 95 ºC for 15s, 50 ºC for 30 s, and 72 ºC for 30 s (Li et al., 2021a).

The bacterial community structure was analyzed using high-throughput sequencing. The v4 hypervariable region of the 16S rRNA gene was amplified with the set of primers 515F/806R (5′-GTGCCAGCMGCCGCGGTAA -3′/ 5'-GGACTACHVGGGTWTCTAAT-3′) (Li et al., 2020a) using Phusion® High-Fidelity PCR Master Mix (New England Biolabs). The cycling conditions consisted of an initial denaturation at 98°C for 1 min, followed by 30 amplification cycles at 98°C for 10 s, 50°C for 30 s, 72°C for 30 s, and then a final extension cycle of 72°C for 5 min. Following PCR, the amplicons were visualized by electrophoresis and purified with the Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) and were sequenced on the Illumina NovaSeq platform (Novogene Corp., China). Sequences with 97% similarity were assigned to the same operational taxonomic units (OTU) using UPRSE (v7.0.1001) (Edgar, 2013). The annotation of representative sequences of each OTU was taxonomically analyzed against the SILVA138 (http://www.arb-silva.de/) database. Based on the results of OTU, bacterial diversity was evaluated using the well-used α−diversity indexes of Good's Coverage, Shannon index, and Simpson index described below (Hill et al., 2002; Ma et al., 2022):

$$C_{Good's\ coverage} = 1 - \frac{n_1}{\mathrm{N}} \tag{1}$$

$$H_{Shannon} = -\sum_{i=1}^{S_{obs}} \frac{n_i}{N} \ln \frac{n_i}{N} \tag{2}$$

$$D_{Simpson} = \frac{\sum_{i=1}^{S_{obs}} n_i\,(n_i - 1)}{N\,(N-1)} \tag{3}$$

where, n_1 is the number of OTUs with only one sequence; N is the total number of sequences; S_{obs} is the number of OTUs, and n_i is the number of sequences in the ith OTU.

The absolute abundance of a bacterial taxon in each sample was calculated with the relative abundance (obtained based on the results of high-throughput sequencing) times the abundance of total bacteria (measured based on 16S rDNA quantified by qPCR) (Dannemiller et al., 2014). The formula is as follows:

$$B_{Taxa}=\%B_{Taxa}\times T_{qPCR} \tag{4}$$

where, B_{Taxa} is the absolute abundance of a bacterial taxon [cell equivalent g^{-1}], $\%B_{Taxa}$ is the relative abundance of the bacterial taxon, T_{qPCR} is the abundance of total bacteria.

2.3 POTENTIAL ECOLOGICAL RISK FROM HEAVY METALS

2.3.1 Metal concentrations

The concentrations of heavy metals (As, Cd, Cr, Cu, Ni, Pb, and Zn) were determined following Method 3052 (U.S.EPA, 1996). Briefly, for each soil sample, 0.5g in dry was digested in solution of 9 mL HNO_3 (65-68%, w/w) and 3 mL HF (40%, w/w), and the solution after digestion was filtered and subjected to quantification by inductively coupled plasma mass spectrometry (ICP-MS, 7800, Agilent, USA). To implement quality assurance and control (QA/QC), we used blanks, duplicated samples, and standard reference materials (obtained from the Center for National Standard Reference Material of China). The relative standard deviation (RSD) for all duplicates was $< 5\%$.

2.3.2 Risk estimation

The potential ecological risk caused by heavy metals in soil was estimated based on the following formulas (Hakanson, 1980):

$$RI_o = \sum_{i=1}^{n} RI_i = \sum_{i=1}^{n} (T_{r,i} \text{ x } C_{f,i}) \tag{5}$$

$$C_{f,i} = C_{D,i}/C_{R,i} \tag{6}$$

where, RI_o is the overall potential ecological risk index of the targeted heavy metals, n is the number of heavy metals, RI_i is the potential ecological risk index of individual heavy metals, Tr is the heavy metal toxic response factor (Zn = 1, Cr = 2, Cu = Pb = Ni = 5, As = 10, Cd = 30) (Hakanson, 1980), the higher value indicates higher toxicity to ecosystem. C_f is the heavy metal pollution index, C_D is the measured heavy metal concentration, C_R is the geochemical background value. In this study, C_R of heavy metals in Shanxi Province, where the coal mines are located, was used, as shown in **Table 2.2**. The evaluation standard for potential ecological risk indexes (Hakanson, 1980) is shown in **Table 2.3**.

Table 2.2 Geochemical background value (mg/kg).

Metals	Cr	Pb	Ni	Cu	Zn	Cd	As
Shanxi Province[a]	59.1	15.5	30.8	25	72.4	0.11	9.4
Chinese soil criteria[b]	350	200	190	100	200	0.6	25
Coal mine soil[c]	92.89	41.01	47.10	33.18	79.4	0.52	29.28

[a] Mean heavy metal concentrations of soil (0-20cm) for Shanxi Province, China (Chinese soil element background value, China Environmental Science Press, Beijing, China, 1990).

[b] Soil Environmental Quality Risk Control Standard for soil contamination of agricultural land, China (GB 15618-2018).

[c] Average heavy metal concentrations in soil of 50 coal mines (Liu et al., 2019).

Table 2.3 Evaluation standard for potential ecological risk indexes.

RI_i	RI_o	Level of ecological risk
<40	<150	Low risk
40-80	150-300	Moderate risk
80-160	300-600	Considerable risk
160-320	>600	Very High ecological risk

RI_i represents the individual potential ecological risk index of each metal. RI_o represents the overall potential ecological risk index of all the metals.

2.4 PHYSICHEMICAL PROPERTIES

pH, EC, water content, organic matter (OM), total phosphorus (TP), and total nitrogen (TN) were measured based on the methods reported by Li et al. (2020b). Water content was determined by drying the samples to a constant weight at 105 °C (at least 5 h) in an oven. The organic matter content (loss on ignition) was measured by combusting the ried samples in a muffle furnace (Yamato, Japan) at 600 °C for 2 h. The pH and electrical conductivity (EC) were measured by using the aqueous solution of pulverized dry sample with deionized water (w/v = 1/10) after shaken for 2 h. Total carbon and total nitrogen were measured by using a nitrogen and carbon analyzer (Huang et al., 2013).

2.5 DATA ANALYSIS

Statistical analysis of the differences in the soil physicochemical properties, heavy metal concentrations, potential ecological risk, and bacterial abundance and diversity in different coal mines was conducted using one-way variation analysis (ANOVA) (STATISTIC software 10.0). Bacteria those contribute mostly to the differences in bacterial community were determined by Linear Discriminant Analysis (LDA) effect size

(LEfSe) method (http://huttenhower.sph.harvard.edu/lefse/) using an LDA score threshold > 3.0.

Network analysis of co-occurrence patterns was conducted to identify the potential host bacteria of metal resistance genes, where the top 100 species and targeted genes acted as nodes and the correlations between species and genes acted as edges in the network. The network was considered robust when Pearson's correlation coefficient was $r > 0.9$ with a significance of $^{***}p < 0.001$, analyzed with "psych" in R (version 4.0.3). Networks were constructed using "igraph" packages in R and visualized with the interactive Gephi platform (version 0.9.2).

The spatial distribution of heavy metals, soil physiochemical properties, and natural factors was identified and mapped using the Inverse Distance Weighted (IDW) method in ArcGis 10.2. Analysis based on Geographical detector (GeoDetector) model was performed to estimate the influencing of natural factors (DEM, slope, NDVI, precipitation, and distance to mine) and soil physicochemical properties (water content, total phosphorus, total nitrogen, EC, pH, and total organic matter) on the spatial distribution of heavy metals in coal mine soil. GeoDetector is an innovative and robust quantitative tool designed to assess the explanatory power of a driving factor (*X*) on the geographical spatial distribution of a dependent variable (*Y*) by evaluating their spatial consistency based on the principle of spatial stratified heterogeneity (SSH) (Wang et al., 2010, 2016; Qiao et al., 2019; Zeng et al., 2022). The spatial consistency (q) is estimated using the following formula:

$$q = 1 - \frac{1}{\mathrm{N}\sigma^2} \sum_{i=1}^{L} N_i \, \sigma_i^2 \tag{7}$$

where, N represents the number of samples, σ^2 stands for the variance of Y. L is the number of stratifications for X. $q \in [0,1]$, $q=1$ indicates the spatial distribution of Y was completely determined by the influencing factor *X*. q=0 indicated that there was no association between *Y* and *X*. In this model, each stratification requires at least two sample points, and larger the sample size can lead to more accurate calculation results.

Analysis based on Positive matrix factorizing (PMF) model (EPA's PMF 5.0), developed by the United States Environmental Protection Agency (EPA) was applied to identify the sources of heavy metals in coal mine soils. It is an efficient multivariate factor analysis receptor model, which quantifies the contribution of sources to samples based on the composition or "fingerprints" of sources determined by decomposing the sample concentration data matrices into the factor contribution and profile matrices (Men et al., 2018; Cheng et al., 2020). This model utilizes both sample concentration and the inherent uncertainty associated with the sample data to weight the individual points. This feature ensures the confidence of the measurement. The formula are as follows:

$$X_{i,j} = \sum_{k=1}^{p} g_{i,j} f_{i,j} + e_{i,j} \tag{8}$$

where, the concentration of chemical species j measured on sample i (X_{ij}) is decomposed into the contribution of factor k to sample i ($g_{i,j}$), the concentration of species j in factor k ($f_{i,j}$), and the corresponding residual ($e_{i,j}$). i, j, and p represent the number of samples, the chemical species, and the number of factors, respectively. Moreover, PMF model derives the factor contributions and profiles by minimizing the objective function Q (Men et al., 2018):

$$Q = \sum_{i=1}^{n} \sum_{j=1}^{m} \left(\frac{e_{i,j}}{u_{i,j}} \right) \tag{9}$$

where, $u_{i,j}$ is the uncertainty calculated using a fixed fraction of the MDL (method detection limit) as follows:

$$u_{i,j}=\frac{5}{6}\times MDL\ (x_{i,j}\leq MDL) \tag{10}$$

$$u_{i,j}=\sqrt{(Error\ Fraction\times concentration)^2+(0.5\times MDL)^2}\ (x_{i,j}>MDL) \tag{11}$$

For getting the final solution, the PMF model was ran 500 in this study. Based on the factor contribution and profile information, and the emission or discharge inventories, the sources were identified.

The relations of bacterial abundance and diversity with the potential ecological risk from heavy metals were examined by Spearman correlation analysis using “psych” packages in R (version 4.0.3). The relations of bacterial community structure and potential ecological risk from heavy metals together with the soil physicochemical properties were investigated by Redundancy analysis (RDA) using “vegan” packages in R (version 4.0.3). Linear models were established following the flow chart as shown in **Fig. 2.3**. Linear regression analysis was conducted using Origin 2021.

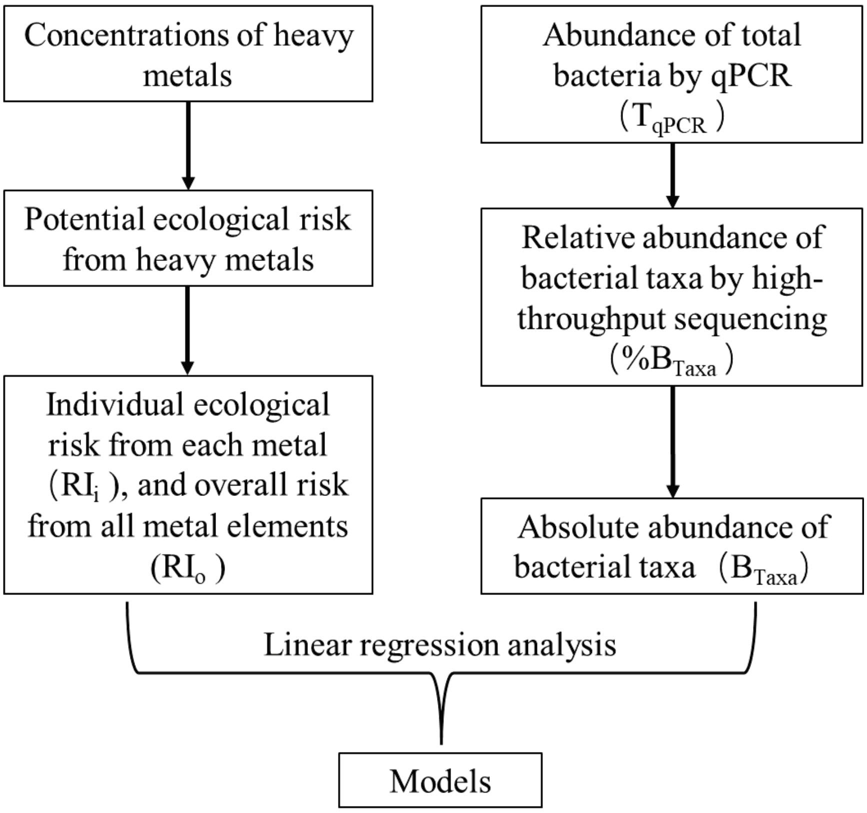


Fig. 2.3 Flow chart of establishing models

Chapter 3

BACTERIAL COMMUNITY IN SOILS OF COAL MINES

3.1 INTRODUCTION

Bacteria are the most abundant and diverse group of soil organisms, driving the geochemical cycle of elements necessary for a healthy ecosystem in soil. A comprehensive understanding of the bacterial community response to heavy metals in coal mine soils is necessary. This requires an integrated evaluation based on bacterial abundance, diversity, and community structure. Bacterial abundance is an important criterion for assessing the health of soil ecosystems. Contamination of heavy metals can reduce the total microbial biomass in sewage sludge and soil (Fließbach et al., 1994; Stefanowicz et al., 2010; Song et al., 2018), and the abundance of specific bacterial populations such as rhizobia (Chaudri et al., 1993) and mycorrhizae (Koomen et al., 1990), because of their toxicity on cellular metabolic functions including protein synthesis, chromosome replication, and DNA synthesis (Kandeler et al., 2000; Tang et al., 2019; Yan et al., 2020). In the overburden soil of the Kakanj lignite mine, the bacterial count was found to be 6 to 18 folds lower than that of soil covered with rich vegetation (Hamidović et al., 2020). The difference might be much bigger if bacteria were quantified based on quantitative PCR, rather than by the plate culture method that could only detect 1-15% of the bacteria (Lok, 2015) used by the authors in their study. In addition to bacterial abundance, bacterial diversity and community structure are also very sensitive to heavy metals. Laboratory studies proved that bacterial diversity in sediment and soil was significantly narrowed, and the bacterial community structure was changed when incubated with the presence of high concentrations of heavy metals (Du et al., 2018; Song et al.,2018). In field studies, the relationships between heavy metals and bacterial diversity are much more complex, therefore, as a result no consistent conclusion can be obtained. Under the long-term stress of Cr, Cu, and Zn, the soil bacterial diversity was reduced (Desai et al., 2009; Singh et al., 2014). However, there were studies that demonstrated positive correlation between bacterial diversity and heavy metals (Pan et al., 2020), or no correlations (Beattie et al., 2018). The contrary findings can be explained probably by factors other than heavy metals, such as soil organic matter, nutrients, moisture, and pH (Boivin et al., 2006; Kenarova et al., 2014; Song et al., 2018; Luo et al., 2021).

3.2 BACTERIAL COMMUNITY

3.2.1 Abundance

The abundance of total bacteria quantified based on 16S rDNA in the soils of the six coal mines is shown in **Fig. 3.1** and **Fig. 3.2**. **Fig. 3.1** showed the spatial distribution of the abundance of total bacteria. The abundance values with mines M1, M2, and M6 were relatively higher than those with M3, M4, and M5, indicating differences among the mines investigated. The highest bacterial abundance was associated with the soil surrounding M1. For the four mines of M1, M2, M4, and M5, an increasing trend of bacterial abundance with increasing distance from the center of the mines were revealed; however, for the mine M6, the trend was decreasing. Judging from the average abundance values for total bacteria plotted in **Fig. 3.2**, significant differences existed among the six coal mines, with the abundance value following the decreasing order of M1 > M2 > M6 > M3 > M4 > M5. The higher bacterial abundance in the mines M1, M2, and M6 implies that the soils in these mines may have relatively healthier soil ecosystems.

3.2.2 Diversity

The differences in bacterial diversity evaluated based on the indexes of Good's coverage, Shannon, and Simpson for the six coal mines are displaced in **Table 3.1**. Judging from the mean values of the three indices, differences among the mines were relatively small. Good's coverage values of all the mines were higher than 98%, and the rarefaction curves (**Fig. 3.3**) tended to reach saturation, indicating that the sequencing depth was sufficient and the bacterial OTUs of the corresponding mine were well captured. For mine M1, Shannon index for the samples showed variation in the range of 7.37-10.09, indicating wide variation of bacterial diversity. Judging from the values of Shannon and Simpson indexes in soil samples of the coal mines, a unique changing trend of bacterial diversity with increasing the distance from the center close to the excavation site was not found.

3.2.3 Structure

86 bacterial phyla and 983 bacterial genera were identified from the soil samples of all six coal mines. The top 20 dominant taxa at the phylum level and genus level are shown in **Fig. 3.4**. Proteobacteria, Acidobacteriota, Actinobacteria, Firmicutes, and Bacteroidota were 5 dominant phyla (**Fig. 3.4 I**); and slight differences relating to their presence levels among the six mines were revealed. Proteobacteria was most abundant phylum in all six mines; and its presence levels in soils of mine M1, M5 and M6 were higher than those of mines M3 and M4. If bacterial community structure was evaluated based on the 16th-20th

phyla in the top 20 dominant phyla of each mine, differences were also existent. As could be seen (**Fig. 3.4 II**), for mines M1 and M3, Thermoplasmatota, Armatimonadota, Planctomycetes, Desulfobacterota, and Bdellovibrionota existed; however, for mines M4, M5 and M6, Elusimicrobiota, Cyanobacteria, and NB1-j existed, respectively. At the genus level, the composition of the top 20 dominant taxa varied greatly among the six coal mines (**Fig. 3.4 III**). Pseudomonas belonging to Proteobacteria, dominated in the mine M1, whose abundance was significantly higher than that in the mines M2, M3, M5, and M6; while, in mines M4, this genus was not found. As common representative bacterial genera, *MND1, Arthrobacter, Sphingomonas, Massilia, RB41, Subgroup_10, Lysobacter, Lactobacillus, Ellin6067, Bacillus, Dongia, Haliangium,* and *Steroidobacter* existed in the soils of all six coal mines, even if their presence levels differed with the mines.

