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Moss biomonitors for heavy metal pollution in soils of Manganese Carbonate Mine across ecological succession stages

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ABSTRACT: Many plants have been widely used in monitoring and assessing heavy metal pollution in soil, air and water. However, few studies have considered the unique value of bryophyte communities in monitoring manganese ore pollution and the diversity characteristics of bryophytes in different natural succession stages. Tongluojing Manganese Mine in Guizhou Province, China, was chosen for identifying bryophytes and statistical analyses. In total 61 species of mosses in 21 genera of 7 families were identified, including 8 dominant species, primarily representing the turf life-form. Following the successive stages from bare rock to woodland, single-species moss communities decreased while multi-species communities increased, with increment of the α diversity index. However, the β diversity index showed the opposite trend. The moss similarity index was the highest (0.43) and Cody index lowest (7.5) on bare rock, while the indices were the lowest (0.17) and the highest (18), respectively, in woodland. The Nemerow pollution index decreased gradually with the successive stages, with the bare rock area being the most polluted. The soil was polluted by Pb, Cr, Zn, Cd and Mn to varying degrees, among which Mn was the biggest pollutant with a concentration 129 times higher than the background value of the soil in Guizhou Province. There was a positive correlation between the contents of Cd, Cr, Zn and Mn in *Weissia planifolia* Dix and those in the substrate, suggesting that *W. planifolia* can be used as an indicator plant. This study highlighted multiple effects of mosses on heavy metal absorption, which could be used as pioneer plants for vegetation restoration in the manganese ore waste rock accumulation area.

KEY WORDS: Biomonitors, heavy metal pollution, manganese carbonate mine, moss diversity.

INTRODUCTION

Guizhou is rich in manganese resources, accounting for about 60% of China's total manganese resources, of which Zunyi area is one of the main concentration areas (Hu *et al*., 2021). Manganese mining brings huge economic benefits, but also produces a large number of mine waste, tailings, electrolytic manganese slag and other solid wastes (Ren *et al*., 2020). It does not only occupy the land and destroys the landscape, but also the metal and semi-metal residues become more active under the influence of external factors, and easily migrate into groundwater, surface water, soil and organisms, causing serious damage to the surrounding ecological environment (Nath *et al*., 2021). According to reliable statistics, heavy metal pollution is the most serious among many soil pollution problems in China (Ávila-Pérez *et al*., 2019). Heavy metal contamination of soils refers to metals with a density greater than 4.5g/cm^3 (Ma *et al*., 2021), such As, Pb, Cd, Hg, Cr, and As, which are highly toxic and can affect the composition (Monni *et al*., 2000), structure, function, biomass and diversity of soil microbial community (Lopez *et al*., 2017), and can enter the human body through biological amplification of the food chain, thus endangering human health (Chang *et al*., 2019). Long-term consumption of food contaminated by heavy metals will lead to mutations in human DNA repair genes and metabolic enzymes (Khan *et al*., 2008), increase the incidence of diseases of esophagus, blood, kidney, nervous system, cardiovascular and cerebrovascular system and urinary system, and induce cancer and organ failure (Lin *et al*., 2016). Lopes *et al*. (2015) showed that metal concentration in contaminated soil may be 100-1000 times higher than its background level, and heavy metals are persistent. At present, heavy metal pollution is one of the most serious environmental problems prevalent in abandoned mining areas (Ruan *et al*., 2021). Therefore, it is of great significance and urgent to find a sensitive indicator plant that can effectively monitor the soil heavy metal pollution in the waste rock accumulation area of manganese ore.

At present, some studies have been done on the monitoring and remediation of heavy metal pollution by vascular plants (Ortiz-Oliveros *et al*., 2021; Beringui *et al*., 2021; Turkyilmaz *et al*., 2018; Sevik *et al*., 2018). Ortiz-Oliveros *et al*. (2021) showed that the succulent plant *Echeveria elegans* Rose had a strong cumulative effect on heavy metals; Mani *et al*. (2021) showed that *Dendranthema morifolium* (Ramat.) Tzvel. had a significant purification effect on Pb contaminated soil; Guo *et al*. (2021) found that *Acorus calamus* L. and *Raphanus sativus* L. have strong enrichment ability for Cd. Zhu *et al*. (2021) in their research on soil contaminated by heavy metals in coal mining area showed that species of *Melilotus luteus* Gueldenst, *Trifolium* L. and alfalfa, as well as *Pteris vittata* L. significantly reduced the degree

