Bioindication of heavy metals using bryophyte communities in the Songtao manganese carbonate ore region, China

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Abstract

Mining and mineral processing are often associated with heavy metal pollution. To reflect the environmental conditions of manganese carbonate ore deposits and of the area surrounding an electrolytic manganese plant, we analyzed the composition and species diversity of bryophyte communities and determined the differences in bryophyte communities related to the content of heavy metals in the substrate. We found that Pottiaceae and Bryaceae were the dominant families in the study area. The species abundance index of bryophyte communities at different locations was in order: vicinity of the mine > mine site > herb-shrubland > electrolytic plant > electrolytic waste dump. The species diversity of bryophyte communities at different locations was in order: herb-shrubland > vicinity of the mine > electrolytic waste dump > mine site > electrolytic plant. Significant differences between heavy metal contents of the substrate were found among the different locations with the exception of Co ($p < 0.05$). Mn contents were significantly different at each location ($p < 0.05$). CCA showed that Mn, Cd, Hg, and Pb content in the substrate were the major factors affecting the characteristics of the bryophyte communities. From the combination of CCA with the correlation heat map, it can be inferred that Hg, Mn, and Cu all have an adverse effect on bryophyte communities in the vicinity of the mine and in the herb-shrubland. Thus the determination of the characteristics of bryophyte communities allows ready identification of the impact of heavy metals on the environment, in turn providing a mechanism for decision-making in terms of pollution management and biodiversity protection.

1 Introduction

Manganese is one of the most important raw materials in the iron and steel industry where it is used as a deoxidizer and desulfurizer in production of iron, steel and industrial alloys (CMDEC, 1989). Manganese is principally sourced from manganese ore. China's iron and steel industry has developed rapidly in recent years driving an increasing demand for, and exploitation of, manganese ore that is increasing every year, and in the process, posing a serious threat to the natural environment and to the health of residents living in proximity to the manganese mines. China has vast deposits of manganese ore, but resources of low-grade ore are far more extensive than deposits of high-grade ore. More than 70% of the manganese ore deposits in China are comprised of manganese carbonate with less than 25% manganese (Liu et al., 2015; Deng et al., 2019), with the result that production of a given quantity of manganese requires the processing of significantly more manganese ore, eventuating in the production of even more slag and waste rock and further damage to the environment. When slag and waste rock are exposed to the environment, weathering and eluviation release heavy metals into the soil, water and atmosphere, ultimately ending in the food chain where they pose a serious threat to human health (Muhammad et al., 2011). The Songtao manganese mine is a very large mine with substantial processing capacity located in the core area of China's "manganese triangle", an area with the largest manganese carbonate ore reserves in China (Liu et al., 2019). In addition to the direct impact on the local environment, heavy metals can also follow watercourses to reach other areas, vastly expanding their impact range. In the study area, the Huayuan River flows through the electrolytic manganese plant, then into the Qiushui River, a tributary of Yuanjiang River, impacting a much greater area than the immediate vicinity of the mine.

When compared to vascular plants, bryophytes are extremely sensitive to environmental changes. The leaf structure of most bryophytes is relatively simple, composed of a single layer or, at most, few layers of cells, and in the absence of a waxy cuticle on the leaf surface, environmental pollutants can readily enter both upper and lower leaf surfaces where they remain (Wu, 1998; Wu et al., 2001). Bryophytes also have a capacity for robust cation exchange (Büscher et al., 1990) and can adsorb metal ions and metal particles from the surrounding environment into the leaves (Richardson, 1981). Thus, bryophytes are often used as indicator plants to monitor environmental pollutants; not only the degree of pollution, but the nature of, and changes in, pollutants. The sensitivity of bryophytes to their environment varies depending on their substrates. Epiphytic bryophytes are more sensitive to atmospheric conditions than either lithophytic or terrestrial bryophytes (Rao, 1982), both of which have the potential to entrap greater quantities of soil and dust particles than epiphytic bryophytes (Bargagli, 2016).