To investigate the soil bacterial community surrounding coal mines in different climate regions, we conducted a comparative analysis of the dominant bacteria in phylum level in the soils of coal mines located in semi-arid, semi-humid, and humid regions, as shown in Table **3.2**. The results revealed variations in the bacterial community structure among coal mines located in different regions. Based on the average values of the relative abundance of bacterial phyla in each region, Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi comprised over 70% of the total bacteria. The relative abundance of Proteobacteria and Actinobacteria was similar in the three climate regions, probably a result of long-term disturbance from heavy metals released during coal mining activities (Sahoo et al., 2016; Frossard et al., 2017; Ngugi et al., 2018). Previous studies have reported that Proteobacteria and Actinobacteria possessed high resistance to heavy metals (Pan et al., 2020; Zhao et al., 2019; Song et al., 2018; Tseng et al., 2021; Johnson et al., 2019).

Among different regions, differences in the relative abundance of Acidobacteria and

Chloroflexi were observed. The relative abundance of Acidobacteria in semi-arid region was almost two times higher than that in humid regions, while the relative abundance of Chloroflexi in semi-arid region was about two times lower than that in semi-humid and humid regions. These variations may be attributed to the differences in their tolerance to water stress. Acidobacteria was reported to exhibit high resistance to water stress (Curiel Yuste et al., 2014), and the relative abundance of several groups (Acidobacteriia, Solibacteres, Chloracidobacteria) increased under long-term water limitation in semi-arid pine forest soil (Hartmann et al., 2017). Conversely, Chloroflexi may have a lower tolerance to water stress, as it was found to be more abundant in wetlands than in meadows (Li et al., 2017). Besides water content, the heterogeneity in soil pH, total carbon, nitrogen, and phosphorus of different climate regions may also contribute to these differences (Chen et al., 2020; Luo et al., 2021; Xiao et al., 2021), which needs further investigations.

3.3 METAL RESISTANCE GENES AND HOST BACTERIA

3.3.1 Abundance of metal resistance genes

Metal resistance genes (MRGs) are the genetic basis for bacteria adaption under heavy metals' stress. The transfer of MRGs from host bacteria will provide information for an in-depth understanding of the mechanism on the response of bacteria to heavy metals. The absolute abundances of MRGs (*arsB*, *pbrT, chrB*), ARGs (*tetG*, *tetM*, *sul1*), mobile gene *intl 1* in soil of the six coal mines are shown in **Fig. 3.5**. The result of the relative abundance of MRGs, as shown in **Fig. 3.6** demonstrated that abundance of MRGs is mainly contributed by *arsB*.

3.3.2 Host bacteria

Network analysis of taxon co-occurrence patterns with MRGs was conducted, where the species and genes acted as nodes and their correlations acted as edges in the network. The network was considered robust when Pearson's correlation coefficient was $r > 0.9$ with a significance of $^{***}p < 0.001$, analyzed with "psych" in R (version 4.0.3). Networks were constructed using "igraph" packages in R and visualized with the interactive Gephi platform (version 0.9.2). The top 20 species according to the magnitude of the absolute abundance were chosen for Network analysis of the co-occurrence patterns. The potential host bacteria of MRGs in M1 are different from M2-M5 (**Fig. 3.7**). Based on the data of the six coal mines, eight potential host bacteria of *arsB* and *pbrT*, two poential host bacteria of *chrB* were identified (**Fig. 3.8**). The MRGs (*arsB*, *pbrT, chrB*) and ARGs (*tetG*, *tetM*, *sul1*) were categorized into different classes.

Table 3.1 Bacterial diversity evaluated using different α−diversity indexes for the six coal mines (M1-M6) based on the result of High-throughput sequencing.

Coal mine	Index	Good's coverage	Shannon	Simpson
M1	range	0.985-0.988	7.373-10.087	0.986-0.996
	mean	0.986^a	9.389^a	0.987^a
M2	range	0.985-0.987	9.275-9.958	0.988-0.995
	mean	0.986^a	9.692^a	0.992^a
M3	range	0.985-0.987	9.05-10.155	0.976-0.997
	mean	0.986^a	9.781^a	0.990^a
M4	range	0.985-0.988	9.673-10.065	0.987-0.996
	mean	0.987^a	9.77^a	0.993^a
M5	range	0.985-0.987	9.143-9.605	0.978-0.985
	mean	0.986^a	9.373^a	0.982^a
M6	range	0.985-0.987	9.439-10.163	0.989-0.997
	mean	0.986^a	9.748^a	0.993^a

Different letters show the differences between the groups in the same index are statistically significant ($p<0.05$).

Table 3.2 Bacterial community in the soils of coal mines of this study and previous studies evaluated through high-throughput sequencing.

Location	Region	Dominant taxa in phylum level and the corresponding relative abundance to the total bacteria	References
Luliang, Shanxi Province, China	Semi-arid	Proteobacteria (39%), Acidobacteriota (12%), Actinobacteria (7%), Firmicutes (5%), Bacteroidota (4%), Myxococcota (3%), Verrucomicrobiota (2.5%), Chloroflexi (1.9%)	This study
Datan, Qilian Mountain, Gansu Province, China	Semi-arid	Proteobacteria (74%), Actinobacteria (11%), Bacteroidetes (5%), Gemmatimonadetes (2%), Chloroflexi (2%), Acidobacteria (1%), Patescibacteria (1%), Firmicutes (0.8%)	Kong et al., 2021
Southeast of Zhungeer Banner,Inner Mongolia Autonomous Region, China	Semi-arid	Proteobacteria (38%), Actinobacteria (30%), Acidobacteria (8%), Gemmatimonadetes (6%), Bacteroidetes (7.5%), Chloroflexi (5%), Nitrospirae (0.3%), Verrucomicrobia (0.5%), Saccharibacteria (0.5%), Cyanobacteria (0.5%)	Li et al., 2019
Ordos City, Inner Mongolia Autonomous Region, China	Semi-arid	Proteobacteria (26%), Acidobacteria (22%), Actinobacteria (23%), Chloroflexi (8%), Bacteroidetes (6%), Gemmatimonadetes (6%), Firmicutes (2%), Patescibacteria (1.8%), Verrucomicrobia (1%), Nitrospirae (1%)	Guo et al., 2022
North of Shanxi Province, China	Semi-arid	Actinobacteria (23%), Proteobacteria (19%), Firmicutes (16%), Acidobacteriota (15%), Chloroflexi (8%), Gemmatimonadota (8%), Bacteroidota (3%), TM7(2%)	Li et al., 2021b
Between Yulin City, Shannxi Province and Inner Mongolia, China	Semi-arid	Actinobacteria (30%), Proteobacteria (20%), Acidobacteria (16%), Chloroflexi (9%), Planctomycetes (4.5%), Gemmatimonadetes (4.5%), Bacteroidetes (2%), Thaumarchaeota (1%), Cyanobacteria (1.8%), Euryarchaeota (1.5%), Armatimonadetes (0.5%)	Zhang et al., 2021
Daliuta Town, Shaanxi Province, China	Semi-arid	Actinobacteria (34.7%), Chloroflexi (16.2%), Proteobacteria (15.9%), Acidobacteria (13.9%), Gemmatimonadetes (7%), Nitrospirae (8%)	Guo et al., 2020
Pingdingshan City, Henan Province, China	Semi-arid	Acidobacteria (35%), Proteobacteria (27%), Gemmatimonadetes (10%), Actinobacteria (9%), Chloroflexi (9%), Rokubacteria (3%)	Sun et al., 2020
Peixian City, Jiangsu Province, China	Semi-arid	Proteobacteria (38.3%), Firmicutes (13.9%), Crenarchaeota (9.1%), Acidobacteria (7.5%), Bacteroidetes (6.4%), Planctomycetes (5.5%)	Tan et al., 2021
Xiangyuan County, Shanxi Province, China	Semi-humid	Proteobacteria (20%), Actinobacteria (16%), Chloroflexi (9.8%), Acidobacteria (9.3%), Gemmatimonadetes (5.6%), Bacteroidetes (3%)	Li et al., 2021c
Huaibei City, Anhui Province, China	Semi-humid	Proteobacteria (30%), Acidobacteria (20%), Actinobacteria (20%), Bacteroidetes (18%), Chloroflexi (12%), Planctomycetes (12%), Gemmatimonadetes (10%), Verrucomicrobia (10%)	Wang et al., 2022
West of Bohemia, Czech Republic	Humid	Proteobacteria (52%), Actinobacteria (17%), Bacteroidetes (10%), Firmicutes (5%), Acidobacteria (5%), Verrucomicrobia (2.5%), Planctomycetes (2.5%), Chloreflexi (1.2%), Gemmatimonadetes (1%)	Harantová et al., 2017
Northwest of Jiangsu Province, China	Humid	Proteobacteria (34%), Chloroflexi (14%), Actinobacteria (9%), Acidobacteria (8%), Planctomycetes (7%), Gemmatimonadetes (5%), Bacteroidetes (5%)	Li et al., 2014a
North of Jiangsu Province, China	Humid	Proteobacteria (35%), Acidobacteria (16%), Actinobacteria (14%), Chloroflexi (10%), Bacteroidetes (7%), Planctomycetes (6 %), Firmicutes (4%), Gemmatimonadetes (3%), Nitrospirae (3%)	Li et al., 2014b
Palmito, Barrancas municipality in La Guajira Department, Colombia	Humid	Acidobacteria (-), Actinobacteria (-), Bacteroidetes (-), Chlorolexi (-), Firmicutes (-), Gematimonadetes (-), Planctomycetes (-), Proteobacteria (-), Thaumarchaeota (-)	Arcila-Galvis et al., 2022
Mpumalanga Province, South Africa	Humid	Chloroflexi (25%), Actinobacteria (20%), Proteobacteria (18%), Acidobacteria (7%), Planctomyces (3.5%), Verrucomicrobia (1.3%), WPS-2 (3.5%)	Ezeokoli et al., 2020

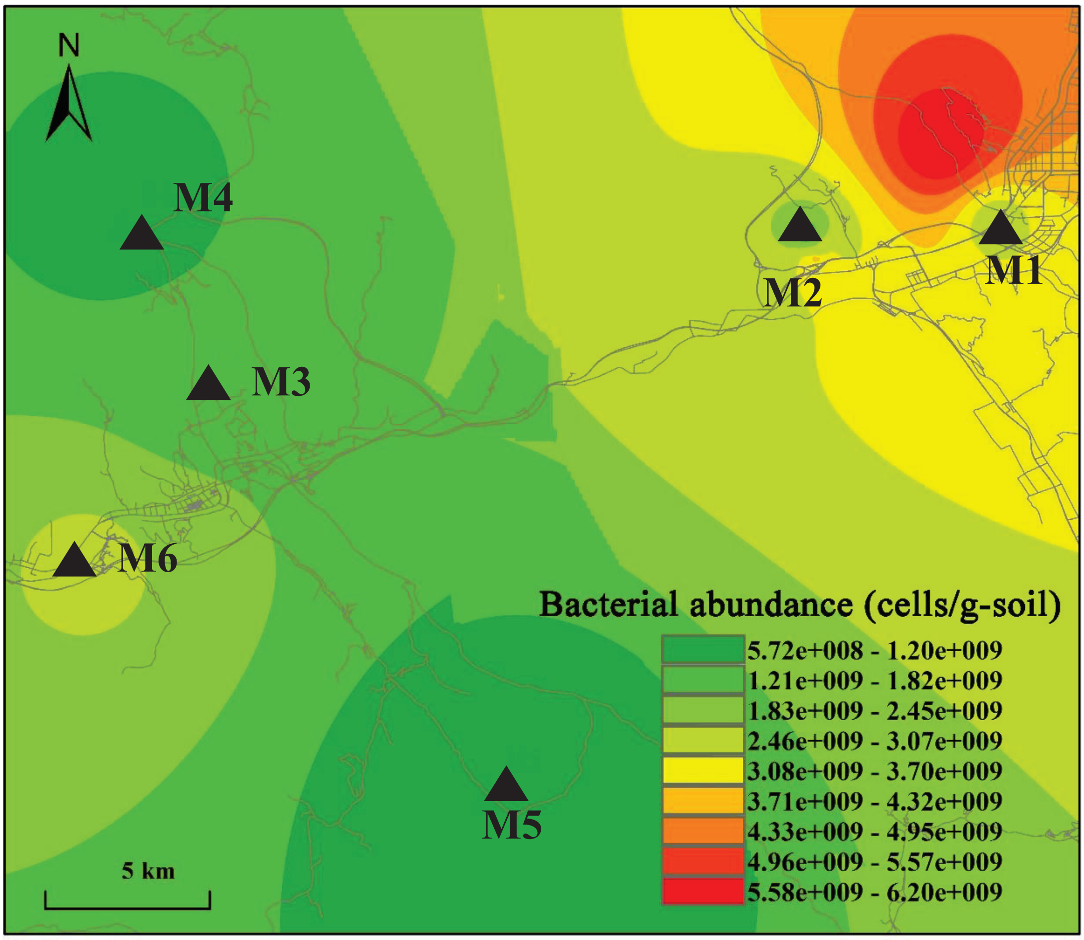


Fig. 3.1 Spatial distribution of the abundance of total bacteria based on 16S rDNA by qPCR in the soils of coal mines (M1-M6). Distribution was identified and mapped using the Inverse Distance Weighted (IDW) method in ArcGis 10.2.

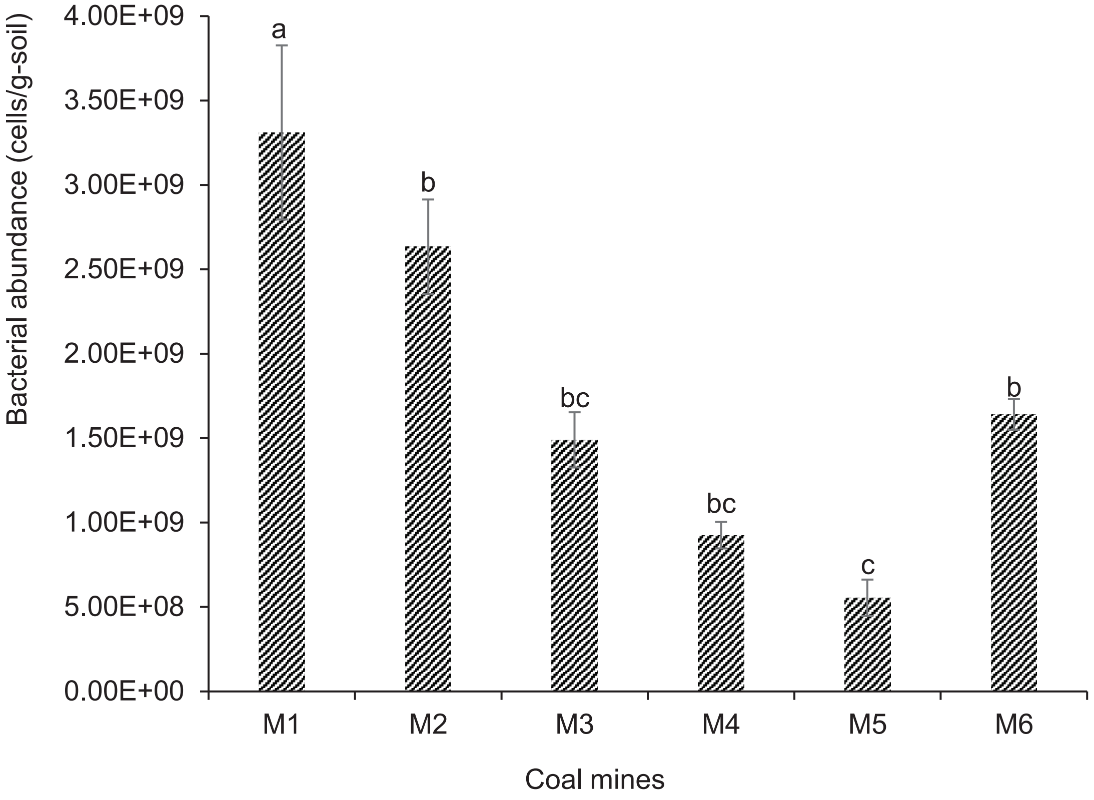


Fig. 3.2 Average abundance based on 16S rDNA by qPCR in the soils of coal mines (M1-M6). Different letters (a, b, c) indicate that the abundance difference between coal mines is statistically significant (*p*<0.05).

