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Study on the Improvement of Coal Gangue and Soil Composite Matrix by Organic Acid Erosion of Plant Roots

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Abstract: Coal gangue is a kind of solid waste produced in the process of coal mining and washing, which a large amount of stockpiling poses a serious threat to the environment in Guizhou karst area. Coal gangue contains a certain amount of organic matter, clay minerals and other soil components, which creates favorable conditions for coal gangue preparation. Organic acids secreted by simulated plant roots were used to treat coal gangue, and scanning electron microscope was used to analyze the surface microstructure after acid etching. The result showed that organic acid soak of simulated root system could effectively etch that surface of coal gangue and destroy its compact mineral structure. After acid etching, BET specific surface area increased by 37.81%, average pore size of adsorption and desorption increased by 60.94% and 49.11%, respectively, and pore volume increased greatly, indicating that organic acid could significantly promote the transformation of coal gangue into soil. The pot experiment was carried out with shallot, strawberry, garland chrysanthemum and ryegrass as plants. The results showed that the biomass of different plants was significantly different due to different optimum mixing ratio. Compared with the control group, the water content of strawberry was 81.36g, and the change was obvious. The results show that heavy metals in coal gangue distribute to different parts through plant roots, and the contents in different parts are different. The enrichment in roots of different plants is the highest, and the enrichment in stems and leaves (edible parts) varies with different plants. Ni, Hg and Zn have the strongest mobility among the four plants, while Pb and Cu have the weakest mobility. Through high-throughput sequencing analysis of soil microbial community composition, diversity and species difference analysis under the optimal coal gangue doping conditions for plant growth, coal gangue doping in soil can improve soil microbial community composition, diversity and species difference, promote soil micro-ecological virtuous cycle, and provide theoretical basis and experimental support for coal gangue agricultural resources and soil improvement.

Key words: coal gangue, organic acids, acid corrosion, heavy metal migration, microbial colonies

1. INTRODUCTION

Coal is the main fossil fuel in the world, which is juxtaposed with oil and natural gas. It is very important to energy production and economic development(Ma et al., 2021). And it will still be the main energy source in China for a long time. Coal gangue is the main solid waste produced in the process of coal mining and washing(M. Li et al., 2020), and the annual discharge is about 15%~20% of coal output(Cheng et al., 2018; Yaofei et al., 2024). At present, the accumulated accumulation of coal gangue has exceeded 7 billion tons, and the emission has increased year by year. Long-term open storage not only occupies a large amount of land resources, but also releases heavy metals, sulfides and other pollutants under the action of rain leaching and weathering erosion, causing ecological safety problems such as soil and water pollution(Ruud, 2006), dust emission, spontaneous combustion(Querol et al., 2008) and landslide(Chen et al., 2025; Gao et al., 2024; D. Li et

al., 2025; M. Zhang et al., 2024), which seriously restricts the ecological environment governance of coal production Kuangqu and the green and low-carbon transformation process of the industry(X. Li et al., 2022; Shi et al., 2023). Therefore, how to realize the bulk, resource and harmless utilization of coal gangue has become a key issue for the sustainable development of the coal industry(Ma et al., 2019). However, the scale and capacity of harmless disposal and resource utilization of coal gangue are not enough to meet the needs of ecological protection and clean and efficient utilization of coal(Zheng et al., 2024). It is of great practical significance to deeply study the basic properties of coal gangue and develop efficient and low-cost recovery technology for comprehensive utilization of coal gangue.

In recent years, phased progress has been made in resource utilization of coal gangue at home and abroad. At present, comprehensive resource utilization of coal gangue is mainly divided into two categories: bulk utilization and high-value utilization. Bulk utilization mainly includes building materials, agricultural production and underground goaf filling(Guo et al., 2019; R. Liu & Wang, 2026); high-value utilization mainly includes extraction of valuable metals(Y. Zhang et al., 2023), high-performance ceramics and extraction of chemical raw materials(X. Liu, 2023; L. Zhou et al., 2019). Therefore, it is necessary to research and develop advanced technology and sustainable production technology to comprehensively utilize coal gangue in large quantities, so as to realize circular economy, relieve the pressure of resource shortage and solve the problem that coal gangue occupies a large amount of land for stacking.

At the same time, the quality of cultivated land and soil in different regions of China is quite different. The reason is that the long-term use of chemical fertilizer leads to the gradual depletion of organic matter and humus in soil, which leads to land degradation problems such as soil compaction, salinization, poor ventilation and permeability, structural hardening, lack of organic matter and mineral nutrients, weak water retention capacity, low soil microbial activity, etc(Marchuk & Marchuk, 2018; Zhao et al., 2023). which directly affects farmland productivity and quality and safety of agricultural products. At present, soil matrix improvement and improvement of physical and chemical properties of saline-alkali soil, reduction of soil pH, salt concentration, bulk density, and improvement of organic matter, total nitrogen, total phosphorus, total potassium, hydrolytic nitrogen and available potassium contents in soil(X. Li et al., 2023) are important hands for high-quality development of modern agriculture. Coal gangue is mainly composed of quartz, feldspar, clay minerals, etc(Sun et al., 2025), rich in silicon, calcium, magnesium, potassium and other mineral nutrients required for plant growth, and has natural potential as soil improvement material(Halecki & Klatka, 2018; Song et al., 2023). The application of coal gangue resource utilization in agricultural soil improvement can not only realize the effective consumption of bulk industrial solid wastes, relieve the ecological environment pressure of Kuangqu, but also provide new low-cost materials for agricultural soil improvement, which conforms to the concept of coordinated development of solid waste resource utilization and agricultural ecological optimization under the “double-carbon” goal, and has remarkable ecological, economic and social benefits(Sun et al., 2025).

In recent years, scholars at home and abroad have carried out a lot of research on the utilization of mineral solid wastes for agriculture, and have made some progress in improving soil physical and chemical properties and promoting crop growth, but there are still many technical bottlenecks and theoretical gaps in the existing research. Zuo et al(Zuo et al., 2025) carried out research on ecological restoration of copper tailings. The results showed that the addition of fermented yak dung (YD) and the growth of plants significantly reduced the pH value and bioavailability of heavy metals in copper tailings, increased the nutrient content of copper tailings, promoted the transformation of primary minerals into secondary

minerals, and improved the water stability of copper tailings. However, natural coal gangue has compact mineral structure, low porosity, small specific surface area and slow nutrient release rate, which makes it difficult to achieve ideal effect when directly used for soil improvement. Activation treatment of coal gangue can enhance the release of nutrients, thus improving soil physical and chemical properties(Ye et al., 2022). On the contrary, related researches show that plant roots can secrete organic acids, which can etch coal gangue particles. With the advantages of mild reaction conditions, green environment protection and remarkable modification effect, coal gangue has gradually become a research hotspot.

In the study of coal gangue soilification, its blending ratio is one of the core parameters determining soil improvement effect. If the blending amount is insufficient, it cannot effectively improve the soil matrix structure of coal gangue and improve soil fertility(Motesharezadeh et al., 2017); if the blending amount is too high, it may change the soil colloidal properties, reduce the water retention rate of coal gangue soil matrix and increase the accumulation risk of heavy metals, especially in acidic conditions, it will promote the release of heavy metals in coal gangue and potentially pollute the surrounding environment(Qin et al., 2023). When heavy metals are dissolved in soil environment, if any of these heavy metals exceeds the standard, they are not easily decomposed by microorganisms in the environment, but can only be transformed, dispersed and enriched in various forms(Khalid et al., 2017). These heavy metals may be bioconcentrated and biomagnified along the food chain(Chilikwazi et al., 2023). Therefore, the environmental ecological risk of trace heavy metals in coal gangue is a key factor restricting its safe agricultural use(Chu et al., 2020). Heavy metal soil pollution is widespread, hidden, delayed, cumulative, heterogeneous, and difficult to degrade, which greatly increases the challenge and cost of soil pollution control(Paz-Ferreiro et al., 2018). Traditionally, heavy metal remediation involves the use of physico-chemical methods, which are limited in their wide application due to their advanced technology, expensive costs, and adverse effects on soil biological structure(Zheng et al., 2024). Phytoremediation is a technology that can be used to remove pollutants from soil. Plants can enrich one or more toxic and harmful substances in the environment according to their physiological characteristics, and carry out normal metabolism in the body to achieve the effect of degrading pollutants in the environment(Dubey et al., 2018). Although scholars at home and abroad have explored the leaching characteristics of heavy metals, the rules of enrichment, migration and transformation of heavy metals in mineral solid waste soil matrix-plant growth system(Santibáñez et al., 2008), especially the enrichment mechanism in crop root-stem-leaf (fruit), and the ecological risk assessment system for long-term agricultural use are not perfect.