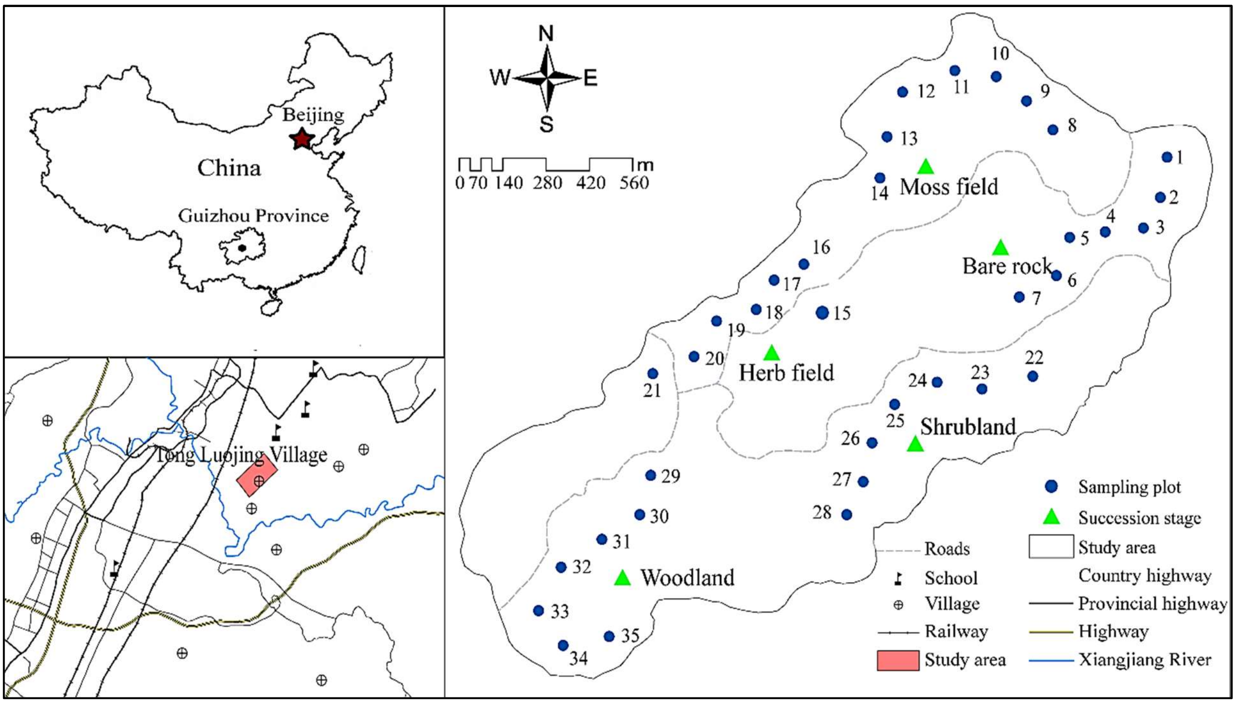

Fig. 1. Schematic diagram of sampling locations in the study area

of heavy metal pollution in soil and had significant ecological benefits. However, the monitoring of carbonate manganese ore environment by analyzing bryophyte communities is rarely reported. Compared with vascular plants, bryophytes have a single layer of cells on their leaves and lack a protective cuticle on their surfaces (Angelovska *et al*., 2016). Bryophytes are more sensitive to pollutants in air or water than vascular plants and are often used as indicators of environmental pollution (Li *et al*., 2020). In addition, bryophytes have strong adaptability to harsh environments (Angelovska *et al*., 2016), and bryophytes are often used as ecological monitoring materials in some areas with low plant coverage and serious pollution, such as the Toluca Valley in Mexico (Zarazúa-Ortega *et al*., 2013; Caballero-Segura, 2014) and roads (Beringui *et al*., 2021) in rocky desertification areas.

This study takes Tongluojing Manganese ore and its surrounding environment in Guizhou Province as the research area. Moss vegetation and underlying soil in different natural succession stages were selected as research objects. Through the combination of field investigation and laboratory study, on the one hand, we contribute the hitherto missing data about bryophyte communities in Guizhou carbonate manganese. On the other hand, by studying the correlation between moss community structure and heavy metal pollution, the indicator role of moss community on heavy metal pollution of carbonate manganese ore and its value in heavy metal monitoring and evaluation are explored, so as to accumulate basic data for environmental monitoring.

MATERIALS AND METHODS

Overview of the region

Tongluojing Manganese Mine produces a sedimentary manganese carbonate ore (Li and Xie, 1984; Weng and Zhou, 1984). The manganese ore primarily consists of siderite and manganocalcite, followed by manganese calcite and iron siderite (Wu *et al*., 2012). The waste rock tailings dump is located in Hetaowan, Minzhu Village, Honghuagang District, Zunyi City, Guizhou Province (N23°53'05" – 23°57'55", E106°17'13" – 106°21'37"), an area alternating between low mountains and trough valleys (Weng and Zhou, 1984; Zhang, 1987). It has a semi-humid spring and summer, distinct seasons, warm winters and a cool summer subtropical monsoon climate; the mean annual temperature is 12.2℃ – 16.9℃; average frost-free period is 227.4 – 330.3 days (Hristozova *et al*., 2020). The climate is mild, with abundant precipitation. The distribution of sampling points is shown in Fig.1. Pictures of habitats at each successional stage are shown in Appendix I, Figure S1.

