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Original Research

Levels, Spatial Distribution, and Sources of Heavy Metals in Karst Caohai Wetlands and Their Impact on Bacterial Community Diversity and Symbiotic Patterns, China

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Abstract

Karst wetlands play a crucial role in heavy metal transport and biogeochemical cycles. This study analyzed heavy metal contents and morphological distribution in sediment samples from Caohai wetlands, Guizhou Province, and investigated bacterial community composition and its interaction with heavy metals using 16S rRNA sequencing. Results showed severe heavy metal pollution in Caohai wetlands, with high bacterial diversity. Bacterial community structure significantly correlated with heavy metal content. The microbial covariance network demonstrated more coexistence than exclusion among bacterial taxa. This study revealed heavy metal pollution characteristics in Caohai wetland sediments and provided a theoretical basis for ecological risk assessment and microbial remediation of heavy metal contamination in karst wetlands.

Keywords: Caohai lakes, heavy metal patterns, sediment, microbial co-occurrence network

Introduction

Karst landscapes are widely distributed in the south of China [1]. It is a unique topography formed mainly by erosion, accumulation, dissolution, and deposition of surface water [2]. China's Guizhou, the core of East Asian karst, is one of the world's three largest karst regions [3]. Karst wetlands are a special, complex, and fragile class of wetland types in this terrain. As a result of dissolution, rocks in karst areas form irregular cracks, caves, and channels, and the network structures they form [4]. Therefore, there is unevenness and diversity in the flow and storage of water bodies in karst wetlands. Because of the connectivity and high permeability of karst space, water bodies can be transported to various places by channels, so karst aquifers are more susceptible to pollution than non-karst aquifers [5]. It

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leads to the shrinkage or disappearance of karst wetlands and irreversible harm to the water environment.

With the rapid development of the economy comes a variety of environmental problems. Water pollution is a problem that all countries in the world are facing and needs to be solved urgently [6]. Heavy metals, in particular, have become a serious pollution problem due to their nonbiodegradability, stability, and persistence [7, 8]. Heavy metals are widespread in the environment and can be toxic and harmful to organisms above a certain concentration range, and some heavy metals in high concentrations can interact with biological enzymes to adversely affect cellular function [9]. Heavy metals also pose a potential threat to human health through the food chain [10]. Concentrations of heavy metals can be exacerbated by human activities such as urban discharges and industrial and agricultural processes, thereby exacerbating the hazards to the aquatic environment [11]. Heavy metals that enter the water column can be present in sediments [12, 13]. Sediments can often be thought of as sinks for heavy metals, and if environmental conditions change, heavy metals stored in sediments may be released, causing secondary pollution of water bodies [14], so sediments are, at the same time, a potential source of heavy metals. Heavy metal pollution is now becoming more and more serious [15]. Karst aquifers have karst pipes and fissures, which can lead to a closer connection between surface water and groundwater, which may affect the precipitation of dissolved heavy metals and the dissolution of heavy metal-containing particles [16]. The remediation of karst aquifers contaminated with heavy metals is difficult and takes a long time to recover, and many articles have reported on the problems related to heavy metal contamination of water bodies in karst areas [2, 11, 17]. However, these articles mainly focus on issues such as the health risks and ecological risks of heavy metals and less on the effects of heavy metals in water sediments on microbial communities and symbiotic patterns.

Microorganisms are important components of water ecosystems and are involved in many ecological processes [18]. They participate in biogeochemical cycles [19], have an important role in food web structure [20], and act as alternatives to primary production [21] and as indicators of ecosystem health [22]. It has been shown that certain microbial communities are suited to survive in nutrient-poor karst environments [23]. In karst environments, microorganisms are highly phylogenetically diverse, and iron oxidation is associated with increased microbial diversity [24]. Compared to other organisms, microorganisms are able to rapidly resist environmental stresses through mutation and evolution when faced with them [15]. Changes in the environment affect the microbial community structure and influence the material cycling process, and different species of microorganisms respond differently to different environmental stresses. When the water body is polluted by heavy metals, the microorganisms in the water body sediments are also seriously affected, and the community composition of microorganisms changes. After being polluted by heavy metal elements, the microbial community in the water body sediments will undergo obvious changes, and its ecological function will also be affected. Studies have shown that microorganisms have different response mechanisms in the face of heavy metal stress; for example, certain microorganisms can metabolize heavy metals into less toxic or inactive forms [25, 26], or in the form of ion exchange [27], or by intracellular accumulation [28]. Since microorganisms are very sensitive to environmental changes, the study of the interaction between heavy metal elements and microorganisms in water sediments is of great significance for a deeper understanding of the environmental quality and ecological safety of water bodies. Many scholars have also carried out studies on the effects of heavy metals on microorganisms in water body sediments: It has been found that new dominant colonies emerge under heavy metal stress, and the number of newly emerged dominant colonies can offset the reduced colonies so that the size of the whole bacterial community remains relatively stable [29]. If, under heavy metal stress, the number of dominant colonies increases more rapidly than the number of other colonies decreases, then, taken as a whole, the entire bacterial community can even remain on the rise. Academics Li et al., who studied the remediation of sites contaminated with heavy metals using microorganisms, found that some microorganisms develop symbiotic relationships when contaminated to increase their resistance in the face of heavy metal contamination [30]. In a survey of microorganisms in lake sediments, Custodio found that *Proteobacteria* and *Actinobacteria* were the dominant flora in the area and that the bacteria were prevalent in sediments high in the heavy metals Cd and As [31]. Yang found that through human interference and the addition of exogenous microorganisms, indigenous microorganisms and exogenous microorganisms established a synergistic relationship to counteract the adverse effects of heavy metals and the environmental changes caused by human interference, and that the contaminated environment would gradually recover over time, and the relationship between the indigenous microorganisms and the exogenous microorganisms would become stronger and stronger, thus facilitating the removal of heavy metals [32]. For the relationship between the effects of heavy metals on microorganisms, it has been concluded that heavy metals inhibit microbial diversity [33]. Some studies have also concluded that heavy metals can promote the abundance and diversity of microorganisms [34]. However, there are fewer studies on microorganisms in karst areas contaminated by heavy metals, and the principles and mechanisms of how heavy metals affect the microbial flora, especially how the communities of microorganisms endemic to karst environments change when they are contaminated by heavy metals, have yet to be investigated.

The Caohai Wetland in Weining, Guizhou, is a typical karst wetland into which a large amount of urban wastewater was discharged every day until 2017 [35]. The Caohai Wetlands used to be a distribution center for old-fashioned zinc smelting [36]. A large amount of heavy metal pollution has been generated, and some studies have explored the response mechanism of microorganisms in the face of heavy metal stress [37-39]. However, studies have mainly focused on the resistance mechanisms of microorganisms to heavy metals, while the interactions of microbial communities on heavy metals in karst wetland sediments have been less studied. In this study, we sampled the Caohai Wetland in Guizhou and analyzed the morphological and distributional characteristics of heavy metals, the community composition of microorganisms, α-diversity analysis, and co-occurring network diagrams with RDA analysis and thermograms. The objectives of this study were to (1) investigate the distribution and causes of total heavy metals and their forms in the surface sediments of typical plateau lakes on the Yunnan-Guizhou Plateau, (2) reveal the characteristics of bacterial communities in karstic environments, (3) explore the interaction relationship between bacterial communities, and (4) explore the interactions between heavy metals and bacteria and their mechanisms.

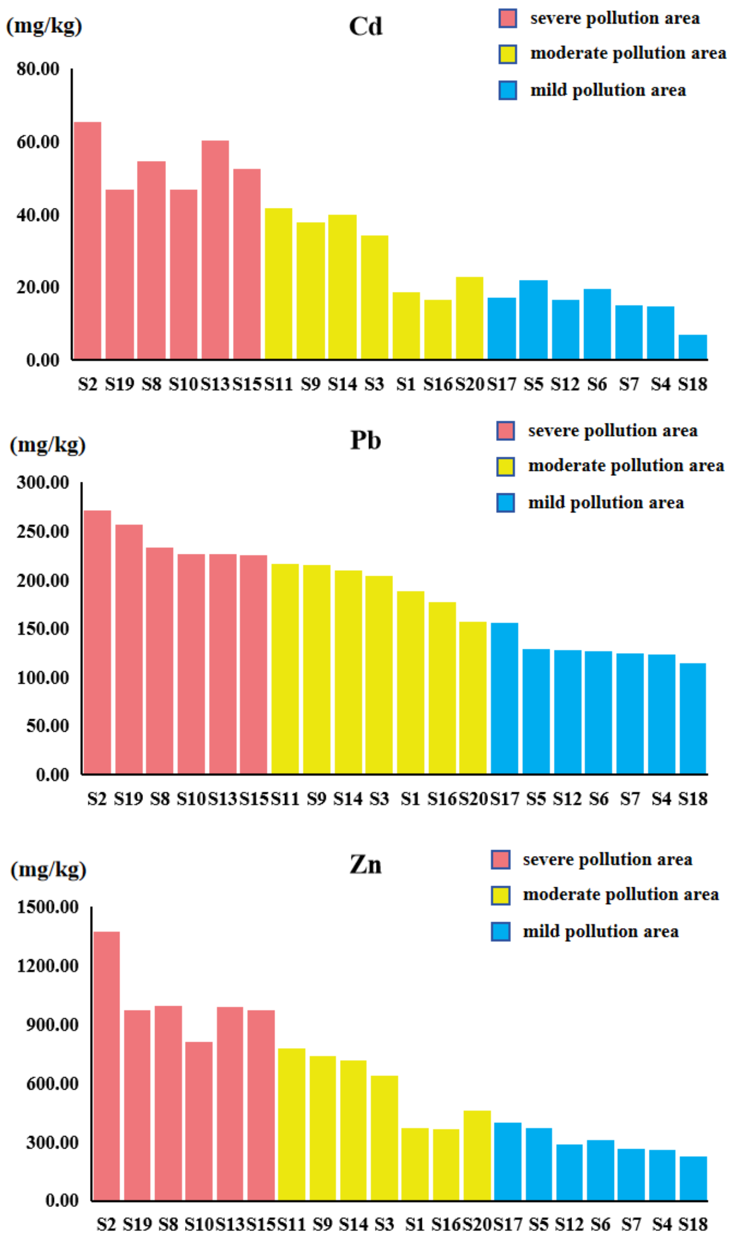


Fig. 1. Metal content in A, B, and C regions (mg/kg).

Materials and Methods

Overview of Study Area

Guizhou Province is located in the centre of the Southeast Asian karst region, the largest in the world [40]. Caohai is the largest natural freshwater lake and typical plateau wetland ecosystem in Guizhou, with its extensive area and rich species. Weining Caohai is located in the western flank of the Qianxi Mountain Reflection Arc, the top part of the northward bend of the Weining Shuicheng Great Dorsal Slope, with an elevation of 2172 m, a lake area of 25 km^2, an average annual precipitation of 950 mm, an average annual sunshine hours of 1,805 h, and an average annual temperature of 10.5 ℃, which belongs to the subtropical plateau monsoon climate [41]. Weining Caohai is currently a relatively well-preserved plateau wetland ecosystem in China and is also a transit point for the wintering migration of many bird species. It is known as the sapphire in the crown of Guizhou tourism. As the kidneys of the earth, wetlands are an important part of the ecosystem, playing an irreplaceable role in regulating the climate, protecting biodiversity, protecting water resources, etc., and are an important aspect of China's ecological security and sustainable development strategy [42]. The topography of the lake area as a whole shows a gradual decrease from the southeast corner to the northwest corner, and atmospheric precipitation is the main water source of the Caohai Wetland. The Caohai watershed is a famous distribution center for indigenous zinc refining in China, with a very long history [17]. The mining and smelting of zinc ore have led to the transformation of heavy metal elements, such as zinc and cadmium, from the primary phase to the secondary phase, and pollutants around the wetland have entered the water body through surface runoff. Currently, the Caohai Wetland is facing more serious zinc and cadmium pollution.