Yang et al(Yang et al., 2020) found that microorganisms in soil can be used to improve the tolerance, removal and degradation ability of plants to pollutants when using plants to remediate soil. Zhang et al(S. Zhang et al., 2026) showed that the establishment of plants accelerated the early soil sample transformation of coal gangue, and jointly modified its structure, chemical and biological assembly. Soil microbial community is also the core carrier of soil ecological function, and its community structure, diversity(Y. Li et al., 2016), soil fertility and crop growth status are closely related(Dixon, 2009). Zhou et al(W. Zhou et al., 2020) found that the nutrient concentration of surface soil is positively correlated with the relative abundance of some fungi. Secondly, the research results also show that increasing plant diversity can improve the diversity of related rhizosphere microorganisms to a certain extent, which is important for improving tailings reclamation efficiency. Bonneville et al(Bonneville et al., 2011) showed that in soil, plant mycorrhizal nutrition obtained from organic matter or litter degradation is limited and recycling of this nutrition is unlikely to be complete(Jackson et al., 2019). Secondly, microbial

activation treatment can significantly improve the availability of nutrients in coal gangue(Lu et al., 2024). It was found that coal gangue was activated by Stenotrophomonas maltophilia YZ1 strain. After treatment with YZ1 strain, the contents of soluble phosphorus, potassium and silicon in coal gangue increased significantly(Zhu et al., 2022). Fungal hyphae symbiotic with plant roots are biological interfaces for plants to obtain nutrients needed for growth and maintenance from rocks and organic matter. Mycorrhizal mycelium network is several times longer and much thinner than plant roots(Schmalenberger et al., 2015), and plays a central role in soil. Dou et al(Dou et al., 2024) showed that diversity, structure and function of bacterial community were significantly enhanced and physicochemical properties were improved after bauxite waste residue was mixed with different modifiers. Soil pH value, exchangeable Na, organic carbon, available N, available K and available P were the main factors affecting bacterial community, and had significant effects on bacterial community composition, structure and diversity.

Based on the dual requirements of resource utilization of coal gangue and improvement of physical and chemical properties of cultivated soil, this study systematically clarified the effect of organic acid on the etching of coal gangue on its soil transformation; through pot experiments, the influence of coal gangue content on plant growth, ecological safety characteristics of heavy metals and soil micro-ecological response mechanism were explored, with the aim of perfecting the theoretical system of coal gangue agricultural soil improvement and providing scientific basis and technical support for local consumption and safe agricultural use of large amount of coal gangue in mining area. The research results have important theoretical value and practical significance for promoting the utilization of solid waste resources in coal industry, improving the ecological environment of agricultural soil in mining area, enhancing cultivated land fertility and ensuring the sustainable development of agriculture.

2. TEST MATERIALS AND METHODS

2.1 Test materials

Coal gangue: from a mine gangue hill in Panzhou City, Guizhou Province, after natural air-drying, hammer crusher crushing and sieving, take the particle size below-8mm. Particle size distribution and characteristics are shown in Fig. 1.

Soil: farmland surface tillage soil, after natural air-drying, crushing and sieving-8mm.

Coal gangue and soil samples are ground to less than 0.075mm, XRD mineral composition and trace heavy metal content of coal gangue are determined and analyzed, and XRD mineral composition test of soil is carried out. The test results are shown in Fig. 2 and Table 1. Coal gangue is mainly composed of SiO_2 and $CaCO_3$, and contains a small amount of heavy metals such as Cu, Zn, Pb, C, etc; soil is mainly composed of SiO_2 and kaolinite, etc. According to test requirements, nutrient test of coal gangue and soil is carried out, and the test results are shown in Table 2.

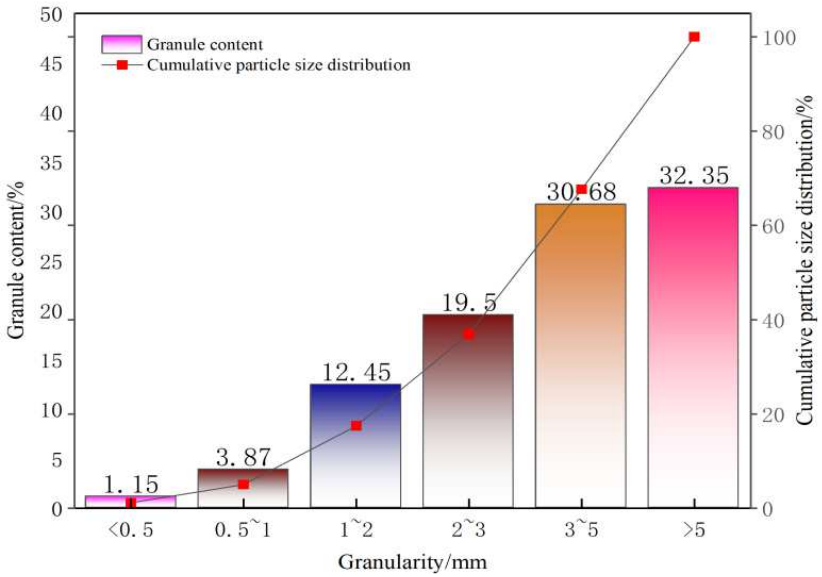


Fig. 1 Particle size distribution and characteristics of coal gangue

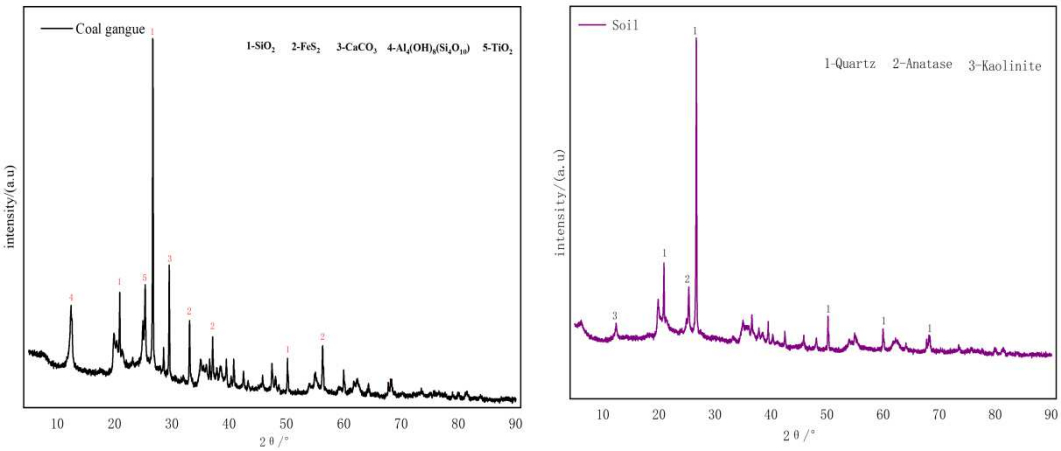


Fig. 2 XRD composition determination of coal gangue and soil

TABLE 1 Determination of heavy metal content of coal gangue

matter	Pb (mg/kg)	Cd (mg/kg)	Cr (mg/kg)	Hg (mg/kg)	As (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Ni (mg/kg)
coal gangue	25.3	0.362	134.0	0.277	15.0	169.0	228	70.0

TABLE 2 Coal gangue and soil nutrient test

matter	pH	organic matter (g/kg)	available nitrogen(mg/kg)	rapid available phosphorus(mg/kg)	rapidly available potassium(mg/kg)
gangue	7.56	271.98	7.00	0.40	93.00
soil	6.09	75.98	21.35	0.42	88.60

Simulating organic acids secreted by roots: referring to organic acid components of plant roots, preparing mixed acid solution (citric acid: malic acid: oxalic acid =1:1:0.5, concentration 3.0mmol/L) to simulate the alteration effect of organic acids secreted by plant roots on coal gangue under natural conditions.

Plants planted: ryegrass, strawberry, garland chrysanthemum, shallot.

2.2 Experiment design

11 groups of coal gangue soil matrix were prepared according to different mass ratios with farmland surface soil as the basic matrix, and the coal gangue content was 0%(control group), 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%. After fully mixing evenly, they were put into flowerpots (inner diameter: 20cm, height: 13.5 cm), and stood for 7d. Relevant tests were carried out after the soil properties were stable, as shown in Table 3.

TABLE 3 Soil matrix of coal gangue with different mass ratio

serial number	material ratio/%	
	coal gangue	soil matrix
1	0	100
2	10	90
3	20	80
4	30	70
5	40	60
6	50	50
7	60	40
8	70	30
9	80	20
10	90	10
11	100	0

2.3 DETERMINATION ITEMS AND METHODS

2.3.1 Acid etching coal gangue by simulating plant root system

8g of coal gangue powder is weighed, added into a simulated root mixed organic acid solution according to a solid-liquid ratio of 1:50, placed in a glass of a room temperature stirrer, stirred and soaked for 30 days; after soaking, filtered, washed by deionized water to neutrality, dried at 105 °C to constant weight to obtain simulated root acid corrosion modified coal gangue, and sealed for standby.

2.3.2 Characterization of organic acid etching coal gangue

The surface morphology of raw coal gangue and simulated root acid etched coal gangue powder samples were observed by scanning electron microscope(SEM) after gold spraying treatment, and the surface compactness, pore structure and particle morphology of coal gangue before and after acid etching were compared and analyzed to explain the effect of simulated root acid on the soil transformation process of coal gangue.

The specific surface area and porosity of raw coal gangue and simulated root etched coal gangue powder samples were analyzed by BET.

2.3.3 Effects of soil matrix with different coal gangue content on plant growth

Sowing equal amount of ryegrass, strawberry, shallot and garland chrysanthemum in each pot, watering regularly, harvesting after cultivation under natural conditions, observing crop growth and growth, measuring fresh weight and dry weight, and comprehensively determining the optimum amount of coal gangue.

2.3.4 Test on migration law of conventional heavy metals in plants

The root, stems and leaf (fruits) of plants with that optimum dosage were digested by microwave, the content of Cu, Zn, Pb, Cd, Cr, Ni, As and Hg were determined, and the transfer coefficient (TF) was calculated to analyze the migration degree of heavy metals from coal gangue soil matrix to different parts of plants.