Sample collection and species identification

Field investigation and sample collection of Tongluojing Manganese Mine and its surrounding environment in the Zunyi area were carried out from 1 September 2019 to 5. Supplementary samples were carried out in the study area from April 7 to 10 of the following year. Field investigations showed that the slag accumulation time varied greatly in different areas of Tongluojing Manganese mine. Therefore, the space-for-time substitution was chosen in order to use sites of different age for assessing the temporal sequence. For this purpose, the slag accumulation area was divided into 5 stages of natural succession according to the time of ore dumping (Fig. 1): bare rock (less than half a year); moss field (six months to a year); herb field (one to two years); shrubland (two to five years); woodland (more than ten years). Habitat profiles and dominant species at each successional stage are shown in Appendix, Table S1. Seven sampling points were set out at each successional stage, and 5 quadrats for each sampling point, each quadrat measured 10 cm × 10 cm. All the mosses in each quadrat were collected, placed into specimen bags and taken back to the laboratory. A 10 cm × 10 cm metallic square was used to determine moss cover. A total of 175 samples was collected. Cover height, light level, temperature, longitude and latitude, habitat and other information were recorded for each point. Surface soil from 5 quadrats (10 cm × 10 cm × 2 cm) was collected using a sampling knife, and the sampling knife was cleaned at all times to ensure purity of the sample. The surface soil of 5 quadrats was taken by quartering, a total of 1000 g, as the soil samples of one sampling point, and the samples were numbered and put into sealed bags.

The morphological characteristics of the moss collections prior to drying were examined and observed. A small number of samples was selected and soaked in water. The plants were removed from water, and with leaves carefully extended for examination, placed on a glass slide and covered with a cover glass (Huang and Zhang, 2017). The samples were dissected with HWG-1 dissecting microscope (Hangzhou No.2 Radio equipment Factory) to obtain the cross section of moss stems and leaves, and investigated using a BDS-200 inverted biological microscope (Chongqing Otter Optical Instrument Co, LTD). Identification of mosses was carried out with reference to 6 editions of *Chinese Mosses* (Li, 2006; Hu, 2005; Wu, 2002, 2004; Gao, 1994, 1996), and the results were recorded.

Sample treatment and content determination

After separating the moss and soil, the soil from each sampling plot was mixed into a new soil sample, resulting in a total of 35 soil samples. For the bryophyte samples, excess debris was removed, they were rinsed with ionized water, then dried and ground, and 0.2 g of each sample removed for digestion. The soil samples were dried to a constant weight at 60℃ and ground to a fine powder using an agate mortar. The samples were then filtered through a 100-mesh sieve. 0.2 g soil samples was weighed into digestion tubes, and nitric acid (6 ml), hydrofluoric acid (3 ml), and perchloric acid (1 ml) were added to the digestion tubes for analysis. The digestion apparatus was heated to 100℃ for 1 h under reflux and then cooled to room temperature. After cooling, the digestion apparatus was heated to 140℃, retaining only 0.5 ml of acid. Measurements were then taken after adding 2% nitric acid to maintain a constant volume of 50 ml. Metal content of the samples was analyzed using an AutoDigiBlock (Beijing Laibotec Instruments Co., Ltd.) fully automated digestion instrument and a NexION 300X inductively coupled plasma mass spectrometer (Platinum Elmer or PerkinElmer). The instrument was set to: RF power 1150 W; cooling gas 18 L min^{-1}; auxiliary gas 1.2 L min^{-1}; atomizing gas 0.81 L min^{-1}; sampling depth 11 mm; peristaltic pump 20 r•min^{-1}; measurement mode STD; internal standard element 103 Rh. The operating instructions for the instrument were carefully followed: a mixed standard working solution was adopted and internal standard method was added online. Quality of reagents used were 68.0 – 70.0% nitric acid (Suzhou Jingrui Chemical Co., Ltd.), 48.8 – 49.2% hydrofluoric acid (Suzhou Jingrui Chemical Co., Ltd.), 70.0 – 72.0% perchloric acid (Sinopharm Holding Chemical Reagent Co., Ltd.), 99.99% argon gas, and ultrapure water.

Data analysis

The Shapiro-Wilk test was completed in stats package of R to evaluate the normal distribution of data. One-way ANOVA was completed in multcomp package of R to analyze the differences in metal content along a pollution gradient. Pearson correlation coefficient analysis and Significance test were performed using psych package of R.

Measurement of α diversity

The moss diversity index was calculated for the 5 stages of natural succession in order to understand the composition and distribution of mosses in the area of the mine tailings dump. This was calculated using the Patrick index, Shannon-Wiener index and the Pielou index.

Patrick index (S_j):

$$S_j = \sum_{j=1}^{n} \frac{X_{ij} - \overline{X_{ij}}}{\overline{X_{ij}}} \tag{1}$$

In this formula, X_{ij} is the data of the jth taxon in the n taxon of the ith region in k regions; $\overline{X_{ij}}$ is the average value of the data of the jth taxa in n taxa in k regions; n is the number of classification levels.

Shannon-Wiener index (H):

$$H = -\sum_{i=1}^{s} \left(\mathrm{P}_i \ln \mathrm{P}_i\right) \tag{2}$$

Pielou index (J):

$$\mathrm{J} = \mathrm{H} / \ln S \tag{3}$$

In the formula, $P_i = N_i/N$, N_i is the coverage of the ith species in each quadrat, N is the sum of the coverage of S species, and S is the number of species per sampling point.

Measurement of β diversity

The species replacement rate between quadrats was obtained by calculating the similarity coefficient of each other's species composition at five different natural successional stages.