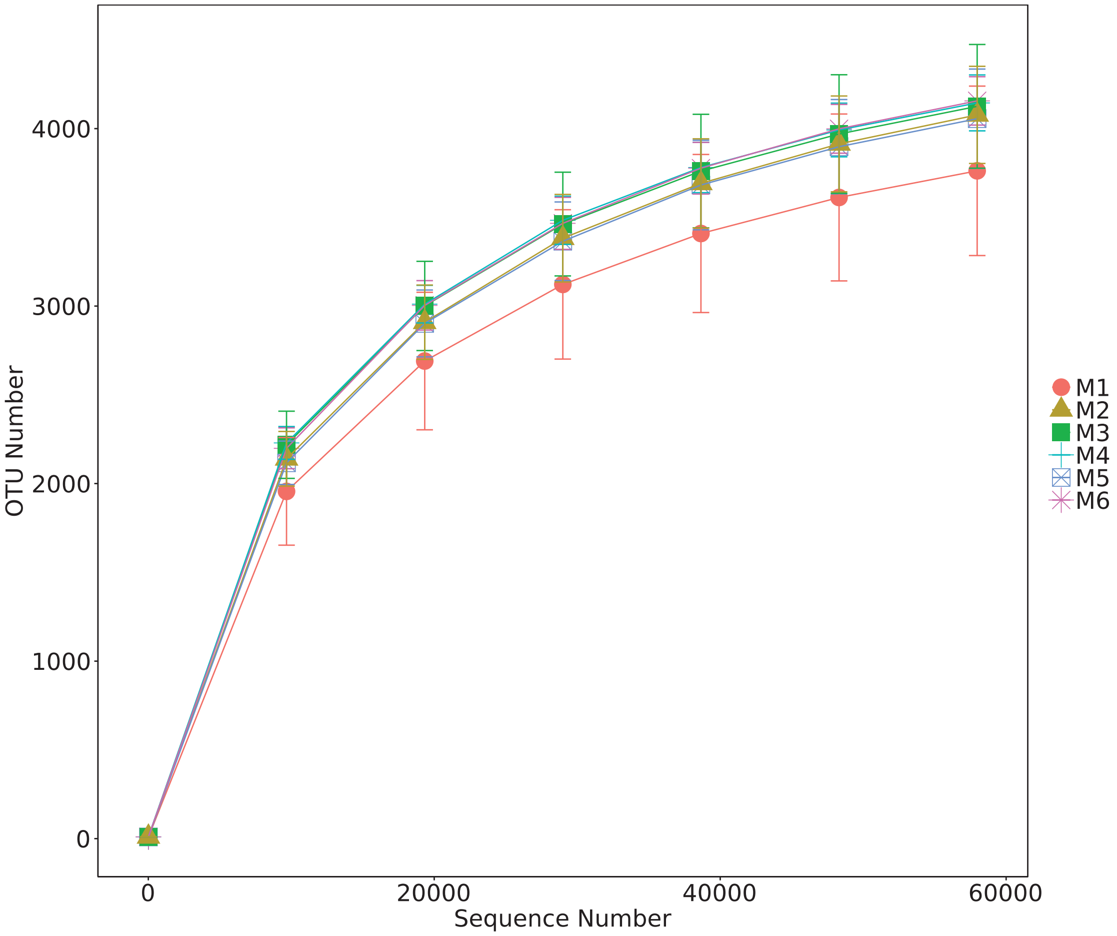


Fig. 3.3 Rarefaction curves for the six coal mines (M1-M6).

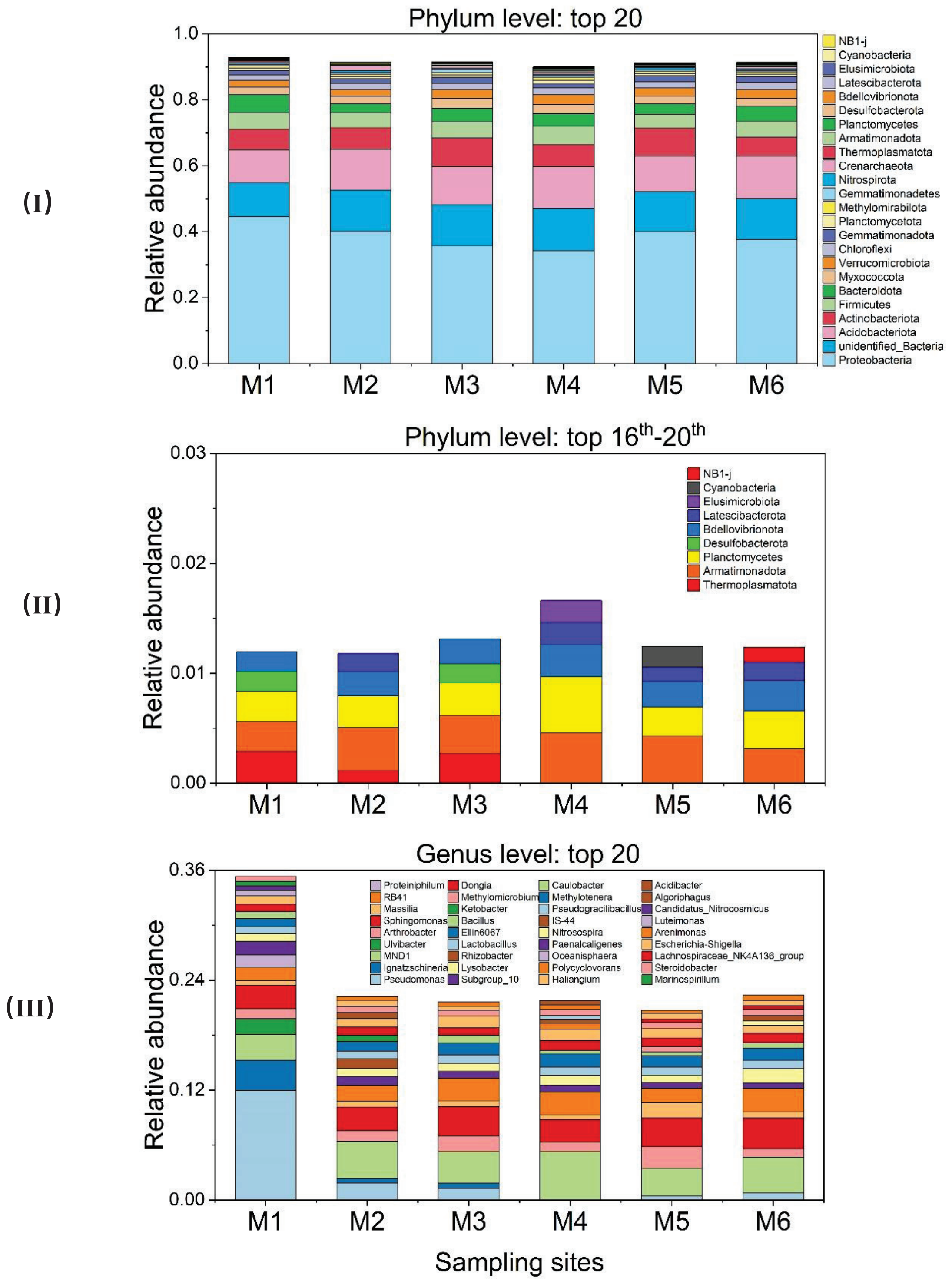


Fig. 3.4 Relative abundance of dominant bacteria based on the result of High-throughput sequencing for the soil of the coal mines (M1-M6): (I) the top 20 dominant taxa at the phylum level; (II) the taxa of 16th-20th in the top 20 dominant taxa at the phylum level; (III) the top 20 dominant taxa at the genus level.

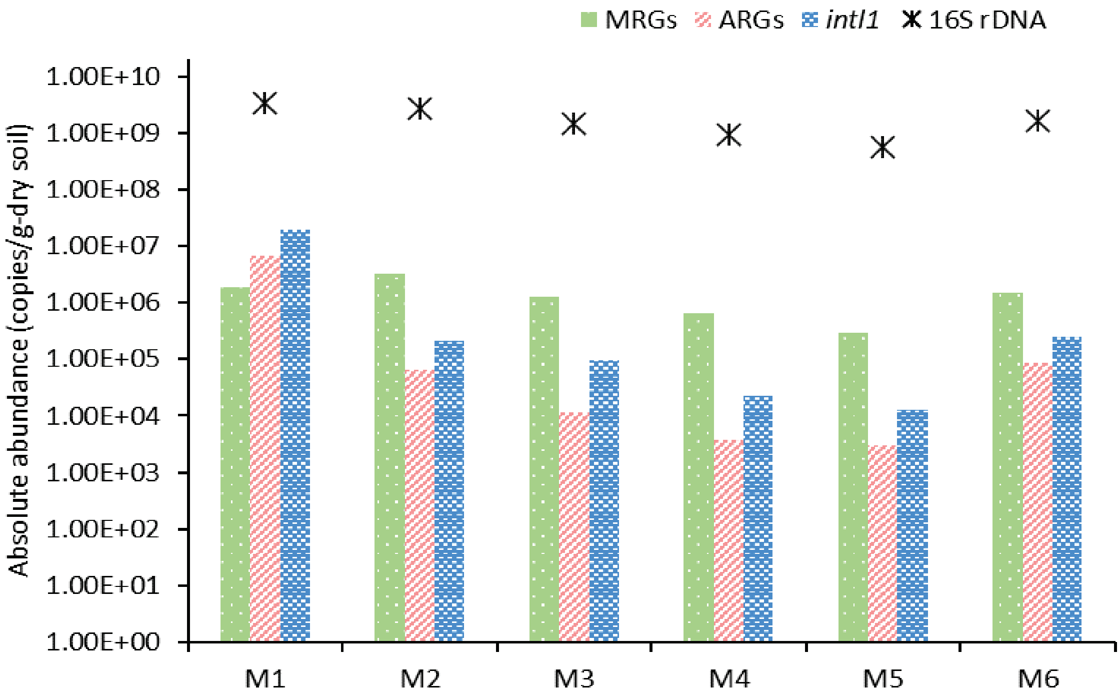


Fig. 3.5 Absolute abundance of Metal Resistance Genes (MRGs), Antibiotic Resistance Genes (ARGs), mobile gene (*intl 1*) and 16S rDNA in soil of the coal mines (M1-M6).

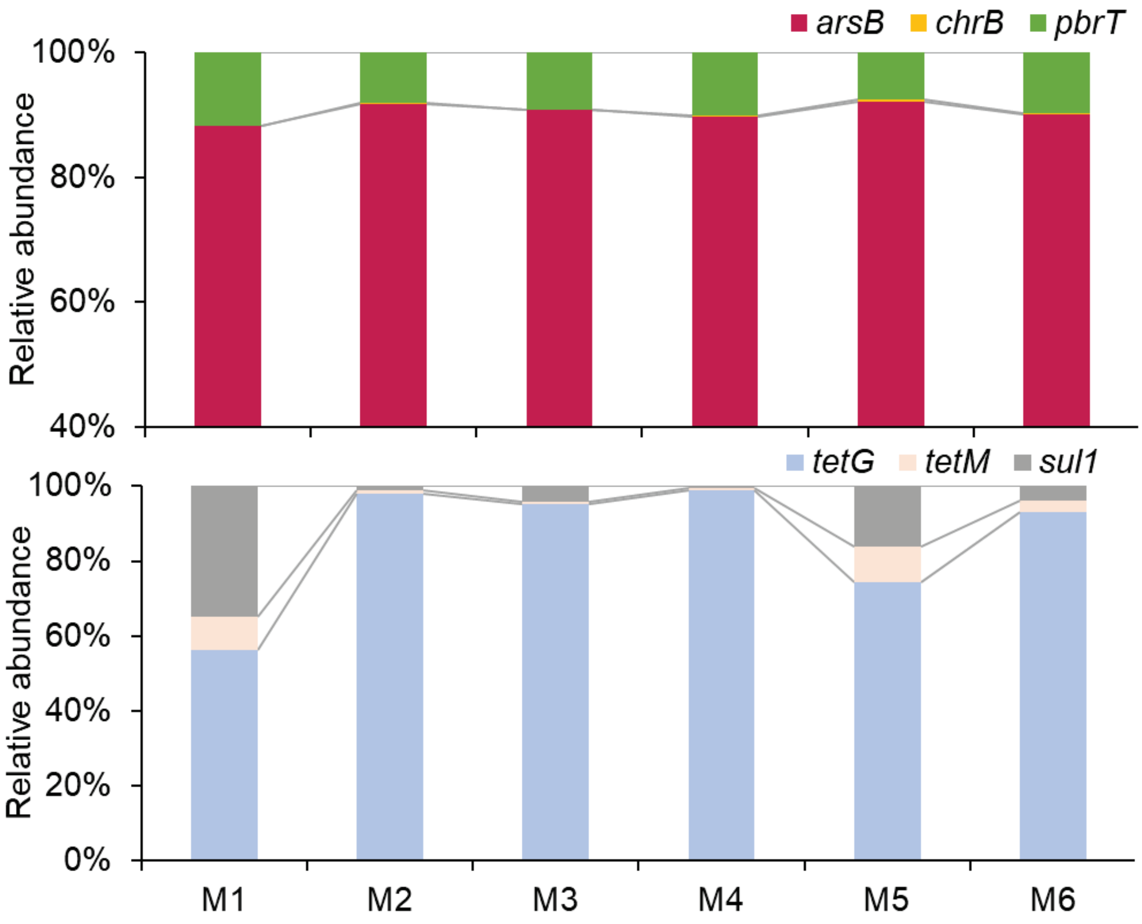


Fig. 3.6 Relative abundance of MRGs (*arsB, chrB* and *pbrT*) and ARGs (*tetG, tetM,* and *sul 1*) in soils of the coal mines (M1-M6).

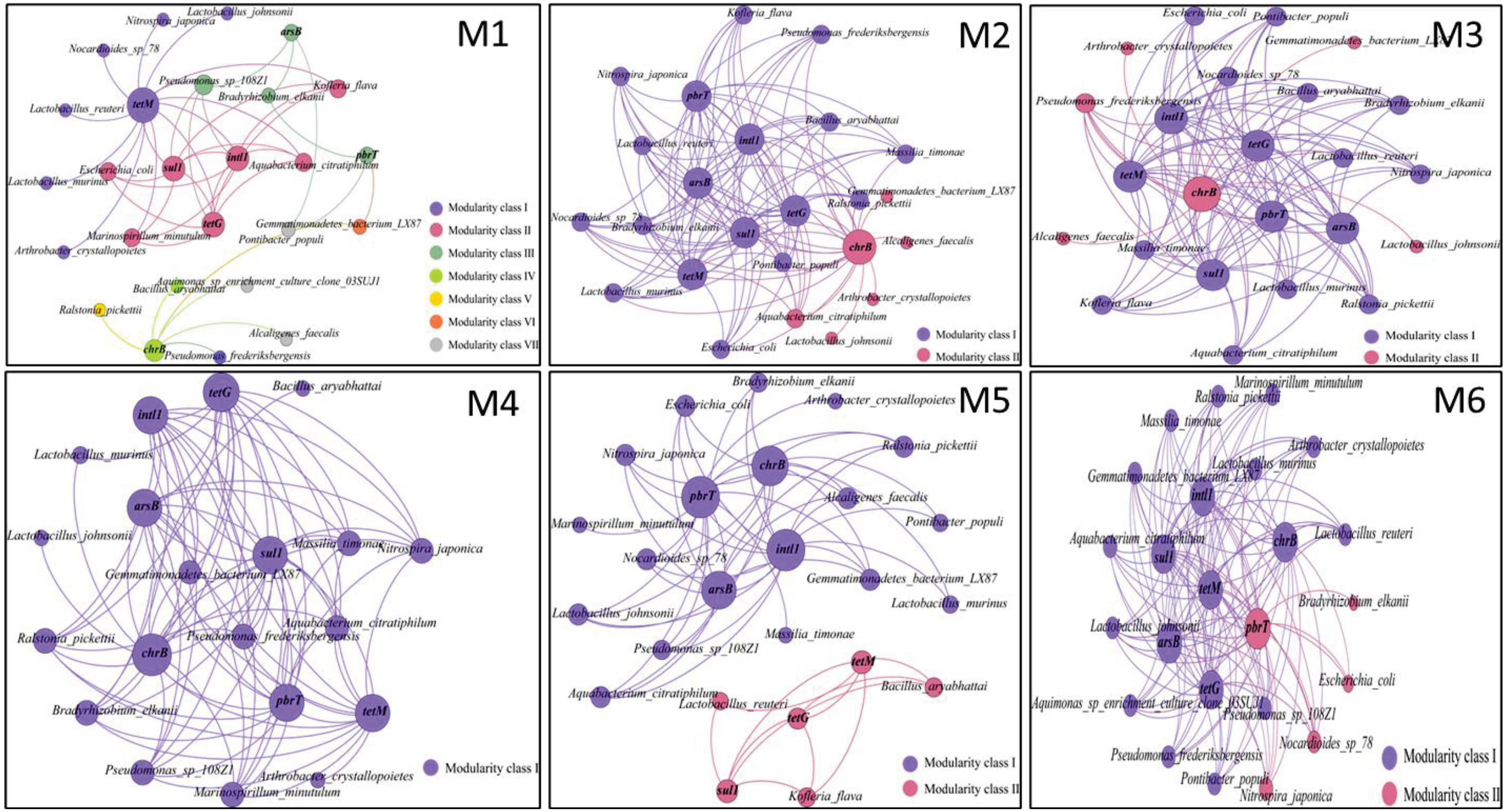


Fig. 3.7 Potential host bacteria of Metal Resistance Genes (*arsB, pbrT* and *chrB*) varied greatly in soil of different coal mines (M1-M6). Host bacteria were identified through network analysis. Circles represent genes and bacterial species. The size of circle indicates degree, and a larger size indicates a higher degree. Lines between circles represent their correlations. Circles with the same color represent genes or bacterial species classified into the same module.