As shown in Fig. 1, according to the pollution degree of Cd, Pb, and Zn, we divided Caohai wetland into severe pollution area, moderate pollution area, and mild pollution area, which are represented by A, B, and C.

Collection of Samples

A grid distribution method was used to set up 20 sampling points, as shown in Fig. 2, according to the topography of the Caohai wetland. Surface sediments were collected in April 2023 using a grab sampler, and three parallel samples were collected from each sampling point, removing plant roots, stones, and other debris, mixing them to form a composite sample, and sealing and preserving them in self-sealing bags. Soil samples were net air-dried, ground and sent to the laboratory for testing. Another copy was taken in a sterile sampling bag, placed in an insulated box with ice packs, quickly brought back to the laboratory, and freeze-dried and preserved in a -80°C refrigerator for DNA extraction.

Determination of Heavy Metal Content and Morphological Analysis of Sediments

With reference to the microwave digestion method for total soil sediment metals (HJ 832-2017), the total amount of six heavy metals, cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn), was extracted from the sediment samples using 6:5:2 HCl:HF:HNO3 (V/V/V), the morphology of the six heavy metals was extracted using a modified BCR continuous extraction method [43]. Each heavy metal gets four different forms: acid-soluble fraction (F1), reducible fraction (F2), oxidizable fraction (F3), and residual fraction (F4).

The total amount and morphology of heavy metals obtained were determined by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher, USA). The standard substance GBW07428 (GSS-14, Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences) was used for quality control, and the recoveries were between 85% and 105%. The water used in the experiments was ultrapure water, the reagents were all of guaranteed reagent, and the vessels used in the experiments were soaked in 10% nitric acid for more than 24 h. The recoveries were between 85% and 105%.

Bacterial Testing of Sediments

Genomic DNA was extracted from 20 sediment samples using the Power Soil® DNA Extraction Kit (Mo Bio Laboratory). Extracted DNA (A260/A280 ratio) was identified for purity by UV spectrophotometer. The V3-V4 variable region of the 16S rRNA gene was amplified using bacterial-specific primers 338F/860R, targeting the V3-V4 variable region of the 16S rRNA gene. PCR amplification was performed using Q5® High Fidelity DNA Polymerase (New England Biolabs). Amplification products were purified using a gel extraction kit (Axygen USA) and their concentrations were analyzed by quantitative fluorescence analysis using the Quant-iT PicoGreends DNA Detection Kit (Invitrogen). PCR products were separated and purified for high-throughput sequencing using the Illumina MisSeq PE300. The sequencing work was entrusted to Shanghai Meiji Bioinformatics Company, where the raw sequences were pre-processed, and high-quality sequences were selected for analysis. Operational taxonomic units (OTUs) were clustered using UPARSE version 7.1 with a similarity threshold of 97%. Chimeric sequences were identified and screened using USEARCH to obtain OTU representative sequences and used for subsequent taxonomic analyses. The taxonomic classification of each OTU was performed by the Ri bosom Database Project (RDP) classifier. The α-diversity indices, including Chao1, Ace, Shannon, and Simpson, were calculated using the normalized dataset from the Mothur software.

Table 1. Statistics of Heavy Metal Elements in Surface Sediment of Caohai Wetland.

Metal	Maximum (mg/kg)	Minimum (mg/kg)	Means ± SD (mg/kg)	Background value 1) (mg/kg)	Certified value 2) (mg/kg)	Exceeded rate 3) (%)
Pb	271.28	113.74	185.30±49.74	19.91	140	70
Cd	65.25	6.8	32.44±17.66	0.22	0.6	100
Cu	61.47	17.58	31.07±9.03	33.7	100	0
Zn	1,373.50	223.25	614.45±329.44	88.88	250	95
Fe	46,167.75	13,309.50	32,008.49±9,525.04	38,700	—	—
Mn	1176.5	383.5	664.01±197.52	668.7	—	—

Note: 1) refers to the average background value of soil elements in Guizhou Province; 2) is the secondary standard limit of GB 15618-2018 (pH is 6.5-7.5); 3) The proportion of the number of sampling points exceeding the standard value in the total.

Data Analysis

Bacterial interspecies co-occurrence networks were constructed using the ‘WGCNA’ R package based on Spearman’s correlation coefficient, and online R software was used to construct the co-occurrence network structure of bacterial genus communities in each ecological zone. Visualization of co-occurrence networks at the OUT (gate) level using Gephi, co-occurrence networks at the OUT (gate) level were visualized using the Gephi software, and bacterial genera with a relative abundance share of ≥0.1% and occurring in ≥50% of the samples were retained in order to reduce the complexity of the network, with Spearman correlation coefficients of $r \geq 0.6$ and significance of $P<0.05$. Finally, the topological features of the co-occurring networks were calculated using the psych and igraph packages in R as well as the Gephi interactive software platform, and additional plots were drawn using Origin 2022 and R 3.3.3 software; Origin 2022 was used for iconography; the study area and sampling points, contour maps were completed with surfer 23 and ArcGIS 10.8; Canoco 5 software was used for RDA (redundancy analysis) to analyze the effects of heavy metals on the bacterial community.

Table 2. Heavy metals content in different sediments reported by other studies.

River/lake/marine	Heavy metal content (mg/kg)						References
	Cd	Pb	Zn	Cu	Fe	Mn	
Caohai Lake,China	32.44	185.30	614.45	31.07	32008.49	664.01	This study
Haizhou Bay,China	0.11	14.58	67.12	16.18	-	550.08	[106]
Ulleung Basin (East Sea)	0.40	24.00	115.00	29.00	3.84	438.00	[107]
Meghna River, Bangladesh	0.28	12.48	42.41	6.22	1290.00	-	[108]
Hooghly River Estuary and Sundarban,India	0.17	14.27	53.76	33.88	25050.00	517.00	[109]
Jiaozhou Bay, China	0.304	38.54	76.00	27.31	-	-	[110]
Ozomu Lake, Nigeria	0.09	1.14	31.44	14.19	1159.00	20.00	[111]
Zhanjiang Bay,China	0.20	20.07	-	18.24	-	-	[112]
Wanghu Lake,China	0.37	34.11	124.20	59.23	-	-	[113]
Dongping Lake, China	0.23	26.14	68.52	37.28	-	-	[114]
Taihu Lake,China	0.61	29.70	109.32	35.53	-	689.78	[115]

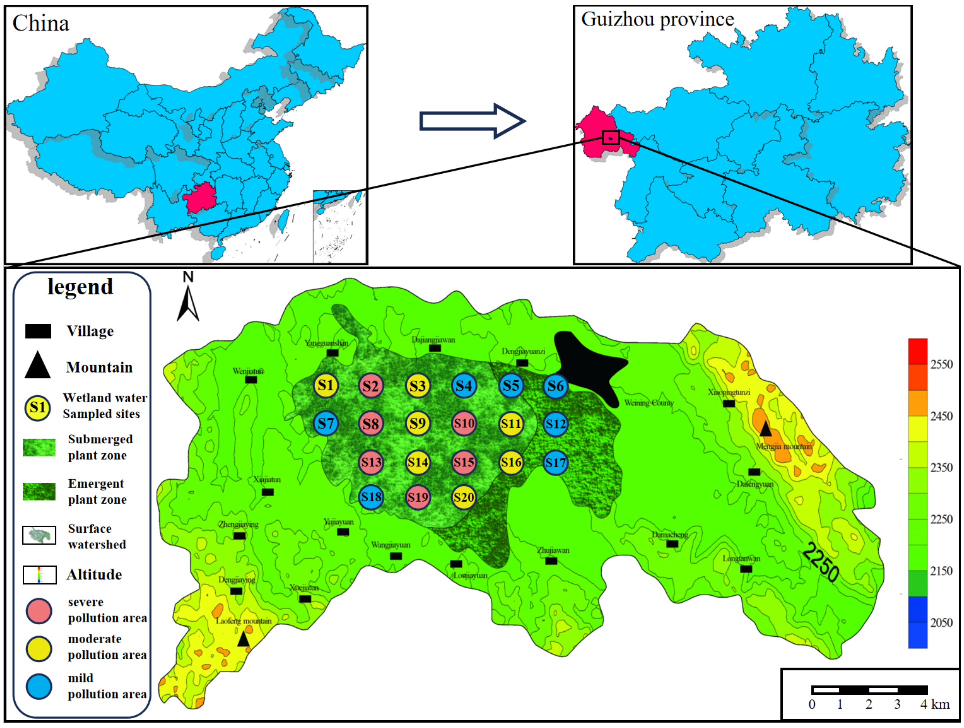


Fig. 2. Study area and sampling points.

Results and Discussion

Characteristics of Heavy Metal Distribution

Characteristics of Total Heavy Metal Distribution

The statistical information of Cd, Zn, and Fe elements in the surface sediments of the Caohai Wetland is shown in Table 1. The distribution of heavy metal element contents in sediments was highly variable, and the mean values of Cd, Zn, Pb, Fe, Cu and Mn were 32.44, 614.45, 185.30, 32,008.49, 31.07 and 664.01 mg/kg, respectively, which were 147.45, 6.91, 9.31, 0.83, 0.92 and 0.99 times, of which the concentrations of Fe, Cu and Mn exceeded the background values in 6, 6 and 11 sampling points, respectively.

In order to understand the severity of heavy metal pollution in this study area, the pollution levels recorded in other areas are provided in Table 2 for comparison, and the data show that the levels of Cd and Zn are much higher than those of the other sites listed in the table, and the levels of Pb are higher than those of most of the sites, indicating that the Caohai Wetland has a higher concentration of Cd, Pb, and Zn, and that this pollution is likely to be closely related to historical zinc smelting in Hezhang area [44]. Referring to the national soil environmental quality standard (GB 15618-2018), the six heavy metals studied, Cd, Zn, and Pb exceeded the secondary standard limits, with exceedance rates of 100%, 95%, and 70%, respectively. Referring to the European Union standard (EU Directive 86/278/EEC), Cd and Zn exceeded the EU standard limits, with exceedance rates of 100% and 75%, respectively, and the results of the study indicate that the Caohai wetland is facing serious heavy metal pollution.

As can be seen from Fig. 3, the high concentration areas of Cd, Pb and Zn were mainly concentrated in the center of the lake, and the changes in the distribution of the concentrations of the three were very similar, with the highest concentration of Cd reaching 65 mg/kg, the highest concentration of Pb reaching 271 mg/kg, and the highest concentration of Zn reaching 1,373 mg/kg; The concentration of Cu had the smallest range, the concentration of Cu reached a maximum of 61 mg/kg in the north-east corner, and the concentration reached 36 mg/kg at two points in the center of the lake and the south-west corner of the lake, and the concentration gradually decreased from these two points to the surroundings; The greatest range of Fe concentrations was found in the mid-northern part of the lake and in the southern part of the lake, where the highest concentration of 46,000 mg/kg was found, and in the southern part of the lake there was a large range of Fe distribution, which was characterized by a gradual decrease towards the north; The distribution of Mn is small, and its concentration reaches a maximum of 1100 mg/kg in the south of the lake, and its distribution is higher in the southeast than in the northwest, and the concentration gradually decreases in both directions from the south position in the lake.

