Ecotoxicology and Environmental Safety 227 (2021) 112897

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Distribution characteristics and risk of heavy metals and microbial community composition around the Wanshan mercury mine in Southwest China

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ARTICLE INFO

Edited by Dr. Hao Zhu

Keywords:
Wanshan mercury mining
Heavy metal
Microbial diversity
Pollution load index
Potential ecological risk index

ABSTRACT

The Wanshan mercury (Hg) mine in Guizhou Province is one of the main Hg-producing mines in China, resulting in serious Hg pollution in soil and wastewater. Therefore, the present study is mainly aimed to investigate the current degree of heavy metal pollution and compared the microbial diversity in the Wanshan Hg mine and its surrounding environment. The results showed the distribution of the pollution load index values was low in the west and high in the east. The northwestern (Aozhai River), northern (Meizi Stream), and southwestern parts of the study area and the area surrounding Erkeng did not reach moderate pollution. Mercury accounted for the majority of the potential ecological risk index values, reaching 67.62%, while the proportions of Cd and As were 15.75% and 10.75%, respectively. Mercury was found mainly in a residual state, which had an average proportion of 71.09%. In the three regions, Proteobacteria and Actinobacteria had the highest relative abundances. According to linear discriminant analysis effect size, the indicator species in the Hg mining area, woodland and cultivated land was f_67–14 (belonging to a family of Solirubrobacterales), Reyranellales and Reyranellaceae, Intrasporangiaceae, respectively. In summary, this study for the very first time estimated that the higher Hg, Cd and As pollution existed in Wanshan Hg mine since their concentration in the all soil samples totally exceeded the standard value (GB15618-2018), while Cd and As pollution in soil was commonly ignored by the previous study. The cultivated land had higher community richness than the mercury mining district and woodland. Our results suggested that the relevant local departments need to take more active measures to solve the problem of high levels of Hg, Cd, and As in the local soil, and prevent their adverse effects on humans.

1. Introduction

With the accelerating industrialization and urbanization in China, extensive mining activities have led to severe environmental issues in recent decades (Wu et al., 2020a; Zhang et al., 2020). In particular, China has vast and diverse mineral resources, and mining activities are an important source of waste and environmental pollution (Chen et al., 2014a, 2014b). The pollution of soil, air and water has improved gradually since China formulated strict environmental management regulations. Furthermore, related departments have paid attention to

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https://doi.org/10.1016/j.ecoenv.2021.112897
Received 8 July 2021; Received in revised form 9 October 2021; Accepted 11 October 2021
Available online 14 October 2021

enhance resource utilization efficiency by several methods, such as gradually standardizing the exploitation and utilization of mine resources, closing black coal kilns and illegal mines and other measures (Chen et al., 2019; Li et al., 2020). Nevertheless, continuous pollution occurred for long periods around mining areas after their shutdown, especially due to the overflow of mine tailings (Saavedra-Mella et al., 2019). Therefore, there is an urgent need to determine the level of risk from pollution in the local environment to allow relevant remediation measures to be taken.

The Wanshan mercury mine, located in northeastern Guizhou Province, Southwest China, is currently abandoned. However, long-term mining activities destroyed the original ecological conditions of this area and seriously affected local residents (Cheng et al., 2018; Wu et al., 2020b). In particular, heavy metal pollution, which is carefully monitored by the local environmental protection department, was a prominent threat to local residents. According to the previous published study, the Hg contents in soil were approximately between 14.15 and 65.37 mg/kg (Cai et al., 2019; Søvik et al., 2011; Zeng et al., 2012; Zhao et al., 2014; Hu et al., 2015; Lin et al., 2017; Song et al., 2019). Zhang et al. (2004) suggested that higher concentration of Hg in mine-waste calcines and alkaline surface water had threatened to the surrounding environment. Li et al. (2015) found that the Hg and Cd were the most abundant trace metals in the Wanshan mining area and there was a substantial content of toxic methylmercury in brown rice. The authors concluded that contaminated rice from the Wanshan mining area poses a potential threat to human health. Chang et al. (2020) considered that the bioaccumulation of atmospheric Hg in rice leaves can facilitate the uptake of soil Se, which may be through the formation of Hg–Se complexes in rice leaves. Wang et al. (2011) reported that Cd and Hg were the most important contributors to potentially harmful element contamination in Wanshan. Vegetables were found to be the main dietary source of potentially harmful elements. Therefore, the relatively higher concentrations of Hg and other heavy metals in soils and crops can pose extreme threats to local residents.

The above researches only focused on the total content and speciation of heavy metals in soil or plant in the Wanshan mining area. There are many reports on the relationship between soil heavy metals and microbial communities in mining areas, such as Wang et al. (2019a, 2019b) and Bai et al. (2021). In soil environment, Hg mainly forms complexes with a variety of inorganic and organic ligands in the soil, accumulates in crops and enters the human body through the food chain (Addai-Arhin et al., 2021). However, many factors affect the uptake of mercury by crops from contaminated soils, such as soil properties, microbial community, and soil fertility, etc (Bai et al., 2021). Among them, soil organic matter forms humus with the aid of microorganisms in soil, which can combine with inorganic particles such as clay minerals and oxides in soil to form organic colloids and organic-inorganic composite colloids (Wang et al., 2019a, 2019b). The process can increase the surface area and surface activity of the soil, and change the cation exchange capacity of the soil, and thus increasing the exchangeable adsorption capacity of the soil for Hg (Pei et al., 2021). In addition, under appropriate conditions (moisture, temperature, pH value, etc.), the microbial community in the fertilizer and the original beneficial microorganisms in the soil form a dominant community to promote the virtuous cycle of carbon, nitrogen, oxygen and other elements in the soil ecosystem, and thereby restoring the soil ecological environment system destroyed by Hg (Yao et al., 2020; Song et al., 2021). Unfortunately, the relationship between the microbial community composition and heavy metals in soil around the area is rarely reported.

The soil microbial community is an important and sensitive indicator, reflecting soil stability and ecological mechanisms (Yong et al., 2015). A healthy microbial population is necessary for adaptation to external factors and the maintenance of soil fertility (Pan et al., 2020). In most cases, heavy metal pollution will adversely affect the inherent community structure and activity of microorganisms (Ojuederie, 2017). In soils polluted by heavy metals for long periods, microbial community structure will change and species diversity will decrease to a large extent (Moffett et al., 2003). However, tolerant species with strong activity levels of key metabolic processes (e.g., denitrification and heavy metal resistance) will survive to form new communities, and the abundance of these species will increase (Matsuzaka et al., 2003). Zheng et al. (2019) indicated that the addition of Hg alone would reduce the number of bacteria but increase the abundance of fungi, and the bacterial community rather than the fungal community was changed by heavy metals. Harris-Hellal et al. (2009) showed that high concentrations of mercury have a lasting effect on the genetic structure of soil microbial communities. These modifications took place during the first week of incubation, when the total concentration of mercury was declining and the concentration of bioavailable mercury was at its highest. Liu et al. (2014a, 2014b) showed that the soil Hg pollution significantly influenced the bacterial community structure. However, the bacterial abundance was significantly correlated with the soil organic matter content rather than the total Hg concentration. The bacterial alpha diversity increased at relatively low levels of total Hg and methylmercury. To our knowledge, there is few reports to investigate heavy metals in soil of Wanshan Hg mining area in terms of microbial community. Moreover, the local ecological environment has been improved gradually because of the remediation by relevant departments. However, the improvement degree of soil heavy metal pollution in Wanshan mercury mining area was not clear after being treated by environmental protection agencies. Overall, the objectives of this study are to 1) investigate the spatial distribution of heavy metal pollution and the speciation of heavy metals (especially Hg) in soil collected from the Wanshan mercury mine area; 2) evaluate the risk from heavy metals using the pollution load index (PLI) and potential ecological risk index (PERI) combined with geographic information system; and 3) understand the impact of this pollution on microbial diversity in the Wanshan mercury mine area by the α diversity index, a LEfSe analysis and a co-occurrence network.