2.3.5 Analysis of microbial colonies in coal gangue soil matrix

The samples of coal gangue soil matrix of shallot, strawberry, garland chrysanthemum and ryegrass planted in the control group and the optimal dosage

group were taken to extract microbial DNA and conduct high-throughput sequencing of bacterial 16SrRNA; the composition, abundance difference and diversity of microbial colonies were analyzed to explore the effect of coal gangue on microbial colonies in coal gangue soil matrix.

3. RESULTS AND DISCUSSION

3.1 Mineralogical Characteristics of Coal Gangue and Changes of Surface Structure after Organic Acid Etching

3.1.1 Mineralogical characteristics and microstructure

Coal gangue heterogeneity is significant, mainly due to its physical properties heterogeneous, chemical and mineral composition complex, ore structure is closely related. According to the apparent characteristics of the coal gangue, texture and cross-section characteristics, the coal gangue block can be roughly divided into 8 categories. According to the classification results, the 8 categories of coal gangue blocks are sorted according to the quality from small to large, and marked as a to h respectively, as shown in Fig. 3.



Fig. 3 Coal gangue blocks after classification (coal gangue-a to coal gangue-h)

Coal gangue-a surface features rough, with many visible pores, cross-section gray, containing less carbon, and dotted with scattered yellow or white particles. Coal gangue-b surface smooth and delicate and black, less flaky, most of the granular. Coal gangue-c cross-section color layered state, plane color is black, metamorphic degree between coal and gangue. The cross section of gangue-d shows a structure consisting of alternating white and black minerals, with less white minerals indicating smooth, fine, granular texture. Gangue-e presents a rough dark gray surface characterized by a softer texture. Gangue-f presents a dark black, hard texture with large particles of pyrite embedded therein. Gangue-g shows smooth and fine, mainly composed of irregular blocks, the overall surface is black, the coal quality is clear, the texture is relatively uniform, and the specific gravity is heavy; gangue-h shows deep black, composed of fragments, small particles and a large amount of coal, the texture is soft, and compared with other types of coal gangue, the coal content is large.

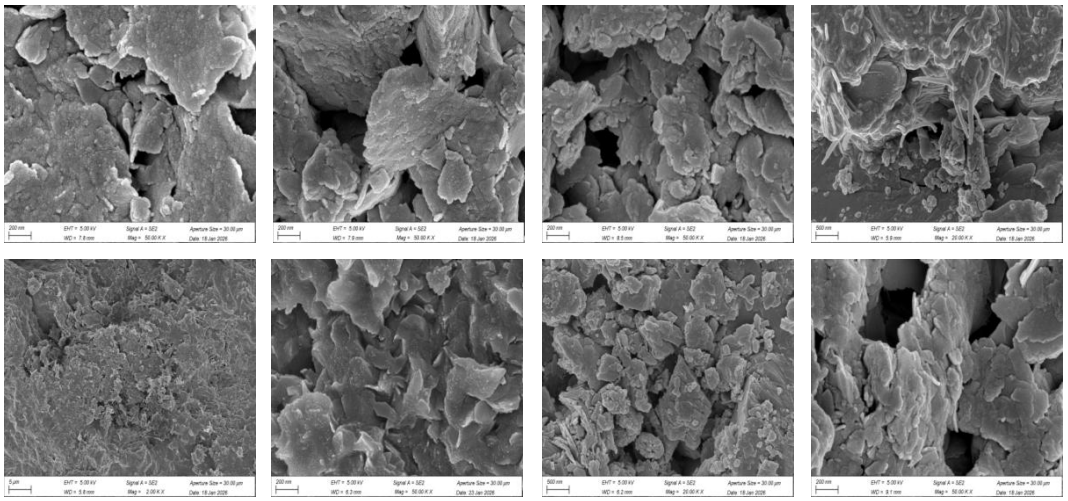


Fig. 4 Microscopic characteristics of different coal gangue

SEM images of various types of coal gangue are shown in Fig. 4. There are significant differences in microscopic morphology, pore characteristics and lamellar/particle aggregation state of different samples. The microstructure of sample a is mainly large-sized thick lamellar stacking, with a large number of cracks and pores, and the overall structure is loose; sample b is obviously finely divided, the compactness of lamellar stacking is relatively high, and the pore size is smaller than sample a, mainly medium and small pores. The structural continuity is weaker than sample a, and the overall porosity is moderate; The microstructure of sample d is fine fibrous and lamellar interwoven morphology, the lamellae are fine and intertwined, the pores are mainly elongated cracks, the structure continuity is good, the uniformity is high, and there is no obvious large size pore; e The microstructure of sample is fine particle dense accumulation type, the particle size is fine and evenly distributed, almost no visible macropores, and the surface is relatively flat, which is the sample with the densest structure and the lowest porosity among all samples; f The sample presents the characteristics of heterogeneous lamellar size, mixed stacking of large and small flakes, wide pore size distribution range, both macropores and micropores, and no obvious regularity of lamellar stacking; g sample is mainly characterized by granular/massive agglomeration, no lamellar structure, prominent particle feeling, pores are intergranular pores, compact and uniform structure, the compactness is second only to e sample; h sample presents thick lamellar stacked structure, there are obvious pores and cracks, lamellar integrity is good, it is the sample with the largest pore size and the most loose structure among all samples, and the interlayer gap is obvious.The results show that the pore size of coal gangue samples a and h is larger than that of other samples, and the surface structure is relatively loose. Therefore, the stacking mode of lamellae and the aggregation morphology of particles are the key factors to regulate the pore structure and compactness of coal gangue samples. According to the significant differences in microstructure of different samples, it can provide microstructure support for the differences in macroscopic properties.

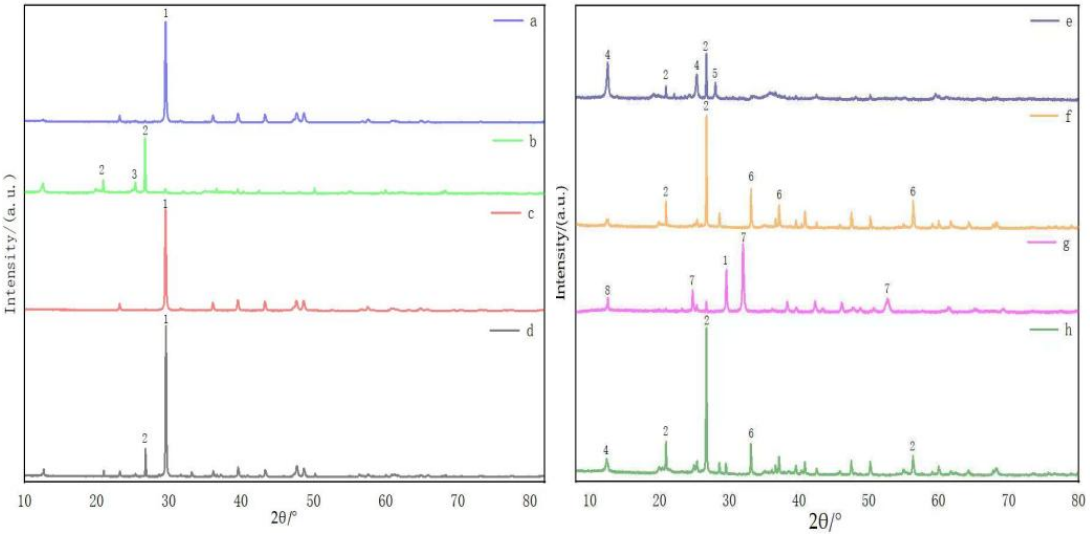


Fig. 5 XRD analysis of coal gangue blocks (1-calcite 2-quartz 3-anhydrite 4-kaolinite 5-kyanite 6-pyrite 7 siderite 8-magnesium chlorite)

As shown in Fig. 5, the mineral composition of coal gangue is complex and varied. Its main minerals include calcite, quartz, anhydrite, kaolinite, kyanite, pyrite, siderite and magnesium chlorite, and a small amount of other minerals. Different types of coal gangue show obvious differences in mineral types, relative contents and microstructure, reflecting the heterogeneity of parent rock properties and formation conditions. It is worth noting that the difference of mineral composition directly affects the etching degree of coal gangue under the action of organic acids: soluble minerals Coal gangue with higher content of calcite and anhydrite is more susceptible to chemical dissolution, while coal gangue rich in stable minerals such as quartz and kyanite shows stronger weathering resistance. Therefore, different types of coal gangue show significant differentiation characteristics in weathering development degree, which leads to differences in soil-forming potential and soil-forming properties.