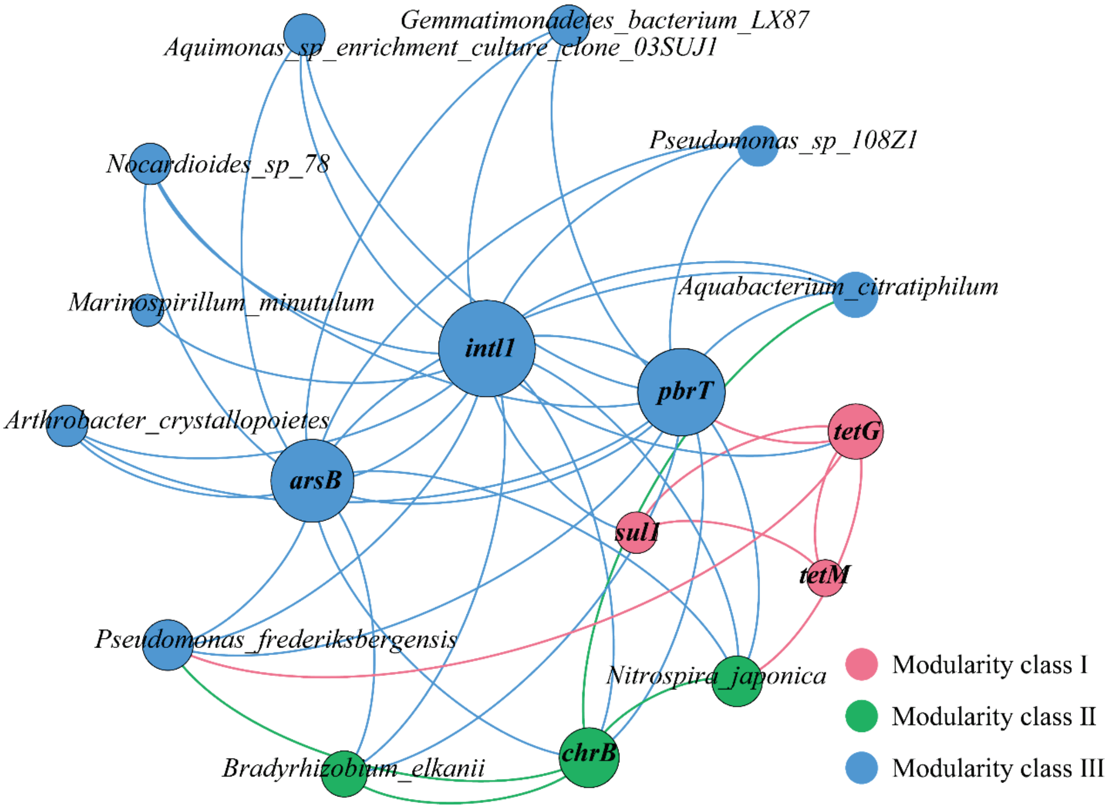


Fig. 3.8 Potential host bacteria of Metal Resistance Genes (*arsB, pbrT,* and *chrB*), Antibiotic Resistance Genes (*tetG, tetM,* and *sul 1*), and Mobile Gene (*intl 1*) in soil of the coal mines identified through network analysis based on data of the six mines. Circles represent genes and bacterial species. The size of circle indicates degree, and a larger size indicates a higher degree. Lines between circles represent their correlations. Circles with the same color represent genes or bacterial species classified into the same module.

3.4 SUMMARY

The soil surrounding different coal mines exhibited significant variations in the abundance of total bacteria. The mines with the smallest and largest production scales displayed a higher bacterial abundance than the other mines. However, no significant differences in bacterial diversity among the coal mines were observed based on the values of Shannon and Simpson indexes. The dominant phyla in the mines were Proteobacteria, Acidobacteriota, Actinobacteria, Firmicutes, and Bacteroidota, which collectively accounted for approximately 70% of the total bacteria, as determined by high-throughput sequencing. Literature review showed that Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi were common bacterial phyla in soil of coal mines. However, the relative abundance of Acidobacteria and Chloroflexi varied among coal mines located in different climate regions. Acidobacteria was enriched in semiarid regions, while Chloroflexi was enriched in humid regions. The potential host bacteria of MRGs in M1 were different from M2-M5. The potential host bacteria of MRGs in the soil 0, 1, and 3km apart from the center of the mine were different. The variations of potential host bacteria of MRGs may be due to the different bacterial compositions. The transfer of MRGs was closely related to the abundance of total bacteria (16S rDNA), and mobile gene (intl1), which negatively associated with heavy metals. The transfer of MRGs may be controlled by heavy metals through inhibiting their vertical and horizontal transfer.

Chapter 4

POTENTIAL ECOLOGICAL RISK FROM HEAVY METALS

4.1 INTRODUCTION

Coal mines in China have different production scales, and their mining technology and management vary greatly. Coal mines with advanced mining technology, and strict management generate less dust and wastewater, and therefore, heavy metals diffuse into the surrounding environment through the dust and wastewater, can be lowered. The diffusion of heavy metals is aided by natural processes such as wind, rain, and erosion, as has been confirmed by serious pollution in the vicinity of coal mines (Zota et al., 2009; Sahoo et al., 2016).

Soils in coal mines are major sink for heavy metals. For instance, Cd, Cu, and Pb were highly concentrated in the surface soil of coal mine brownfield in Beijing, China (Li and Ji, 2017a); and Cu and Zn in the surface soil near coal mining areas of the Jharia coalfields, India (Agrawal et al., 2016); and Cr in the soil of Oltu coal mines, Turkey (Tozsin, 2014). These heavy metals seriously threaten the soil ecology and the inhabitant's health due to their high toxicity, non-biodegradability, and accumulative property (Liu et al., 2019; Li and Ji, 2017a). For monitoring heavy metal contamination in coal mine soils, direct measurement of heavy metal concentrations is a well-used approach. Meanwhile, in recent years, the approach to using potential Ecological Risk (RI) estimated on the basis of heavy metal concentrations is increasing with respect to its application frequency (Liu et al., 2019). However, regarding its relation with organisms in soil, information is limited.

4.2 HEAVY METAL DISTRIBUTION

4.2.1 Concentrations

The results of heavy metal concentrations (As, Cd, Cr, Cu, Ni, Pb, and Zn) and physicochemical properties (pH, EC, water content, organic matter, total nitrogen, and total phosphorus) in soils of the six coal mines (M1-M6) are summarized in **Table 4.1**. The concentrations of heavy metals varied in soil samples of the coal mines. For mines M2-M5, most heavy metals revealed their concentration values higher in the soil sample from the center of the mine. However, for M1, the concentration of As, Cu, and Cd were lowest in the center and for M6 with the largest coal production scale within the targeted 6 coals in this study, for all targeted metals, the lowest concentrations were observed in the center sample (site A). Judging from the mean concentrations of heavy metals, significant differences among the mines could be seen for Cr, Pb, Cu, Cd and As. For the mine M3, the concentrations of Pb, Zn, and Cd in the soil sample were the highest; while, for the mine M5, the concentrations of Cr, Cu and As were the highest. In regard of basic physicochemical properties of the soils, the differences among the targeted coal mines were found smaller.

The average heavy metal concentrations of all soil samples followed the decreasing order of Zn (36.7mg/kg) > Cr (34.7mg/kg) > Ni (20.0mg/kg) > Pb (15.6mg/kg) > Cu (13.8mg/kg) > As (3.7mg/kg) > Cd (0.1mg/kg). Compared with the concentration values in the Soil Environmental Quality Risk Control Standards for agricultural soil in China (GB 15618-2018) and the reported average ones in soils of 50 coal mines located in other regions (Liu et al., 2019), the concentrations observed for soils surrounding the targeted coal mines of this study were much lower. However, if compared with the background values in Shanxi Province (**Table 4.2**), where the targeted coal mines are located, the concentrations of Cd in the samples at site A and B of the coal mine M3 (M3.A and M3.B) and those of Pb in the samples of the mines except M6 (M1.A, M2.A, M3.A, M3.B, M3.C, M4.B, M4.C, M5.A, M5.B) were relatively higher, thus causing concerns on potential ecological risk from these metal elements in soils of these sites.

The soil physicochemical properties, including pH, EC (electrical conductivity), water content, organic matter, total nitrogen, and total phosphorus, for the six coal mines (M1-M6) are presented in **Table 4.1**. Statistical differences were observed among the mines for the mean values of pH, organic matter, and total phosphorus. The variation coefficient indicated low heterogeneity for these properties. The average values for pH, EC, water content, organic matter, total nitrogen, and total phosphorus were 8.52, 164.6 mS/m, 24.2%, 18g/kg, 554 mg/kg, and 2.49 mg/kg, respectively. These values indicate alkaline soil conditions (Sharma & Chowdhury, 2021; Roy et al., 2022; Qi et al., 2022).

4.2.2 Spatial distribution

The spatial distribution of each of the metals (As, Cd, Cr, Cu, Ni, Pb, and Zn) are displayed in **Fig. 4.1**. From this figure, it could be seen that the concentrations differed with the targeted coal mines and with the metals. Among all mines, M3 showed the highest values for Cd, Cu, Pb, Zn, and Cr; M5 the highest value from As and M4 the highest value from Ni, for soils in the corresponding center site of the mines. Generally, the from Cr, Cu, Ni, and Pb were higher in soils surrounding the mines of M3, M4 and M5 than the mines of M1, M2 and M6. For M6, soil in the center (A) showed relatively lower values and soils apart from the center (B and C) showed relatively higher values. A distribution feature of lower values for soil in the center was also revealed for the mine M1 relating to the values of As, Cu and Cd.

The higher concentrations of metals in soils of the mines M3, M4, and M5 were possible to be attributed to the higher levels of metals in the dust resulting from coal mining activities, as well as the influence of natural factors and soil physicochemical properties (Sultana et al., 2022; Qiao et al., 2019). Different from the distribution trend reported in previous studies for soils near a large metal mine and a Pb/Zn smelter (Ding et al., 2017; Li et al., 2015) that heavy metals mainly concentrated in soils 1 km from the mining area and decreased gradually with increasing distance along the wind direction; a unique distribution trend of decreases for the potential ecological risk with increasing the distance from the center close to the excavation site was not revealed for all six mines targeted in the present study. The differences in the extent of involvement among the coal mines by factors such as coal transportation, coal processing, use, soil properties, and geographical and meteorological properties were probable reasons that need further investigation. Since the concentrations of heavy metals in soils surrounding the mines M3, M4 and M5 were higher, direct diffusion from these three mines to other three mines M1, M2 and M6 was also conceivable, with the contribution of which probably changing with the geographical and meteorological conditions, a topic also requires investigation in coming studies.

4.2.3 Factors influencing the spatial distribution

The potential contribution of natural factors (DEM, slope, NDVI, precipitation, and distance to mine) influencing the spatial distribution of heavy metals in soil around coal mines was shown in **Fig. 4.2**. For Cr and Pb, the main influencing factors were the distance apart from the mine and DEM. For Cu, DEM and Slope were the main influencing factors. For Zn, the distance apart from the coal mine was the main influencing factor. For As, precipitation, distance apart from the mine, and DEM were the main influencing factors. For Ni, Se, and Cd, the main influencing factor was DEM. The potential contribution of DEM to the spatial distribution of the eight metals all exceeded 0.2.

The potential contribution of soil physicochemical properties (water content, total phosphorus, total nitrogen, EC, pH, and total organic matter) influencing the spatial distribution of heavy metals in soil around coal mines was shown in **Fig. 4.3**. For Cr, Ni, and Cu, the main influencing factors were EC, moisture content and total phosphorus. For Zn, the main influencing factors were moisture content and TP. For As, total phosphorus was the main influencing factor. For Se, pH and total phosphorus were the main influencing factors. For Cd, moisture content and total nitrogen were the main influencing factors. For Pb, pH, moisture content and total nitrogen were the main influencing factors.

The comparison of the effects of natural factors and soil physicochemical properties on the spatial distribution of heavy metals (sum of the q value of each factor in each aspect) was shown in **Fig. 4.4**. The soil physicochemical properties showed a higher effect on the spatial distribution of heavy metals than that of natural factors, except for As. The interaction of natural factors and soil physicochemical characteristics on the distribution of heavy metals was greater than that of single factor.

To further explore the positive or negative correlations between natural factors and soil physicochemical properties with heavy metal concentrations, Redundancy Analysis (RDA) was performed as shown in **Fig. 4.5**. Two main factors, RDA1 and RDA2 were obtained, of which the sum was greater than 85%, indicating that natural factors and soil physicochemical properties well explained the variations in heavy metal concentrations in the soil of coal mines. The distance apart from the mine and water content with longest lengths showed the greatest impact on the distribution of the heavy metals, which negatively correlated with the concentrations of Cu, Cd, Zn, Pb, Ni, Cr, and As. pH, DEM, Slope, and NDVI showed positive correlations with the concentrations of Cu, Cd, Zn, Pb, Ni, Cr, and As, and DEM possessed stronger impact. Variance partitioning analysis (VPA) analysis demonstrated that natural factors explained 43% of the distribution of these eight elements in the soil, which was higher than the soil physical and chemical properties (12%).

To understand the linear relations between DEM and the concentration of heavy metals, linear regression analysis was performed based on the DEM and metal concentrations of 18 soil samples from the 6 coal mines. The regression fit model of the eight heavy metals were shown in **Fig. 4.6**. There are positive correlations between the concentrations of heavy metals and DEM. The concentrations of As, Cr, and Cu increased obviously with increasing of DEM.

4.3 POTENTIAL ECOLOGICAL RISK FROM HEAVY METALS

The values of the individual potential ecological risk from each heavy metal and its contribution to the overall potential ecological risk from all heavy metals are shown in **Table 4.4**. The overall ecological risk values from all seven metals (RI) fell in the range of 33.76-58.62, indicating potential ecological risk differences among the six targeted coal mines. The largest risk value difference seemed to exist between the two mines M3 and M6. For the individual potential ecological risk from each metal element, comparison of the results in this table revealed an order of Cd (26.8) > Pb (5.06) > As (4.03) > Ni (3.23) > Cu (2.76) > Cr (1.19) > Zn (0.51). Cd was the major element and it accounted for about 47% of the overall ecological risk from the entire seven metals. Comparison with the evaluation standard values as shown in **Table 4.3**, indicated that among all six coal mines (M1-M6) studied, the coal mine M3 has moderate ecological risk for soil at its center site (M3.A), with the potential risk value from Cd being 55.5. Based on the data for 50 coal mines in China, a recent review study (Liu et al., 2019) reported that averagely about 51% of the overall potential ecological risk of heavy metals (Cd, Hg, As, Pb, Ni, Cu, Cr and Zn) was contributed by Cd. Our data support this report, indicating that Cd is a major contaminant for soils surrounding coal mines that requires great attention relating to its presence levels and associated potential ecological risk.

Evaluating the ecological risk indexes of heavy metals may not fully capture the toxicity of heavy metals to microorganisms because it was proposed based on the concentrations of accumulated heavy metals in larger organisms, rather than on the concentrations accumulated in microorganisms. We compared the accumulation capability of metal element-cesium in different bacteria and organism species. Many organisms with relatively bigger sizes than bacteria, such as mammals, amphibians, fish, insects, benthos, fungi, and plants can accumulate metal elements from their living environment (**Table 4.5** and **Table 4.6**). For aquatic plants and algae that play the role as producers in ecosystems, higher concentrations of ^{137}Cs were detected in *Potamogeton crispus*, *Trapa bispinosa*, and Filamentous Alga sampled near the Fukushima Nuclear Power Plant (Sasaki et al., 2016). For benthic organisms that act as primary consumers, such as *Crustacean*, *Asteroidea*, and *Polychaete* collected from the coast of Fukushima, radioactive Cs was also detected (Shigenobu et al., 2015), and the ratio of the concentration of ^{134} Cs and ^{137} Cs in *Flabelligeridae* (belong to *Polychaete*) and sea sediment reached 46% (Shigenobu et al., 2015). For the sedentary demersal fishes that prey on benthic organisms, such as *Sebastes cheni, Hexagrammos otakii,* and *Microstomus achne* captured from the coast of Fukushima, higher concentrations of radioactive Cs were detected from their tissues (Wada et al. 2013). ^{137}Cs was also detected from small epipelagic fishes, such as *Sardine* and *Japanese Anchovy* in the Kanto area (150km from FNPP). In lake Hayama, piscivorous fishes at the top level of the food chain, such as the *Japanese catfish* and *largemouth bass*, accumulated more radioactive Cs on average than other fishes (Matsuda et al., 2015). Radioactive Cs can be gradually accumulated by aquatic organisms through the food web.

Judging from the accumulated concentration per unit mass of organisms, the accumulation capability in bacteria is relatively higher compared to larger organisms (**Table 4.7**). The accumulation capability of bacteria was estimated to reach 8.89 %, 7.00 %, and 9.35 % (w/w) on average for species from the freshwater sediment, and 5.48 %, 9.32% and 15.8 % (w/w) for the species from the coastal sediment of this study under 5, 20, and 35℃, respectively, if the average mass of a bacterium is as the reported value of about 10^{-12} g/cell in wet. Existing research suggested a strong possibility of heavy metal accumulation in microorganisms, indicating higher toxicity of heavy metal to soil bacteria than large organisms.

4.4 SOURCES OF HEAVY METALS

The EPA PMF 5.0 model was utilized to determine the sources and quantify their contributions of metal elements in soils of coal mines. Different iterations of the model with varying numbers of factors (3, 4, and 5) were compared to determine the optimal solution. The three-factor solution yielded the best results with the smallest Q value and residuals falling within the range of -3 to 3. The predicted values estimated by PMF showed strong correlations ($R^2 > 0.6$) with the true values, indicating that the selected factors effectively captured the source information in the original data (Norris et al., 2014).

The contribution of Factor1, Factor2, and Factor 3 to the total heavy metal concentrations, was 32.2%, 39.4%, and 28.4%, respectively (**Fig. 4.7**). Factor 1 had highest contribution to Se (93%), followed by Zn (45%), and Cd (45%), and exhibited highest contributions in the soils surrounding the excavation centers of mines M3 (**Fig. 4.8**), which had higher concentrations of Se, Zn, Cd than other mines corresponding background values. According to the study by Hao et al. (2022), coal mining activities are a significant source of elevated Se concentrations in the soil. Furthermore, many studies have reported that coal mining can result in increased levels of Zn and Cd in the soil through atmospheric sedimentation of metal-enriched dust, as well as leaching from mined rock and coals caused by snow and rain (Cheng et al., 2019; Song et al., 2020; Sahoo et al., 2016). Therefore, Factor 1 is primarily attributed to coal mining activities.

Factor 2 showed highest contributions to Zn (48%), Cd (40%), and Cr (38%). The hotspots of Factor 2 distribution are observed in the soils surrounding coal mines M5, and

M6, which are characterized by higher production scales (**Fig. 4.8**). These mines likely experience intensified coal haulage due to their high production levels. In this study area, trucks are primarily used for coal transportation. The release of Zn, Cd, and Cr into soil can be attributed to automobile exhaust emissions, which infiltrate the topsoil through fuel combustion (Ying et al., 2016; Huang et al., 2018; Jin et al., 2019). Moreover, if the coal in the trucks is not adequately covered, the metals present in the coal can be released into the soil as well. Hence, the higher contributions to Zn and Cd are likely associated with vehicular traffic and coal dust. It can be speculated that Factor 2 represents the contribution of coal transportation traffic.

Factor 3 demonstrated highest contribution to As (53%) and Ni (46%). Several studies have reported that As and Ni in soils originate from soil parent materials (Zhou et al., 2016; Khan et al., 2017; Sun et al., 2019). These metals were present in lower concentrations compared with their corresponding background values. It is inferred that the composition of parent materials and pedogenic processes played significant roles in determining the levels and distribution of As and Ni in the soil. The contribution from Factor 3 was high in the overlapping areas of the mines, and the variation in its contribution within the study areas was much lower than the other two factors. Therefore, Factor 3 was identified as a natural source.