2. Materials and methods

2.1. Study area and Sample collection

The Wanshan mercury mine (E: 109°11′2″–109°14′54″, N: 27°30′14″–27°32′41″) is located in Wanshan district and is under the jurisdiction of Tongren city, eastern Guizhou Province. A typical karst landform is located in the center of a mineralization zone along the Pacific Rim. A random distribution method was used to collect soil samples from the Aozhai River (AZ), the Meizi Stream (MZ), Wukeng (WK), Erkeng (EK), the Wawu River (WW), Zhangjiawan (ZJW) and farming areas around Wanshan (Fig. 1). MZ, EK and WK are located in the abandoned area near the original mine, and AZ, MZ and WW are the three larger rivers that flow through the mining area and around the Wanshan. ZJW is a village near Wanshan. After randomly selecting three 1 m × 1 m sampling points from each location, soil samples were collected by quincunx sampling method, which is suitable for plots with small area, flat terrain and uniform soil. The soil samples collected in the field were divided into two parts. Approximately 5 g (used for 16 S rRNA sequencing) was placed in a sterile sampling bag inside a foam box containing sterile ice bags. The remainder of the 3–5 samples collected from each location was mixed into a composite sample, and the rocks were removed from the surface soil (0–10 cm). The composite soil samples were then sealed in polyethylene (PE) vacuum bags and brought back to the laboratory in a foam box containing ice. The samples were dried in a vacuum freeze dryer (FD-1-50, Beijing Boyikang). After being ground in an agate mortar and passed through a 100-mesh nylon sieve, the samples were sealed and stored away from light for the next test. The other part of soil samples was naturally dried, and the dried samples were subsequently used to determine the total amount and speciation of heavy metals. Before collecting the samples, the sampling tools were placed in an acid bath ($K_2Cr_2O_7$) for at least 24 h and washed with ultrapure water to minimize their interference in the results.

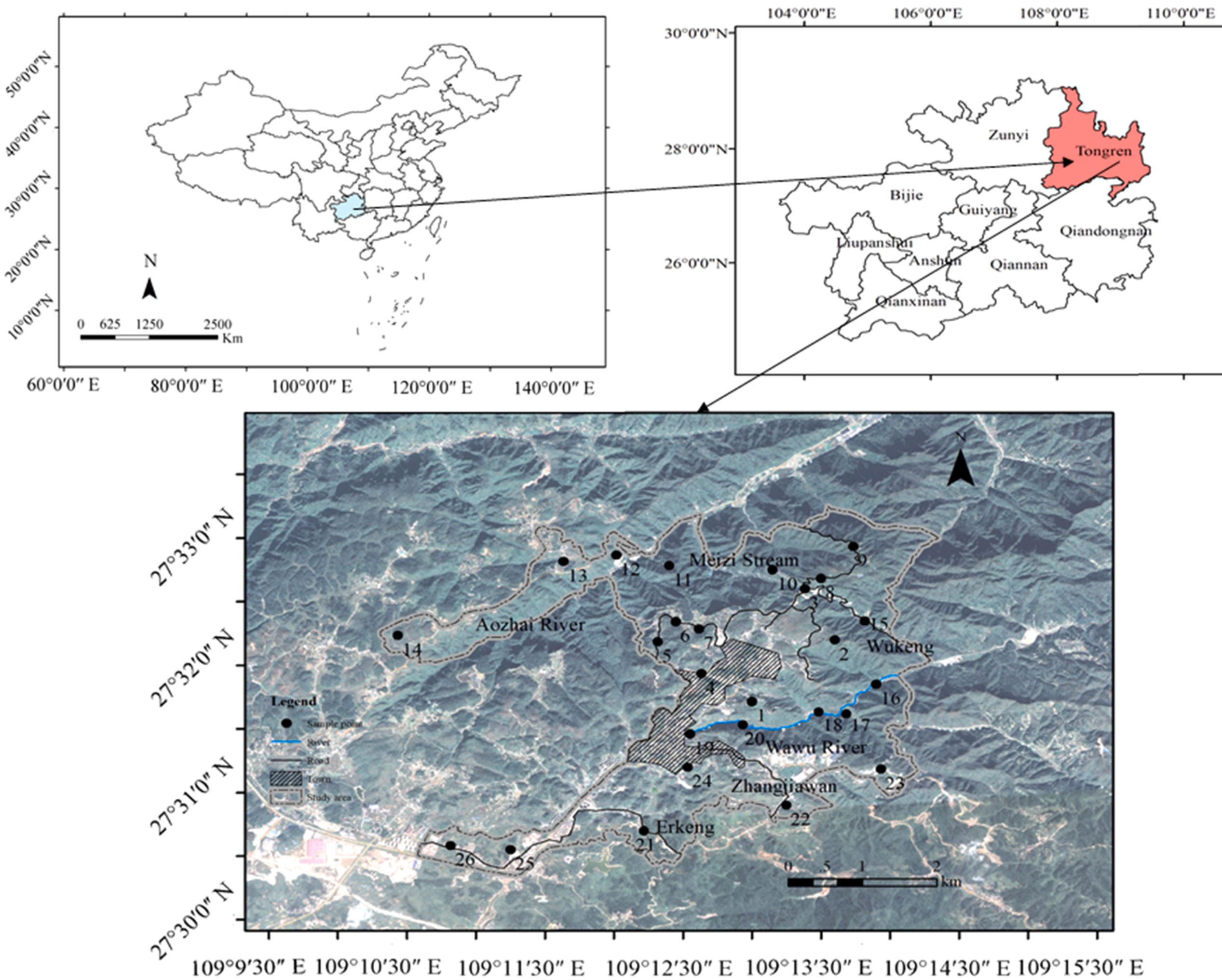

Fig. 1. The information of sampling points from Wanshan Hg mine area.