Sorensen index (Wang and Zhang, 2020):

$$S_c = 2c/(a+b) \tag{4}$$

Cody index:

$$\beta_c = [g(H)+l(H)]/2 = (a+b-2c)/2 \tag{5}$$

In these formulae, *a* is the number of common species in the two succession stages, *b* and *c* are the number of unique species in each two succession stages. If diversity ranges from 0 – 1, then communities are very similar if values range from 0 – 0.25; communities are moderately similar if values range from 0.25 – 0.50; communities are not similar if values range from 0.50 – 0.75; communities with values between 0.75 and 1.00 are very dissimilar (Wos *et al*., 2018).

Nemerow index

The Nemerow single factor index (Tang *et al*., 2019) was used to determine which heavy metals contaminated the soil of the manganese tailings dump. In terms of the pollution index:

$$P_i = \frac{S_i}{B_i} \tag{6}$$

P_i is the single pollution index; S_i is the measured value of heavy metal element *i* in the soil surface layer; and S_i is the background value of heavy metal element *i* in surface soil of Guizhou Province (Song and Shen, 2017). Soil heavy metal pollution is divided into 5 levels based on the pollution index (Balabanova *et al*., 2017; Long and Zhang, 2016).

RESULTS

Analysis of moss diversity in the tailings dump of the Tongluojing manganese mine

Moss species distribution and composition

In order to understand the bryophyte species composition in the study area, all species were analyzed statistically (Fig. 2). A total of 61 species of mosses in 26 genera and 7 families were identified in the tailings dump of the manganese mine (Appendix, Table S2). Pottiaceae and Brachytheciaceae were the dominant families, accounting for 32% and 39% of species, respectively. *Bryum* Hedw. was the dominant genus, accounting for 32% of all species. Dominant species (species occurrence frequency greater than 5 times were identified as dominant species) included *Ditrichum pallidum* (Hedw.) Hampe, *D. difficile* (Duby) Fleisch., *Bryum argenteum* Hedw., *B. blindii* Bruch & Schimp., *Weissia planifolia* Dix, *Barbula subcontorta* Broth. and *Trichostomum barbuloides* (Broth.) chen. *Bry. argenteum* and *Bry. blindii* are mainly distributed in the bare rock area, *Bar. subcontorta* in the moss field, *Tri. barbuloides* in the herb field, while *Dit. pallidum* and *Dit. difficile* are mainly distributed in shrubland and woodland. *W. planifolia* is widely distributed in all five stages of natural succession. Vegetation cover gradually increased along successional stages, as did the number of moss taxa: the trend for families: bare rock (3 species) < moss field (4) < herb field (4) <shrubland (5) < woodland (6); genera: bare rock (4) <moss field (5) < herb field (8) < shrubland (10) <woodland (13); and species: bare rock (5) < moss field (18) < herb field (22) < shrubland (23) < woodland (31) (Fig. 2).

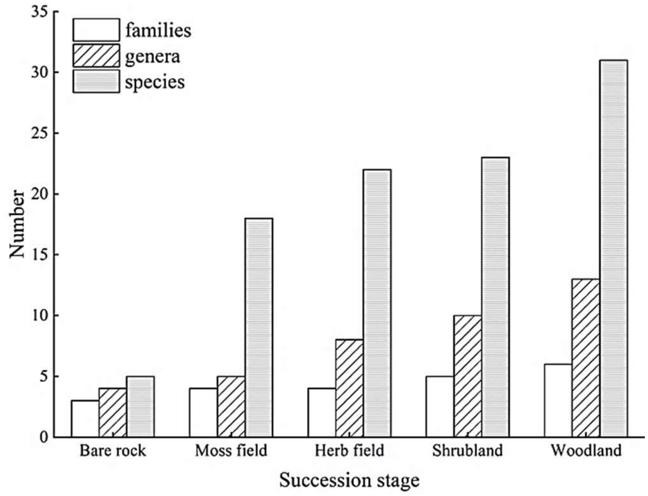

Fig. 2. Quantitative statistics of mosses community in different stages of natural succession

Distribution of life forms and structural composition of mosses

In order to understand the life forms and community structure composition of moss communities in different natural succession stages, percentage histogram analysis was performed on all species. The proportion of short turfs was highest in each natural succession stage, followed by tall turfs and finally wefts. Turfs dominated all successional stages (Fig. 3), particularly bare rock, moss field and herb field; wefts were present in the shrubland and woodland where they accounted for 12% and 27% of life forms, respectively. In the successional stages of this study, the number of single-species moss communities gradually decreased with a corresponding increase in multi-species communities (Fig. 3). Numbers of single-species communities recorded for each successional stage were: bare rock (7); moss field > (4); herbfield > (2); shrubland > (1); woodland > (0). The number of multi–species communities increased from: bare rock (0); moss field (3) < herb field (5); shrubland (6) < woodland (7).