3.1.2 Effect of simulated root organic acid etching on surface structure of coal gangue

(1) SEM test

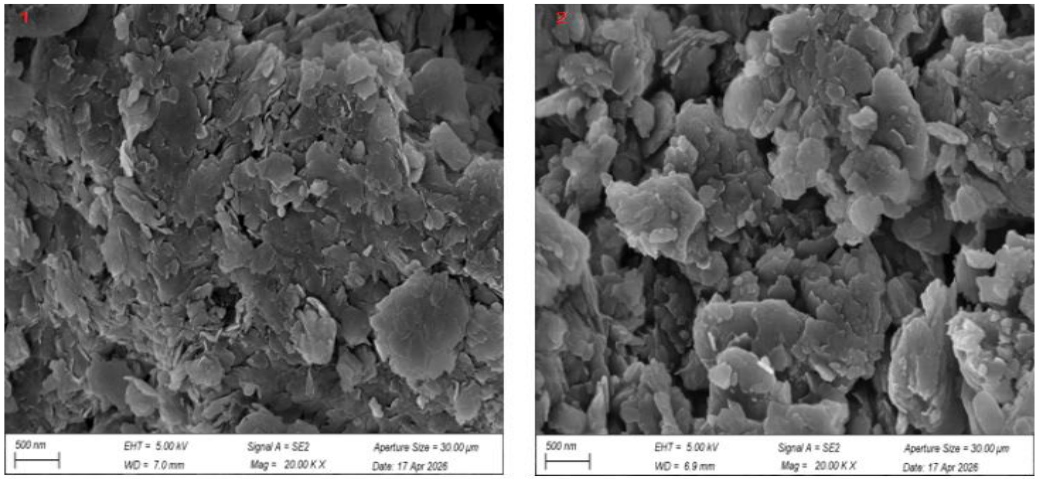


Fig. 6 Microstructure characteristics of coal gangue before and after organic acid soaking (1: before soaking;2: after soaking)

In order to simulate the environment of organic acid exudation by roots during plant growth, an organic acid solution with a concentration of 3.0mmol/L was prepared, and coal gangue powder was immersed in the solution and reacted for 30 days under continuous stirring to observe the acid corrosion characteristics of coal

gangue surface.

As shown in Fig. 6, although both groups of samples in Fig.6 a and Fig. 6 b show typical layered silicate mineral characteristics, the particles are mainly flaky and scaly, which is consistent with the crystal structure of kaolinite and other clay minerals in coal gangue, but there are significant differences between them in microscopic morphology. In Fig. 6 a, the lamellar structure of the sample is relatively dense and smooth, the mineral particles are closely combined, the crystal edges are clear, the lamellar stacking is orderly, the number of pores is small and the pore size is small, and the original mineral morphology is relatively complete. This structure is not conducive to water adsorption, and the soil degree is relatively low. In contrast, the lamellar structure of the sample in Fig. 6 b obviously tends to be scattered and fluffy, the surface is significantly etched, the dense structure is destroyed, the roughness is significantly improved, a large number of pores and grooves of different sizes are developed on the surface, the particle edges tend to be rounded, and obvious particle agglomeration is accompanied. The lamellar pore structure is more developed. The above characteristics indicate that after soaking in organic acid, the surface of coal gangue has been greatly altered, the degree of grain refinement is improved, the primary lamellar structure is broken and redistributed, and the specific surface area and porosity are significantly increased. In addition, organic acids react with coal gangue minerals to dissolve impurities such as carbonate, which makes the particle structure loose and has similar characteristics to soil, thus providing favorable conditions for subsequent soil improvement and plant planting.

(2) Specific surface area and porosity test

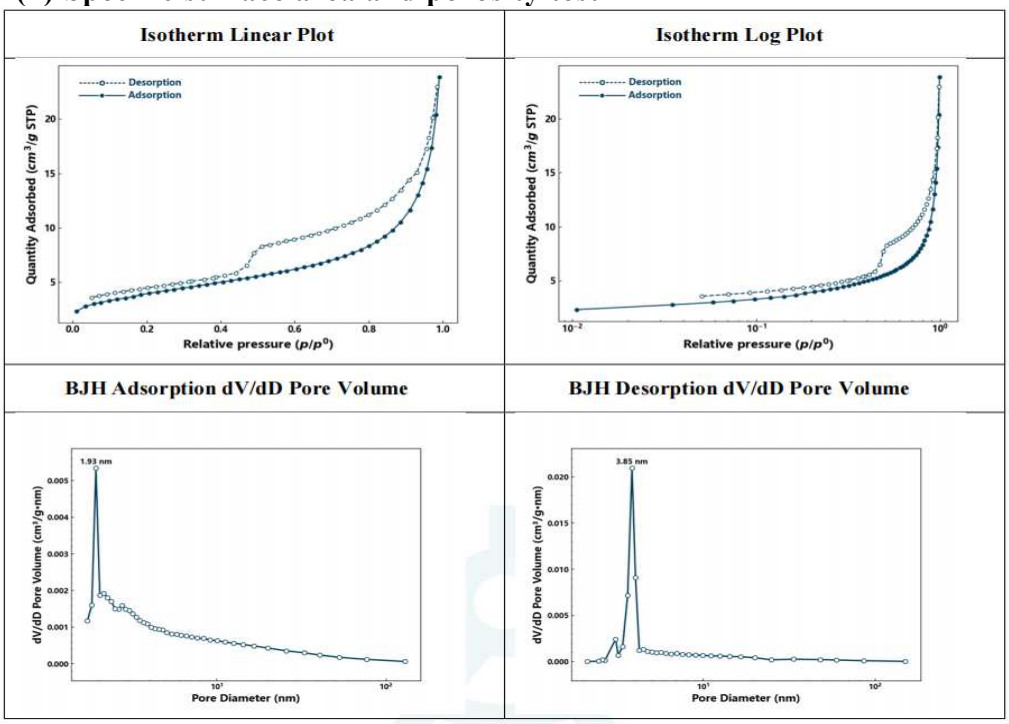


Fig. 7 Analysis of specific surface area and porosity of coal gangue before acid etching

As shown in Fig. 7, nitrogen adsorption-desorption isotherm presents typical IV isothermal characteristics, adsorption and desorption curves form clear hysteresis loop, nitrogen slowly adsorbs in monolayer and multilayer at low pressure section, and capillary condensation suddenly increases at middle and high pressure section, indicating that a large number of connected mesopores and a small number of macropore fractures develop in acid-etched coal gangue; The pore volume decreases rapidly with the pore size increasing. The slight difference between the two pore size curves comes from the capillary hysteresis effect of ink-bottle pores. The data repeatability is good. It shows that a large number of

mesoporous structures are formed in the coal gangue after the organic acid etching dissolves the primary minerals, and the pore development degree and specific surface area are significantly improved.

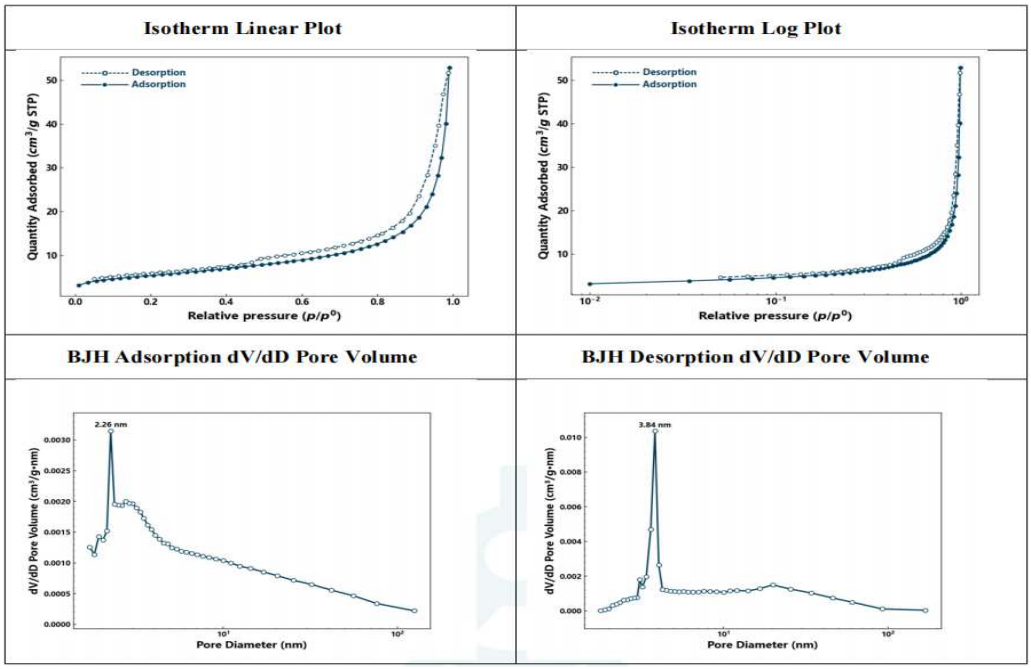


Fig. 8 Analysis of specific surface area and porosity of coal gangue after acid etching

As shown in Fig. 8, nitrogen adsorption-desorption isotherm shows that the sample presents typical IV type isotherm characteristics, adsorption branches and desorption branches form obvious hysteresis loop, nitrogen slowly adsorbs in monolayer and multilayer in low pressure interval, and capillary coagulation occurs in steep rise curve in middle and high pressure interval, indicating that a large number of connected mesoporous structures are developed in coal gangue matrix and accompanied by a small number of macroporous cracks; The main peak position of pore size distribution curve is basically consistent with that of BJH adsorption and desorption pore size distribution curve. The most probable pore size of sample is concentrated in mesoporous area, and the proportion of micropores is very low. The slight difference between the two pore size curves is caused by capillary hysteresis effect caused by ink-bottle pore. The data repeatability is good, indicating that rich mesoporous pores are formed after organic acid etching coal gangue primary minerals, which effectively improves the specific surface area of matrix and pore connectivity.