2.1.1. *Concentration of heavy metals and speciation analysis*

The pH values of the soil samples were determined with an electrode (PHS-3E, REX) (Zhang et al., 2020). The contents of soil organic matter (SOM) were detected by an oxidation capacity test with $K_2Cr_2O_7$ and high external heating. The total amounts of Pb, Cd, Cu and Zn in soil samples were determined by digestion with HNO_3-$HClO_4$-HF (3:1:1, v-v), and those of Hg and As were determined by digestion with HNO_3-HCl (1:3, v-v). The extraction process of the selected heavy metal speciation was exhibited in Table S1. The concentrations of Pb, Cd, Cu, and Zn in the soil samples were measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Prodigy XP, Leeman Labs, USA) (RF power: 1150 W; Cooling air flow: 1.0 L/min; Injection cleaning time: 30 s; Integration time: 30 s; Flushing pump speed : 45 r/min; Analyzing pump speed: 45 r/min). The Hg and As contents were measured by an atomic fluorescence spectrophotometer (AFS-2100, Beijing Haiguang). According to the soil distribution characteristics of Guizhou Province, the eastern Guizhou region is characterized by subtropical yellow soil, for which the Chinese soil standard material GBW07408 (GSS-8) is used as the reference material. The extraction processes of exchangeable forms, carbonates, iron/manganese oxides, organic matter-bound heavy metals and heavy metal residuals referred to the study of Zhang et al. (2020), and the specific method was exhibited in Table S1. To ensure the precision of the methods, a blank control and three repeated experiments were also used. For each heavy metal, the accuracy and precision of the methods and results were checked using a certified standard reference material (GSS-3/GBW07403) purchased from the State Bureau of Technical Supervision of China. Meanwhile, certified standard reference materials were used for the recovery study, and the recovery rates of the measured heavy metals in the soil samples ranged from 93.2% to 103.6%. The linear correlation coefficients of all heavy metals were greater than 0.999, and the relative standard deviations ($n = 3$) varied between 1.0% and 6.2%. The limits of detection (LODs) and limits of quantification (LOQs) were as follows. Hg: $\lambda = 253.7$ nm, LOD = 1.75 mg/kg and LOQ = 5.25; As: $\lambda = 189.04$ nm, LOD = 2.15 mg/kg and LOQ = 6.45; Cd: $\lambda = 214.438$ nm, LOD = 0.01 mg/kg and LOQ = 0.03; Cu: $\lambda = 324.754$ nm, LOD = 0.15 mg/kg and LOQ = 0.45; Pb: $\lambda = 220.351$ nm, LOD = 2.5 mg/kg and LOQ = 7.5; Zn: $\lambda = 213.856$ nm, LOD = 0.04 mg/kg and LOQ = 0.12. (The LODs of 1.000 g mineralized soil were calculated, and the LOQs were considered to be three times the LODs.).

2.1.2. *DNA extraction and PCR amplification*

Based on the manufacturer's protocols, the E.Z.N.A.® Soil DNA Kit (Omega Biotek, Norcross, GA, USA) was used to extract microbial DNA from soil samples. A NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was utilized to detect the final DNA concentration and purity, and DNA quality was checked by 1% agarose gel electrophoresis. A thermocycler PCR system (GeneAmp 9700, ABI, Applied Biosystems, Foster City, CA, USA) and primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were employed to amplify the V3–V4 hypervariable regions of the bacterial 16 S rRNA gene. The PCR amplification procedure was as follows: 3 min of denaturation at 95 °C; 27 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C; and a final extension at 72 °C for 10 min. PCRs were carried out in triplicate in a 20 μL mixture containing 4 μL of 5 × FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu polymerase, and 10 ng of template DNA.

Based on the manufacturer's protocol, a 2% agarose gel was used to extract the resulting PCR products, which were further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, Madison, WI, USA). The purified amplicons were pooled in equimolar amounts and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to standard protocols by Majorbio Bio-pharm Technology Co. Ltd. (Shanghai, China).

2.2. Assessment of heavy metal pollution in soil

The pollution load index is a simple and practical tool for assessing the level of pollution from heavy metal compounds (Mohammad et al., 2019):

$$C_f = \frac{C_{heavymetal}}{C_{background}} \tag{1}$$

$$PLI = (C_{f1} \times C_{f2} \times \cdots \times C_{fn})^{1/n} \tag{2}$$

The contamination factor (C_f) is defined as the ratio of the concentration of heavy metals in the soil to the control concentration (Soil Environment Quality Risk Control Standard for Soil Contamination of Agricultural Land in China, GB15618–2018) (Table 1). n is the number of samples. Sediment is considered to be polluted if the PLI value > 1; otherwise, it is not polluted.

The potential ecological risk index considers conversion patterns of heavy metals in sediments and evaluates the sensitivity of regional heavy metals. The formulas for PERI are as follows:

$$C_f^i = C_s^i \big/ C_n^i \tag{3}$$

$$E_r^i = T_r^i \times C_f^i \tag{4}$$

$$RI = \sum_{i=1}^{n} E_r^i \tag{5}$$

C_f^i is the pollution coefficient of a single heavy metal relative to the reference value of that heavy metal, C_s^i is the measured concentration of a heavy metal (mg/kg), C_n^i is the reference value of a heavy metal (mg/kg), and E_r^i is the PERI of a single heavy metal. T_r^i is the toxicity response coefficient of a heavy metal, and the toxicity coefficients of Pb, Cd, Hg, As, Cu and Zn are 5, 30, 40, 10, 5 and 1, respectively (Liu et al., 2017). RI is the PERI of composite heavy metals. The reference values (C_n^i) were determined by the soil heavy metal background values in Guizhou Province. The values of E_r^i and RI are found in Table S2.

2.2.1. Data analysis

Soil bacterial 16S rRNA data processing: The original sequences were spliced by Quantitative Insights Into Microbial Ecology (QIIME) and DADA2 (Huang et al., 2019) software to remove the barcodes and primers. According to a similarity level of 0.97, a naive Bayesian algorithm was used to train a feature classifier based on the Silva database (SSU132 version, Max Planck Institute for Marine Microbiology and Jacobs University, Bremen, Germany). Species annotation files were obtained by further training on behalf of the sequence and feature classifier. Sequence variation files (amplicon sequence variant (ASV) tables) were matched with annotation files to allow classification at the species level.

In this study, ArcGIS 10.2 was used to draw the sampling graph and kriging interpolation graph. The remaining graphs were produced using Origin 9.0 and Excel 2016. The igraph package was used to analyze the co-occurrence network of bacterial community structure, and Gephi 0.9.2 software was used to draw the network map (Csardi and Nepusz, 2006). LEfSe of bacteria was performed by Python 2.7 software (National Institute of Mathematics and Computer Science, city, Netherlands).

3. Results and analysis

3.1. Distribution of Pb, Cd, Hg, As, Cu and Zn concentrations in soil in study area

The contents of mercury, cadmium and arsenic in the soil of Wanshan district generally exceeded those outlined by the Soil Environmental Quality Risk Control Standard for Soil Contamination of Agricultural Land (GB15618-2018) (Table 1), of which the exceeding multiple of Hg to the standard values was the largest. Zn concentrates in soil merely outnumber the local criterion value. Pb and Cu contents did not exceed the local and national limited value. The soil pH value was 7.06 ± 0.81, and the SOM content was 36.95 ± 26.04 g/kg. Table 1 showed that the mercury content in the soil of mercury mining areas was higher than that of cultivated land and forest land, and its value was much higher than the local and national limit concentration.