Trend in variation of moss α diversity index

In order to comprehensively evaluate moss diversity at each stage of natural succession, the Shannon-Wiener

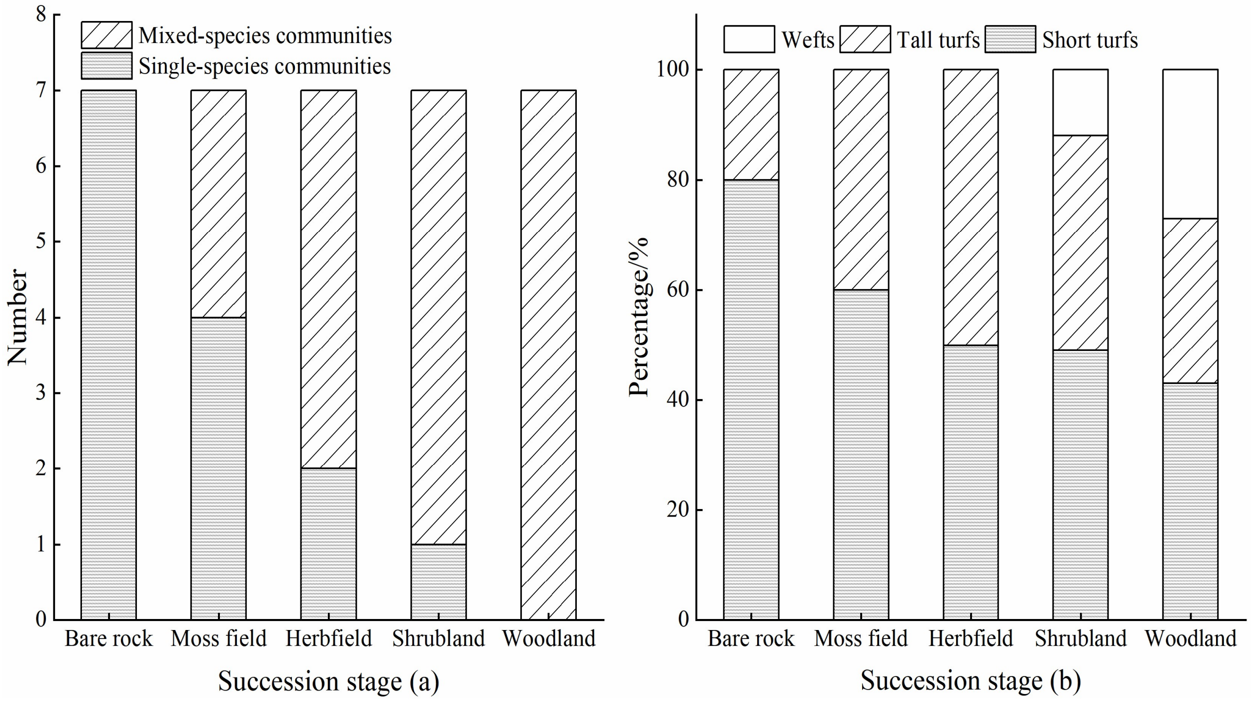

Fig.3. Community structure and life forms of mosses in different stages of natural succession

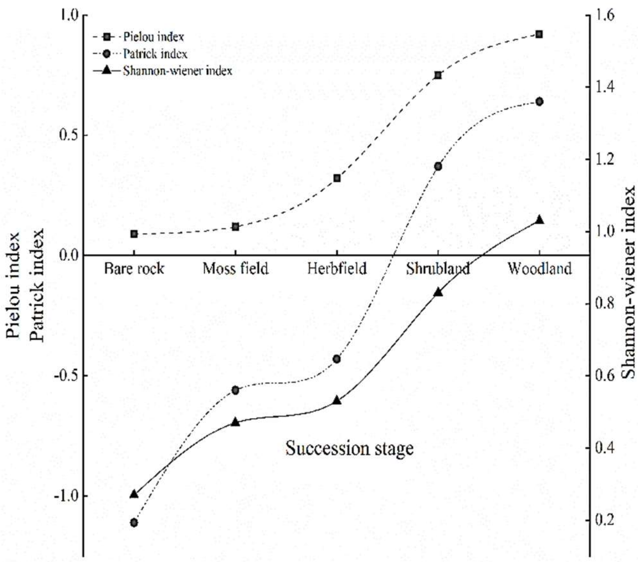

Fig. 4. Changes in α-diversity indices of mosses in each stage of natural succession

index was used to represent species diversity, Pielou index to represent evenness, and Patrick index species richness (Fig. 4). The trend in variation of all three indices was roughly the same; all gradually increased in line with successional stages; Patrick index: bare rock < moss field < herbfield < shrubland < woodland; Shannon-Wiener index: bare rock < moss field < herbfield < shrubland < woodland; Pielou index: bare rock < mossfield < herbfield < woodland < shrubland.

Table 1. Changes in *β*-diversity indices of mosses in the various stages of natural succession

	Succession stage	1	2	3	4	5
	1	-	**0.43**	0	0	0
Rensen	2			**0.4**	0.14	0.18
similarity index	3				**0.22**	0.20
	4					**0.17**
	5					-
	1	-	**7.5**	12	14	8.5
	2			**12**	14	8.5
Cody index	3				**12.5**	8.5
	4					**18**
	5					-

Note：1&2, 2&3, 3&4 and 4&5 represent the adjacent natural succession stages. 1 Bare rock；2 Moss field；3 Herbfield；4 Shrubland；5 Woodland.