Since the changes in the concentration distributions of Cd, Pb and Zn are very similar, they are likely to have the same source, Presence of lead and zinc deposits in the Caohai watershed, the historical context of the Caohai watershed as a distribution center for

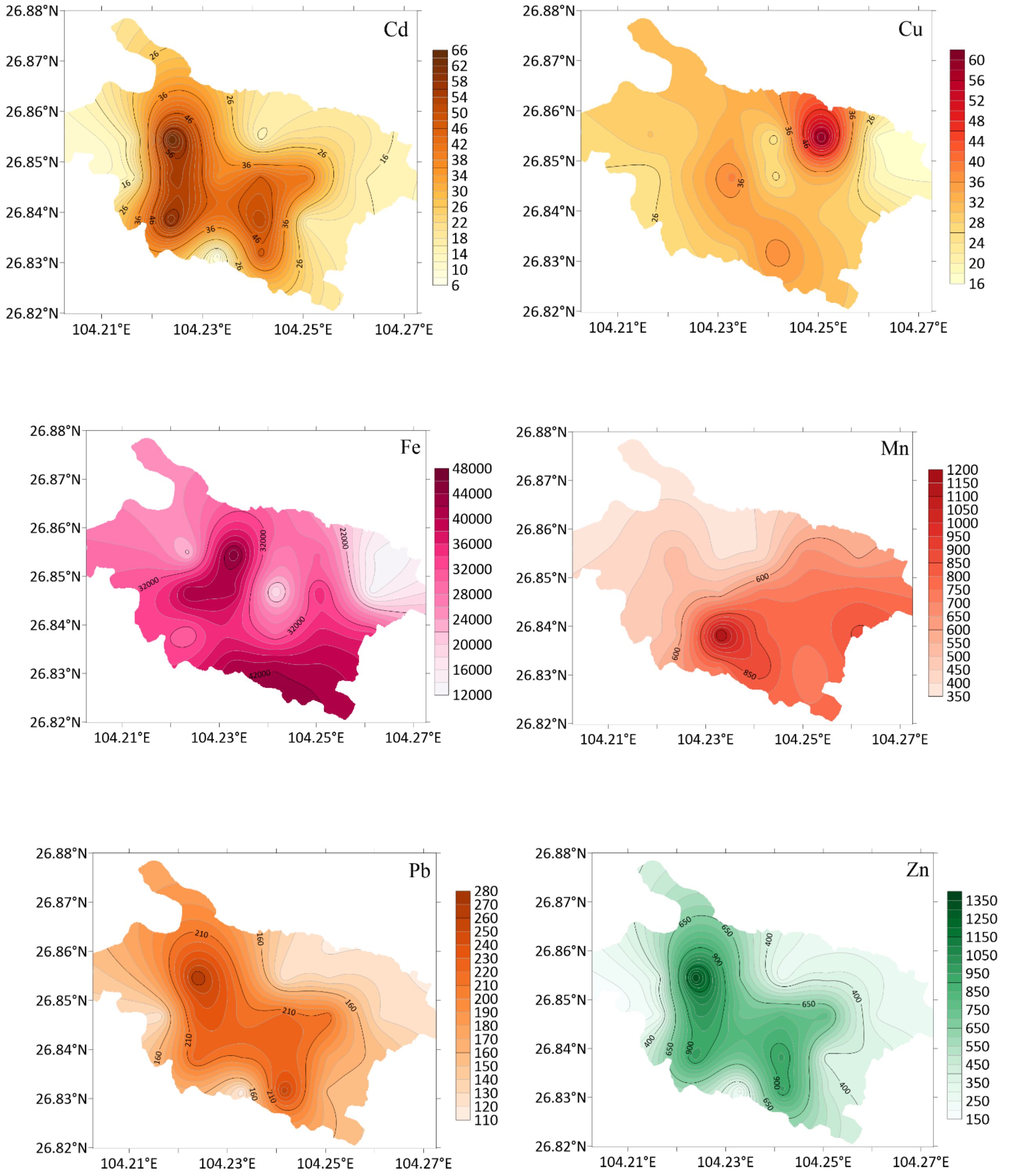


Fig. 3. Contour map of heavy metal concentration in Caohai surface sediments (mg/kg).

indigenous zinc smelting, this method of zinc refining produces liquid zinc at high temperatures and releases many heavy metals, in particular Pb, Zn, Cd, often at levels tens to hundreds of times higher than the limits, which become major pollutant metals and which enter the surrounding environment through atmospheric deposition [45]. This is likely one of the reasons for the high concentrations of these three heavy metals in the Caohai watershed. The sources of six heavy metals, Cd, Zn, Pb, Fe, Cu and Mn, in sediments from the Caohai were analyzed by principal component analysis (Fig. 4), among them, the content of Mn in the 20 sediments is the closest to the background value, which means that Mn has no obvious anthropogenic contamination and

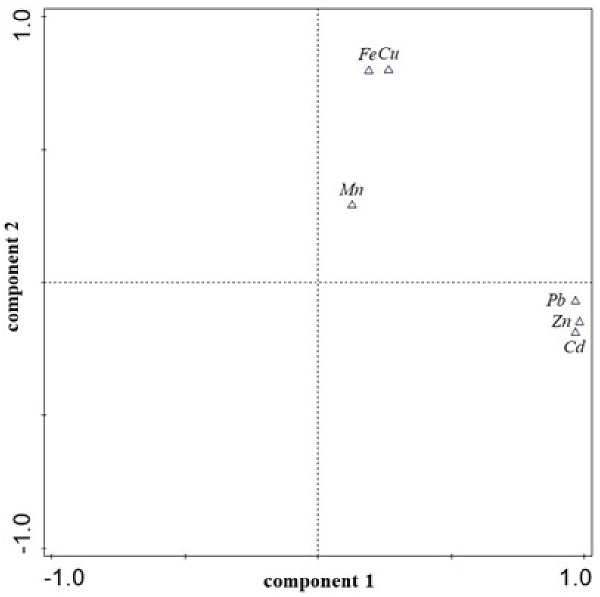


Fig. 4. Principal component analysis diagram of Caohai surface sediment.

basically represents the natural content, so the second principal component can represent the natural factors such as rock weathering, the first principal component represents the source of human activity; From Fig. 4, it can be seen that Cd, Pb and Zn may have similar sources. The Caohai Wetland used to be the main site of zinc refining, and many pollutants, including heavy metals, were released to the environment during the process of zinc refining, and it is very likely that the sources of Cd, Pb, and Zn came from the process of zinc refining. It has been pointed out that the concentration of heavy metal elements in the sediments of the C has shown an increasing trend in recent years [41]. This may be related to the zinc smelting slag and abandoned zinc smelting tanks left in the area around the Caohai, which have been washed away by rainwater after the rainy season. It is also possible that the current rapid economic development of the Caohai, such as the impact of agricultural pollution and rubbish from the tourism industry, the expansion of Weining County in the north-eastern part of the Caohai, the construction of a new port in the north, and the large areas of arable land around the Caohai are related [46]. The highest concentration of Cd in the sediments of the Caohai reached 65 mg/kg, with areas of high values occurring mainly in the center of the lake. It is generally accepted that heavy metals in natural waters originate mainly from natural geological processes and human activities. In the case of metal sulphides such as pyrite, for example, Cd, Pb, Zn, and Cu can lead to diffusion into karst systems due to their oxidation and mineral dissolution [47]. Among other things, geological processes such as rock weathering and soil erosion release large amounts of heavy metal elements into the water column, and the transport and transformation of Cd is influenced by pH, redox conditions, and microorganisms, under acidic conditions, Cd is more readily released and transported to the water column [48]. Under alkaline conditions, heavy metals are usually present in a solid phase, thus limiting the transport of Cd in the water column, and this alkaline environment contributes to the adsorption of Cd on secondary minerals and to the precipitation of Cd-rich carbonates in sediments [49]. According to the sampling results, the water body of the Caohai Wetland is weakly alkaline, which may result in the Cd being trapped in the water body sediments and reduce the movement to the water body, and in a reducing environment, the Cd usually exists in a soluble form and is more easily transported [50]. In contrast, in oxidizing environments, heavy metals are usually present in solid phase with a low risk of migration. The waters of the Caohai Wetland are mainly an oxidizing environment, which reduces the movement of heavy metals such as Cd, and this is likely to be one of the reasons why the three heavy metals, Cd, Pb, and Zn, have similar concentration distributions [46]. Microorganisms in the soil can participate in the transformation processes of heavy metals, such as reduction, oxidation, and complexation. Microbial activity can alter the morphology and bioavailability of heavy metals in sediments [51]. The high concentration area of Pb is mainly distributed in the center of the lake, which reaches 271 mg/kg, and its migration and transformation is a complex process affected by many factors. Like Cd, the migration of Pb is also affected by pH, redox conditions and microorganisms, and it is easy to be released under acidic and reducing conditions, which is more conducive to migration, while it is easy to be immobilized under alkaline and oxidizing conditions, which slows down its migration, it has been shown that in sediments contaminated with heavy metals over a long period of time, anthropogenic activities or bioturbation can disrupt the equilibrium of heavy metal adsorption in the water column and sediments, resulting in the reintroduction of heavy metals from the substrate back into the overlying water column, with relatively static water bodies being the most affected [52]. The distribution of Zn in the Caohai Wetland is roughly the same as that of Cd and Pb, with a maximum concentration of 1,373 mg/kg in the lake. In addition to being affected by pH, redox conditions, and microorganisms, Zn is also readily bound to organic matter through adsorption, precipitation, ionic interactions, and complexation [53], it has also been found that heavy metals such as Zn are predominantly distributed in the surface layer of sediments rich in plant roots, and that oxygen released from plant roots promotes the precipitation of metals [54], heavy metals are also immobilized in plant roots or taken up by the iron film on the root surface [55].

The transport and transformation of heavy metals are affected by the plants in the water body and the redox potential. The wetland has a large number of aquatic plants, and the eastern part of the lake area is dominated by emergent plants, which is a reducing environment, while the western part is dominated by submerged plants, which is an oxidizing environment [36], differences in redox conditions will inevitably affect the transport and transformation of heavy metals in the lake area. It has been shown that Zn in sediments is released under oxidizing conditions and remobilized under reducing conditions, while ferromanganese oxides (hydroxides) in sediments remobilize Zn^{2+} released from oxidizing environments [56]. So Zn in the surface sediments of the Caohai gradually migrated to the eastern lake area. Similarly, the distribution characteristics of Pb and Cd were also influenced by plants and sediment iron and manganese elements.

Characteristics of the Morphological Distribution of Heavy Metals in Surface Sediments

The proportions of different forms of the six heavy metals, Cd, Cu, Fe, Mn, Pb, and Zn, present in the surface sediments of the Grassy Sea are shown in Fig. 6. Continuous BCR extraction is an important method to study the bioefficacy of heavy metals, which can reveal the transport pathways of heavy metals in sediments and is of great significance [57]. The amended BCR method states that different extractants are to be used for extracting the different forms of heavy metals in the sediments, which are classified as: acid-soluble fraction, reducible fraction, oxidizable fraction, and residual fraction. The first three of these are collectively known as the effective states [58]. Heavy metals in the acid-soluble fraction are weakly bound to the sediment and are readily released into the overlying water [59]. Heavy metals in reducible and oxidizable states have potential toxicity under changes in redox conditions [60]. Heavy metals in the effective state have high bioavailability and certain environmental risks [61]. In contrast, heavy metals, which are predominantly in the residue state, do not pose an environmental risk to biological communities [62]. The average percentage composition of the effective state of Cd at all sampling points in Caohai reached 76.6%, the average effective state percentage of Zn in all sampling points reached 64.53%, and the average effective state percentage of Fe and Mn reached 50.36% and 57.37%, respectively. The residual state of Cu and Fe had obvious advantages, reaching 58.42% and 49.64%, respectively, and the residual state of Cu at all sampling points was up to 87%, among all the sampling points, Cd accounted for the least proportion of residual state, accounting for only 23.40%; the weak acid extractable percentage of Cu, Pb, Fe and Zn is almost 0, indicating that these four heavy metals may form complexes or precipitates with other substances in Caohai wetland water, and have low bioavailability to organisms. The concentration of weak acid extractable state of Mn is the highest among the six metals, accounting for 32.13%, and the maximum value in the sampling point is 44.69%. Among the six heavy metals, the percentage of the oxidizable state of Mn is the lowest, 9.37%, followed by Pb, which is 23.67%, and the percentage of the other four metals is not much different, which is 30%-35%. The proportion of the reducible state of Pb is the highest among the six metals, at 35.48%, and that of Cu is the lowest, at 6.30%. Among the six metals, the distribution of residual, oxidizable, and reducible forms of Cd is the most uniform, and the concentration of these three forms at each sampling point does not change much.