The average Pb content was lower than the soil background value in Guizhou Province and the Soil Environment Quality Risk Control Standard for Soil Contamination of Agricultural Land in China (GB15618-2018), and the soil Cu content exceeded the background value but was lower than the control value. Notably, the contents of Cd, Hg and As exceeded the soil background value, and the Zn content fluctuated slightly relative to the control value. The spatial distribution of heavy metals shown on Fig. 2 indicates that there is a possibility of composite heavy metal pollution. Within the study area, Pb was concentrated mainly near the northwestern parts of the AZ and southeastern ZJW; the maximum values of Pb were located to the west of the AZ. Cadmium accumulated along the WW and MZ, and the upstream and downstream reaches of the WW were close to areas with high accumulations of Cd, i.e., WK (2.58 mg/kg) and Wanshan (3.81 mg/kg), respectively. The concentrations of Hg, As and Cu were 24.36, 36.26 and 30.37 mg/kg, respectively; high accumulations of these heavy metals were found near Wanshan Mercury Mine National Park, which is the core area of smelting in the original mercury mine. Moreover, relatively high Hg

Table 1
Concentrations of heavy metal in soils in Wanshan Hg mine (mg/kg).

Study area	Pb	Cd	Hg	As	Cu	Zn
Mercury mining area	23.55 ± 8.50[a]	2.17 ± 1.14[a]	16.94 ± 6.97[a]	34.52 ± 2.80[b]	39.72 ± 13.51[a]	342.92 ± 10.60[a]
Cultivated land	30.33 ± 8.18[a]	0.61 ± 0.16[b]	9.30 ± 10.34[b]	39.07 ± 6.40[a]	39.85 ± 11.12[a]	197.31 ± 14.97[a]
Woodland	31.03 ± 9.24[a]	0.61 ± 0.15[b]	2.90 ± 2.33[ab]	45.56 ± 6.46[ab]	45.51 ± 14.02[a]	194.24 ± 13.06[a]
Background values (Guizhou Province)	35.2	0.7	0.11	20	32	99.5
Control value (GB15618–2018)	120	0.3	2.4	30	100	250

Note: Mean ± SD, n = 5. Different superscript letters in each row represent significant differences between different treatments (ANOVA, $P < 0.05$).

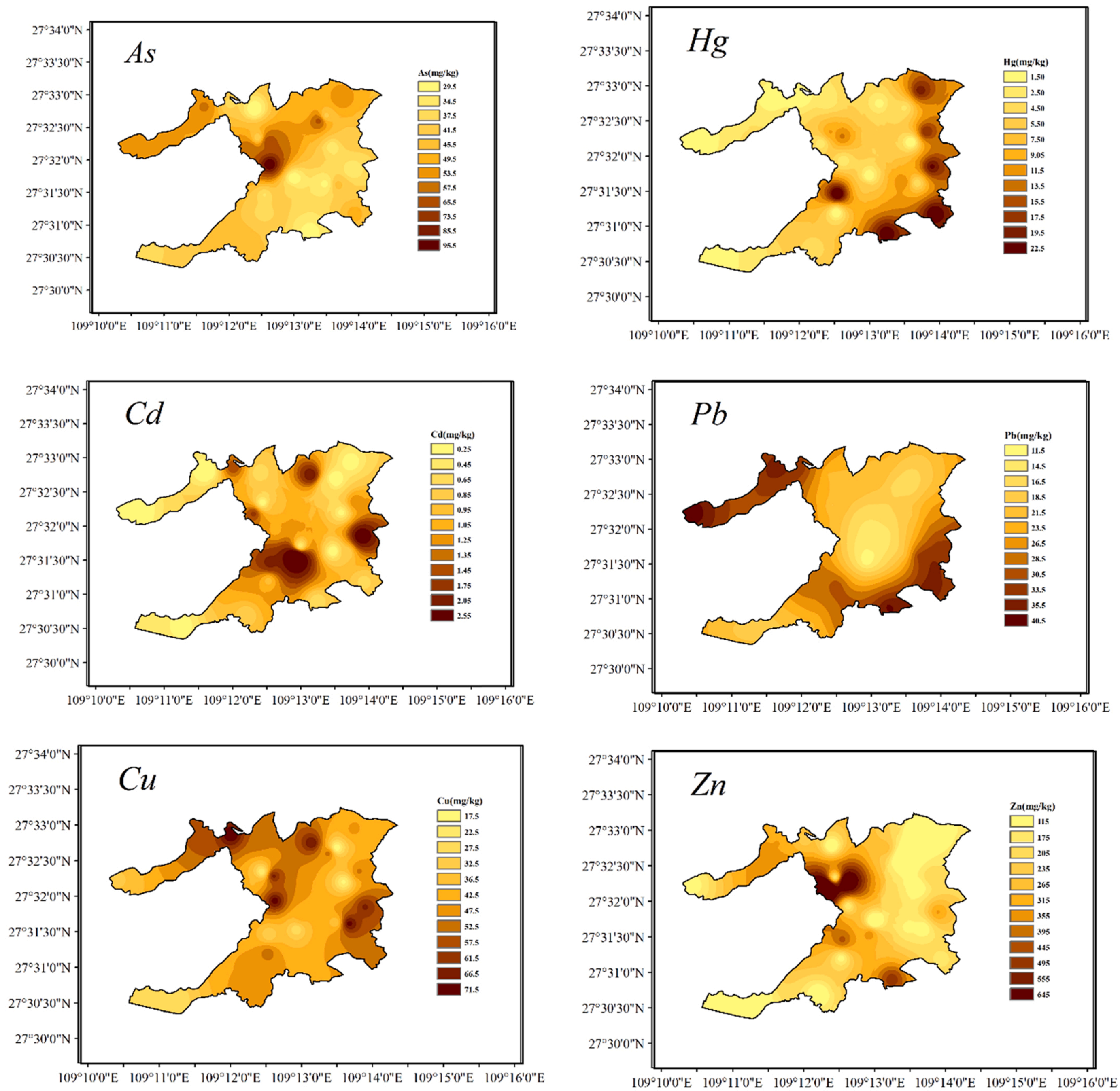

Fig. 2. Heavy metal distribution of the soil in the Wanshan area (mean, n = 5).

contents were found in the eastern and southeastern parts of the study area, including near the MZ and WK, upstream of the WW and ZJW. As and Cu accumulated to a lesser extent in the northern part of the study area, and Zn was primarily concentrated near the road in northern Wanshan.