Trend in variation of moss β diversity index

In order to determine the correlation between the composition of moss communities at each successional stage, the Rensen index was used to represent the similarity coefficient of species composition in adjacent successional stages, and the Cody index was used to represent the replacement rate of species in adjacent successional stages (Table 1). The difference between the two indices is significant and there is an overall opposite trend in change. The Rensen similarity index varied from 0.17 to 0.43, with the highest similarity index (0.43) recorded on the bare rock and in the moss field; the lowest similarity index was recorded in the herbfield and shrubland (0.17). The Cody index varied between 7.5 and 18; the replacement rate was lowest (7.5) on bare rock and

Table 2. Pearson correlation coefficient of heavy metal content in ***Weisia planifolia*** and matrix.

	Cd_1	Cd_2	Cr_1	Cr_2	Pb_1	Pb_2	Zn_1	Zn_2	Mn_1	Mn_2	Cu_1	Cu_2
Cd_1	1											
Cd_2	0.984^{**}	1										
Cr_1	0.937^{*}	0.901^{*}	1									
Cr_2	0.916^{*}	0.916^{*}	0.968^{**}	1								
Pb_1	0.931^{*}	0.945^{*}	0.959^{**}	0.991^{*}	1							
Pb_2	0.931^{*}	0.939^{*}	0.746	0.735	0.776	1						
Zn_1	0.936^{*}	0.875	0.987^{**}	0.922^{*}	0.908^{*}	0.755	1					
Zn_2	0.976^{**}	0.976^{**}	0.951^{*}	0.974^{**}	0.976^{**}	0.866	0.922^{*}	1				
Mn_1	0.962^{**}	0.931^{*}	0.933^{*}	0.860	0.891^{*}	0.871	0.943^{*}	0.907^{*}	1			
Mn_2	0.979^{**}	0.959^{**}	0.923^{*}	0.867	0.900^{*}	0.913^{*}	0.929^{*}	0.927^{*}	0.995^{**}	1		
Cu_1	0.947^{*}	0.948^{*}	0.780	0.780	0.805	0.991^{**}	0.789	0.901^{*}	0.864	0.907^{*}	1	
Cu_2	0.956^{*}	0.906^{*}	0.962**	0.933^{*}	0.908^{*}	0.815	0.972^{**}	0.958^{*}	0.900^{*}	0.907^{*}	0.864	1

Note: 1 represents the content of heavy metals in ***W. planifolia***; 2 represents the content of heavy metals in the soil. $^{*}P<0.05$ (bilateral); $^{**}P<0.01$ (bilateral).

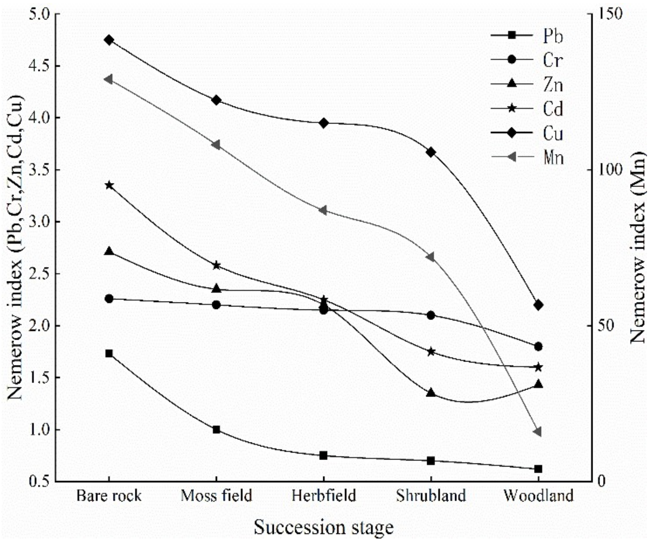

Fig.5. Nemerow single factor pollution index in different stages of natural succession

in the moss field and highest (18) in the shrubland and woodland.

Analysis of heavy metals in mosses and underlying soil

Analysis of soil heavy metal element content and evaluation of metal pollution

In order to understand the degree of heavy metal pollution in the tailings dump, heavy metals in the underlying substrate soils were evaluated using the Nemerow index with reference to the background values and pollution classification standards for Guizhou Province. The study areas of the tailings dump were contaminated by heavy metals to varying degrees, with bare rock most seriously polluted with content of six metals exceeding the standard (Fig. 5): Pb (1.73); Cr (2.26); Zn (2.71); Cd (3.35); Mn (129); Cu (4.75). On bare rock, the level of Pb was light, at level III; the levels in the remaining four successional areas were even lower, but not at levels that could be ignored. The pollution levels of Cr and Zn in soil were recorded at level IV (moderate pollution), indicating serious soil pollution in the different successional stages. Mn pollution was the most serious and all five successional stages were severely polluted. The content of heavy metals on bare rock was determined at 129 times higher than the background value of soil in Guizhou Province.

Relationship between mosses and metal element content in soil

In order to explore the relationship between moss and the contents metal elements in the soil, Pearson correlation analysis was first performed on them (Table 2), with the results that Zn-Pb, Mn-Pb, Mn-Zn, Cd-Pb, Cd-Zn, Cr-Mn and Cr-Cd showed significant positive correlation in *Weissia planifolia* ($P < 0.05$); Cr-Pb, Cr-Zn, and Cd-Mn in *W. planifolia* showed extremely significant positive correlation ($P < 0.01$).