The heavy metals in the weak acid extractable state are extremely sensitive to the changes of external water environment conditions and can be released under weak acid and neutral conditions [63]. It has extremely high bioavailability, the greatest impact on the environment, and high toxicity. From 20 sampling points, Mn has a higher proportion of weak acid extractable concentration than the other five metals, so the migration ability and bioavailability of Mn in the Caohai wetland are strong. At the same time, Mn is an essential element for plant growth. Low concentration can promote plant growth. If exposed to high concentration for a long time, it will cause irreversible damage to plants [64]. Higher levels of the weakly acidic extractable state of Mn in the Caohai may be related to aquatic plant growth, while excess Mn causes oxidative stress, leading to cell death and a reduction in the number of immune cells [65]. Mn can directly affect the aquatic organisms in Caohai. Mn can accumulate in organisms through the food chain through bioaccumulation, which is harmful to the organisms. Some studies have pointed out that plants in the Caohai area enrich heavy metals in the soil by absorption, thus reducing the content of heavy metals in the soil surface [66]. Thus, the migration ability of heavy metals is improved.

The reducible state refers to the Fe / Mn oxide-bound state, which is the part of the metal element that is closely related to the iron-manganese oxide or that itself becomes the hydroxide precipitate [67]. This form

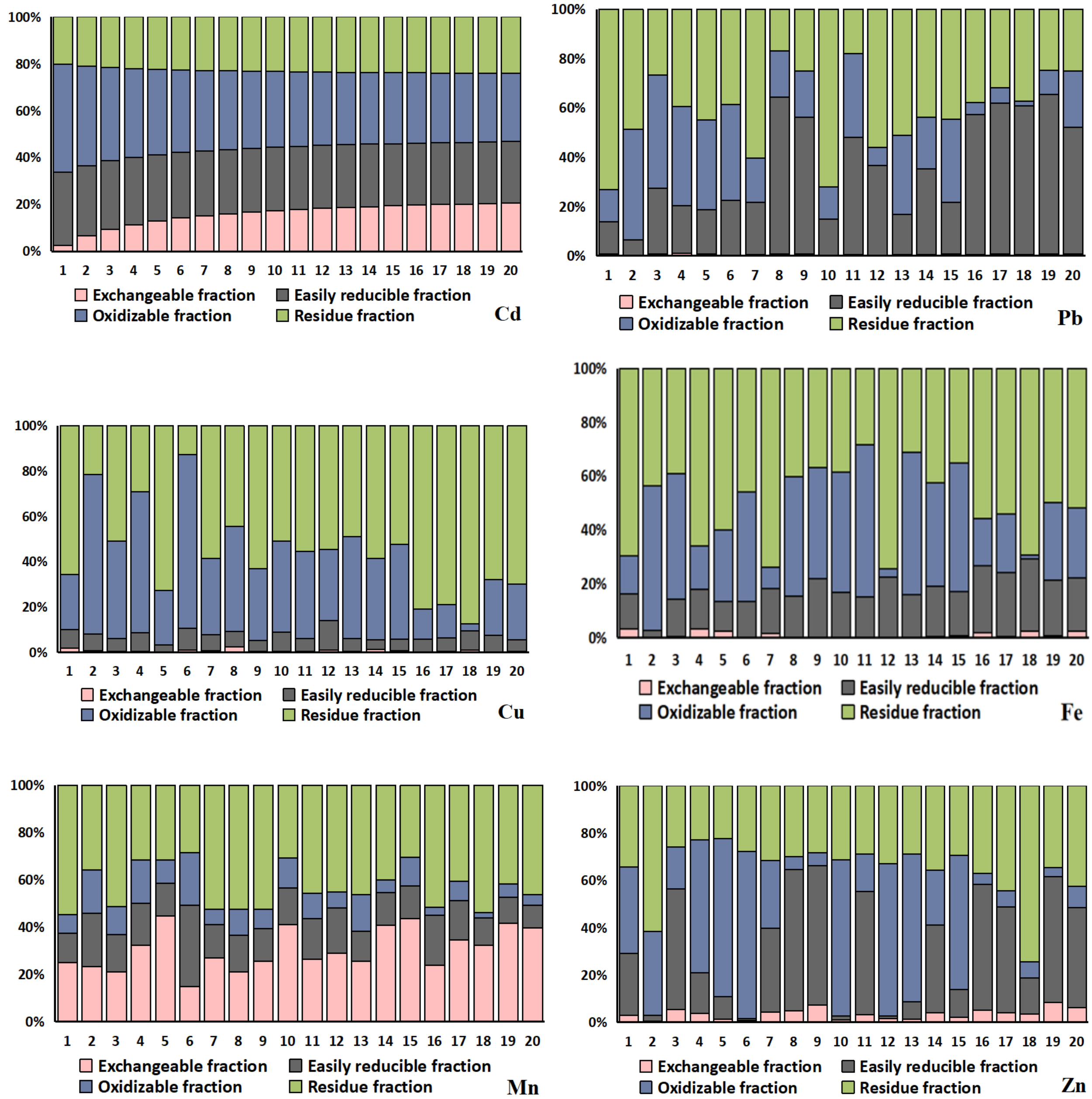


Fig. 5. Mass fraction of heavy metals in Caohai sediments.

of metal is very stable in water and will not be released easily. Iron-manganese oxide has a large specific surface area and a large ion exchange and adsorption capacity for heavy metals. It can adsorb and fix some heavy metal ions and change the redox properties of metal ions under the action of environmental microorganisms [68]. Therefore, Fe plays an important role in the formation of the reducible state of heavy metals. From the study of Wang Qin et al. on the analysis of heavy metals in the sediments of the lower reaches of Xiangjiang River, it is concluded that Zn and Pb are mainly present in iron minerals [69]. Prusty et al.. found that Fe / Mn hydroxides are important binding sites for heavy metals such as Cu and Pb [70]. It can be seen from Table 2 that the content of Fe in Caohai is much higher than that of Mn, and the effect of Mn is neglected. Therefore, it can be inferred that Fe may be one of the important reasons that affect the formation of the reducible state of Cd, Pb, and Zn metals. As shown in Fig. 6, the morphological distribution maps of Cd, Pb, and Zn and the distribution maps of Fe concentration are superimposed to obtain the morphological distribution maps of Cd, Pb, and Zn under Fe concentration. It can be seen intuitively that Cd, Pb and Zn are mainly in the reducible state under the environment of high concentration of Fe. Microorganisms use MnO_2 and $Fe(OH)_3$ for anaerobic

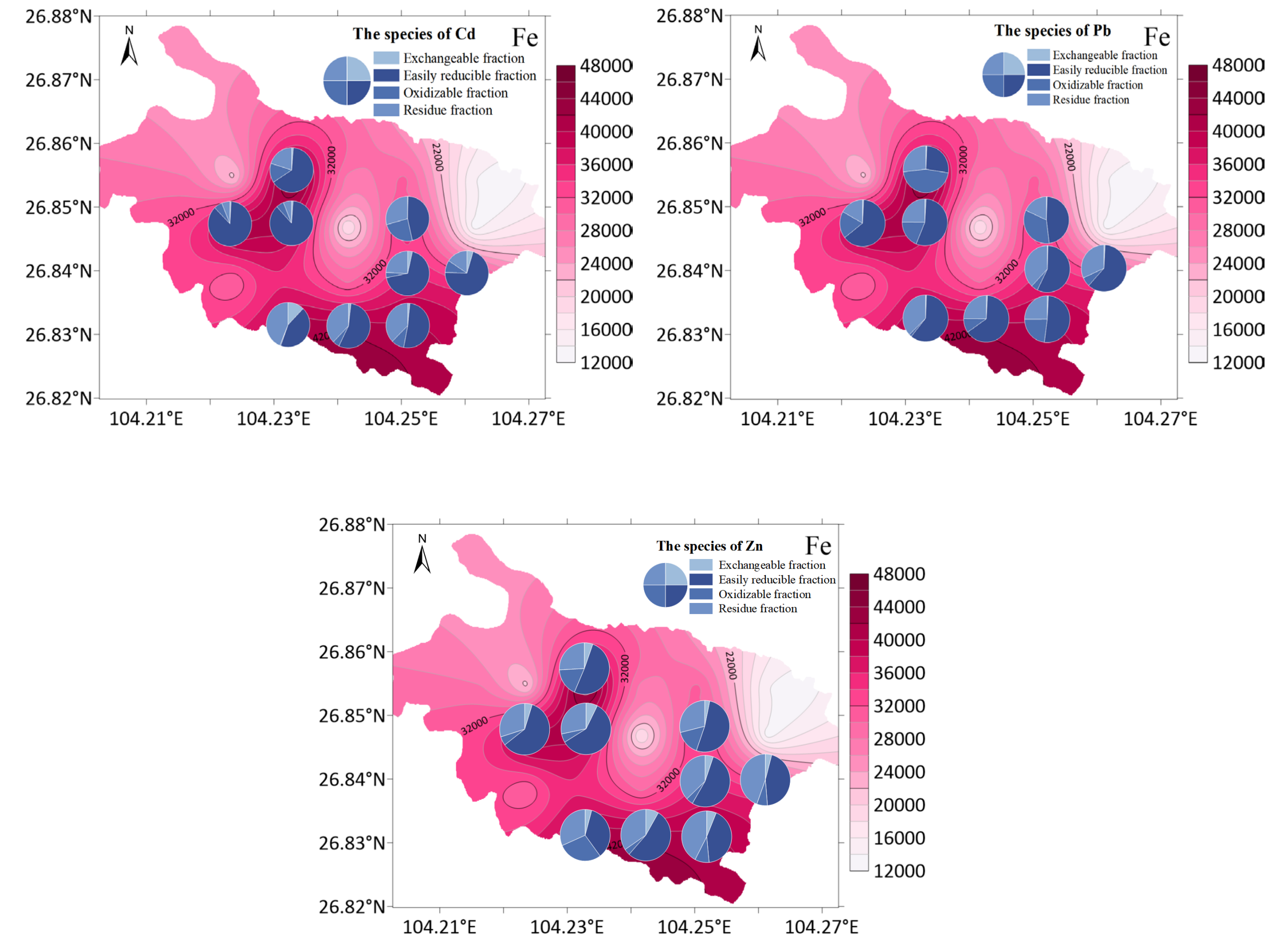


Fig. 6. Fe Overlay of concentration distribution and Cd Pb Zn morphology distribution.

respiration under reducing conditions and reduce MnO_2 and $Fe(OH)_3$ to Mn^{2+} and Fe^{2+}, which are dissolved in interstitial water or adsorbed on particulate matter. Microorganisms always tend to use higher potential electron acceptors to oxidize organic matter to obtain relatively high energy and efficiency [71]. Microorganisms preferentially use MnO_2 for anaerobic respiration, so Mn can diffuse and deposit in the eastern lake area, so the diffusion capacity of Mn is greater than that of Fe. Studies have shown that some bacteria can use Fe (III) as an electron acceptor for iron reduction [72]. This may be one of the reasons for the showing heavy metal reduction state of Cd, Pb, and Zn at the sampling sites with high Fe concentrations.