3.2. Evaluation of heavy metal pollution and speciation in soil around the Wanshan mercury mine

The PLI was determined for each heavy metal in the present study (Fig. 3). The PLI index values of Pb, Cd, Hg, As, Cu and Zn were 0.25–1.18, 0.23–5.44, 0.40–221.44, 1.32–4.88, 0.48–2.3 and 0.69–11.58, indicating no-to-moderate pollution, very strong pollution, very strong pollution, moderate-to-strong pollution, moderate pollution and moderate pollution, respectively. The PLI values in the study area ranged from 0.79 to 4.39, with an average of 2.35. Overall, the distribution of the PLI values was low in the west and high in the east. The northwestern (AZ), northern (MZ), and southwestern parts of the study area and the area surrounding EK had no to moderate pollution; heavy pollution was found in the cities and towns in the center of the study area, north of Wanshan, near WK (to the east), and downstream of the WW; and southwestern and southern ZJW had severe pollution.

The assessment of the total amount of an element may render an inaccurate risk evaluation since the content of a certain element in a specific mining area is often high. Therefore, in this study, the non-residual state of Hg was evaluated (Fig. S1). The assessment of the potential risk of single heavy metals showed that the PERI values of Pb were 1.27–5.90 (average value: 3.60), indicating a slight risk. The Cd

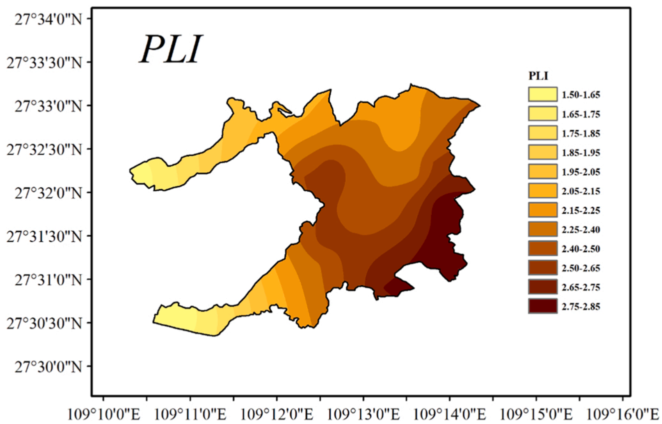

Fig. 3. Distribution of PLI in Wanshan area (mean, n = 5).

content in the soils posed a moderate risk, with PERI values from 6.88 to 163.31 (average value: 40.29). There was a very high risk from the nonresidual Hg in the soil; the PERI values for this element were between 6.95 and 1869.2, with an average value of 425.55. The PERI values of As in the soil were from 13.22 to 48.81 (average value: 22.59), indicating a slight risk. The PERI values of Cu and Zn in the soil were 2.42–11.51 (average value: 7.10) and 0.69–11.58 (average value: 2.57), respectively, representing a slight risk. Overall, the RI values of the heavy metals ranged from 56.70 to 1961.12, with an average value of 528.70, indicating a relatively high risk. From the perspective of $E_{i\ r}$, the proportion contributed by Hg accounted for the majority (67.62%) of the PERI values, followed by Cd and As, which accounted for 15.75% and 10.75%, respectively (Fig. S1).

Fig. S2 shows the speciation of heavy metals based on an improvement of the Tessier sequence extraction procedure, which was applied to explore the characteristics of heavy metal migration and transformation. The residual states of As and Cu in the soil collected from the study area accounted for a relatively high proportion of the elements, with average proportions of 98.20% and 92.58%, respectively. The contents of these heavy metals may be derived mainly from the geological background and are relatively stable. Non-residual states accounted for a significantly higher proportion of Pb, Cd and Zn than of As and Cu. Among the non-residual states, the average proportion of iron-manganese oxide-bound Pb was 40.26%, and the highest proportion of this state was 69.92%. Among the nonresidual states of Cd in soil, the average proportion of iron-manganese oxide-bound Pb was 27.83%, and the highest percentage of this state was 51.70%. As a relatively stable heavy metal, Zn was found mainly in residual states (average proportion: 87.17%). Additionally, the average proportion of iron-manganese oxide-bound Zn was 9.39%, and the highest proportion of this state was 32.45%. The major form of these three heavy metals is the residual state, followed by the iron-manganese oxide-bound state, indicating that there is a certain degree of correlation among Cd, Pb, and Zn. The large amount of mining for Hg, the main mineral in the study area, has caused damage to the surrounding environment. Mercury was mainly found in a residual state, with an average proportion of 71.09%. The average and maximum percentages of the organic matter-bound state of Hg were 24.82% and 84.04%, respectively. Interestingly, the proportion of non-residual Hg to the total Hg content was cultivated land > forestland > mercury mining area.

3.3. Relationship with community structure and bacterial function

The differences in the Sobs, Chao and Shannon indexes of the different soil samples at a classification level of 97% were not significant (Table 2). The Chao index can reflect the richness of a community, and the value of this index was highest in the cultivated land and lowest in the mercury mining area (cultivated land > forestland > mercury mining area). The Shannon index, which is an indicator of species richness and evenness, was highest in the woodlands. The Simpson index measures the dominance of species in a community, and its value was similar among the three regions. Coverage reflects whether sequencing results accurately represent the conditions in the sampling area. The higher the coverage value is, the lower the probability that a sequence in the sample is not detected; therefore, the coverage values in this study demonstrate a relatively high representativeness of the sampling areas. The higher the ACE index is, the higher the richness of the community; thus, the cultivated land had higher community richness than the mercury mining area and woodland.

Taxonomic analysis was performed on representative sequences of OTUs, which were established based on a similarity level of 97% (Fig. 4). A total of 33 phyla were found in this study, among which Proteobacteria, Acidobacteria, Chloroflexi and Actinobacteria were dominant (relative abundances > 1%) in the three regions. Proteobacteria and Actinobacteria had the highest relative abundances in the three regions. To further analyze the bacterial community structure, LEfSe of 535 genera with relative abundances greater than 1% was performed. Fig. S3 shows only the indicator species meeting the significance threshold of the linear discriminant analysis (LDA) (i.e., those with values greater than 3.0). According to LEfSe, there were 21 different indicator species in the four regions analyzed, including 13 species far from the mercury mining area (control), 2 species in the mercury mining area, 5 species in the woodland and 1 species in the cultivated land (Fig. S3). The indicator species in the control area belonged mainly to the phylum Bacteroidia and classes Bacteroidia and Chitinophagales. Additionally, f_67–14, Reyranellales and Reyranellaceae, and Intrasporangiaceae

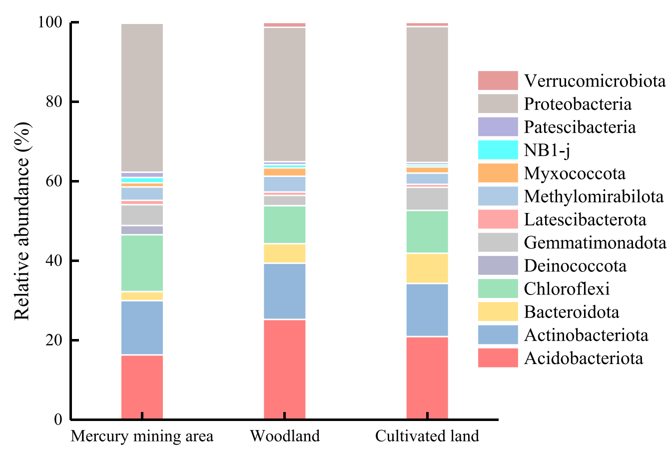

Fig. 4. The relative abundance of bacterial communities in mercury mining area, woodland and cultivated land at the phylum level (mean, n = 3).