Table 4.1 Heavy metal concentrations and physiochemical properties of soil samples from the targeted six mines (M1-M6).

Coal mine		**M1**		**M2**		**M3**		**M4**		**M5**		**M6**	
Index	unit	range	mean	range	mean	range	mean	range	mean	range	mean	range	mean
pH	-	8.28-8.53	8.47^{b}	8.39-8.65	8.51^{ab}	8.64-8.94	8.79^{a}	8.19-8.76	8.34^{a}	8.42-8.76	8.51^{ab}	8.34-8.62	8.5^{ab}
EC	mS/m	158-214	179^{a}	141-178	154^{a}	68-186	107^{a}	154-195	177^{a}	151-174	165^{a}	144-243	168^{a}
Water	%	22-27.4	24.3^{a}	23.6-25.5	24.6^{a}	22.5-25.1	23.8^{a}	23.2-26.4	25.5^{a}	21.8-24.6	23^{a}	21.2-29.5	23^{a}
OM	g/kg	15.4-17.7	16.8^{c}	15.1-17.9	16.3^{c}	18.4-21.5	20.2^{a}	16.9-20.1	19.1^{abc}	17-19.2	17.9^{abc}	18.3-20.5	19.1^{ab}
TN	mg/kg	520-620	537^{a}	503-528	514^{a}	516-613	558^{a}	498-587	532^{a}	532-632	585^{a}	519-620	562^{a}
TP	mg/kg	2.4-2.8	2.6^{ab}	2.5-3.0	2.6^{ab}	2.2-3.2	2.7^{ab}	2.1-2.2	2.1^{b}	2.1-2.4	2.3^{ab}	2.3-3.0	2.8^{a}
Cr	mg/kg	29.8-33.8	31.7^{bc}	21.2-36.2	27.5^{c}	36.1-47.9	38.4^{ab}	35.6-37.8	36.6^{abc}	38.8-43.9	41.9^{a}	22.2-31.9	29.7^{c}
Pb		14.7-15.8	15.3^{ab}	10.1-16.4	12.6^{b}	15.6-22.8	18.3^{a}	15.4-16.3	15.8^{ab}	14.6-17.4	16.3^{ab}	10.3-15.0	14.2^{b}
Ni		16.9-21.2	20.0^{a}	14.7-20.9	17^{a}	15.2-26.0	20.0^{a}	20.5-26.1	23.7^{a}	19.0-23.7	21.8^{a}	14.1-20.2	18.6^{a}
Cu		12.1-14.0	13.2^{ab}	12.8-14.3	13.6^{ab}	13.8-18.2	14.8^{a}	13.2-14.6	13.8^{ab}	12.8-16.6	15.1^{a}	9.2-12.7	12.1^{b}
Zn		28.8-43.7	35.1^{a}	27.4-33.5	29.8^{a}	35.4-59.5	44.9^{a}	33.8-34.9	34.2^{a}	36.1-40.8	38.9^{a}	19.8-53.7	35.5^{a}
Cd		0.08-0.10	0.09^{b}	0.07-0.10	0.08^{b}	0.09-0.20	0.13^{a}	0.09-0.10	0.09^{b}	0.09-0.10	0.09^{b}	0.06-0.10	0.09^{b}
As		3.32-3.75	3.44^{ab}	2.15-5.06	2.97^{ab}	2.04-4.95	3.23^{ab}	3.36-4.63	4.14^{ab}	4.54-5.39	5.12^{a}	2.30-3.38	3.02^{b}

*pH and EC represent the measurement result for soil added to pure water in the ratio of 1:10 (w/v). TN, TP and OM represent total nitrogen, total phosphorus and total organic matter in soil. Different letters (a, b, and c) show the differences between the groups in the same index are statistically significant ($p<0.05$). The letter ‘ab’ in the group of coal mines means the difference of the value with the letters of ‘a’ and ‘b’ is not statistically significant. The letter ‘abc’ in the group of coal mines means the difference of the value with the letters of ‘a’, ‘b’ and ‘c’ is not statistically significant.

Table 4.2 Geochemical background value (mg/kg).

Metals	Cr	Pb	Ni	Cu	Zn	Cd	As
Shanxi Province[a]	59.1	15.5	30.8	25	72.4	0.11	9.4
Chinese soil criteria[b]	350	200	190	100	200	0.6	25
Coal mine soil[c]	92.89	41.01	47.10	33.18	79.4	0.52	29.28

[a] Mean heavy metal concentrations of soil (0-20cm) for Shanxi Province, China (Chinese soil element background value, China Environmental Science Press, Beijing, China, 1990).

[b] Soil Environmental Quality Risk Control Standard for soil contamination of agricultural land, China (GB 15618-2018).

[c] Average heavy metal concentrations in soil of 50 coal mines (Liu et al., 2019).

Table 4.3 Evaluation standard for potential ecological risk indexes.

RI_i	RI_o	Level of ecological risk
<40	<150	Low risk
40-80	150-300	Moderate risk
80-160	300-600	Considerable risk
160-320	>600	Very High ecological risk

RI_i represents the individual potential ecological risk index of each metal. RI_o represents the overall potential ecological risk index of all the metals.

Table 4.4 Individual potential ecological risk from each heavy metal and the overall ecological risk (RI) from all heavy metals of the six mines (M1-M6).

Sampling site	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**Average M1-M6**	**Average contribution (%)**
As	3.78 ± 0.14^{ab}	4.20 ± 0.90^{ab}	3.97 ± 0.89^{ab}	4.27 ± 0.40^{ab}	5.15 ± 0.26^{a}	2.83 ± 0.33^{b}	4.03	7.09
Cr	1.06 ± 0.04^{bc}	1.10 ± 0.15^{c}	1.42 ± 0.13^{ab}	1.23 ± 0.02^{abc}	1.40 ± 0.05^{a}	0.91 ± 0.10^{c}	1.19	2.09
Pb	5.10 ± 0.15^{ab}	4.73 ± 0.59^{b}	6.10 ± 0.69^{a}	5.20 ± 0.10^{ab}	5.18 ± 0.27^{ab}	4.06 ± 0.49^{b}	5.06	8.91
Ni	3.02 ± 0.21^{a}	3.09 ± 0.29^{a}	3.50 ± 0.51^{a}	3.66 ± 0.27^{a}	3.43 ± 0.22^{a}	2.68 ± 0.29^{a}	3.23	5.69
Cu	2.61 ± 0.11^{ab}	2.75 ± 0.09^{ab}	3.25 ± 0.27^{a}	2.84 ± 0.09^{ab}	2.93 ± 0.22^{a}	2.18 ± 0.23^{b}	2.76	4.86
Zn	0.52 ± 0.06^{a}	0.44 ± 0.02^{a}	0.66 ± 0.10^{a}	0.48 ± 0.01^{a}	0.53 ± 0.02^{a}	0.45 ± 0.14^{a}	0.51	0.90
Cd	24.41 ± 0.44^{ab}	23.63 ± 2.13^{b}	39.72 ± 9.38^{a}	28.17 ± 2.03^{ab}	24.20 ± 1.01^{ab}	20.65 ± 3.55^{b}	26.80	47.15
RI	40.49 ± 0.46^{ab}	39.94 ± 3.93^{b}	58.62 ± 9.35^{a}	45.85 ± 2.09^{ab}	42.82 ± 1.82^{ab}	33.76 ± 5.01^{b}	43.58	-

The values were presented as mean ± standard error. Different letters (a, b, and c) show the differences between the groups in the same metal are statistically significant ($p<0.05$). RI represents the overall potential ecological risk of the seven metal elements. Average contribution is the average risk value of each metal divided by the average value of the overall risk.

Table 4.5 The concentrations of metal element (Cesium) accumulated in animals.

Taxon	Species		Cs concentration	Reference
Mammals	Bull	Japanese black beef bull	572 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Yamashiro et al., 2013
	Monkey	Macaca fuscata	2.5×10^4 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Hayama et al., 2013
Insects	Butterfly	*Zizeeria maha*	31.2 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Nohara et al., 2014
Amphibians	Frog	*R. ornativentris and R. tagoi tagoi*	4.73×10^4 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Matsushima et al., 2015
Fish	Epipelagic fish	Sardine	7.89 Bq/kg-ash (^{134}Cs and ^{137}Cs)	Morita et al., 2015
		Japanese anchovy	5.69 Bq/kg-ash (^{134}Cs and ^{137}Cs)	Morita et al., 2015
	Piscivorous fish	Japanese catfish	2911 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Matsuda et al., 2015
		Largemouth bass	2708 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Matsuda et al., 2015
	Demersal fish	*Sebastes cheni*	3200 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Wada et al., 2013
		Hexagrammos otakii	3000 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Wada et al., 2013
		Microstomus achne	1140 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Wada et al., 2013
Arthropod	Crustacea	Crangonidae	1.09 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Shigenobu et al., 2015
		Paradorippe granulata	4.37 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Shigenobu et al., 2015
Echinoderms	Asteroidea	*Philyra syndactyla*	3.58 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Shigenobu et al., 2015
		Luidia quinaria	2.65 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Shigenobu et al., 2015
Annelids	Polychaeta	Eunicidae	11.2 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Shigenobu et al., 2015
		Flabelligeridae	99.4 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Shigenobu et al., 2015
		Terebellidae	30.2 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Shigenobu et al., 2015
		Polynoidae	12.1 Bq/kg-wet (^{134}Cs and ^{137}Cs)	Shigenobu et al., 2015

Table 4.6 The concentrations of metal element (Cesium) accumulated in fungi, plants and algas.

Taxon		Species	Cs concentration	Reference
Fungal	Wild mushroom	*Boletopsis leucomelas*	871 Bq/kg-dry (^{137}Cs)	Kuwahara et al., 2005
		Tricholoma portentosum	1960 Bq/kg-dry (^{137}Cs)	Kuwahara et al., 2005
Plant	Aquatic plant	*Potamogeton crispus*	2.69×10^{4} Bq/kg-dry (^{137}Cs)	Sasaki et al., 2016
		Trapa bispinosa	1.96×10^{4} Bq/kg-dry (^{137}Cs)	Sasaki et al., 2016
Alga	Filamentous Alga	*Spirogyra* sp.	7.11×10^{3} Bq/kg-dry (^{137}Cs)	Sasaki et al., 2016

Table 4.7 The concentrations of metal element (Cesium) accumulated in microorganisms.

Taxon	Species	Cs concentration	Reference
Cyanobacteria	*Anabaena sp.,* and *Microcystis sp.*	1.01×10^{3} Bq/kg-dry (^{137}Cs)	Sasaki et al., 2016
	Nostoc commune	1.02×10^{6} Bq/kg-dry (^{134}Cs and ^{137}Cs)	Sasaki et al., 2013
	Synechocystis PCC 6803	6.78×10^{-5} ng/ cell (Cs)	Avery et al., 1991
Bacteria (from soil)	*Streptomyces sp. K202*	200 µmol/g-dry (Cs)	Kuwahara et al., 2011
	R. erythropolis CS98	690 µmol/g-dry (Cs)	Tomioka et al., 1994
Bacteria (from freshwater sediment)	Heterotrophic bacteria	3.95×10^{-6} - 5.68×10^{-4} ng/cell (Cs)	Li et al., 2022c
Bacteria (from coastal water sediment)	Heterotrophic bacteria	1.52×10^{-6} – 7.41×10^{-4} ng/cell (Cs)	Li et al., 2022c

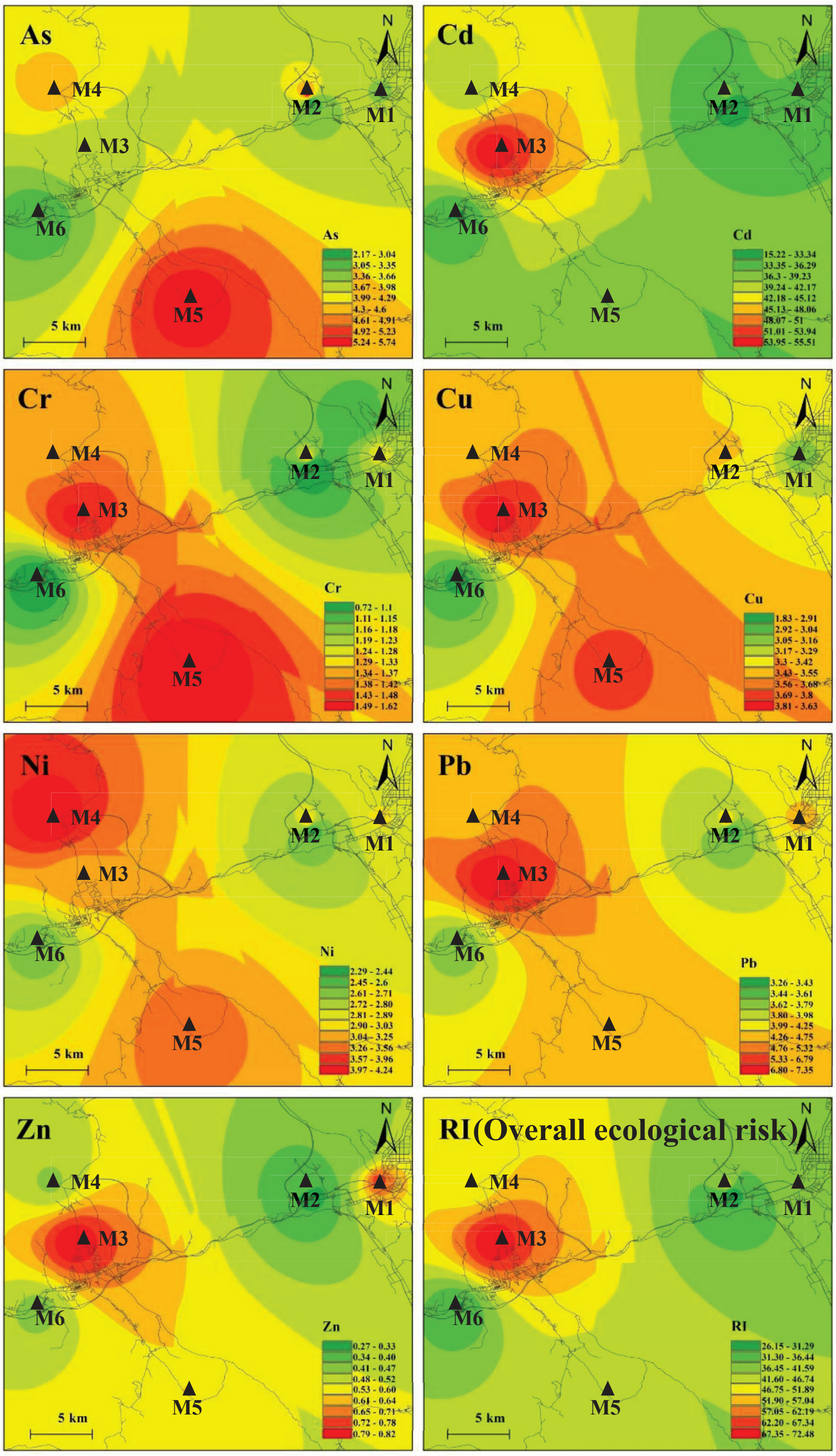


Fig. 4.1 Spatial distribution of the individual potential ecological risk from each of the heavy metals (As, Cd, Cr, Cu, Ni, Pb, and Zn), and the overall ecological risk from all seven metals (RI) around the coal mines (M1-M6). Distribution was identified and mapped using the Inverse Distance Weighted (IDW) method in ArcGis 10.2.

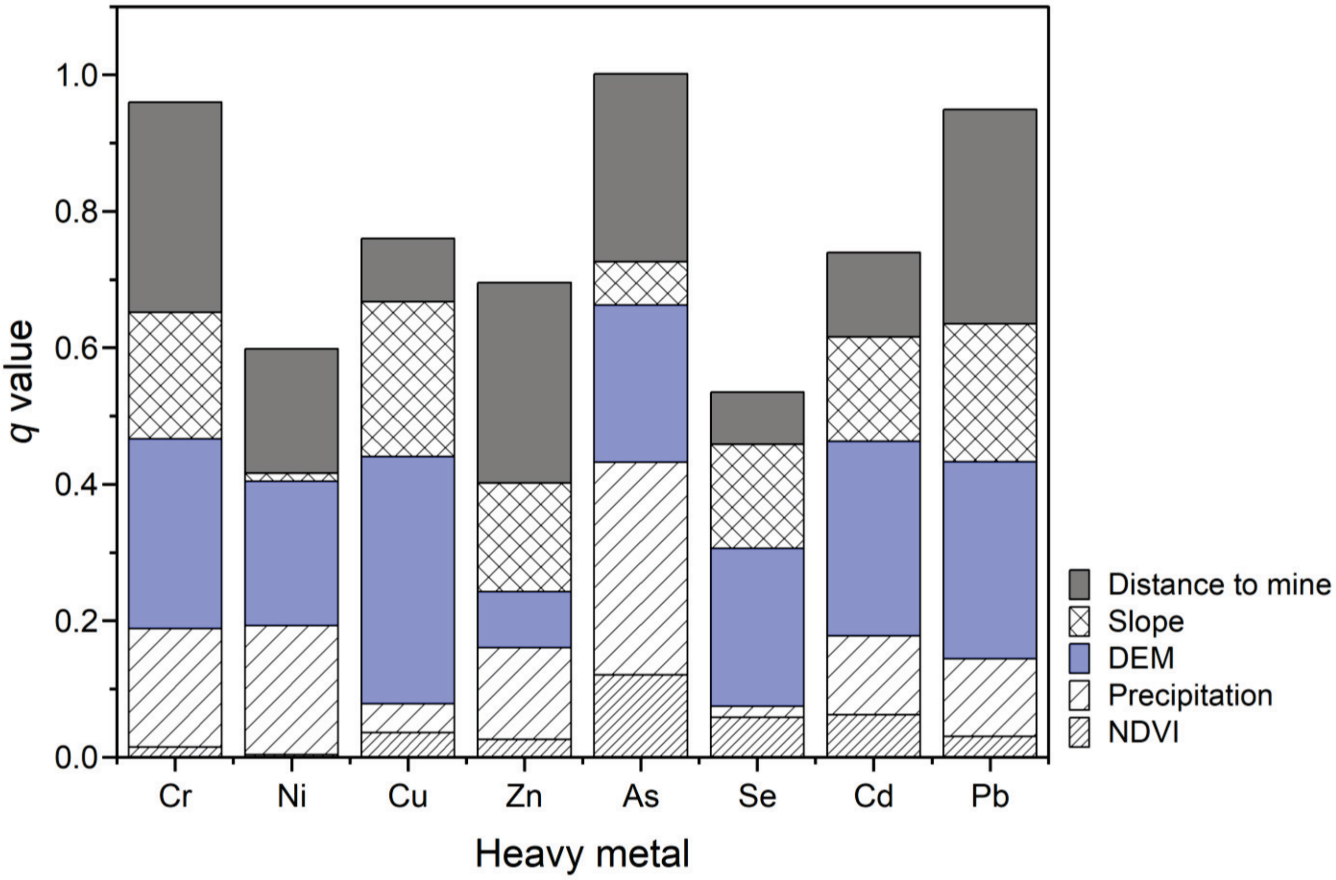


Fig. 4.2 Contribution of natural factors (DEM, slope, precipitation, NDVI, and distance to mine) influencing heavy metal spatial distribution.