The oxidizable state refers to the combination of organic matter and sulfide, which refers to the combination of heavy metal ions as the central ion and the active group of organic matter as the ligand or the formation of insoluble substances in water by sulfur ions and heavy metals [73]. Heavy metals in the oxidizable state can form complexes and chelates with organic matter such as humic acid and alkanes in sediments and can also form co-precipitation with sulfides. Under oxidizing conditions, organic matter is oxidized and decomposed by microorganisms in the water-sediment system. Sulfur is converted into S^{6+}, and heavy metal elements are released in the form of ions [74]. Therefore, the heavy metals in this form need to be decomposed in a strong oxidizing environment; that is, the heavy metals in this form are not easily released in a weak oxidizing environment in Caohai sediments. The bioavailability of heavy metals in this form is the lowest, so it poses the least threat to the quality of the water environment. The organic matter, such as animal and plant residues in the Caohai wetland, can form stable and insoluble metal chelates with Cd, Cu, Pb, Fe, and Zn, which can effectively reduce the diffusion of heavy metals in water. Cu is a heavy metal with strong binding ability in this form because Cu has a strong affinity with organic particles and their covering layer [75]. Therefore, Cu is not greatly affected by Fe / Mn oxides, and its distribution characteristics are greatly affected by organic matter in the Caohai wetland.

The residual state is a very stable form of heavy metals. It means that heavy metals are present in crystalline minerals in the form of silicates and are extremely difficult to be released [63]. Because it is not easy to be released under natural conditions, the residual content of heavy metals in Caohai can be regarded as the background value of heavy metals in Caohai. In general,

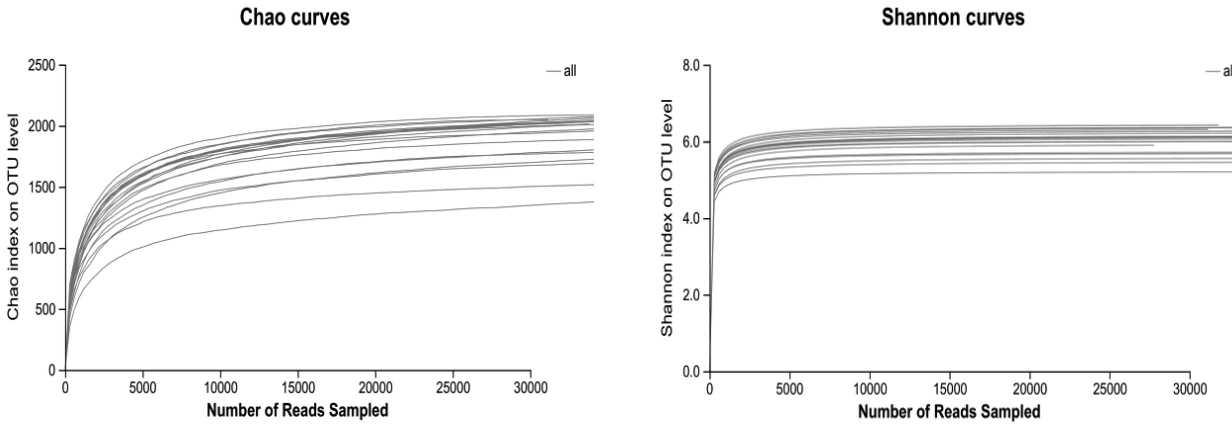


Fig. 7. Shannon and Chao index dilution curves of Caohai sediment samples.

it will not change with the change of water environment, and it is the smallest risk coefficient in the studied form, which has almost no impact on the environment.

Bacterial Diversity and Community Composition in Sediments

The dilution curves of Chao index and Shannon index of 20 samples collected in Caohai wetland tended to be gentle (Fig. 7), indicating that the detection rate of microbial community in the samples collected in Caohai wetland was close to saturation, and the current sequencing amount could cover most of the species in the samples.

The results of microbial α diversity analysis index of Caohai sediments are shown in Fig. 8. The overall change of Chao index and Shannon index is not large, indicating that the microbial diversity of 20 sampling points is not very different. However, from the histogram of 20 sampling points of the Chao index, the richness of microbial community in S1, S4, S5, S7, S13, and S18 is slightly lower than that of other sampling points. Combined with Fig. 2 and Fig. 3, it is not difficult to find that these six points are located near the sampling points with the highest concentration of heavy metals. It can be seen that the high concentration of heavy metals in the sampling area may slightly inhibit the activity of some bacteria and reduce the number of these bacteria. Heavy metal pollution in soil has a great influence on microorganisms [76]. That is to say, in the soil environment, microorganisms are very sensitive to heavy metal stress, but they are relatively less sensitive to heavy metal pollution in freshwater bodies than in soil [77]. This is a little similar to our above results. The diversity and abundance of bacteria have not changed significantly. This may be because the Caohai wetland was once a famous zinc smelting distribution center in China, releasing a lot of heavy metals. The heavy metals produced have entered the water environment, and the number of microorganisms sensitive to heavy metals has decreased sharply. Subsequently, the microorganisms that are relatively insensitive to heavy metals have gradually adapted to the environment and survived as locally dominant strains. So far, several sampling points with the highest concentration of heavy metals still have the effect of inhibiting microorganisms. Therefore, at these points, the abundance and diversity of microorganisms we obtained will be slightly lower than those with low concentration. Although on the whole, the Chao index and Shannon index of the 20 sampling points did not fluctuate much, when we divided it into three regions to study, we found that there were some trends, as shown in Fig. 9, the Chao index showed A > B > C, which indicated that the abundance of microbial species was the highest and the total number was the highest in the heavily polluted area of heavy metals. That is to say, under the stress of a certain concentration of heavy metals, the number of microbial populations can be promoted. Studies have shown that toxic pollutants can harm the common microbial flora, thereby inhibiting their growth and reducing their number, but the flora that can well adapt to the heavy metal environment can be more abundant in the polluted environment than in the normal environment [78], The abundance of some bacteria such as *Proteobacteria* and *Chloroflexi* increased with the increase of pollution [34], this is highly consistent with our results. Shannon index showed B > A > C, indicating that heavy metal stress at a certain concentration can promote microbial diversity, but too high concentration is not conducive to microbial diversity, that is to say, for microbial diversity, heavy metal concentration is too low or too high cannot have a beneficial effect on microorganisms.

Among the 20 sediments collected in Caohai, a total of 3406 OTUs were generated. The OTU annotations were classified from phylum to genus, and a total of 53 phyla, 119 classes, 279 orders, 442 families and 729 genera were obtained. As shown in Fig. 10, at the phylum level, *Proteobacteria* had the highest average proportion of 29.01% in Caohai sediments; followed by *Chloroflexi*, with an average proportion of 23.44%; *Cyanobacteria*, 9.07% were also detected in sediments. *Actinobacteria*, 8.34%; *Acidobacteria*, 6.93%; *Bacteroidetes*, 6.67%; *Firmicutes*, 2.83%; *latescibacteria*, 1.82%; *spirochaetes*,

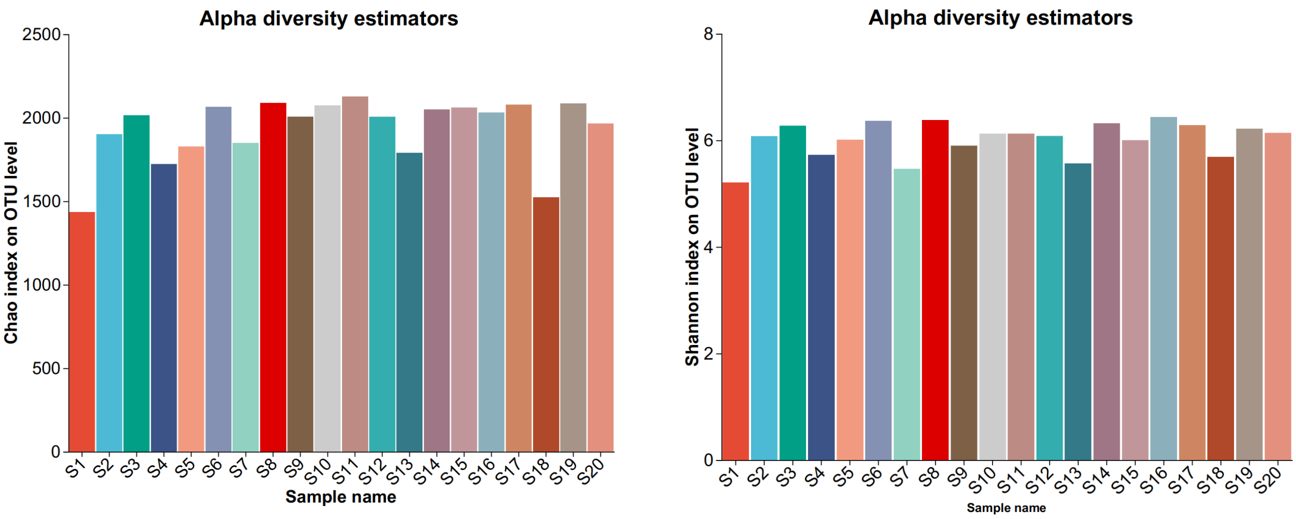


Fig. 8. Microbial community α diversity index in Caohai sediment samples.

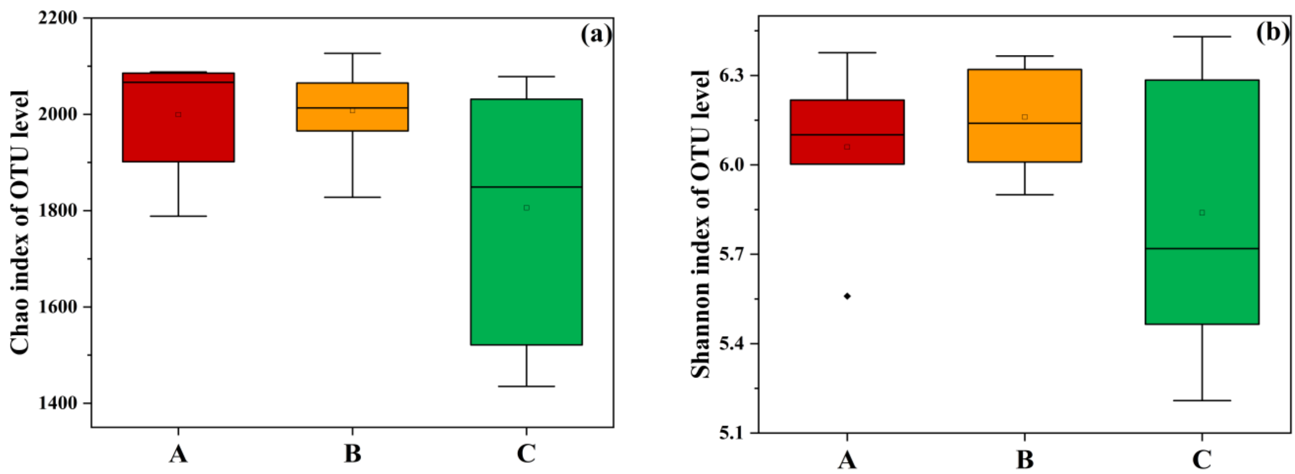


Fig. 9. α diversity index of microbial communities in A, B, and C regions in Caohai sediment samples.

1.73%; patescibacteria, 1.30%; *Chlamydiae*, 0.25%, the rest of the small number of flora accounted for 8.62% of the total.