Since the contents of Cd, Cr, Mn and Zn were correlated in both *W. planifolia* and soil, they were used to do linear fitting (Fig. 6), which showed similar changes in content of heavy metals in *W. planifolia* and soil, demonstrating an overall increasing trend. As the content of heavy metals in the soil increased, so too did the content of heavy metals in *W. planifolia*. The Mn fitting degree between the soil and *W. planifolia* was the best, and the two were positively correlated ($P < 0.01$). The Mn fitting equation of *W. planifolia* and the soil was $Y = -101.66 + 0.55X, R^2 = 0.99,\ P < 0.01$.

DISCUSSION

Characteristics of moss diversity at different stages of natural succession

The external morphology of each moss taxon is manifested through long-term adaptation to environmental conditions and is referred to as its life form (Ma *et al.*, 2018). Life forms have been classified according to Mägdefrau (Angelovska *et al.*, 2016), and

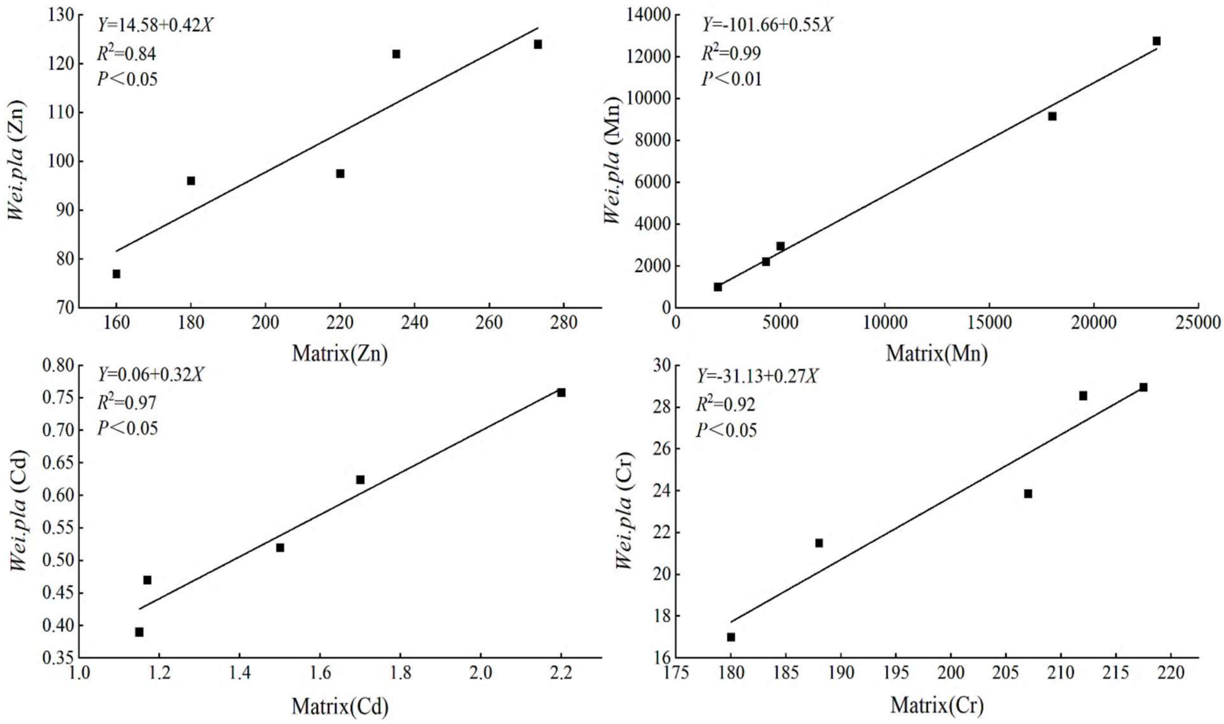

Fig. 6. Linear fitting of heavy metal content between ***Weissia planifolia*** (*W. pla*) and soil.

divided into short turfs (< 1cm), tall turfs (≥1cm) and wefts. Moss communities were mainly distributed according to the ecological environment in which they were growing and by the characteristics of the underlying soil substrate (Dawes *et al*., 2020) so that moss communities are the result of the combination of the morphological characteristics of mosses and environmental factors. Moss communities are also well suited to reflect the characteristics of ecological environments. The structure of this study shows that the short turfs are dominant in the primary succession stage. This is consistent with the research results of many scholars (Liu and Zhang, 2016; Long and Zhang, 2016). Since hort turfs help bryophytes to absorb water and improve their adaptability, they are usually dominant in arid, extreme, harsh and polluted environments (Ren *et al*., 2020). Different characteristics of plant communities can reflect environmental conditions in different succession stages, and environmental changes can also affect the distribution of plant communities (Coelho *et al*., 2021). The increase in diversity of the moss communities along successional stages was accompanied by a gradual increase in the number of moss taxa. These results are different from those of other mines in Guizhou, for example, of the tailings piles from bauxite mines in Guizhou where there was firstly an increase in species number, followed by a decline (Huang and Zhang, 2017). This can be explained by the relatively short period of accumulation (Gao and Zhang, 2008), the fact that the associated woodland is not totally covered by trees and shrubs, and although there is both tree and shrub cover, there are few individuals so the canopy density is low and there is no decline in species diversity in these later stages of succession (Chen *et al*., 2013; Tucker and Farge, 2021). This may also be related to the presence or absence of particular minerals and this aspect needs further study.