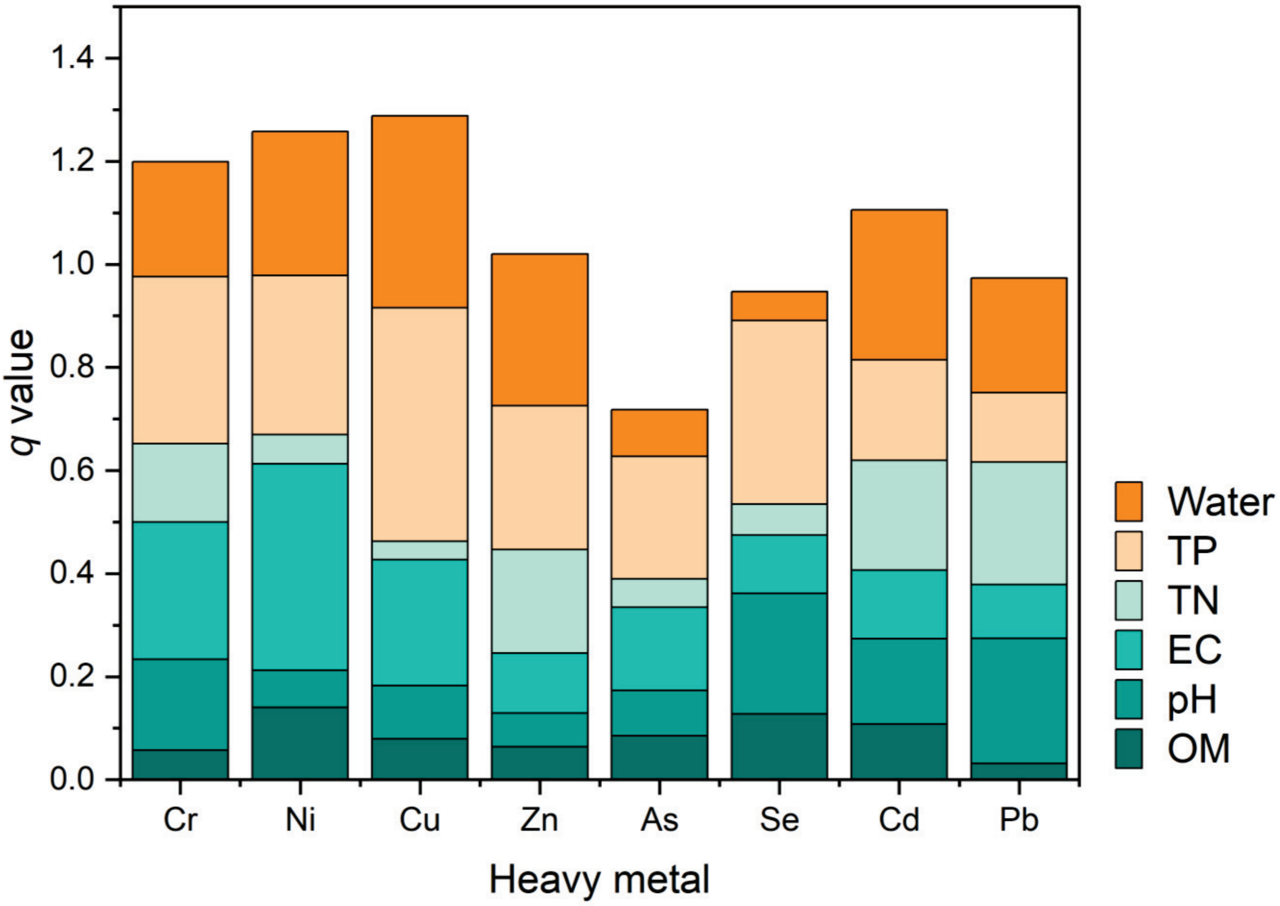


Fig. 4.3 Potential contribution of soil physiochemical properties (water content, total phosphorus, total nitrogen, EC, pH, and total organic matter) influencing heavy metal spatial distribution. TN, TP and OM represent total nitrogen, total phosphorus and total organic matter in soil.

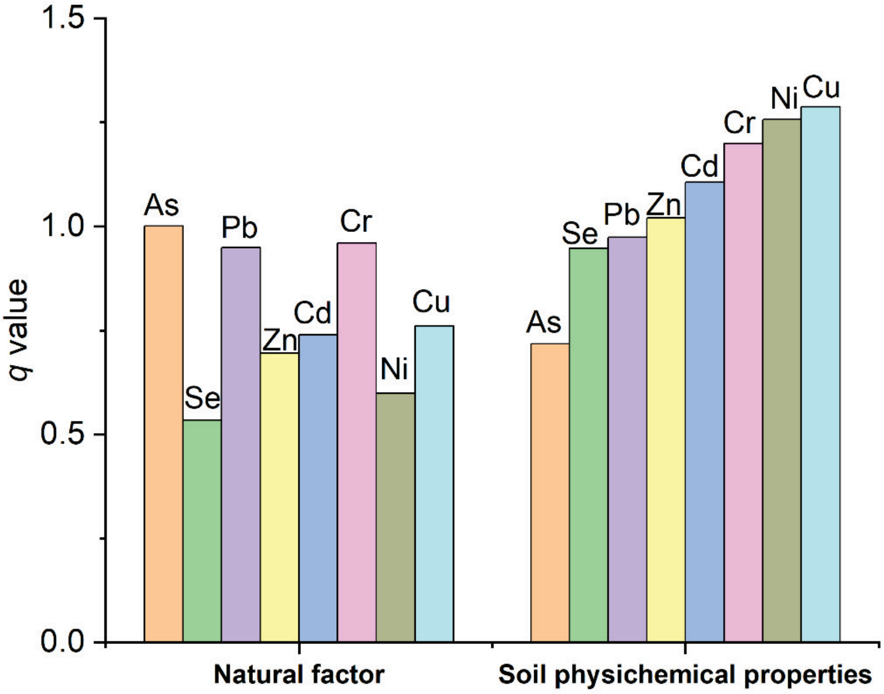


Fig. 4.4 Comparation of potential contribution of natural factors and soil physiochemical properties influencing heavy metal spatial distribution.

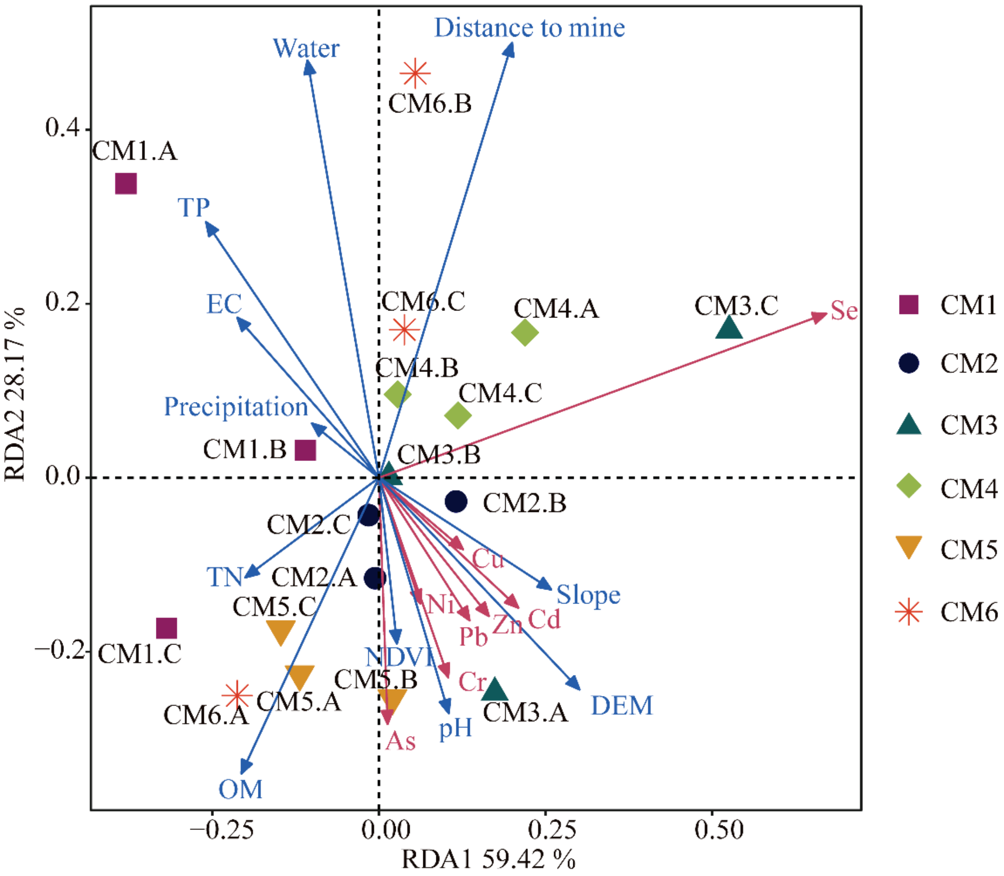


Fig. 4.5 Redundancy analysis (RDA) of the relations between concentrations of heavy metals, and natural and geographical factors (DEM, slope, precipitation, NDVI, and distance to mine) and physiochemical properties (pH, EC, water content, organic matter, total nitrogen, and total phosphorus) in soil of the coal mines (M1-M6). TN, TP, and OM represent total nitrogen, total phosphorus, and total organic matter in soil.

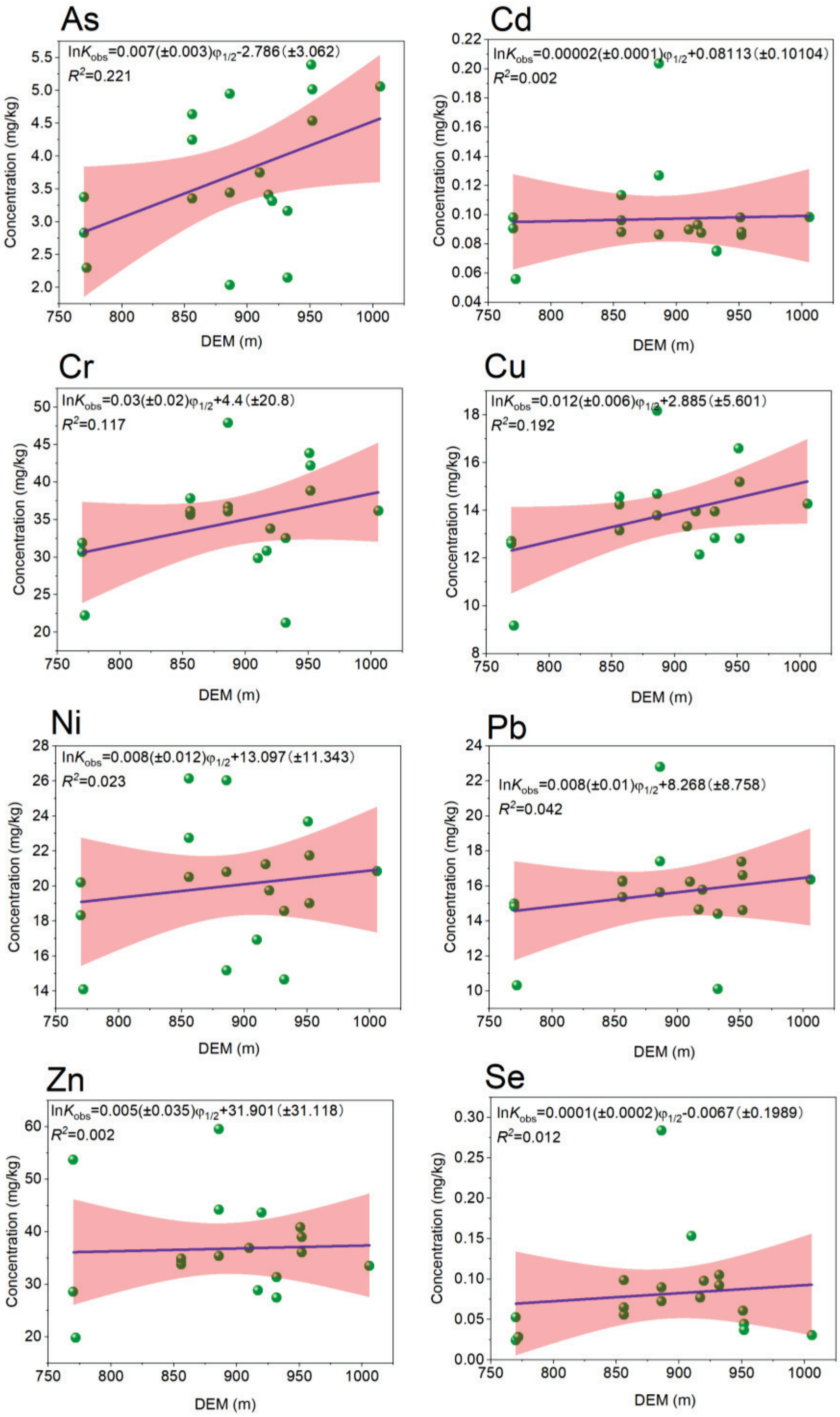


Fig. 4.6 Linear regression models between concentration of heavy metal (As, Cr, Pb, Ni, Cu, Zn, Cd and Se) and DEM of the sampling sites in the coal mines (CM1-CM6). Solid lines indicate the regression fit, and the shaded area denotes 95% confidence intervals.

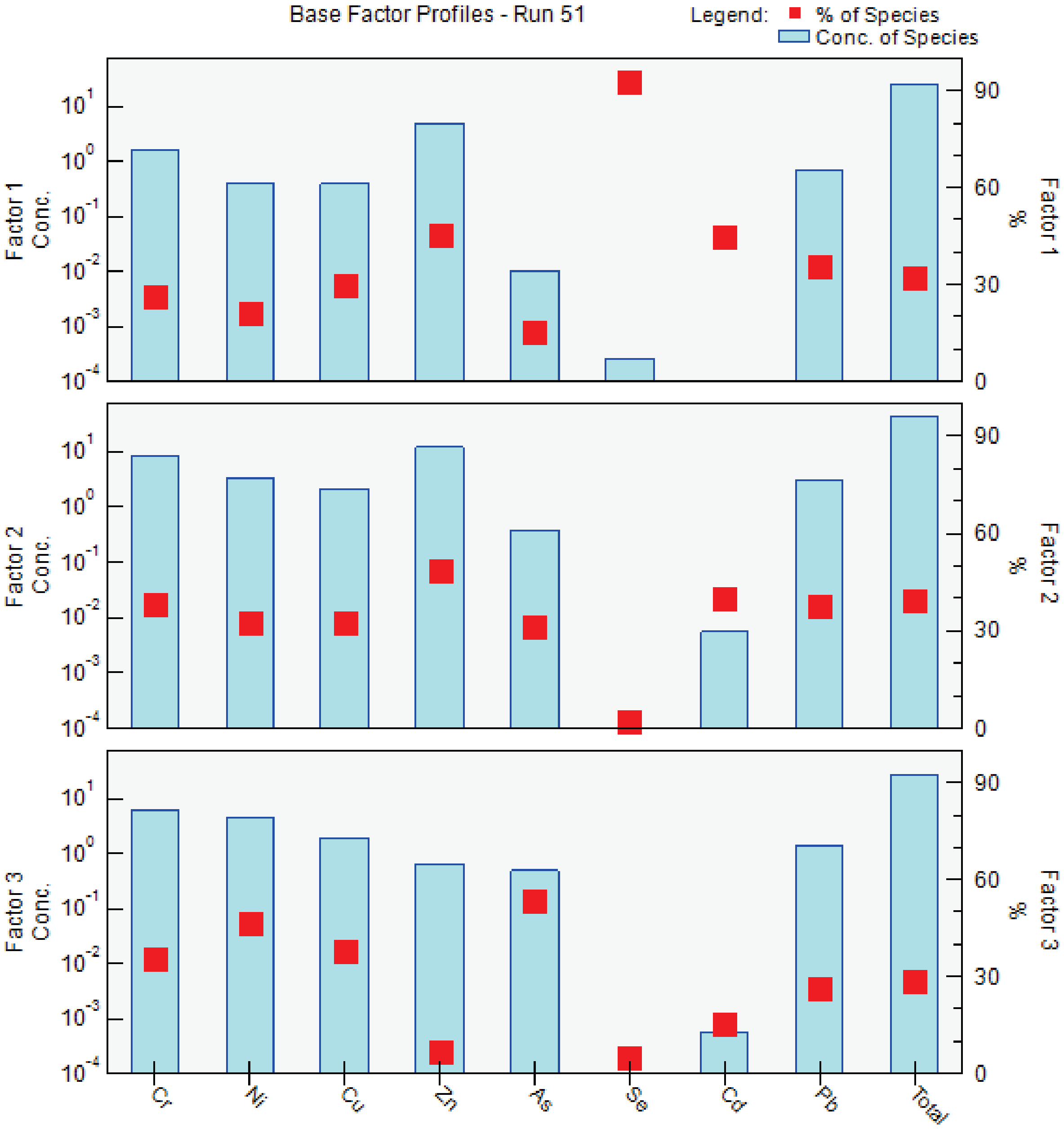


Fig. 4.7 Profiles and contributions of sources of the heavy metals of the sampling sites in the six coal mines.