Proteobacteria is one of the most diverse phyla and the largest one in microorganisms. It contains many pathogenic bacteria such as Escherichia coli, Helicobacter pylori, Salmonella, etc., and also includes many bacteria that can fix nitrogen. *Proteobacteria* are widely distributed throughout the world, even in the polar regions and the deep sea can be found in their tracks, in most microbial populations is a larger advantage [79], as Zhou said, it is reasonable to detect the highest proportion of *Proteobacteria* in Caohai wetland samples because of its wide distribution, many species and strong adaptability. Like *Proteobacteria*, *Chloroflexi* is widely distributed and abundant in the free-living microbial community because the flora under its phylum includes heterotrophic, phototrophic, and inorganic nutrients and can adapt to both aerobic and anoxic environments [80]. Therefore, the proportion of this phylum in Caohai wetland and *Proteobacteria* are high, and they occupy a dominant position in all phylums. *Cyanobacteria* are a group of microorganisms with far-reaching implications because the phylum *Cyanobacteria* is the only prokaryote on Earth that has evolved oxygen-containing photosynthesis. Throughout its evolutionary history, *Cyanobacteria* has played an important role in the global carbon cycle [81], it is they that have evolved the great ability of photosynthesis, using CO_2 as a carbon source to produce O_2, which makes the earth change from anaerobic environment to aerobic environment and increases the oxygen content of the earth's atmosphere and ocean. *Actinobacteria* is also a larger branch of microorganisms. The microorganisms in *Actinobacteria* are mostly free-living organisms, which are widely present in aquatic and terrestrial ecosystems [82]. Long-term Cd pollution will lead to the emergence of specific microbial communities such as *Latescibacteria*. Studies have shown that *Latescibacteria* usually exist in extreme environments

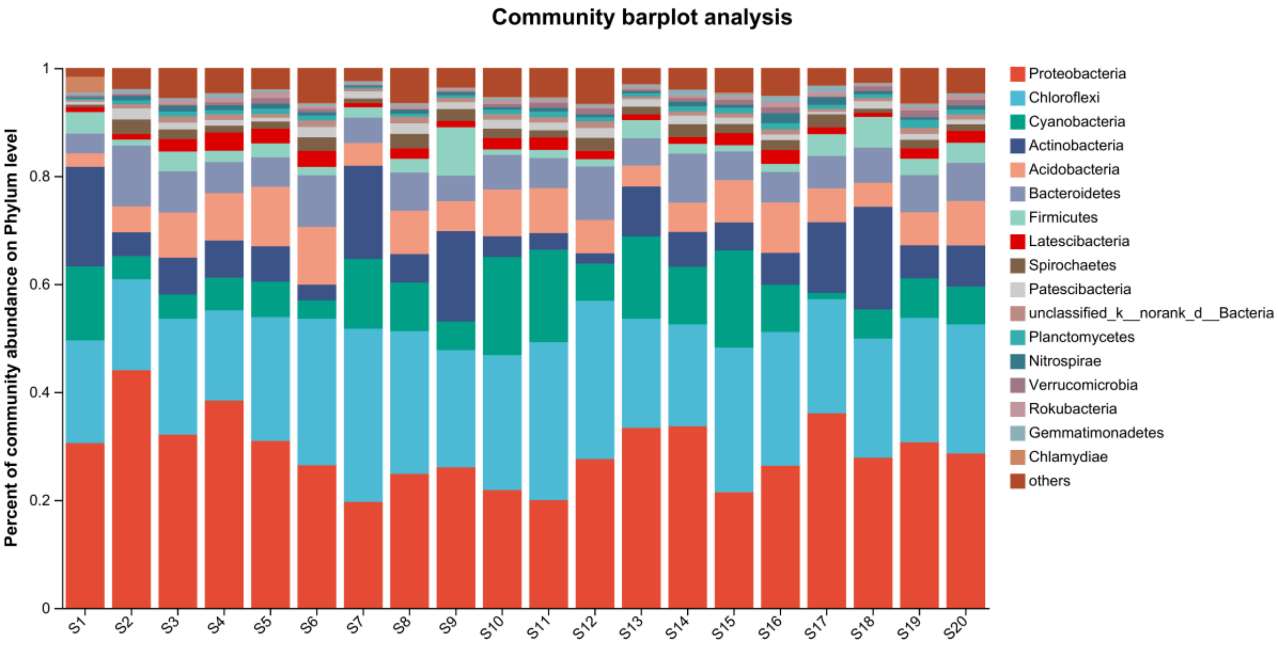


Fig. 10. Composition of bacterial species at each sampling point at the Phylum level.

and show strong tolerance in extreme environments [83]. This is consistent with the background of long-term Cd pollution in Caohai. *Patescibacteria* are a group of diverse bacteria with many unusual characteristics. Studies have shown that most *Patescibacteria* adhere to and proliferate on other bacteria as obligate epiphytes [84].

As shown in Fig. 11, in order to further explore the effects of Cd, Pb, and Zn on the bacterial community in the Caohai wetland, the microbial species composition of the three contaminated areas, A, B, and C, was compared. It was found that *Proteobacteria* and *Chloroflexi* were still the dominant species in the Caohai wetland, indicating that these two species have strong adaptability to heavy metals. This result is consistent with the research of many scholars [85-86]. *Proteobacteria* is often dominant in places contaminated with heavy metals, research has found [87]. From the study of Kuppusamy and Chen, it can also be seen that the survival rate of *Proteobacteria* in the environment containing heavy metals is still very high [88-89].

This shows that *Proteobacteria* can adapt well to an environment with heavy metals. *Chloroflexi* and *Proteobacteria* became the dominant species in Caohai because of their strong adaptability; this is consistent with the findings in the sediments of Baiyangdian Lake, which are also long-term affected by heavy metals [90].

In Area A, which was most seriously polluted by heavy metals, except for *Proteobacteria* and *Chloroflexi*, *Cyanobacteria* accounted for the highest proportion, reaching 12.00%, which may be due to the rapid recovery of the phylum after heavy metal stress, studies have shown that *Cyanobacteria* do have a strong resistance to heavy metals [91], in Yan's study, *Proteobacteria* were almost exclusively found near the heavy metal leakage site, but *Cyanobacteria* gradually became more prevalent as we got further away from the heavy metal leakage site and became the largest group of organisms other than *Proteobacteria*. The reason for this may be that many strains of this phylum have the ability of photosynthetic autotrophy and can quickly restore the population and adapt to the changing environment in a short time when they encounter environmental pressure. This view is the same as Barthès' [92]. The other flora were not well adapted to this change in environment, resulting in a decrease in numbers, and *Cyanobacteria* became the most predominant flora besides *Proteobacteria* and *Chloroflexi*. *Bacteroidetes*, *Acidobacteria*, *Actinobacteria,* and *Firmicutes* also ranked behind *Proteobacteria* and *Chloroflexi* as the dominant phyla of heavy metal pollution [93].

In area B, which is slightly less polluted by heavy metals, the inhibitory ability of heavy metals to microorganisms becomes weaker because the concentration of heavy metals is lower than that in area A. However, compared with area A, the number of *Cyanobacteria* in area B does not increase but decreases. It may be because the number of bacteria in other phyla becomes larger, and the competitive relationship between the flora leads to a decrease in the number of this phylum. In this region, except for *Proteobacteria* and *Chloroflexi*, the proportion of other secondary dominant bacteria is basically the same.

Area C was the least polluted by heavy metals, and *Actinobacteria* became the largest flora except *Proteobacteria* and *Chloroflexi*. It may be because the phylum is dominant among the microbial populations in places that are not or less polluted by heavy metals. In

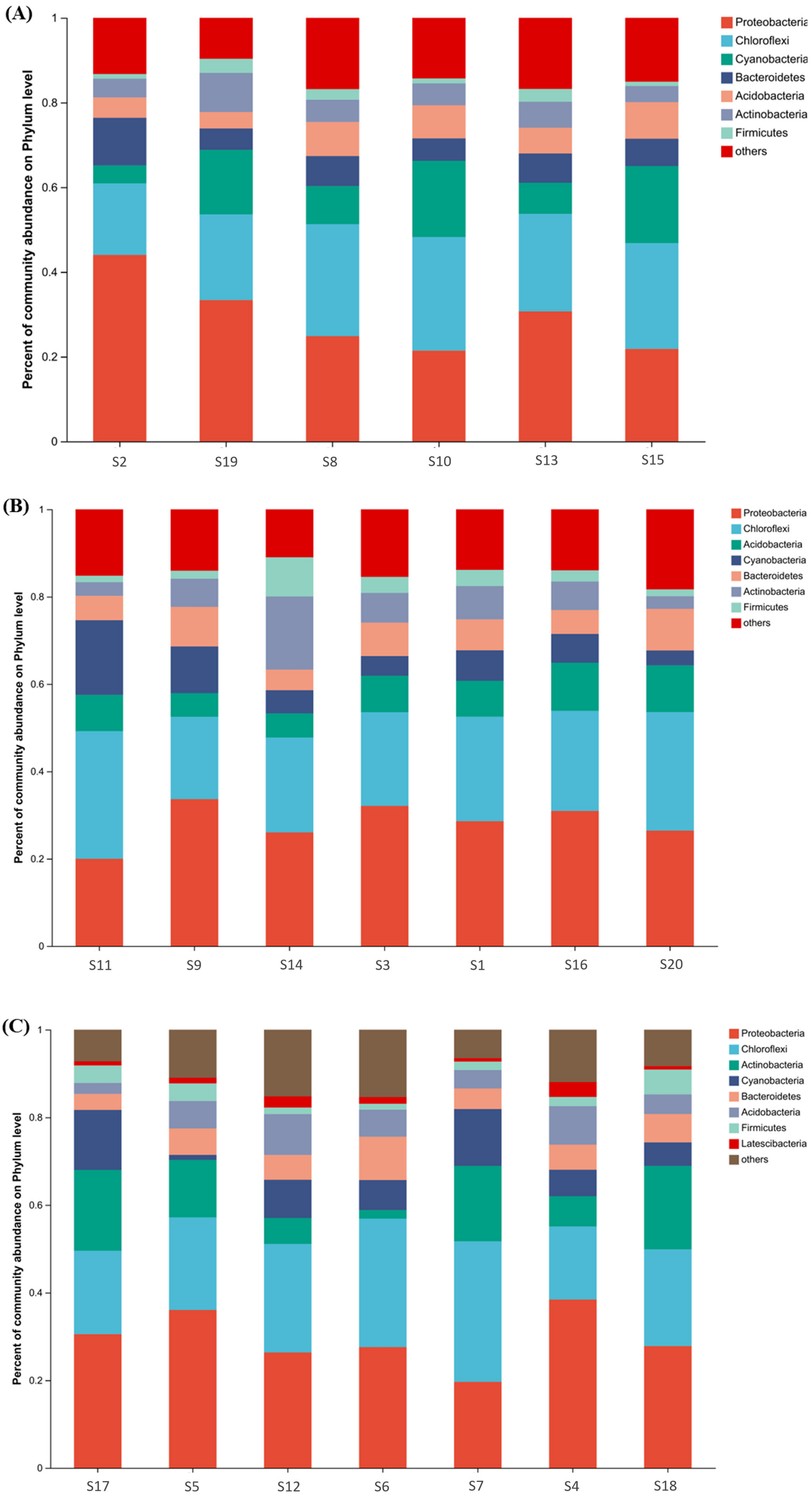


Fig. 11. Composition of bacterial species in areas A, B, and C at phyla level.

normal water bodies, the proportion of *Actinobacteria* can even exceed the number of *Proteobacteria* [94].