Table 2
Community abundance and diversity index of different areas.

Different areas	ACE	Chao	Coverage	Shannon	Simpson
Mercury mining area	2598.47 ± 417.28[a]	2573.59 ± 365.93[a]	0.93 ± 0.04[a]	9.62 ± 0.56[a]	0.20 ± 0.08[a]
Woodland	3182.31 ± 825.33[b]	3218.76 ± 858.47[a]	0.96 ± 0.02[a]	10.02 ± 0.54[a]	0.20 ± 0.06[a]
Cultivated land	4080.2 ± 563.39[a]	4120.02 ± 619.75[b]	0.95 ± 0.04[a]	10.53 ± 0.30[a]	0.20 ± 0.08[a]

Note: Mean ± SD, n = 3. Different superscript letters in each row represent significant differences between different treatments (ANOVA, $P < 0.05$).

were the indicator species in the mercury mining area, woodland and cultivated land. A co-occurrence network was employed to visually display the relationships between soil bacterial genera and functional enzymes in the mercury mining area (Fig. 5). The modularization coefficient of the developed model was 1.38, the average clustering coefficient was 0.026, and the average path length as 5.168. The functions of the nodes indicated that the co-occurrence network had six functional modules, which contained the majority of the nodes in the whole network.

4. Discussion

4.1. Distribution characteristics of heavy metal contents in Wanshan

The spatial distribution analysis showed that the higher Pb contents were concentrated mainly near the northwestern and southeastern parts of the AZ and ZJW, respectively, and that the Pb contents around Wanshan were low. Lead pollution generally comes from urban automobile exhaust and industrial emissions. Lead contents may be relatively low around towns because of terrain and wind direction (Weissenstein et al., 2011; Sharma et al., 2008). The towns of Wanshan district are located in the valleys between the mountains to the north and south, and upward winds form readily during the day. Lead was transported to the north and south of Wanshan district during the day by the smoke and dust produced by the smelting process, and the Pb then settled on the soil surface at night because of the gradual decrease in temperature. The smelters in Wanshan district were originally concentrated in cities and towns. The pollutants produced by smelting activities were typically discharged into the natural environment without any treatment. These pollutants were not treated after the mining areas were closed, and the continuous accumulation of strata may have caused the Pb content in the topsoil to be lower than that in the subsoil. This unique

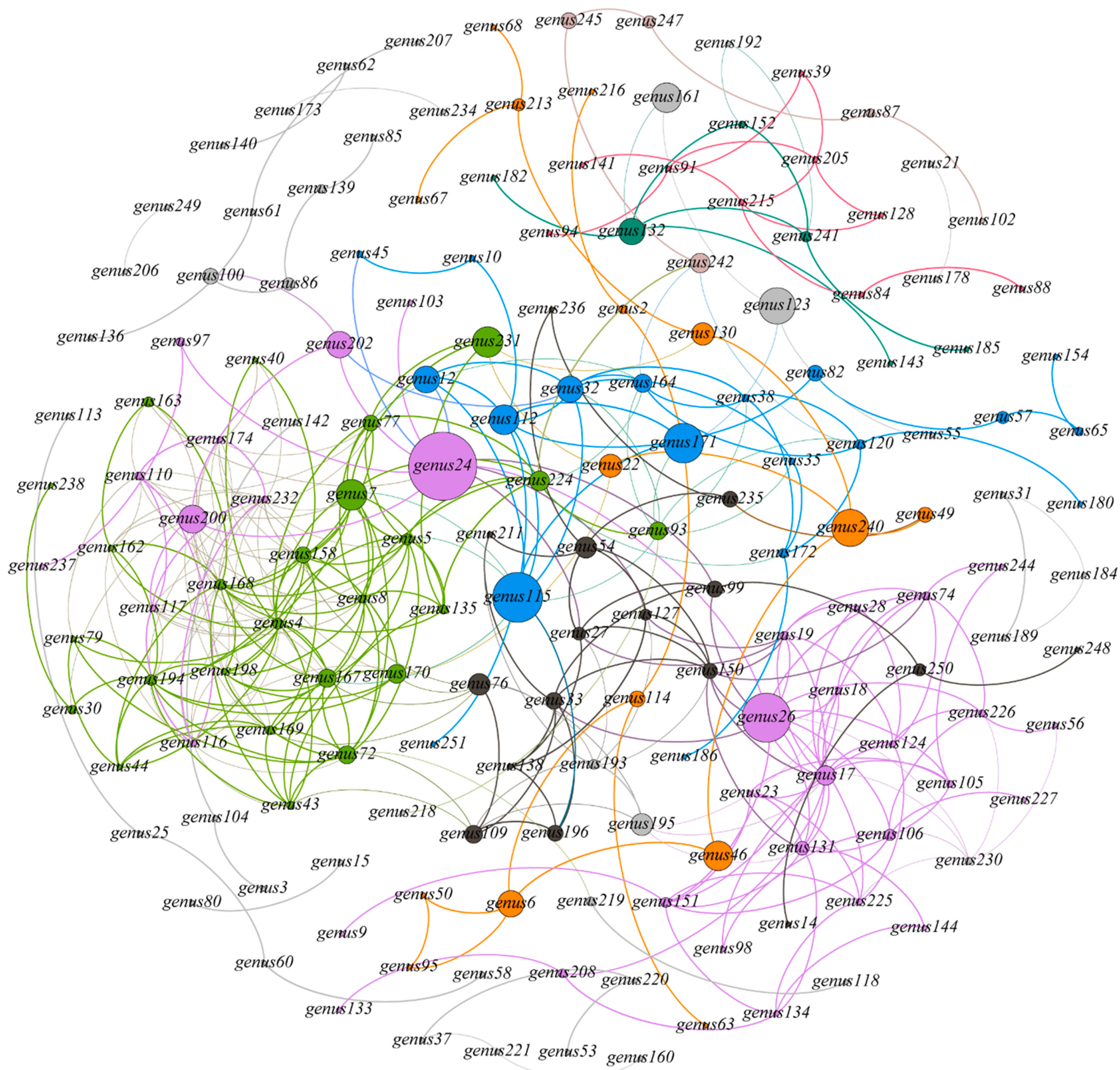

Fig. 5. Co-occurrence network analysis showed the correlations among species of bacterial genera at the taxonomic level in soils of Hg mining area. The edge only showed the Spearman correlation of strength ($|r| > 0.8$) and significance ($p < 0.05$) (mean, $n = 3$); the size of each node was proportional to the number of connections (degree); the thickness of edge between two nodes was proportional to the value of Spearman correlation coefficient, ranging from $|0.8|$ to $|1|$; and the symbiotic network was colored by modularization.

distribution pattern has also been reported for Pb in previous studies (Olobatoke et al., 2016; Liu et al., 2014a, 2014b). The higher Zn contents were mainly concentrated along the northern part of the highway (close to the MZ), which was the main slag storage site in the past. The upstream and downstream regions of the MZ and WW had relatively high Cd contents. There were significant accumulations of Hg in the eastern part of the study area, near the MZ, WK and southern ZJW. The slag left in large pits near WK and the MZ still had high Hg contents. Studies have shown that the slag formed from ore after smelting is a soluble mercury-rich secondary mineral. In the Wanshan area, which belongs to the equatorial rainy zone, the annual precipitation is 1300 mm and there is sufficient moisture content in the air. Following long-term surface runoff and rainwater leaching, mercury was shown to continue to expand into the surrounding environment and organisms, causing mercury pollution (Rytuba et al., 2003).