The characteristics of communities and ecosystems can be expressed by species diversity, which directly or indirectly reflects the types of structure, stages in development and differences in habitat of communities and ecological systems (Hurlbert, 1971; Zheng, 1998; Gao and Zhang, 2008). Statistical analysis of moss diversity indices showed consistency in the variation trend of the Shannon-Wiener diversity index, Pielou index and Patrick index. The greater the uniformity index at each successional stage, the more habitable the environment and the better suited to facilitate the characteristics of a diversity of moss species: the greater the species richness, the higher the diversity index. In sites with low levels of evenness, environments are more extreme, but also more uniform, providing habitats which are only habitable by relatively few species. Competition amongst communities also leads to lower species richness and a lower diversity index. However, the β diversity index of the Cody and Rensen indices showed an opposite trend, so that the greater the similarity index of adjacent successional stages, the lower the replacement rate. Greater distance between successional stages is reflected in fewer of the same species of mosses being present and the reduced similarity in species between communities. In the later stages of succession, shrubland and woodland, vegetation cover was complex and mosses were more abundant and more diverse.

Survival of mosses is highly dependent on environmental humidity (Kaufmann and Berg, 2014) and life forms of moss communities can reflect the local environmental conditions. Most short turfs are distributed in exposed, barren and dry areas (Wu, 1998), while most wefts are found in sheltered, shaded areas with high humidity (Chen *et al*., 2013). In all five successional stages of this study, from bare rock to woodland the life forms of mosses were dominated by short turfs. Each successional change was accompanied by a gradual change in vegetation and habitat, wefts appeared in both herbfield and shrubland. Short turf mosses are well able to adapt to extreme habitats, including exposed rock surfaces, extremely high light levels, heavy metal pollution, and with little shelter afforded by sparse vegetation (Liu and Zhang, 2016).

Analysis of heavy metal elements in moss and soil

The Nemerow single factor pollution index confirmed high levels of Mn, Cr, Zn and Pb in the area of the tailings dump, which is consistent with those of Lu *et al*. (2014) and Xie *et al*. (2007). Analysis of heavy metals contents in mosses and the underlying soil showed a significant correlation between Cd, Cr, Mn and Zn, and that the increase in these heavy metals in the mosses could be apportioned to the content of heavy metal elements in the soil, a result consistent with the studies of Long and Zhang (2016) and Bargagli *et al*. (1987). The degree of contamination of metal elements in mosses and soil can be predicted by monitoring the content of metal elements in mosses. Mosses can then be used as biological monitors to reflect the content of heavy metals in the underlying soil, and indicating the degree of heavy metal pollution in the local soil, and as a scientific basis for monitoring and treatment of heavy metal pollution in the area of the tailings dump.

The regression diagnosis of the linear model enrichment coefficient was used to reflect the effect of metal absorption of the bryophyte communities. Due to synergistic or antagonistic effects, different elements in plants may promote or inhibit the absorption of elements, and the correlation between different elements can reflect whether such a relationship exists between elements (Woods *et al*., 2021; Zeng *et al*., 2002). The significantly positive correlation between two elements implied synergistic effect between them, and antagonistic effect verse vice (Yuan *et al*., 2006; Jiang and Zhang, 2013). Positive correlation indicates that the sources of the two elements may be similar; weak negative correlation or no correlation indicates that the combination of the two elements has an antagonistic effect, or that the combined pathways of the elements may be different (Gulanet *et al*., 2020; Coelho *et al*., 2021). The levels of Cr-Pb, Cr-Zn and Cd-Mn in mosses were highly significantly correlated, leading to speculation that the sources of these elements are similar, and that there is a synergistic effect when the elements are absorbed.

CONCLUSIONS

This study explored moss species and heavy metal (Mn, Cu, Pb, Zn, Cr, Cd) contents in soil samples obtained from the waste slag accumulation area of Tongluojing manganese mine. The findings of this study provide valuable references for other manganese mining areas with similar levels of heavy metals, and help in control and monitoring of heavy metal pollution. The study key findings are as follows:

(1) Species composition of mosses in different natural succession stages in Tongluojing manganese slag accumulation areas differed significantly. The bryophyte species were most abundant in woodland, while the bryophyte species were poor in bare rock area. The dominant species were different at each stage, but *W. planifolia* was widely distributed and distributed at all stages.

(2) Correlation analysis showed that bryophytes have multiple effects on heavy metal uptake, and can be regarded as the pioneer plants in the restoration of heavy metal contaminated areas. At the same time, heavy metal elements in moss were enriched in different degrees at each succession stage. There was a positive correlation between the heavy metal elements in moss and its growth matrix. The linear fitting of heavy metals Cd, Cr, Mn and Zn in *W. planifolia* and its growth matrix is well, so it can be considered as an indicator plant for monitoring heavy metals in the soil of manganese ore residue accumulation area.

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