The isotherms of the two groups of coal gangue are IV type mesopore curves with adsorption desorption hysteresis loop and mesopore structure, the hysteresis loop of the acid-etched sample is wider, the adsorption capacity and pore volume peak value of BJH are higher, the mineral etching is more complete, and the specific surface area, mesopore number and pore connectivity of the acid-etched sample are much better than those of the compact primary coal gangue. After acid etching, the BET specific surface area of gangue increases by 37.81%, the average pore size of adsorption and desorption increases by 60.94%, 49.11%, and the pore volume also increases greatly, indicating that organic acid can promote the soil transformation process of gangue.

3.2 Effect of Coal Gangue and Soil Matrix on Plant Growth

In the pot planting experiment of coal gangue soil, setting different coal gangue content gradient, planting shallot, strawberry, garland chrysanthemum and ryegrass respectively, observing the difference of plant growth and biomass (fresh weight and dry weight), evaluating the response characteristics of different plants to coal gangue improved soil.

3.2.1 Effect of Different Coal Gangue Content on Plant Growth

The test results show that the sensitivity of different plants to coal gangue content is obviously different, and there is a specific optimal content interval for each plant, as shown in Fig. 9 to Fig. 12.

The sensitivity of shallot to coal gangue was low, and the growth and development of shallot were not promoted or inhibited obviously by different proportion of coal gangue. This was closely related to its physiological characteristics: shallot had developed roots, outstanding ability of bearing barren and stress, and could adapt to the slight fluctuation of soil physical and chemical properties after coal gangue improvement, showing strong environmental adaptability. The growth and fruiting performance of strawberry were significantly affected by coal gangue addition, showing obvious “optimal addition interval”. When the dosage of 50% was added, the soil structure was significantly improved, the vegetative growth and reproductive growth of strawberry were promoted, the number of leaves, the number of red fruits and the size of fruits reached the maximum, which was the best proportion under this experimental condition; when the dosage was lower than 20%, the improvement effect was not obvious; when the dosage was higher than 60%, the stress effect was obvious, and the growth of strawberry was seriously inhibited. The best dosage of garland chrysanthemum in coal gangue improved soil was 30%. Under this dosage, the comprehensive performance of roots was the best: The main root is thick and full of elongation, the lateral root is dense, the fibrous root is abundant, the root system is complete, the growth is obviously better than other treatments, there is no browning and rot phenomenon, the root depth is sufficient, the root system activity is at a high level, and effectively supports the healthy growth of the shoot. When the dosage was increased to more than 30%, root growth was inhibited significantly, taproot shortened, lateral roots reduced, fibrous roots sparse, root structure incomplete development, browning and necrosis appeared, root activity decreased significantly, water and nutrient absorption capacity was seriously damaged, which directly restricted shoot growth. When the coal gangue content was between 20% and 60%, the soil bulk density decreased, the aeration porosity increased, the soil environment was significantly improved, the growth of ryegrass in the early stage changed little, but the effect was significant in the late stage. When the coal gangue content was 60%, the ryegrass plant reached the maximum, the erect character was strong, the leaves were thick and green, and the growth trend was optimal. When the coal gangue content was more than 60%, the stability of water retention in the substrate decreased, the yellow leaves and slight yellowing appeared in the late stage of ryegrass, and the vegetative growth was obviously inhibited.

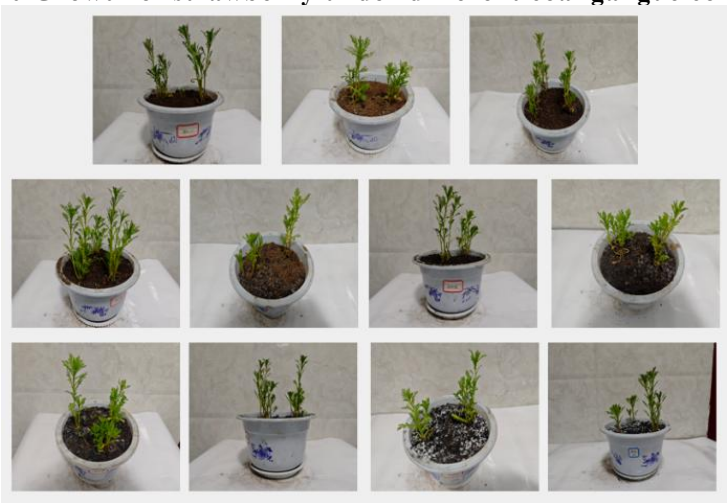
According to the indexes of plant growth cycle, the optimum dosage of coal gangue corresponding to different plants was: shallot 50%, strawberry 50%, garland chrysanthemum 30%, ryegrass 60%. It should be noted that the plants did not grow best in the pure gangue test group, mainly due to the obvious shortage of water retention capacity of pure gangue matrix, which restricted its application effect in ecological reclamation.



462
463 **Fig. 9 Growth of shallot under different coal gangue content**



464
465 **Fig. 10 Growth of strawberry under different coal gangue content**



466
467 **Fig. 11 Growth of garland chrysanthemum under different coal gangue content**

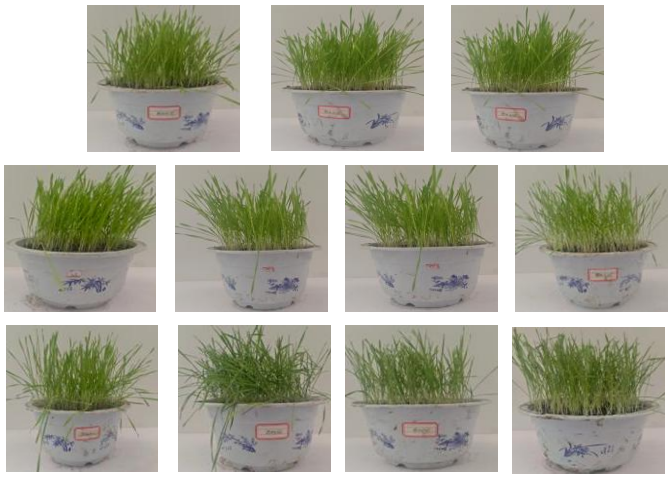


Fig. 12 Growth of ryegrass under different coal gangue dosage

3.2.2 Difference between fresh and dry plant weight

Fresh weight and dry weight of plants are core indicators reflecting biomass accumulation and growth status. The difference of fresh weight and dry weight of plants under different coal gangue-compound soil matrix treatments is shown in Fig. 13, showing a trend of "higher fresh weight, richer dry matter accumulation" as a whole.

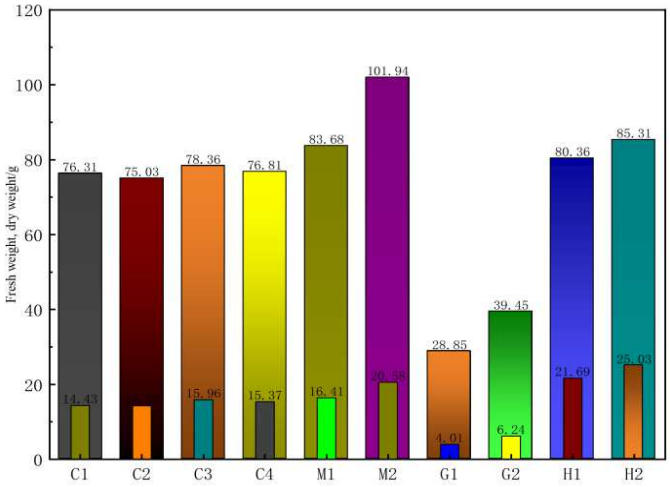


Fig. 13. The optimum dosage of coal gangue and composite soil matrix

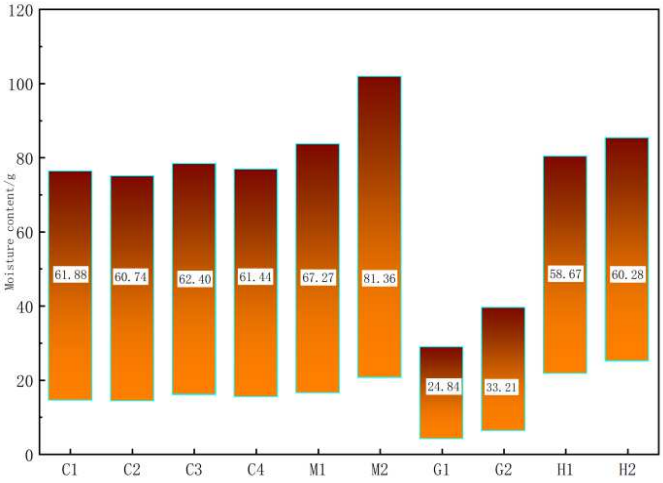


Fig. 14 Coal gangue-composite soil matrix group

As shown in Fig. 14, no matter what coal gangue content is added, the fresh weight and dry weight of cultivated shallot (C1 to C4 groups) have little difference, indicating that shallot is less sensitive to coal gangue content, and different addition ratios have no obvious promotion or inhibition effect on its normal growth

and development. Compared with strawberry planting (M1 to M2), M2 group showed the best performance, with fresh weight of 101.94g and dry weight of 20.58g, which were significantly higher than those of control group, indicating that strawberry biomass accumulation was vigorous and growth was good under this substrate formula. The fresh weight and dry weight of ryegrass (H1 group and H2 group) had little difference, which indicated that ryegrass had weak sensitivity to coal gangue addition and had no obvious inhibition and promotion effect on plant growth.