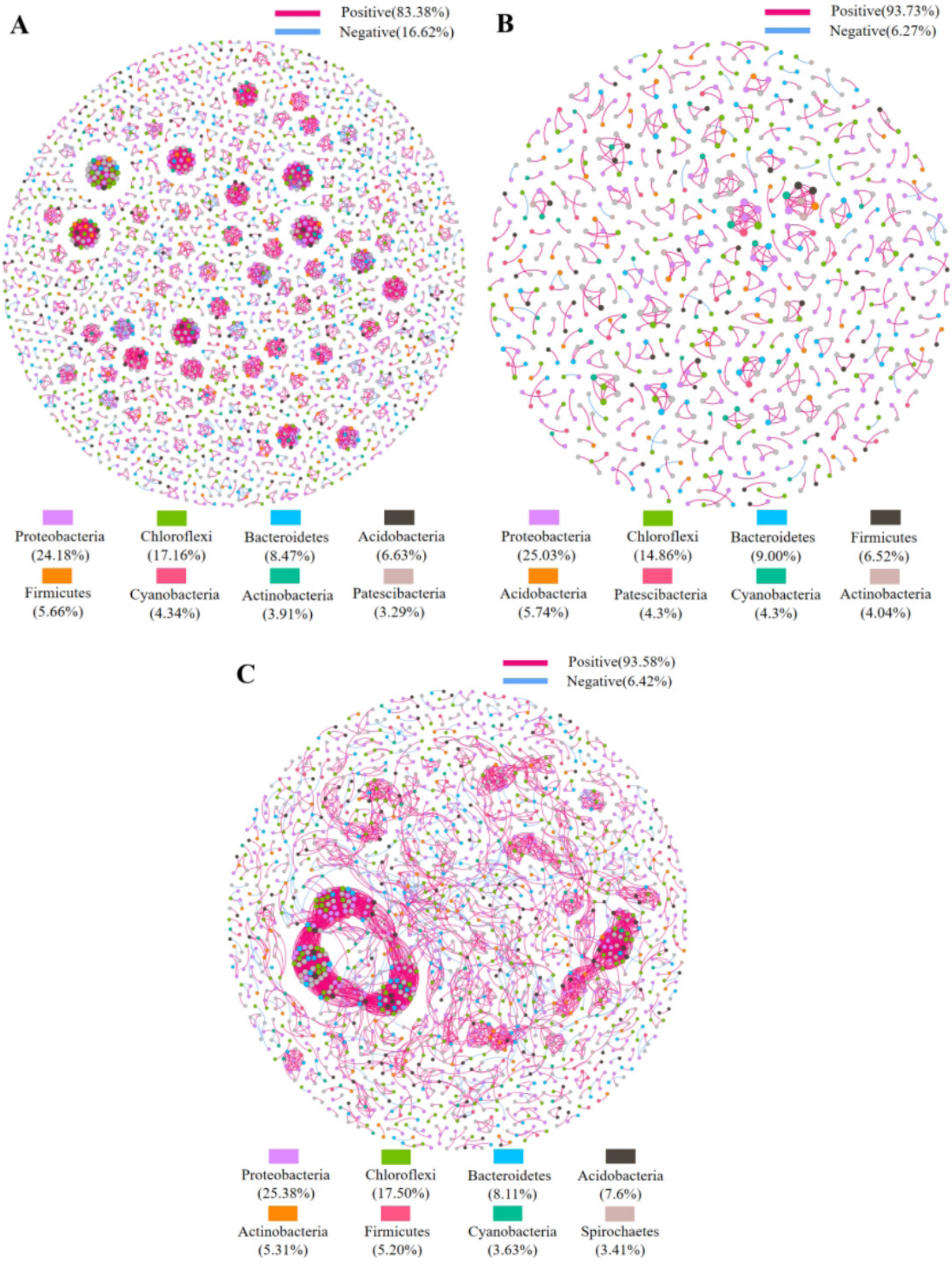
The Collinearity Network Analysis of Bacterial Community

Based on the 16S rRNA gene taxonomic units (OTUs), a microbial collinearity network was constructed. As shown in Fig. 12, the heavily polluted area includes 2279 nodes and 5218 edges, of which 4351 (83.38%) are positive correlation networks and 867 (16.62%) are negative correlation networks, indicating that the coexistence between different bacterial groups in this area is greater than the repulsion. There are five modules in the collinearity network. The topological structure parameters of the network are: average degree 4.579, average weighting degree 4.579, network diameter 1, modularity 0.975, and average clustering coefficient 1. *Proteobacteria* was the most dominant phylum in this area, accounting for 24.18% of the total, followed by *Chloroflexi* (17.16%), *Bacteroidetes* (8.47%), *Acidobacteria* (6.63%), *Firmicutes* (5.66%), *Cyanobacteria* (4.34%), *Actinobacteria* (3.91%) and *Patescibacteria* (3.29%). The moderately polluted area includes 767 nodes and 622 edges, of which 583 (93.73%) are positive correlation networks and 39 (6.27%) are negative correlation networks. It can be seen that in this area, the same coexistence effect is far greater than the exclusion effect. The network topology parameters of this region are: average degree 1.622, average weighted degree 1.622, network diameter 1, modularity 0.992, and average clustering coefficient 1. *Proteobacteria* was the most important phylum, accounting for 25.03% of the total, followed by *Chloroflexi* (14.86%), *Bacteroidetes* (9.00%), *Firmicutes* (6.52%), *Acidobacteria* (5.74%), *Patescibacteria* (4.30%), *Cyanobacteria* (4.30%), *Actinobacteria* (4.04%). The slightly polluted area included 1789 nodes and 5668 edges, of which 5304 (93.58%) were positive correlation networks and 364 (6.42%) were negative correlation networks. Similarly, in this area, the coexistence effect between bacteria was greater than the repulsion effect. The network topology parameters in this area are: average degree 6.337, average weighted degree 6.289, network diameter 27, modularity 0.855, and average clustering coefficient 0.722. *Proteobacteria* was also the most important phylum, accounting for 25.38% of the total, followed by *Chloroflexi* (17.50%), *Bacteroidetes* (8.11%), *Acidobacteria* (7.60%), *Actinobacteria* (5.31%), *Firmicutes* (5.20%), *Cyanobacteria* (3.63%), and *Patescibacteria* (3.41%).

Overall, the synergistic effect among the bacterial populations in the three zones was much greater than the competitive effect, suggesting that the exclusion effect among the bacteria would be reduced when subjected to heavy metal stress; among them, the low concentration zone had the highest level of complexity, which represented the most stable microbial community system in this zone. The lowest level of complexity was found in the middle concentration zone, which may be due to the inhibition of the activity of most of the bacterial groups by the higher concentration of heavy metals, thus reducing the connection between the groups and gradually appearing the dominant groups [87]. Symbiotic relationships were established between most of the dominant strains to resist heavy metal stress [30]. In the high concentration area, the dominant bacteria adapted to high concentrations of heavy metal stress and even promoted its abundance [34]. The synergistic effect between different bacteria in the high concentration area was 10% lower than that in the middle and low concentration area, indicating that in the high concentration area, most of the bacteria were eliminated, the number of dominant bacteria gradually increased, but the competitive relationship between different dominant bacteria gradually increased.

The Relationship Between Bacterial Community and Heavy Metals in Sediments

In order to study the relationship between the dominant phylum of microorganisms and heavy metals in the sediments of Caohai Lake, RDA analysis was carried out on the relationship between the top 10 dominant phyla and heavy metals in the three areas of heavy pollution area, moderate pollution area and mild pollution area divided by the pollution degree of heavy metals Cd, Pb and Zn. The results are shown in Fig. 13. Different concentrations of heavy metals have different effects on microorganisms. In the heavily polluted area, the results of the RDA analysis showed that the two axes explained 53.01% and 29.44% of the data, respectively, which could explain 82.45% of the effect of heavy metals on the distribution of microbial communities in this area. Heavy metals Cd, Pb, Zn and *Proteobacteria*, *Bacteroidetes*, *Spirochaetes* showed a strong positive correlation with *Patescibacteria*, among which Zn had the greatest impact on these four bacterial communities; *Firmicutes* and *Actinobacteria* were almost not affected by Pb, Zn and Mn, and Fe, Cd, and Cu had a weak positive correlation with *Firmicutes* and *Actinobacteria*; Mn and *Cyanobacteria*, *Chloroflexi*, *Latescibacteria*, *Acidobacteria* showed a strong positive correlation, Cu and Fe showed a weak positive correlation with *Cyanobacteria*, *Chloroflexi* and *Latescibacteria*, and had little effect on *Acidobacteria*. In moderately polluted areas, the situation is different. The results of the RDA analysis showed that the two axes explained 40.34% and 27% of the data, respectively, which could explain 67.34% of the impact of heavy metals on the distribution of microbial communities in the region. *Proteobacteria*, *Bacteroidetes*, *Spirochaetes,* and *Patescibacteria* only showed a positive correlation with heavy metal Mn and no correlation with heavy metal Cd, Pb, Zn, or even showed a weak negative correlation, and Fe, Cu showed a negative correlation; *Firmicutes* and *Actinobacteria* have strong adaptability in moderately polluted areas. They have a strong positive correlation



A, B, and C are co-occurrence networks of heavily polluted, moderately polluted, and lightly polluted areas in the Caohai wetland, respectively. Nodes are colored according to gate horizontal abundance, and the edge is strongly correlated (Spearman R> |0.6|), which is significant ($P < 0.05$). The red line indicates positive interactions. The blue lines represent negative interactions.

Fig. 12. The collinear network of microbial communities in A, B, and C regions of Caohai sediment samples.