At present, there are two procedures used to determine environmental pollution using bryophytes: firstly, using moss bags (Wu et al., 2008; Salo et al., 2012; Vuković et al., 2013); secondly the collection and analysis of bryophytes growing in an area in question. Content of heavy metals determined in bryophytes of the same species, or the same genus, can be used to reflect the pollution status of a given area (Vincent et al., 2001; Gerdol et al., 2002; Schintu et al., 2005; Guo et al., 2017; Liu et al., 2018; Cowden et al., 2019) and characteristics of bryophyte communities can also be used to monitor environmental pollution (Denayer et al., 1999; Liu et al., 2011; Wang et al., 2015). In harsh environments such as mines, caves and areas of rocky desertification, bryophyte communities are often used to monitor heavy metal pollution (Liu et al., 2018) because of the difficulty of finding and identifying bryophytes of the same species or the same genus in a relatively small-scale area and the problem of locating sufficient materials for investigation. However, the species diversity of a bryophyte community can adequately reflect levels of environmental pollution (Yang et al., 2011). Changes in environmental conditions transform the structure and functional characteristics of animal and plant communities and heavy metals pollution stress reduces the stability of the community and also species diversity (Chen et al., 1989; Xu et al., 2004; Yang et al., 2011; Li et al., 2020). In Guizhou Province, bryophytes have been used to monitor heavy metal pollution in mercury, gold, copper and bauxite mines. The objectives of this study are: 1) to understand the composition, richness and species diversity of bryophyte communities in different locations associated with manganese carbonate mining and processing; 2) to determine the heavy metal content of the substrates underlying bryophyte communities in the vicinity of the mining operations; 3) to explore whether composition of bryophyte communities on heavily polluted mining processing areas can be used to determine the heavy metal composition of the underlying substrates.

2 Materials And Methods

2.1 Study area

The study area is located in Songtao Miaozu Autonomous County, Tongren City, Guizhou Province, China (Fig.1). The region has a mild-subtropical, humid, monsoonal climate with very high rainfall, high temperatures in summer, and very low temperatures in winter. The mean annual temperature is 16.5°C: the daily average temperature in July is 27.3°C; 4.3°C in January. The mean annual precipitation is 1378.3 mm, with a mean of 183 rainy days each year, and 1228 mean annual hours of sunshine duration. The mine is located on the passive continental margin of the southeastern boundary of the Yangtze Block. The manganese ore is mainly composed of rhodochrosite ($MnCO_3$) and calcimangite, followed by manganese dolomite, manganese-bearing calcite, pyrite, chalcopyrite, quartz, feldspar, clay minerals, carbonaceous organic matter and a small quantity of sulfate including gypsum, barite and other heavy minerals. The content of MnO_2 ranges from 11.02% ~ 32.71%; the content of Fe_2O_3, P_2O_5 and SiO_2 are 2.04% ~ 7.48%, 0.08% ~ 0.43% and 20.90% ~ 65.90% respectively (Gao et al., 2018; He et al., 2013).

Table 1. Basic information for the five sampling locations

Location	Longitude	Latitude	Elevation	Sampling plot
Vicinity of the manganese mine (a)	109° 06' 42"E	28° 06' 42"N	525.20±3m	plots a1-a5
Manganese mine site (b)	109° 06' 42"E	28° 06' 42"N	525.20±3m	plots b1-b5
Electrolytic plant (c)	109° 11' 57"E	28° 11' 01"N	374.88±6m	plots c1-c5
Electrolytic waste dump (d)	109° 10' 39"E	28° 11' 19"N	401.95±4m	plots d1-d5
Herb-shrubland (e)	109° 10' 35"E	28° 14' 30"N	514.81±6m	plots e1-e5