By calculating the moisture content (fresh weight-dry weight) of plants, it was found that the moisture content of shallot changed little between 60.5 and 62.5 g under different coal gangue content, while for strawberry, the moisture content was 81.36 g when coal gangue content was 50%, which was 14.09 g higher than that of control group, indicating that the growth of strawberry was promoted in this proportion matrix. Because garland chrysanthemum is sensitive to coal gangue, it can only be planted with low content of coal gangue, and its water content is lower than that of other three plants in the same growth cycle, so it grows poorly. According to the growth condition of plants, it is preliminarily judged that there is a specific optimal dosage interval of coal gangue among different plants, and during the optimal dosage interval, the fresh weight and dry matter accumulation of plants can be promoted, which can be used as the optimal substrate for ecological restoration of coal gangue dump.

3.3 Migration of heavy metals in gangue-soil matrix in plant system

In order to investigate the migration, distribution and accumulation of heavy metals in coal gangue soil matrix, pot experiments were carried out with shallot, strawberry, garland chrysanthemum and ryegrass as test crops under the optimal coal gangue dosage. The contents of Pb, Cd, Cr, Cu and Zn in roots, stems and leaves (fruits) of plants were determined respectively, and the distribution differences, enrichment abilities and transport characteristics of heavy metals in different parts of plants were systematically analyzed, and the ecological safety risks of soil matrix with optimum coal gangue content were evaluated accordingly.

3.3.1 Heavy metal migration differences

The content of heavy metals in the roots, stems and leaves (fruits) of Allium fistulosum, strawberry, garland chrysanthemum and ryegrass were determined under the optimum dosage conditions. The results showed that there were obvious interspecific differences and position differences in the distribution of heavy metals in different plants and organs, as shown in Fig. 15.

The heavy metal contents in all organs of Allium sativum L. showed a very significant distribution law, which was root> stem> leaf. As the primary part of Allium sativum L. contacting soil directly, the root was the main place for heavy metal absorption and accumulation, and its contents were significantly higher than those in all organs above ground, showing a strong heavy metal accumulation ability. This law might be closely related to the precipitation of root cell wall, the complexation of root exudates and the retention mechanism of root in plant. For strawberry, except Cr, the distribution of other heavy metals in different organs followed the same rule of root> stem> leaf, while garland chrysanthemum showed a similar trend, except for As, Pb and Cu. From the migration characteristics of heavy metals, Zn, Cu and other elements essential for plant growth are generally higher in all organs than other heavy metals, indicating that they are easy to transport from roots to shoots. These differences may be closely related to the chemical properties of heavy metal ions, the uptake pathways of plant roots, and the selective transport mechanisms of transport proteins in vivo.

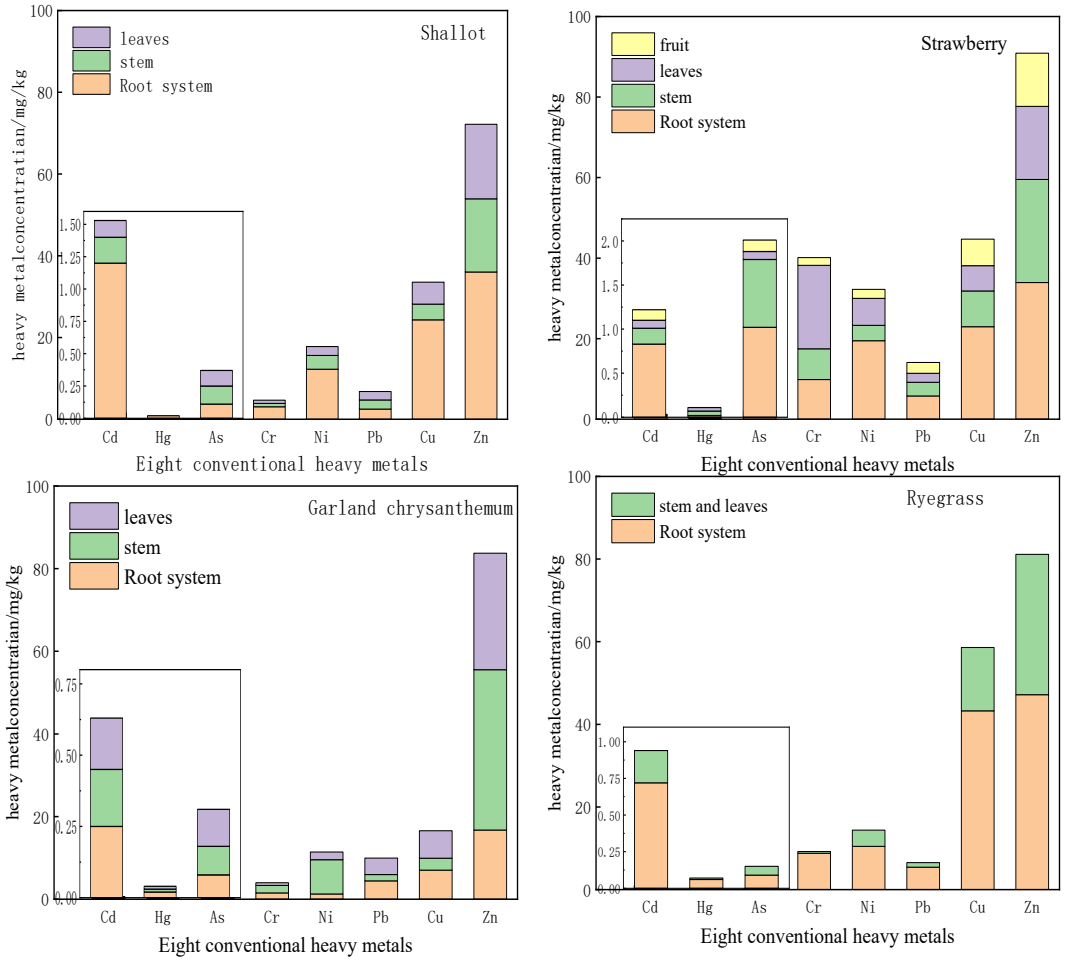


Fig. 15 Heavy metal contents in different parts of plants under the optimum conditions of gangue content

3.3.2 Transfer coefficient

As shown in Fig. 16, there are obvious differences in the absorption and distribution trends of heavy metals among the four tested crops. The distribution of some heavy metals in plants shows that the root enrichment is the highest, the stem is the second, and the leaf (edible part) is relatively low; while the other heavy metals show the distribution characteristics of the root enrichment is the highest, the leaf is the second, and the stem is the lowest. According to the transport coefficients of eight heavy metals, the transport ability of heavy metals in four tested plants was significantly different, among them, garland chrysanthemum had the strongest transport ability for Ni and Zn, and the transport coefficient of Ni was as high as 801.97%, showing a strong ability to transport heavy metals to the shoot, strawberry had a relatively strong transport ability for Hg and Cr. Shallots are outstanding in the transport of As and Pb; in contrast, ryegrass has the lowest transport coefficients for all types of heavy metals. Heavy metals are mainly retained in the roots and have weak upward migration capabilities.

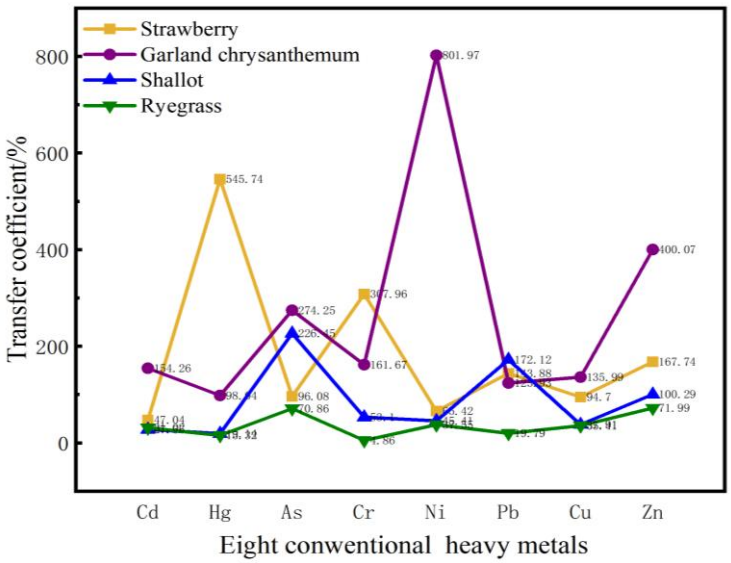


Fig. 16 Plant transport coefficient under the optimum condition of gangue content

In general, Ni, Hg and Zn have the strongest mobility among the four plants, while Pb and Cu have the weakest mobility. This rule is closely related to the ion radius, coordination ability of heavy metals and the selectivity of transport proteins in plants. From the perspective of ecological safety and land use, ryegrass has the best effect on heavy metals retention in its roots, which can effectively reduce the risk of heavy metals entering animals or human bodies through food chains. It is suitable as an ecological restoration plant in coal gangue contaminated areas. Although garland chrysanthemum and strawberry have some potential for remediation due to their easy transportation of heavy metals to the ground, they also bring high food safety risks and should not be planted as crops in coal gangue contaminated areas.

3.4 Analysis of microbial community composition, diversity and species difference in soil matrix

Related studies have shown that microbial community structure is closely related to soil characteristics. Changes in soil organic carbon content can drive geographical differences in soil microbial distribution(J. Liu et al., 2014), heavy metal concentration, soil pH and electrical conductivity (EC), etc(Fierer & Jackson, 2006; Zhalnina et al., 2015). Meanwhile, these factors will significantly affect the composition, diversity and species diversity of soil bacterial communities. Secondly, soil microbial community structure plays an important role in soil quality, which plays a vital role in the natural environment by facilitating material circulation, energy flow and environmental purification through complex network systems(Freilich et al., 2010).