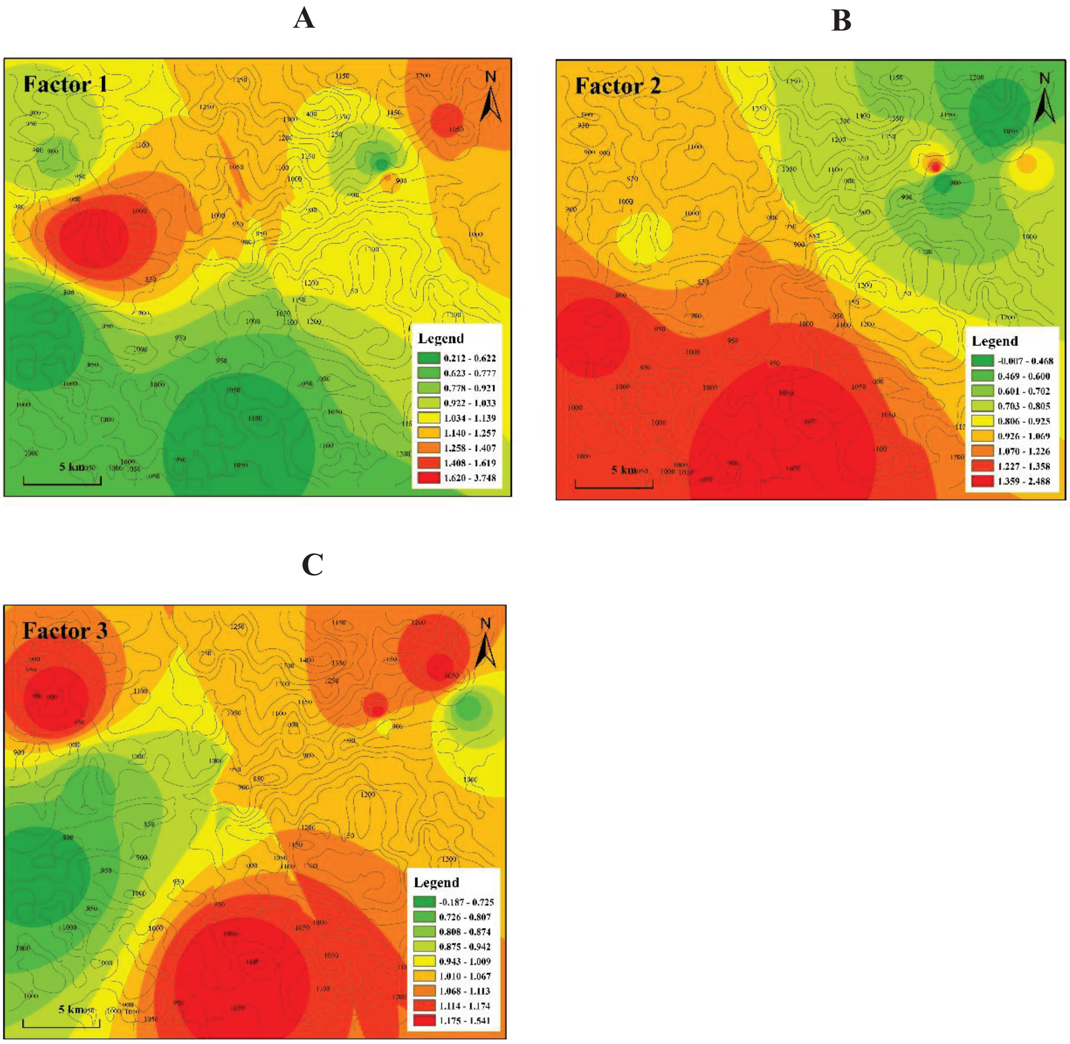


Fig. 4.8 Spatial variation in soils of coal mines for contributions from each of the three sources. Distribution was identified and mapped using the Inverse Distance Weighted (IDW) method in ArcGis 10.2.

4.5 SUMMARY

The study found varying levels of heavy metal contamination in different coal mines. Notably, Mine M6, which had the largest coal production scale among the six mines studied, had a lower ecological risk. Of the seven heavy metals tested (As, Cd, Cr, Cu, Ni, Pb, and Zn), Cd was found to be the most significant contributor, accounting for nearly 50% of the overall ecological risk. Additionally, there were positive correlations observed between the concentrations of heavy metals and Digital Elevation Models (DEM). Linear regression models were developed to establish the relationship between heavy metal concentration and DEM. The study also discovered that soil physicochemical properties had a greater impact on the spatial distribution of heavy metals than natural factors, except in the case of As. The application of the Positive Matrix Factorization (PMF) model identified three potential pollution sources: 1) coal mining activities (32.2%), as suggested by high levels of Se, Zn, and Cd; 2) coal transportation traffic (39.4%), as indicated by higher levels of Zn, Cd, and Cr; 3) natural source (28.4%), as indicated by elevated levels of As and Ni.

Chapter 5

RELATION BETWEEN BACTERIAL COMMUNITY AND POTENTIAL ECOLOGICAL RISK

5.1 INTRODUCTION

Prolonged coal mining activities can result in regular influxes of heavy metals into the soil, significantly impacting bacterial community. Previous research showed that different heavy metals have different effects on large organisms (the toxic factors are Zn = 1, Cr = 2, Cu = Pb = Ni = 5, As = 10, and Cd = 30) (Hakanson, 1980), their toxicity to microorganisms may also differ. To gain insight into the mechanisms underlying the impacts of heavy metals on soil microbial ecology surrounding coal mines in semi-arid region, quantitative evaluations of the relations between bacterial community (abundance, diversity, and structure) and ecological risk from heavy metals are necessary.

The response of the bacteria community to heavy metals is a process of selective growth of the tolerance group of bacteria and selective decay of the sensitive group of bacteria (Diaz-Raviña and Bååth, 1996; Ruyters et al., 2010a; Du et al., 2018). For bacteria sensitive to heavy metals, their growth can be inhibited, or they can survive in a dormant state (Tang et al., 2019), or extinct. For bacteria with strong tolerance against heavy metals or bacteria with weak tolerance but can recover after adapting to the toxicity of heavy metals, they can grow normally and become the dominating ones (Yin et al., 2015), such as metal tolerant bacteria-Proteobacteria and Firmicutes found in heavy metal contaminated soil (Singh et al., 2019; Zhao et al., 2019). Proteobacteria showed a positive relationship with heavy metals in the soil contaminated by heavy metals (Song et al., 2018; Pan et al., 2020; and Li et al., 2017b), while, and opposite relationship was found in soil co-contaminated with heavy metals and rare-earth elements (Luo et al., 2021). The variations in the response to heavy metals may be due to the possibility that bacterial species even in the same phylum may have different lifestyle and require different substrate and nutrient levels for carbon, nitrogen, and energy sources (Bouskill et al., 2010) and are affected differently by surrounding environmental factors (Luo et al., 2021; Song et al., 2018; Pan et al., 2020). This may suggest that compared to studies in phylum levels, studies in species levels are more adequate for evaluation of the response of bacterial community to heavy metals. More comprehensive studies are expected since, within the bacterial community in coal mine soil, there might be some bacterial species that have tolerance against heavy metals and could grow readily and there might be some too sensitive to grow. If such bacterial species exist, they can serve as bioindicators to be used for more effective monitoring of heavy metal contamination in coal mine soils, which needs to be investigated through field studies in soil of different coal mines.

5.2 BACTERIAL ABUNDANCE AND POTENTIAL ECOLOGICAL RISK

The relations of the abundance of total bacteria with the potential ecological risk from heavy metals and the soil physiochemical properties are shown in **Fig. 5.1**. The abundance of total bacteria showed statistically negative relations with the overall ecological risk from all metals, and the individual risk from Cd, Cr, and Zn; and the relation with the overall risk was more significant. For the soil properties, the abundance of total bacteria showed statistically positive relations with water content and total phosphorus (**Fig. 5.2**).

The more significant relation between the abundance of total bacteria and the overall risk suggested that the toxicity of compound heavy metals was stronger than that of individual metal elements, consistent with the research by Song et al. (2018) and Li et al.

(2022a). Many studies have shown that high contamination of heavy metals can decrease soil biomass. For example, when the concentrations of Zn, Cu, and Cd were increased two times the EU mandatory upper limits, the microbial biomass in sandy loam decreased by two times (Chander et al., 1995). In soils with Cd, Cu, and Zn concentrations of 0.3-1.5, 100-500, and 150-300 mg/kg, respectively, negative relations between microbial biomass and metal concentrations were observed (Song et al., 2018).

In our study, the concentrations of Cd, Zn, and Cr (ranges of 0.06-0.20, 19.8-59.5, and 21.2-43.9 mg/kg, respectively) were much lower, even though statistically negative relations between the abundance of total bacteria and Cd, Zn, and Cr still exist. The risk posed by these metals to microorganism may be underestimated, because the toxic response factors (TF) of heavy metals used for calculation were determined without taking into account the accumulated concentrations in microorganisms (Wang et al., 2023). Chen et al., (2020) found that the toxic response factor (TF) of Cu for bacteria was underestimated, together with the findings of this study, highlighting the need for systematic studies on the accumulated concentrations of heavy metals in bacteria for better estimating the toxicity of heavy metals to microorganisms. This can be achieved by conducting contact experiments with heavy metals using bacteria of different sizes and functions, such as those involved in nitrification, denitrification, desulfurization, and methane production, according to the methods described by Li et al. (2022c), which are under preparation. And the accumulation concentration of metals can be calculated and identified as described in **Table 5.1**. Additionally, in semi-arid region, water and nutrient scarcity can cause the bacteria to be more vulnerable to heavy metal disturbance, leading to negative responses under low contamination levels (Li et al., 2022b; Xiong et al., 2017), which also needs further investigation.

In this study, water content and total phosphorus revealed statistically positive relations with bacterial abundance, indicating their crucial roles in regulating the growth of bacteria in soils of semi-arid region. Water is the most limiting factor for soil in semi-arid region (Arau´jo et al., 2004). High pH, alkaline-calcareous, as well as free calcium ions in soil of semi-arid region, can cause poor solubility and availability of phosphorus (Marschner, 2011; Sharma and Chowdhury, 2021). The positive relation between water content and bacterial abundance can also be attributed to the possibility that low water content leads to higher concentrations of heavy metals in capillary water, thereby reducing bacterial abundance. The lower abundance of total bacteria in soils of mines M3, M4, and M5 compared to M1, M2, and M6 can be explained by the higher value of RIo/W (water content) for M3, M4, and M5. The interaction of phosphorus with heavy metals and water content in soils of semi-arid region may be weak. In semi-arid region, differences in water availability caused little effect on phosphorus transformation (Arau´jo et al, 2004), and in soil with high pH value, phosphorus and heavy metals can form precipitates (Adriano 2001; Avudainayagam et al. 2001). However, the correlation analysis showed that total phosphorus negatively corrected with the overall risk from metal elements ($p < 0.05$), indicating their possible interactions, which need to be investigated in further study.

From high to low taxonomic levels, the linear models that can describe the relations between the absolute abundance of bacterial taxa and the potential ecological risk from heavy metals are shown in **Table 5.2.** The twelve identified bacterial taxa belong to Proteobacteria, which was the most dominant phylum in this study. Among these taxa, four - Alphaproteobacteria, Sphingomonadaceae, Nitrosphaeraceae, and Xanthobacteraceae - were found to be enriched in soils with low phosphorus levels, according to previous studies by Oliverio et al. (2020) and Hermans et al. (2017). This suggests that the established models can well describe the relations between bacterial community and heavy metal concentrations in coal mine soils in semi-arid regions. Additionally, our study is unique in that it established models based on the absolute abundance of bacterial taxa, which is a departure from previous studies that used relative abundance (Li et al., 2020a; Tang et al., 2019). This is the first time that such models have been developed in this context. Based on these models, the impact of heavy metals on microbial ecology can be estimated, and the abundance of bacteria can be predicted. However, further validations and testing are necessary to optimize these models and develop their applications in other climate regions.

The result revealed statistically negative relations between the dominant bacterial phyla and the overall ecological risk from all metals, as well as the risks associated with Cr, Zn, and Cd. Proteobacteria, the most abundant phylum, showed high sensitivity to the overall risk and individual risks from Cr and Cd. Acidobacteriota and Chloroflexi were highly sensitive to Cr, Zn, and Cd. These significant correlations suggest potential linear relations between the absolute abundance of specific bacterial taxa belonging to these phyla and the ecological risk posed by heavy metals. Thus, we screened the bacterial taxa dominating in all soil samples**,** from high to low taxonomic levels (from class, order, family, to genus), and identified twelve bacterial taxa that exhibited high sensitivity to the overall ecological risk, and individual risk from Cr, Cd, and Zn. From high to low taxonomic levels, the linear models (at $p < 0.05$) that can reflect the relations between the absolute abundance of bacterial taxa and the potential ecological risk from heavy metals**.** These linear models can predict the level of heavy metal contamination and bacterial abundance based on one of two known variables. Among these screened bacterial taxa, Alphaproteobacteria, Sphingomonadaceae, Nitrosphaeraceae, and Xanthobacteraceae were found enriched in soils with low phosphorus (Oliverio et al., 2020；Hartmann et al., 2017). These models provide valuable quantitative frameworks for estimating heavy metal contaminations in soils in semi-arid regions and in phosphorus-limited soil. Additionally, further validations and testing are necessary to optimize these models and develop their applications in other climate regions.

5.3 BACTERIAL DIVERSITY AND POTENTIAL ECOLOGICAL RISK

The relations of the bacterial diversity with the potential ecological risk from heavy metals and the soil physiochemical properties are shown in **Fig. 5.1**. The bacterial diversity (Shannon and Simpson indexes) had statistically negative relations with Zn. For the adverse relation of bacterial diversity with Zn in this study, Wang et al. (2021) reported similar results in the sediment with low concentrations of Zn (44-400 mg/kg). In soils with high concentrations of Zn (>449mg/kg), the bacterial Shannon index showed positive correlations with Zn (Pan et al., 2020). The contrary findings may be due to the possible threshold of Zn in regulating the bacterial diversity, including the extinction of bacteria sensitive to Zn under low concentrations of Zn and the appearance of new bacteria tolerant to Zn under high concentrations of Zn. The wide variation in Shannon index observed in the soils of mine M1 may be due to the distinct differences in Zn concentrations among different samples.

5.4 BACTERIAL COMMUNITY STRUCTURE AND POTENTIAL ECOLOGICAL RISK

Redundancy analysis (RDA) plot for relations between bacterial community structure and potential ecological risk from heavy metals and soil physiochemical properties is shown in **Fig. 5.2**. The plot showed that water content and total phosphorus are factors that contributed mostly to the variations of the bacterial community structure in the soils of coal mines; and revealed positive relations with the abundance of dominant phyla. The potential ecological risk from heavy metals showed negative relations with the dominant bacteria.

The vital role of water content in structuring the soil microbial community of arid and semi-arid regions has been reported in previous studies (Guo et al., 2021; Che et al., 2018; Hedĕnec et al., 2018). In the Namib Desert, the relative abundance of bacteria changed greatly after precipitation, with increases in Proteobacteria and Actinobacteria, and a decrease in Acidobacteria (Armstrong et al., 2016). Proteobacteria and Actinobacteria were found to be sensitive to water limitation, while Acidobacteria were more tolerant (Hartmann et al., 2017; Curiel Yuste et al., 2014). The high relative abundance of Pseudomonas (a member of Proteobacteria) in mine M1 could be attributed to the relatively high-water content in the soil. For the role of phosphorus in shaping bacterial community structure, Oliverio et al. (2020) studied the bacterial community in soils with broad natural gradients in extractable phosphorus (tropical forests, temperate grasslands, and arid shrublands), and found significant variations in the bacterial community structure in different soils, and Acidobacteria and Alphaproteobacteria were the main taxa that enriched in low phosphorus soil. Spearman analysis was performed to evaluate the relations between the dominant bacteria in phylum level and the potential ecological risk from heavy metals based on the absolute abundance (BT_{axa}), as presented in **Fig. 5.3**. The result revealed statistically negative relations between the dominant bacterial phyla and the overall ecological risk from all metals, as well as the risk associated with Cr, Zn, and Cd. Proteobacteria, the most abundant phylum, showed high sensitivity to the overall risk and individual risk from Cr and Cd. Acidobacteriota and Chloroflexi were highly sensitive to Cr, Zn, and Cd.

5.5 METAL RESISTANCE GENES AND POTENTIAL ECOLOGICAL RISK

The relations between the abundance of MRGs, abundance of total bacteria (16S rDNA), and mobile gene (*intl1*) and potential ecological risk from heavy metals are shown in **Fig. 5.4**. Metal Resistance genes (*arsB, pbrT* and *chrB*), total bacteria (16S rDNA), and Mobile Gene (*intl1*) negatively correlated with all heavy metals. Cd, Cr and Zn significantly correlated with the absolute abundance of 16S rDNA and *intl 1*. The investigation into metal accumulation in various bacteria strongly supports the notion that soil bacteria have a high propensity for accumulating heavy metals (**Table 5.1**). The accumulation of heavy metals, whether on the surface or inside microbial cells, can significantly impact bacterial growth. Specifically, when heavy metals accumulate inside bacteria, they can cause detrimental effects by disrupting vital cellular processes like enzyme activity, DNA stability, and protein function. As a response, cells may activate stress responses, initiate repair mechanisms, or even undergo programmed cell death (apoptosis) to minimize the damage caused by exposure to heavy metals. Consequently, this can lead to alterations in the genes responsible for these essential cellular processes.

Table 5.1 Accumulation capability of metal element in different bacteria.