2.2 Sample collection and processing Samples of substrate soil from 1 cm~3 cm deep were collected from each plot and packed into polyethylene plastic bags. A substrate sample consisted of a mixture of 5 soil samples collected from each plot, providing a total of 25 substrate samples. Plant roots and gravel in the samples were removed after air drying in the shade, the remainder sent to the laboratory, grind and homogenized, and stored in sealed bags for determination of heavy metals. **2.3 Determination of heavy metals**Substrate samples were digested by an automatic digester (AutoDigiBlock, Labtech, Beijing). 0.2g of each substrate sample was weighed, put into a PTFE digestion tube which in turn was placed in a graphite digestion hole, subsequently, a test was conducted with a blank. After the samples in the PTFE digestion tube were moistened with water, 6 ml nitric acid (68.0% ~ 70.0%, UPS), 3 ml hydrofluoric acid (48.8% ~ 49.2%, UPS) and 1 ml perchloric acid (70.0% ~ 72.0%, GR) were added to the digestion tube, which was then well shaken and the tube cover tightened. The digester was first programmed to 100 °C to heat the digestion solution and reflux for 1 h, then cooled to room temperature and the tube cover opened. It was then programmed to 140 °C to force out the acid until 0.5 ~ 1 ml of the digestion solution remained. Finally, the inner wall of the digestion tube was cleaned with a small amount of ultrapure water and diluted with 2% nitric acid to return the volume to 50 ml. The Inductively Coupled Plasma Optical Emission Mass Spectrometer (NexION 300X, PerkinElmer, USA) was used for the determination of Mn, Fe, Cr, Co, Ni, Cu, Zn, Cd, Hg, Pb in the digestion solution.In the determination process, soil standard GBW07405 (GSS-11) was analyzed to check the accuracy and precision of each metal analysis.

2.4 Data analysis

The Abundance index (Zuo, 1990), Shannon-Wiener diversity index, Simpson diversity index, and Pielou evenness index were used to determine the characteristics of the bryophyte communities at each location. This was calculated by the cover to represent the number of individual bryophyte species. The calculation formulas are as follows: **Please see formulas 1 - 4 in the supplementary files.**

m is the number of species in a region; P_i is the important value of species, $P_i = N_i / N$, N_i is the number of the i species among m species, and N is the total number of m species.

ArcGIS was used to draw the sampling area map; the difference analysis of data was completed in Spss 21.0; heavy metal and bryophyte species data were assessed using by Microsoft Excel 2016; diversity data were calculated by 'vegan' package of R (Version 4.0.1); the indicator species was determined by the R package 'labdsv', statistical analysis was performed using the 'ggplot' package, 'corrplot' package and 'pheatmap' package in R (Version 4.0.1) software, CCA analysis was performed using Canoco5.0 software.

3 Results

3.1 Characteristics of the bryophyte communities

3.1.1 Composition of bryophyte communities

A total of 110 bryophyte species, 54 genera in 25 families were recorded at the five locations. These included six liverwort species from six genera in five families and 105 moss species from 48 genera in 20 families. The dominant families were Pottiaceae, Bryaceae and Hypnaceae, accounting for 28.8%, 16.2% and 12.6% of species, respectively. There was a considerable difference in the number of species, genera and families at each location (Fig. 2A): most occurred in the vicinity of the manganese mine, (38 species, 32 genera, 17 families); fewest were recorded at the electrolytic waste dump (14 genera, 4 families); and the lowest number of species at the electrolytic plant. The Abundance index for the five locations was similar to the distribution of genera and families, with the highest Abundance index recorded in the vicinity of the mine and the lowest at the electrolytic waste dump. The species composition of bryophytes at the family level was similar in the vicinity of the mine and at the mine site area. Hypnaceae, Pottiaceae and Leucobryaceae accounted for the largest proportion of species numbers in the vicinity of the mine with percentages of 38.67%, 17.51% and 9.06%, respectively (Fig. 2B). At the mine site, Polytrichaceae, Ditrichaceae and Pottiaceae accounted for 35.65%, 16.95% and 12.92%, respectively. The electrolytic plant and the electrolytic waste dump were characterized mainly by Pottiaceae and Bryaceae, which accounted for 90.5% and 98.9%, respectively. Hypnaceae species were widely distributed in the vicinity of the mine, at the mine site and in the herb-shrubland area, but was not found near either the electrolytic plant or the electrolytic waste dump. Short turf life-forms accounted for 55.82% of bryophyte taxa at all five locations, comprising 39.47%, 67.65%, 83.33%, 92.86% and 35.14% in the vicinity of the mine, at the mine site itself, at the electrolytic plant, the electrolytic waste dump and in the herb-shrubland, respectively.

Five bryophyte species were determined as indicator species for the five locations. *Campylopus coreensis*, *Pogonatum neesii*, *Barbula ehrenbergii*, *Trichostomum brachydontium* and *Pseudosymblepharis angustata* were the indictors for the vicinity of the mine, mine site, electrolytic plant, electrolytic waste dump and herb-shrubland, respectively (Table A1).