3.4.1 Microbial colony composition

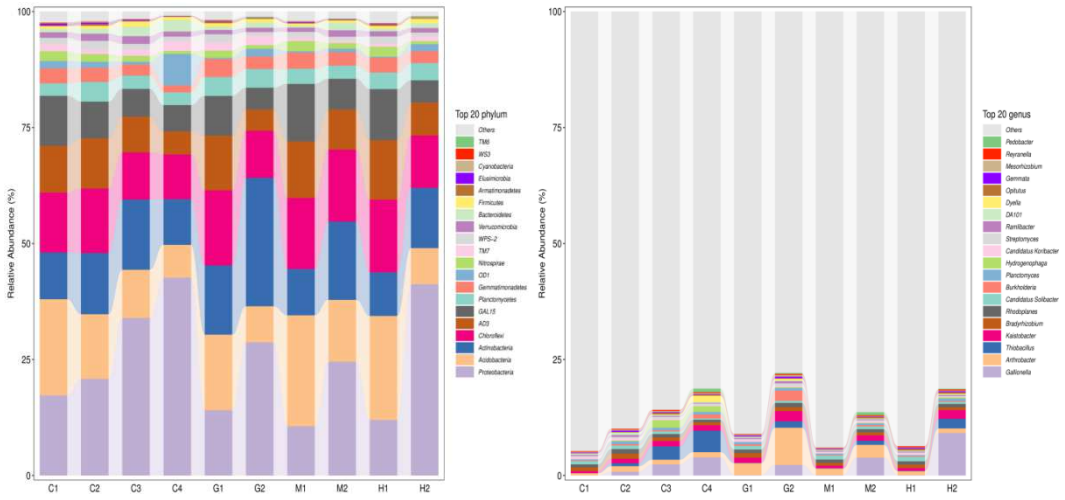


Fig. 17 Histogram of species composition at phylum and genus level

(C1-C4 are the coal gangue soil matrix at different doping levels for growing shallot, G1 and G2 represent the coal gangue soil matrix at different doping levels for growing garland chrysanthemum, M1 and M2 represent the coal gangue soil matrix at different doping levels for growing strawberry, H1 and H2 represent the coal gangue soil matrix at different doping levels for growing strawberry)

The stacked histogram of relative abundance of Top20 dominant genera at phylum and genus level is shown in Fig. 17. The abscissa is coal gangue-composite soil matrix with different content, the ordinate is relative abundance of species (%), and different colors represent different dominant genera.

At phylum level, Proteobacteria, Actinobacteria, Acidobacteriota and Chloroflexota were the dominant flora in each component. In contrast, Proteobacteria abundance increased significantly and Acidobacteriota abundance decreased in the experimental group, indicating that plant rhizosphere effectively improved the physical and chemical conditions of the substrate and promoted the recovery of eutrophic bacteria.

At the genus level, the dominant genera in each group mainly include Gaiella, Arthrobacter, Truepera, Kribbella and Brevibacterium, among which Gaiella, as a typical oligotrophic bacteria under Acidobacteria, its relative abundance increases with the increase of coal gangue blending ratio, while functional bacteria genera involved in organic matter decomposition and heavy metal tolerance In the experimental group, the relative abundance of these functional bacteria increased significantly, and different plants showed different enrichment patterns, indicating that plant root exudates could selectively promote the colonization of beneficial bacteria and improve the nutrient cycle and stress tolerance of the substrate.

For the same plant species, whether coal gangue is mixed in soil and its mixing ratio are the key variables affecting the relative abundance of rhizosphere bacteria. Further comparison of strawberry, garland chrysanthemum and ryegrass showed that there were significant differences in rhizosphere colony composition of different plants in both the control soil without coal gangue and the improved soil with coal gangue, which indicated that the effect of coal gangue addition was not independent, but closely related to plant species. The adaptability of different plants to soil physical and chemical properties, differences in root exudates composition and other factors jointly regulate the response path of microbial communities, and then determine the differences in bacterial genus composition and abundance changes. Secondly, the communities in sediments with extremely high metal content are obviously different from those in sediments with relatively low metal content(Kwon et al., 2015), indicating that metal content is also an important factor affecting microbial composition. Wu et al(Wu et al., 2026) showed that planting pioneer vegetation can reduce the extreme alkalinity of soil, increase

organic carbon, accelerate the alteration of primary minerals and the formation of secondary minerals, and accompany the reorganization of microbial communities and their ecological functions.

Combined with the coal gangue-composite soil matrix test, it is shown that coal gangue admixture significantly changes the soil microbial community structure and reduces the stability and functional diversity of the community; phytoremediation can effectively reverse this trend, which is attributed to the better soil environment created by plant root exudates, fallen root cells and mucus(Langille et al., 2013). Secondly, through rhizosphere effect to regulate the composition of dominant bacteria, improve the abundance of Proteobacteria and key functional bacteria, which also provides important microbiological support for the ecological restoration of coal gangue matrix.

3.4.2 Alpha Diversity Analysis

The microbial species accumulation curve showed that with the increase of sample number, the species accumulation curve gradually tended to be flat, indicating that the sequencing depth was sufficient, and the collected samples could fully characterize the true characteristics of coal gangue-soil microbial community, as shown in Fig. 18. At the same time, the horizontal axis of OTU-Abundance curve has a large span, and the overall downward trend is gentle, reflecting that the microbial species richness in the soil is relatively high, the community uniformity is good, and the microbial community structure is relatively stable, as shown in Fig. 19.