Through the linear regression, the relationship between the weight of each heavy metal and the environmental factors pH and SOM was explored. As shown in Fig. S4, mercury had a significant relationship with SOM. The influence of SOM on the distribution of Hg contents has been reported in many studies. An increase in SOM content will cause a reduction in redox potential, which is conducive to the transition of heavy metals to the residual state. Soil colloids and SOM have a strong adsorption capacity for Hg and are prone to producing chelated compounds (Zhang et al., 2013). Previous studies have shown that a significant relationship between soil Hg and SOM can indicate the possibility of Hg having a natural source; however, high SOM contents often exists in soils with a small particle size (Armid et al., 2014), and heavy metals of human origin are generally concentrated in soils composed of fine particles, not coarse particles (Idris et al., 2008). Therefore, on the basis of the existing research, the present study speculated that the soil Hg in contaminated area came mainly from mining activities and that the remaining slag and pits were not effectively treated after mining and smelting, which leads to the release and diffusion of Hg. Second, Zinc had a significant relationship with pH, indicating that an increase in pH may increase the total amount of Zn, which is similar to the results of previous studies on heavy metals in mines (Wang et al., 2006).

4.2. Risk assessment of heavy metals in Wanshan

The PLI value in the study area was 0.79–4.39, with an average of 2.35 (Fig. 4). Overall, lower and higher PLI values were observed in the western and eastern parts of the study area, respectively. No to moderate pollution was found in the AZ (northwest), MZ (north), and EK (southwest). The central and northern parts of the town and the WK (east) and downstream area of the WW Rivers were polluted. The southwestern part of the study area and ZJW (south) were severely polluted. The MZ, EK and WK were located on the abandoned lands of the original mining area, and the level of pollution in the MZ and EK has decreased compared with previous reports by Hu et al. (2015) and Zhan et al. (2017). Interestingly, the soil in ZJW, a village in town, was severely polluted by heavy metals. Zhou et al. (2018) reported that heavy metal pollution in the soil in the Hg mining area has been significantly improved due to the treatment of this area by relevant departments; however, the pollution of nonmercury mining areas does not seem to have been ameliorated (sampling was performed in July 2016). The higher risk in ZJW was probably due to geological factors, transportation of mercury ore and atmospheric deposition. Dai et al. (2012) reported that dry deposition played a dominant role in total atmospheric Hg deposition in the study area, since the dry deposition fluxes were 10.4–37.9 times higher than the wet deposition fluxes during the whole sample period.

The PERI considers the toxic effects of metals and their measured concentrations in sediment. The contributions of the heavy metals to the PERI was as follows: Hg (67.62%), Cd (15.75%) and As (10.75%). This pattern was consistent with the results of previous studies. Gou and Ruan (2020) demonstrated that the average RI of heavy metals in the soil was 10655.70, indicating a very strong potential ecological risk. Mercury posed the majority of the potential ecological risk. Copper, Ni and Cr in soil are derived mainly from natural activities. Arsenic, Pb and Zn are mainly from coal combustion and transportation pollution. Cadmium is mainly from agricultural pollution; and Hg is mainly from mercury smelting pollution. Li et al. (2015) also found that Hg and Cd were the most abundant trace metals in the Wanshan mining area. Obviously, Hg pollution was mainly from anthropogenic and geological factors, the former of which primarily included the transportation and exploitation of mercury ore and atmospheric deposition. Unlike Hg, the Cd content in the crust in China is low, generally 0.097 mg/kg. Guizhou Province had the highest background value of Cd in soil, mainly because the terrain (typical karst, with the most carbonate in the area) and hydrothermal conditions were different from those in other places. In addition, in this mercury mining area, the main sources of soil Cd pollution were several anthropogenic factors, including mining, mineral processing, nonferrous metal smelting, and electroplating. Moreover, impractical application of pesticides and chemical fertilizers and atmospheric deposition are important pathways by which exogenous Cd enters the agricultural soil in this study area. The background As in the soil comes mainly from soil-forming parent material, and its concentration and distribution are determined by the environmental factors of the soil-forming process. In addition to geological factors, the exploitation of mercury ore is an important contributor to arsenic pollution in the soil of the study area.

Generally, the heavy metals speciation can more accurately reflect practical pollution characteristics and hazards. In the present study, the concentrations of residual As, Cu and Zn were higher than those of the other forms of these heavy metals. The reason for this finding may be that these three heavy metals come mainly from geological sources and are not affected by environmental changes. Additionally, the proportions of non-residual Pb, Cd and Hg were higher than those of the residual states of these heavy metals. The proportion of iron-manganese oxide-bound heavy metals in the study area was lower than that of their residual states only. Iron-manganese oxide-bound Pb and Cd easily form hydroxide precipitates given sufficient oxygen; however, they can be released under hypoxic conditions to cause secondary pollution (Qiao et al., 2013; Li et al., 2016). Therefore, according to the proportions and states of local heavy metals, appropriate soil cover and water isolation may have a positive effect. Among the nonresidual Hg, organic matter-bound Hg had the highest proportion. This finding was similar to the results of previous studies (Gosar et al., 2006; Higueras et al., 2003; Biester et al., 1996; Navarro et al., 2006).

4.3. Microbial community composition in the study area

The different vegetation structures and litter fall affect the composition and quantity of soil microorganisms. The higher the values of the Chao and ACE indexes were, the higher the number of OTUs in the community, suggesting greater community richness. Thus, the cultivated land had higher community richness than the mercury mining area and woodland. The reason for this finding may be that fertilization and cultivation enhanced the microbial activity in the cultivated soil, as suggested by Wen et al. (2018). Concretely, cultivated land has high nutritional value due to the addition of a variety of fertilizers for plant growth, and these nutrients may improve the soil microbial biomass. On the other hand, the cultivated land has better aeration, light, and WHC that can also facilitate microbial growth. Unfortunately, the higher concentration of heavy metals in the soil can greatly affect the microbial diversity (Natasha et al., 2020). The Shannon index is related to the richness and evenness of the community, the higher the index value, the higher the diversity of the community. Indeed, Hg pollution in soil can significantly affect the structure and function of the microbial community and thus reduce the abundance of bacteria; however, it can also increase the diversity of bacteria. Moreover, the LEfSe showed that the phylum Bacteroidia was the main indicator species in the control. It has been reported that bacteria are relatively sensitive to pH value, especially in acidic soil, which can support Proteus, Bacteroides, etc.; this is one of the reasons for the dominant phyla in the control. In addition, Liu et al. (2018) found that mercury was positively correlated with the abundance of Firmicutes and Bacteroides, while methylmercury was positively correlated with the abundance of nitrifying Helicobacter. Yun et al. (2016) demonstrated that the concentrations of mercury and methylmercury in paddy soil were negatively correlated with the relative abundance of nitrifying spirochetes but positively correlated with the relative abundance of δ-Proteus, actinomycetes, Bacteroidetes and Firmicutes. The f_67–14, as belonging to a family of Solirubrobacterales, was the main indicator species in the mercury mining area, which is located in a karst region. Yun et al. (2016) also reported that Solirubrobacterales were relatively abundant in weathered carbonate rocks, which is consistent with the findings of the present study.