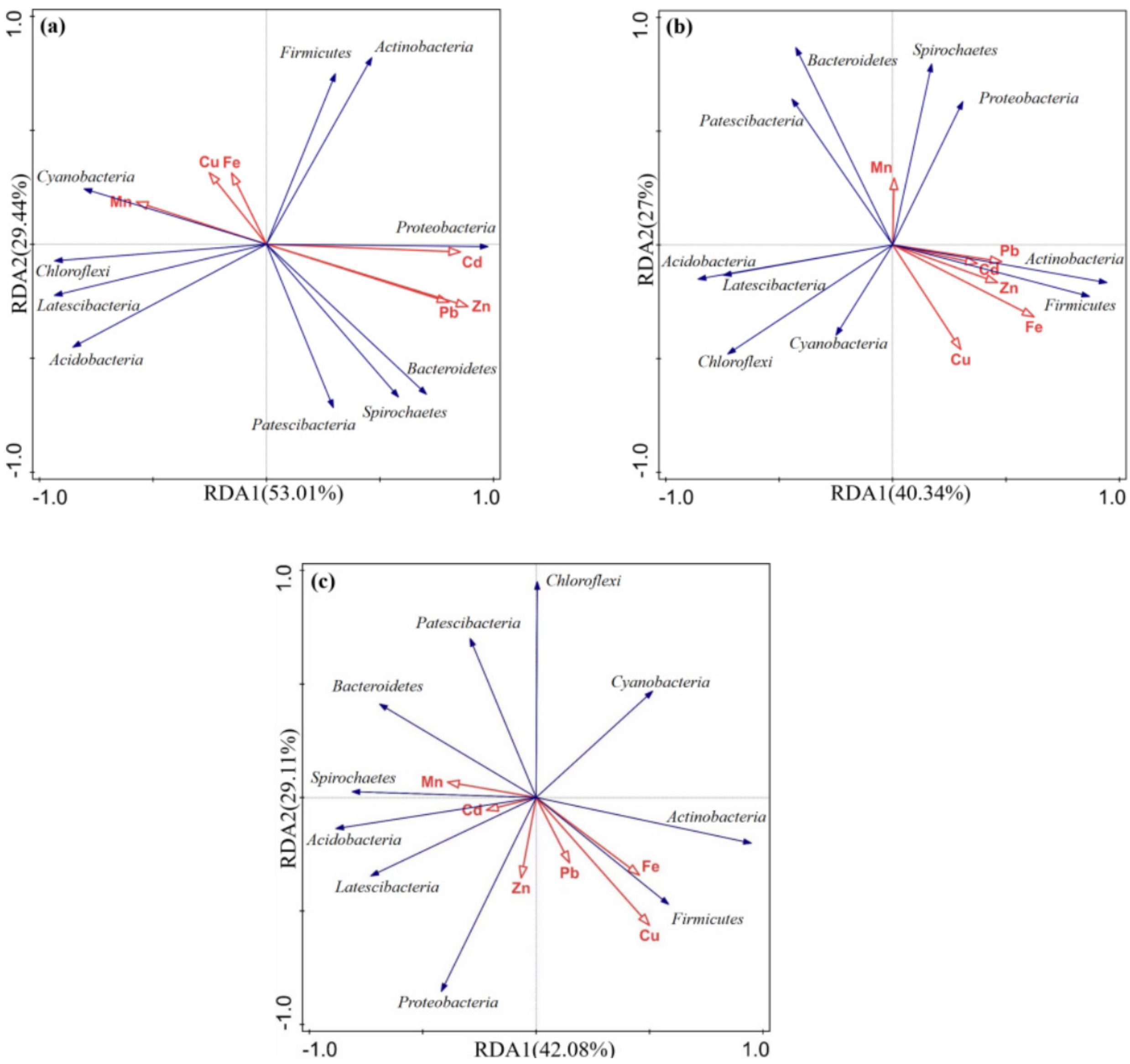
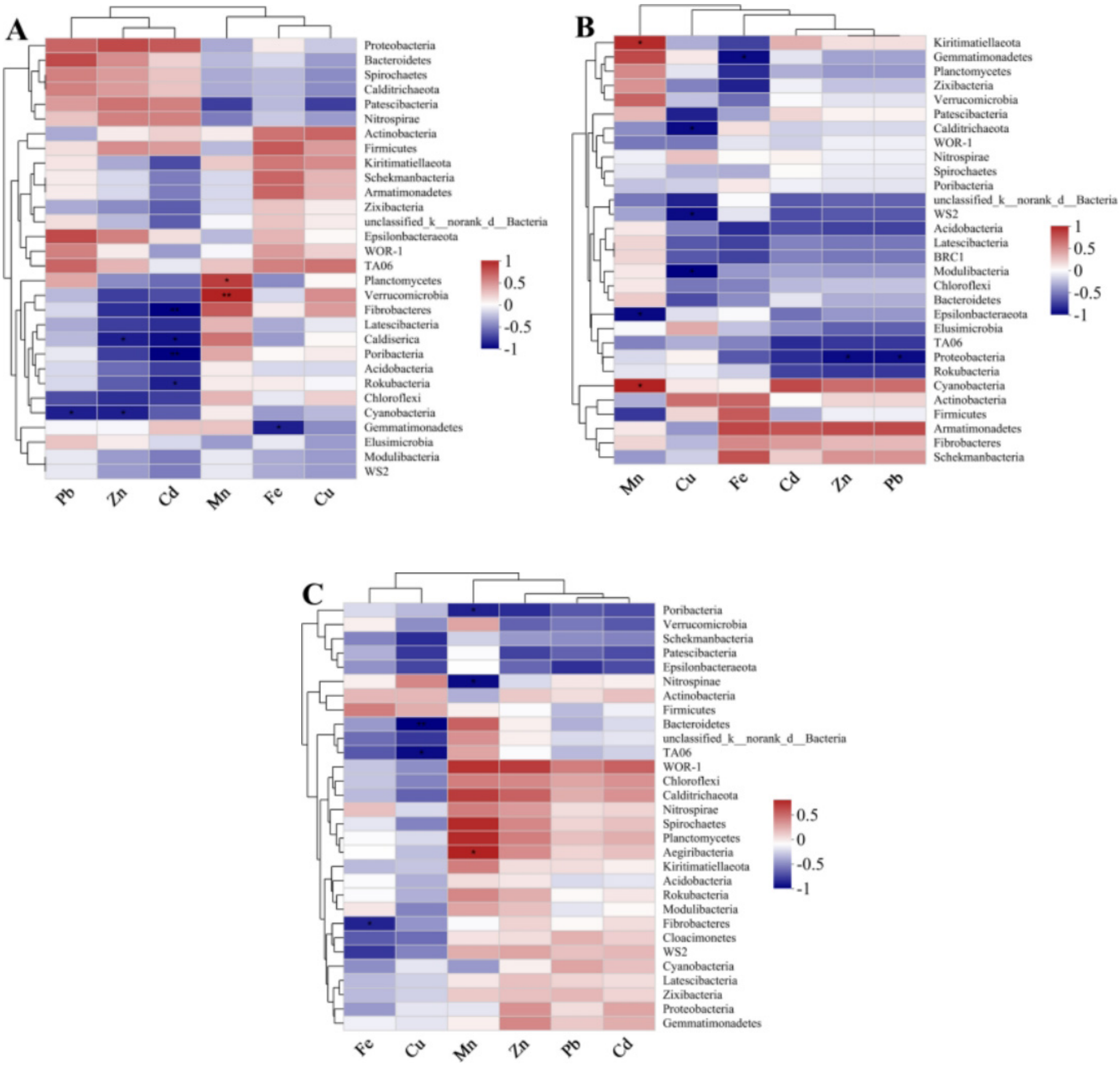


Fig. 13. RDA analysis of the relationship between Caohai sediment microbial communities and heavy metals in three regions.

with Cd, Pb, Zn, Fe, and Cu. Among them, the influence of Fe is the largest. *Cyanobacteria* still show a positive correlation with Cu. *Chloroflexi*, *Latescibacteria,* and *Acidobacteria* cannot adapt well to the environment of moderately polluted areas. All heavy metals do not show a positive correlation with them. In the lightly polluted area, the results of the RDA analysis showed that the two axes explained 42.08% and 29.11% of the data, respectively, which could explain 71.19% of the impact of heavy metals on the distribution of microbial communities in the region. *Proteobacteria*, *Firmicutes,* and *Actinobacteria* showed a positive correlation with Zn, Pb, Fe, and Cu, among which Cu had the greatest influence, and *Proteobacteria* also showed a weak positive correlation with Mn and Cd. *Bacteroidetes*, *Spirochaetes*, *Latescibacteria*, *Acidobacteria,* and Mn, Cd showed a positive correlation. *Patescibacteria* and *Chloroflexi* only had a weak positive correlation with Mn. In the lightly polluted area, *Cyanobacteria* showed inadaptability in this area, and no heavy metals showed a positive correlation with this phylum.

Among all the phyla, *Proteobacteria* is the most adaptable to the environment polluted by heavy metals, and the findings of many studies are consistent with the results of this study [78, 95-97]. It may be that the bacteria have resistance genes to heavy metals and have developed resistance. Studies have shown that bacteria of Proteobacteria reduce heavy metals through redox reactions or export heavy metals through ion channels, transporters, and pumps [98]. In this study, *Proteobacteria* had a positive correlation with heavy metals in three different degrees of pollution areas. Especially in the heavily polluted areas, Cd, Pb, and Zn had a strong positive correlation with this phylum, especially Zn, indicating that Proteobacteria may have some special adaptation mechanisms to heavy metals in heavy metal pollution and can survive and reproduce in the environment with high heavy metal concentrations. *Chloroflexi* showed a positive correlation with Cu and



A, B, and C are the correlation heat maps of the heavily polluted area, moderately polluted area, and lightly polluted area of Caohai Wetland respectively. The color column indicates the correlation degree between bacterial community and heavy metals. If the correlation between the two is positive, it is shown in red; otherwise, if it is negative, it is shown in blue. The depth of the color indicates the strength of the correlation, and the darker the red, the higher the degree of positive correlation; The darker the blue, the higher the degree of negative correlation

* means $P<0.05$,** means $P<0.01$

Fig. 14. Heat map of correlation between the top 30 bacterial communities and heavy metal environmental factors in different polluted areas.

Mn in the heavily polluted area, which indicated that *Chloroflexi* had a certain ability to resist copper [99] and manganese. Zhang et al. found that the relative abundance of *Chloroflexi* in sediments was higher than that in water [100]. *Actinobacteria* is also a kind of bacteria with strong adaptability. In the lightly polluted area, the flora has a positive correlation with Pb, Zn, Fe, and Cu. In the moderately polluted area, it has a positive correlation with all heavy metals except Mn. In the heavily polluted area, it is almost not affected by heavy metals or even slightly promoted. This is because the microorganisms of this phylum have metabolic diversity and are resistant to various environments [101]. *Acidobacteria*, as one of the dominant bacteria, was promoted by heavy metals Mn, Zn, and Cd in the low pollution area in this study. Although in the moderate pollution area, all heavy metals did not show a positive correlation with the flora, the average abundance of *Acidobacteria* in this area was only second to *Chloroflexi*, reaching 8.22%, indicating that *Acidobacteria* can still survive and reproduce in large quantities under the inhibition of all heavy metals, indicating that *Acidobacteria* is suitable for survival in places contaminated by heavy metals [102]. In heavily polluted areas, the average abundance of *Cyanobacteria* is second only to *Chloroflexi*, up to 12%, which shows the strong adaptability of *Cyanobacteria*. Studies have shown that heavy metals provide electron donors for such bacteria to support their survival in harsh environments [103]. In this study, *Cyanobacteria* and heavy metal Mn showed a strong positive correlation in the heavily and moderately polluted areas, which also indicated that *Cyanobacteria* may have the ability to resist copper.

According to the correlation heat map of the dominant bacteria (the top 30 species in abundance) and heavy metals in Fig. 14, except for Fe and Cu, Mn, Cd, Pb, and Zn had a certain correlation with each phylum in the lightly polluted area. The correlation between Cd, Pb, and Zn and each phylum was very similar, and Mn was significantly positively correlated with *Aegiribacteria* ($P<0.05$). In the moderately polluted area, the correlation between Cd, Pb, Zn, and each phylum was still very similar. Pb and Zn were significantly negatively correlated with *Proteobacteria* ($P<0.05$), and Mn was significantly positively correlated with *Cyanobacteria*. In this area, Fe, Cd, Pb, and Zn showed a negative correlation with most of the flora ($P<0.05$). In the heavily polluted area, there were similar correlations between Pb and Zn on microorganisms, where Mn showed a significant positive correlation with *Planctomycetes* ($P<0.05$) and a highly significant positive correlation with *Verrucomicrobia* ($P<0.01$). On the whole, the correlation between bacteria and heavy metals in the moderately polluted area is more negative than that in the other two areas. This result may be that most normal bacteria are eliminated in this area, and new dominant bacteria appear in the heavily polluted area to adapt to the polluted environment [104]. In the study of Geissler, it was found that the number of main bacterial groups in the sample was greatly reduced after the addition of heavy metals, and then the number of microbial groups with high resistance to heavy metals increased. After a long period of cultivation, the heavy metals were fixed by microorganisms. The number of inherent flora in the sample increased and gradually replaced the heavy metal-resistant flora [105]. This is consistent with our results. The pollution of Caohai is a long process, and the degree of pollution is high. The high abundance of bacteria in the heavily polluted area is mainly the high heavy metal resistance bacteria, which can adapt to the polluted environment well.

Conclusions

It was found that among the six metal elements measured, Cd, Pb, and Zn were likely to come from anthropogenic sources and had a high degree of pollution. *Proteobacteria* and *Chloroflexi* are the dominant bacteria that can adapt to heavy metal pollution in the Caohai area. Under the geological background of high Cd in karst soil in Guizhou, long-term Cd pollution will lead to the emergence of specific bacterial communities, such as *Latescibacteria*. In the competition of the survival of the fittest in water sediments, the emergence of heavy metals has broken this competitive relationship, making the bacteria in Caohai sediments cope with the stress of heavy metals in a symbiotic way. With the deepening of heavy metal pollution, the number of most bacteria groups has decreased sharply. At the same time, new bacteria groups with high resistance to heavy metals have emerged. They can adapt to this environment and increase their number. Finally, when the concentration of heavy metals is the highest, they stabilize their respective positions and gradually form a competitive relationship. This study provides useful information for the subsequent understanding of the resistance mechanism of bacterial communities to different concentrations of heavy metals and the changes in community interactions. It is of great significance for assessing environmental quality, providing biological indicators, and exploring ecological restoration.

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Conflict of Interest

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

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