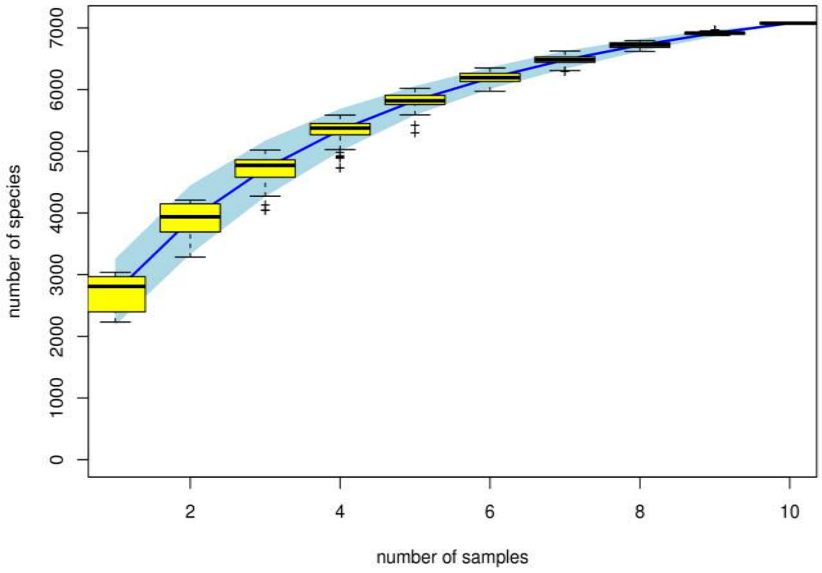


Fig. 18 Specaccum species accumulation curve

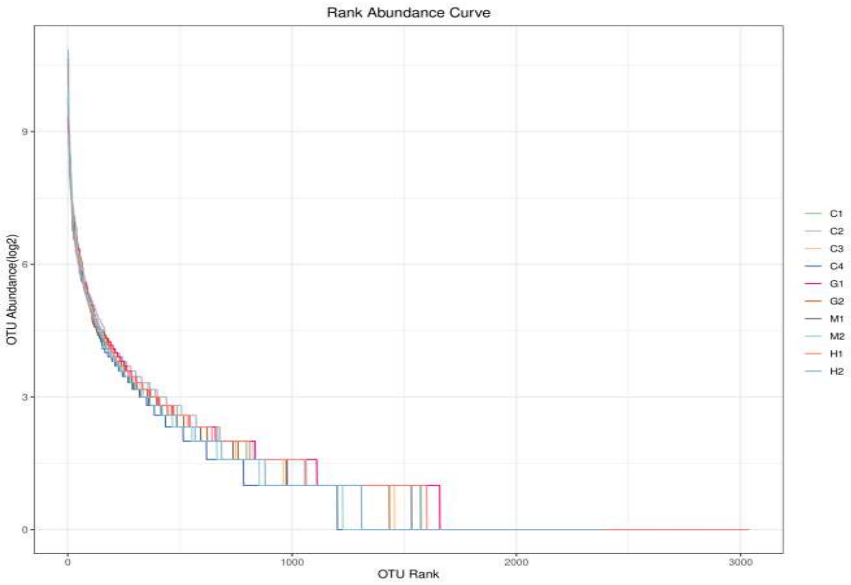


Fig. 19 Abundance grade curve

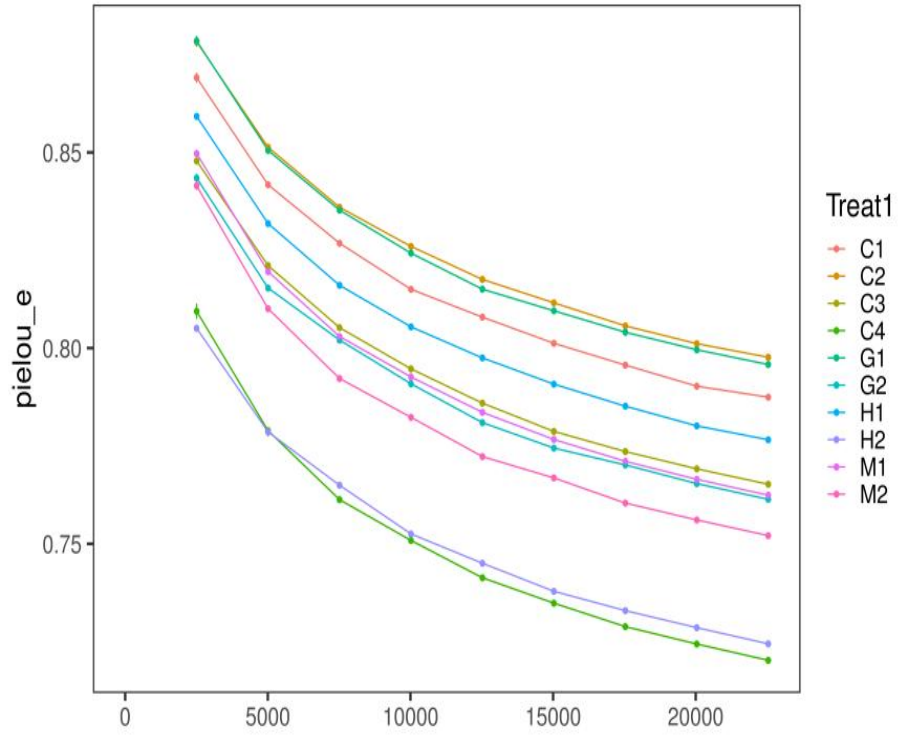


Fig. 20 Alpha diversity analysis

In order to further analyze Alpha diversity of root soils of different plants, correlation tests were conducted. The test results are shown in Fig. 20. It shows that there are significant differences in rhizosphere microbial diversity of four tested plants, and the overall trend is: garland chrysanthemum> ryegrass> strawberry> shallot. This difference pattern may be closely related to the characteristics of rhizosphere environment of different plants and their response mechanism to coal gangue improvement soil. The rhizosphere of garland chrysanthemum coronarium L. was activated by heavy metals, and the selective pressure in rhizosphere environment was enhanced, which promoted the adaptability and differentiation of microbial community and made its diversity the most abundant. However, the rhizosphere microbial species composition of Allium fistulosum is relatively simple and its uniformity is poor, which may be related to its strong selectivity of root exudates and insensitive response to changes in coal

gangue content.Related studies have shown that soil microbial diversity can also enhance soil nutrient cycling(Delgado-Baquerizo et al., 2016) and improve soil physical and chemical structure.

3.4.3 Species difference analysis

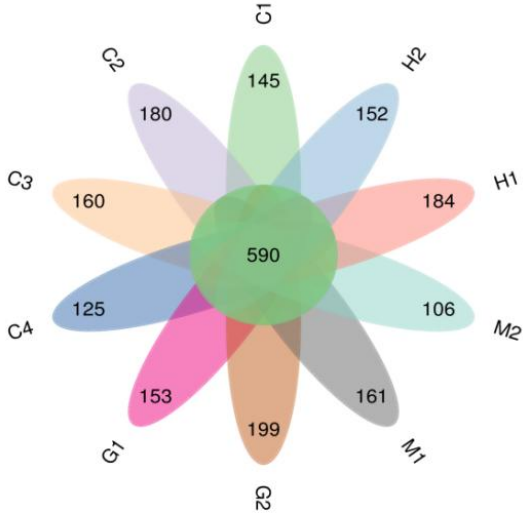


Fig. 21 Petal diagram of ASV/OTU sample (group)

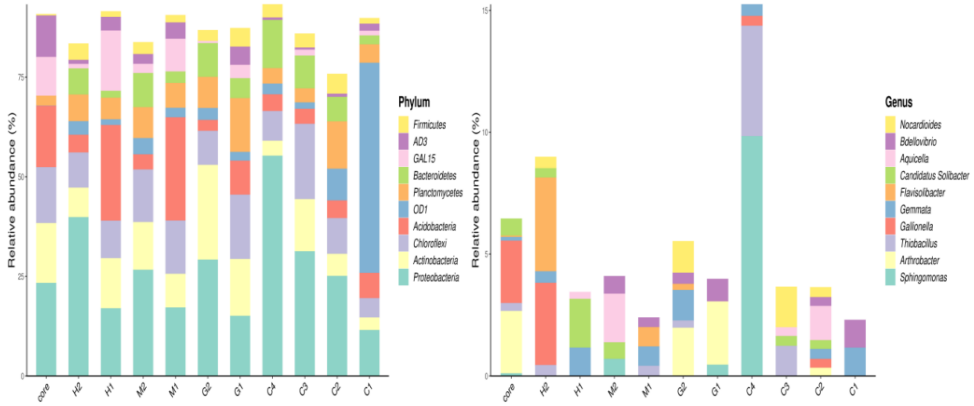


Fig. 22 ASV/OTU Abundance Histogram in Different Regions of Petal Diagram

According to the results of petal species distribution map and species composition at phylum and genus levels, there are a large number of 590 core microorganisms in rhizosphere soil of four plants, which constitute the heavy metal-resistant basic flora stably existing in coal gangue substrate environment. Meanwhile, a large number of unique microbial groups are also screened from different plants, among which the number of unique flora of garland chrysanthemum is the largest, which is consistent with the highest Alpha diversity, as shown in Fig. 21 and Fig. 22.

At phylum level, Proteobacteria, Bacteroides and Actinomycetes were the dominant phylum in each group. It should be noted that the abundance of Proteobacteria and Bacteroides increased significantly in the rhizosphere of garland chrysanthemum, which had the function of activating heavy metals in soil, and formed a synergistic effect with the high transport characteristics of Ni and Zn by garland chrysanthemum itself, thus strengthening the extraction effect of heavy metals. In contrast, the rhizosphere of ryegrass was characterized by the enrichment of actinomycetales and firmicutes, which were beneficial to the passivation and retention of heavy metals, and to the stabilization of heavy metals in the rhizosphere and the reduction of their migration to the shoot.

The rhizosphere of garland chrysanthemum was enriched with a large number of heavy metal-activated functional genera, which further supported its high ability of heavy metal migration; Lolium perenne was dominated by heavy metal-holding

genera, which was highly consistent with its characteristics of low transport coefficient and strong root holding ability; Allium sativum and strawberry had relatively weak flora specificity and less functional differentiation than the former two.

In conclusion, plants can achieve differential responses to heavy metal uptake, transport or retention by regulating the phylum and genus composition of rhizosphere microbial community, which provides theoretical support for plant-microbial remediation of heavy metal contamination in coal gangue matrix at micro level. In addition, proper amount of coal gangue can effectively improve the diversity of rhizosphere microorganisms and the abundance of beneficial bacteria, enhance the activation and supply capacity of soil nutrients, and create a good micro-ecological environment for plant root growth.

3.5 CONCLUSION

(1) The heterogeneity of gangue in Panzhou City, Guizhou Province results in its complex structure and mineral composition. The surface of gangue can be etched obviously by organic acid immersion, and its original compact structure can be destroyed. After acid etching, the BET specific surface area of gangue increases by 37.81%, the average pore diameter of adsorption and desorption increases by 60.94%, 49.11% respectively, and the pore volume also increases greatly.

(2) According to the growth condition of the tested plants, the requirements of different plants for the optimum coal gangue mixing ratio are significantly different: The sensitivity of shallot to the amount of coal gangue is relatively weak, through comprehensive analysis, the best growth ratio is 60%, and the best mixing ratio of other three plants is 50%, 30%, 70%.

(3) According to the four plants tested, the determination results of heavy metal contents in different organs (shallot, strawberry, garland chrysanthemum, ryegrass) showed that heavy metals were mainly enriched in plant roots, and the enrichment degree in stems and leaves varied with plants. In the same plant, different heavy metals had different transport capabilities, but the overall ability to migrate to aboveground parts was weak, which met the safety standards of agricultural soils and agricultural products as a whole, and the ecological risks were within the controllable range.

(4) The appropriate dosage of coal gangue in soil can significantly change the physical and chemical properties of coal gangue-soil matrix, and then regulate the composition, Alpha diversity and species differences of soil microbial community in plant rhizosphere.

Data availability

Raw 16S rRNA amplicon sequencing data were deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession PRJNA1532461. Review-only access link is available upon request.

https://dataview.ncbi.nlm.nih.gov/object/PRJNA1532461?reviewer=urdirsmfq2m1klv0mostvd890k.

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Author contributions

Jingbo Wang: Writing - original draft, Writing - review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. Xianhai Li: Writing - review & editing, Validation, Supervision, Project administration,

Methodology, Investigation, Conceptualization. Wei Cheng: Writing - review & editing, Investigation, Supervision. All authors reviewed the manuscript.

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Sample collection permission statement

All samples have obtained the collection permit from the mining management unit before sampling, and there are no conflicts of interest related issues.

Competing interests

The authAll samples have obtained the collection permit from the mining management unit before sampling, and there are no conflicts of interest related issues.ed the collection permit from the mining management unit before sampling, and there are no conflicts of interest related issues.nancial interest.

Additional information

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