Taxon	**Species**	**Cs concentration**	**Reference**
Bacteria (from soil)	*Streptomyces sp. K202*	200 µmol/g-dry (Cs)	Kuwahara et al., 2011
	R. erythropolis CS98	690 µmol/g-dry (Cs)	Tomioka et al., 1994
Bacteria (from freshwater sediment)	Heterotrophic bacteria	3.95×10^{-6} – 5.68×10^{-4} ng/cell (Cs)	Li et al., 2022c
Bacteria (from coastal water sediment)	Heterotrophic bacteria	1.52×10^{-6} – 7.41×10^{-4} ng/cell (Cs)	Li et al., 2022c

Table 5.2 Linear regression modeling (y = ax + b, $p<0.05$) of the relations between the absolute abundance (y) of dominant taxa (in class, order, family, and genus level) and the ecological risk (x) from heavy metals in the soils of coal mines (M1-M6). Unit: y = cell equivalent g^{-1}

x		y (B_{Taxa})	a	b	r	p-value
RI_o		c_Alphaproteobacteria	-1.1×10^7	7.1×10^8	-0.57	0.013
		o_Sphingomonadales	-4.4×10^6	2.8×10^8	-0.56	0.015
		f_Sphingomonadaceae	-4.4×10^6	2.8×10^8	-0.56	0.015
		g_Sphingomonas	-2.7×10^6	1.7×10^8	-0.57	0.013
RI_i	Cr	o_Dongiales	-3.6×10^7	5.7×10^7	-0.71	0.001
		f_Dongiaceae	-3.6×10^7	5.7×10^7	-0.71	0.001
		g_Dongia	-3.6×10^7	5.7×10^7	-0.71	0.001
	Cd	f_Nitrosomonadaceae	-3.4×10^6	1.8×10^8	-0.52	0.027
		g_MND1	-1.9×10^6	1.1×10^8	-0.48	0.046
	Zn	o_Xanthomonadales	-4.1×10^8	2.7×10^8	-0.62	0.006
		f_Xanthomonadaceae	-3.9×10^8	2.6×10^8	-0.62	0.006
		g_Lysobacter	-1.4×10^8	8.9×10^7	-0.60	0.009

RI_i represents the individual potential ecological risk index of each metal. RI_o represents the overall potential ecological risk index of all metal elements. r: correlation coefficient. c represents the taxonomic level in class, o in order, f in family, and g in genus.

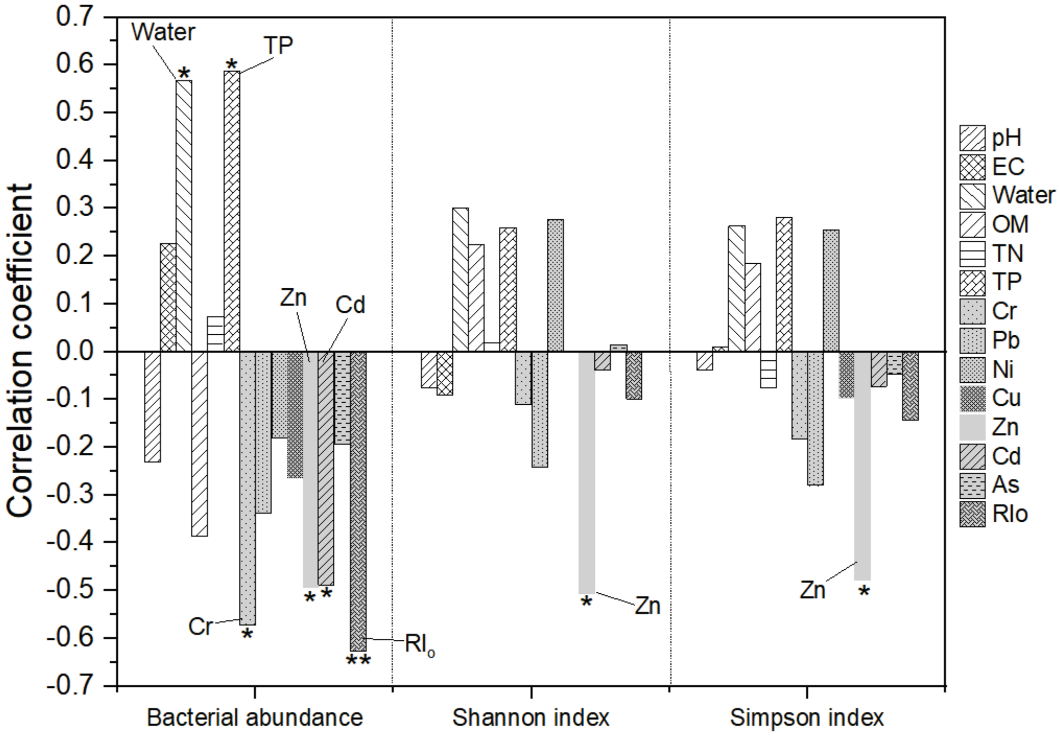


Fig. 5.1 Relations of the abundance of total bacteria based on 16S rDNA by qPCR and bacterial diversity based on the result of high-throughput sequencing with the potential ecological risk from heavy metals (*p<0.05; **p<0.01). TN, TP, and OM represent total nitrogen, total phosphorus, and total organic matter in soil. RI$_o$ represents the overall ecological risk from all heavy metals.

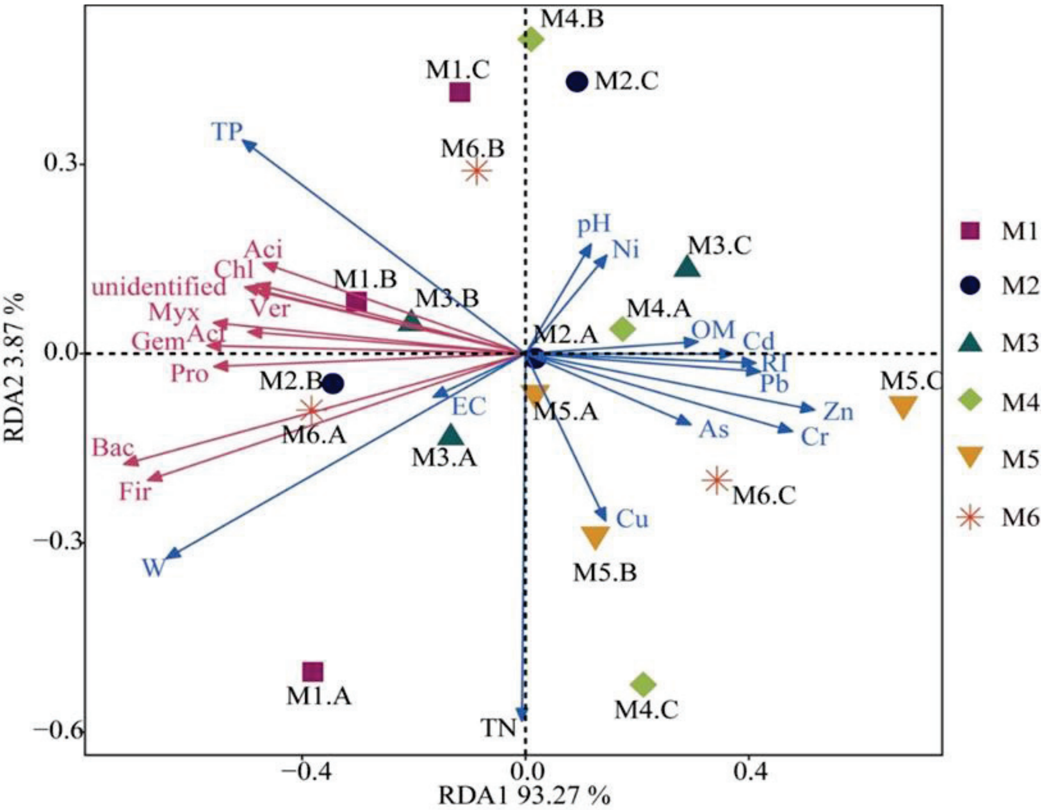


Fig. 5.2 Redundancy analysis (RDA) of the relations between the bacterial community structure and the potential ecological risk from heavy metals based on the absolute abundance of the dominant bacteria (B$_{\text{Taxa}}$) in the soils of coal mines (M1-M6). RI$_o$ represents the overall ecological risk from all seven metals. TN, TP, OM, and W represent total nitrogen, total phosphorus, total organic matter, and water content, respectively, in soil. Pro, Aci, Act, Fir, Bac, Myx, Ver, Chl, Gem, and unidentified were the abbreviations of Proteobacteria, Acidobacteriota, Actinobacteriota, Firmicutes，Bacteroidota, Myxococcota, Verrucomicrobiota, Chloroflexi, Gemmatimonadota， and unidentified_Bacteria, respectively. A, B, and C indicate the soil sampling sites around each mine distanced 0, 1and 3km apart from the center of the mine, respectively.

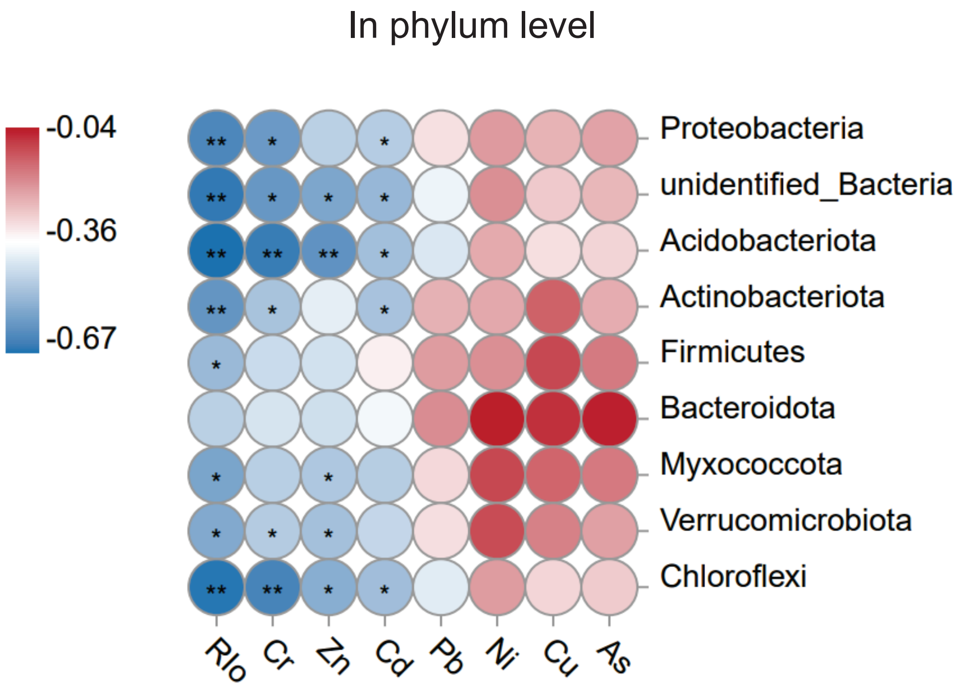


Fig. 5.3 Relations between dominant bacteria in phylum level and the overall potential ecological risk from all heavy metals (RI_o), and the individual risk from each metal based on the absolute abundance (B_{Taxa}) of bacteria in the soils of coal mines (M1-M6) (*p<0.05; **p<0.01).

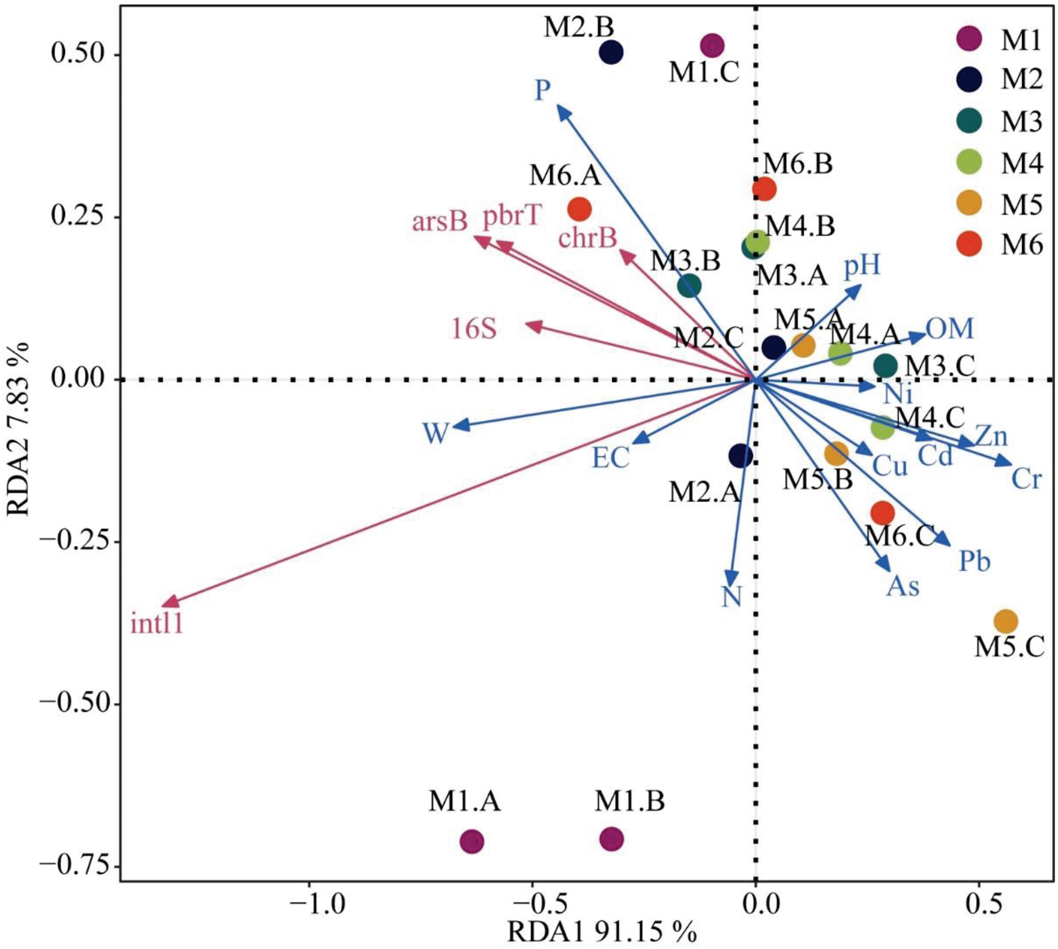


Fig. 5.4 Redundancy analysis (RDA) of the relations between the Metal resistance genes and the potential ecological risk from heavy metals in the soils of coal mines (M1-M6). TN, TP, OM, and W represent total nitrogen, total phosphorus, total organic matter, and water content, respectively, in soil. 16S was the abbreviations of 16S rDNA. A, B, and C indicate the soil sampling sites around each mine distanced 0, 1and 3km apart from the center of the mine, respectively.

5.6 SUMMARY

The bacterial community, with respect to abundance, diversity, and structure, were significantly affected by heavy metals. The abundance of total bacteria revealed negative relations with the overall ecological risk from all metal elements, and the individual risk from Cd, Cr, and Zn. The bacterial diversity showed a negative relation with the individual risk from Zn. Water content and total phosphorus had positive relations with bacterial abundance and significantly affected the bacterial community structure. Bacteria highly sensitive to heavy metals were identified, and linear models based on their absolute abundance and ecological risk from heavy metals were established. Regarding the relations between bacterial community and the production scales of the mines of this study of 0.6–8.0 × 10^6 t/year, a clear trend was not revealed. This probably suggests that the management and operation among the mines under the same administrative jurisdiction do not differ greatly, which needs future comparation with coal mines of other regions. These findings provide information for an in-depth understanding of the impact of coal production on soil environment in semi-arid region, and have important implications for the management and regulation of coal mining operations.

Chapter 6

CONCLUSIONS

The impact of heavy metals on the bacterial community in coal mine soils was investigated in this study through three important aspects: the potential ecological risk from heavy metals, the properties of the bacterial community (abundance, structure, and diversity), and the relations between the bacterial community and the potential ecological risk from heavy metals.

Chapter 2 details the sources and sampling sites, as well as the sampling procedures used to collect sediment samples for this study. The methods used to analyze soil physiochemical properties and heavy metal concentrations were also described. Additionally, the methods for analyzing bacteria using quantitative PCR and high-throughput sequencing were explained. The chapter includes a description of the calculation of potential ecological risk and the statistical data analysis methods used. The procedures used to analyze the relations between bacterial communities and the potential ecological risk from heavy metals were also outlined. Finally, the process for establishing linear models was described.

In Chapter 3, the properties of the bacterial community, such as abundance, diversity, and structure, are described. The study found significant variations in the abundance of total bacteria among the soils surrounding different coal mines. However, no significant differences in bacterial diversity were observed among the coal mines based on the values of Shannon and Simpson indexes. High-throughput sequencing analysis revealed that the dominant phyla in the mines were Proteobacteria, Acidobacteriota, Actinobacteria, Firmicutes, and Bacteroidota, which together accounted for approximately 70% of the total bacteria.

Chapter 4 provides an analysis of heavy metal concentrations in soils around coal mines and their potential ecological risks. The study revealed that heavy metal contamination levels varied among different coal mines, with cadmium (Cd) being the most significant contributor, accounting for almost 50% of the overall ecological risk. Furthermore, positive correlations were observed between the concentrations of heavy metals and Digital Elevation Models (DEM). The study also demonstrated that soil physicochemical properties had a more significant influence on the spatial distribution of heavy metals than natural factors. This chapter also investigates the potential hosts of metal resistance genes (MRGs), with a particular focus on arsB as the dominating MRG. The study identified the hosts of MRGs in soils from coal mines and examined how the transfer of MRGs may be influenced by heavy metals, which could inhibit their vertical and horizontal transfer.

Chapter 5 explores the relations between the bacterial community and the potential ecological risks posed by heavy metals, along with model analyses utilizing bacterial abundance and potential ecological risk. The study found that heavy metals significantly affected the bacterial community in terms of abundance, diversity, and structure. The abundance of total bacteria was negatively correlated with the overall ecological risk from all metal elements, as well as with the individual risks from Cd, Cr, and Zn. The bacterial diversity was also negatively correlated with the individual risk from Zn. Additionally, the study established 12 linear models based on the absolute abundance of bacteria and ecological risks from heavy metals.

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