Mercury was mainly found in a residual state, with an average proportion of 71.09%. Interestingly, the proportion of non- residual Hg to total Hg contents was cultivated land > forestland > mercury mining area. In the oxidizing environment, Hg can be oxidized to Hg^{2+} with the participation of Hg resistant bacteria. Mercuric sulfide may be formed when there is a certain amount of S^{2-} in soil solution. Mercuric sulfide is stable under anaerobic conditions, but when there is a large amount of S^{2-}, a soluble HgS_{2-2} will be formed in the solution (Birane et al., 2019). Mercuric sulfide can also be converted into Hg^{2+} under the action of some special biological enzymes in the oxidizing environment (Natasha et al., 2020). Long term cultivation will acidify the soil, accelerate the soil impoverishment, activate the harmful heavy metals in the soil, and increase the number of harmful microorganisms (Ochmian et al., 2020). Soil acidify may render the microbial Hg methylation by affecting microbial community composition and Hg availability (Natasha et al., 2020). It is generally believed that acidic conditions (possibly including neutral conditions) are conducive to the formation of CH_3Hg, and soil acidification caused by agricultural activities (such as fertilization) may increase the non- residual Hg contents in agricultural soil (Tang et al., 2020).

Proteobacteria is one of the most abundant bacteria in the world. Wang et al. (2020) reported that Proteobacteria tolerated mercury contamination, while at the phylum level, Acidobacteria, Planctomycetes and Chloroflexi were sensitive to mercury pollution. Omnitrophica and Ignavibacteriae microorganisms were very sensitive to mercury contamination and died quickly when exposed to mercury. Wang (2017) reported that at the phylum level, mercury and methylmercury pollution increased the relative abundances of Proteobacteria, Acidobacteria, Planctomycetes and Chloroflexi, which were sensitive to mercury pollution, while Acidobacteria, Planctomycetes and Nitrospirae were sensitive to methylmercury pollution. Ji et al. (2018) showed that microbial diversity and community composition varied among sites and that at varying depths, soil microbes were significantly affected by soil environmental factors such as pH and the contents of Pb, Hg, and total organic carbon. Lead and Hg negatively affected soil microbial diversity, and less polluted soil showed increased microbial diversity and a complex community structure. The community composition analysis showed that Firmicutes, Proteobacteria and Actinobacteria were the dominant microorganisms. Mercury reductase can reduce Hg(II) to Hg(0). Reduced Hg(0) easily enters the atmosphere and global Hg cycle since it has low solubility and volatilization. Currently, the reported Hg-reducing microorganisms include mainly mercury-resistant prokaryotes and several mercury-sensitive bacteria. Hg-tolerant prokaryotes include aerobic heterotrophic bacteria (Proteus and gram-positive bacteria) and archaea, which have high species diversity and are widespread in the environment, especially in ecosystems with high Hg contents. This was the main reason why Proteobacteria was the most abundant bacteria in the present study. According to prediction of the co-occurrence network, genus 24, genus 26, genus 115 and genus 240, corresponding to g_Luteitalea, g_Vicinamibacteraceae, g_JG30-KF-CM45, and g_Lysobacter, respectively, were relatively important in the developed model. The model also supported the finding that Proteobacteria, Acidobacteria, Chloroflexi and Actinobacteria were the dominant phyla (with relative abundances > 1%) shared by the three regions. The accumulation of heavy metals in soil will inevitably damage the structure and activity of the existing soil microbial community, eventually reducing soil fertility and quality. Soil microbial population structure is an important parameter to characterize the community structure and stability of soil ecosystems and can reflect the degree of heavy metal pollution.

5. Conclusion

The PLI values of Pb, Cd, Hg, As, Cu and Zn indicated no-to-moderate, very strong, very strong, moderate-to-strong, moderate and moderate pollution, respectively. Hg contributed the most to the PERI values, reaching 67.62%, followed by Cd and As, with contributions of 15.75% and 10.75%, respectively. Hg was found mainly in a residual state, which accounted for an average proportion of 71.09%. The average and maximum percentages of the organic matter-bound state of Hg were 24.82% and 84.04%, respectively. The cultivated land had higher community richness than the mercury mining area and woodland. According to linear discriminant analysis effect size, the indicator species in the Hg mining area, woodland and cultivated land was f_67–14 (belonging to a family of Solirubrobacterales), Reyranellales and Reyranellaceae, Intrasporangiaceae, respectively. In summary, this study for the very first time estimated that the higher Hg, Cd and As pollution existed in Wanshan Hg mine since their concentration in the all soil samples totally exceeded the standard value (GB15618-2018), while Cd and As pollution in soil was commonly ignored by the previous study. Our results suggested that the relevant local departments need to take more active measures to solve the problem of excessive Hg, Cd and As in local soil and prevent the possible impacts of the pollution on human beings. In addition, in order to prevent and control the mercury pollution, the next research should further explore the effects of different soil environments on the transformation of chemical forms of mercury and take effective measures to intervene and control the migration and transformation of mercury in soil. In conclusion, this is a preliminary study to prepare for the next step of screening Hg-resistant strains to reduce the bioavailability of Hg and repair the Hg, Cd and As pollution.

Funding

This work was financially supported by the Guizhou science and technology support plan project (No. [2020]1Y178), [2019]1217, [2017]1176); Opening fund of the State Key Laboratory of Environmental Geochemistry (SKLEG2021202); the basic condition platform construction project of Guizhou Science and Technology Department (No. [2019] 5701); the Department of Education of Guizhou Province (No. KY[2021]302); the Strategic Pilot Science and Technology (Class A, XDA23040303); the National Natural Science Foundation of China (41601537); the Key Research Program of Frontier Sciences, CAS (QYZDJSSWDQC008); the National Key Research and Development Program of China (2017YFC0404701), and Chinese Government Scholarship Fund (CSC NO. 201904910058).

CRediT authorship contribution statement

X.H., X. W. Z. Z. and J. M. designed the research; X.H., X.W., Z.Z., J. M. and X. T. wrote the paper and performed the experiments; J. Z. and H. L. performed the data analysis.

Compliance with ethical standards

Ethical approval and consent to participate

This article does not contain any studies with human participants or animals.

Consent to publish

Not applicable.

Declaration of Competing Interest

We declare that the manuscript has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The present study data are available from the corresponding author on reasonable request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112897.

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