3.1.2 Species diversity of bryophyte communities in the five locations

Three alpha diversity indices, Shannon-Wiener diversity index, Simpson diversity index, and Pielou evenness index, were calculated to evaluate the diversity of the bryophyte communities (Fig. 2C). The alpha diversity values in herb-shrubland were higher than those of the other four locations with small variation in range and relatively uniform distribution. The alpha diversity values of the electrolytic plant were the lowest, with a large variation range and uneven distribution. In general, the alpha diversity values of each location were observed as herb-shrubland > vicinity of the mine > electrolytic waste dump > mine site > electrolytic plant.

3.2 Analysis of heavy metal contents in substrates underlying bryophyte communities

The total concentrations of ten heavy metals in the substrates underlying the bryophyte communities were measured. Heavy metal contents for each location are the mean of the five plots shown in Table 2. There were significant differences in Mn content among the five locations ($P < 0.05$), in the order: electrolytic plant > mine site > vicinity of the mine > electrolytic waste dump > herb-shrubland. Except herb-shrubland, Mn contents in the vicinity of the mine, at the mine site, at the electrolytic plant, and at the electrolytic waste dump, were 4.80, 7.02, 9.79 and 2.28 times higher than the background values for soil in Guizhou Province, respectively. The contents of all ten heavy metals at the electrolytic plant were higher than the Guizhou background values. With the exception of Co and Hg, the contents of other heavy metals at the electrolytic plant were higher than at any other of the five locations. The contents of Ni, Cu, Zn and Pb at the electrolytic plant were significantly different from the other four locations ($P < 0.05$), but there were no significant differences in Ni, Zn and Pb contents in the vicinity of the mine, at the mine, at the electrolytic waste dump and in the herb-shrubland ($P > 0.05$). With the exception of Cr, the difference in metals contents between the vicinity of the mine and the mine site itself, was not significant, and with the exception of Cd, the contents of other heavy metals at the two locations were higher than Guizhou background values.

Correlation analysis showed that there were significant positive correlations among Co, Mn, Fe, Zn, Cr, Cu, Ni and Pb ($P < 0.01$ or $P < 0.05$) (Fig. 3), while the correlations between Hg and other heavy metals were not significant ($P > 0.05$). Cd element had significant positive correlation with Pb and Cu ($P < 0.01$ or $P < 0.05$), but had no significant correlation with other heavy metal elements ($P > 0.05$).

Table 2
Contents of heavy metals in the substrate underlying bryophyte communities

Element	Vicinity of the mine	Mine site	Electrolytic plant	Electrolytic waste dump	Herb-shrubland	Guizhou background values
Mn ($g\cdot kg^{-1}$)	3.81 ± 0.48b	5.57 ± 0.56ab	7.77 ± 0.69a	1.81 ± 0.10c	0.75 ± 0.05d	0.794
Fe ($g\cdot kg^{-1}$)	52.89 ± 2.98b	55.81 ± 0.37ab	68.94 ± 13.28a	41.72 ± 11.30b	43.40 ± 4.17b	4.17
Cr ($mg\cdot kg^{-1}$)	97.43 ± 6.81bc	108.50 ± 5.38ab	126.01 ± 8.57a	69.82 ± 6.05c	79.47 ± 6.77bc	95.9
Co ($mg\cdot kg^{-1}$)	38.52 ± 11.55a	37.53 ± 4.58a	37.90 ± 4.59a	19.84 ± 2.63a	18.45 ± 2.03a	19.2
Ni ($mg\cdot kg^{-1}$)	52.67 ± 4.74b	40.51 ± 3.23b	80.53 ± 4.29a	51.47 ± 8.45b	43.51 ± 4.87b	39.1
Cu ($mg\cdot kg^{-1}$)	46.18 ± 3.49b	38.55 ± 4.00b	76.41 ± 6.17a	34.74 ± 6.18bc	22.51 ± 2.25c	32.0
Zn ($mg\cdot kg^{-1}$)	160.72 ± 15.57b	176.41 ± 16.48b	257.38 ± 29.10a	151.95 ± 37.69b	148.70 ± 18.57b	99.5
Cd ($mg\cdot kg^{-1}$)	0.54 ± 0.04ab	0.36 ± 0.04b	0.70 ± 0.06a	0.48 ± 0.06b	0.58 ± 0.07b	0.659
Hg ($mg\cdot kg^{-1}$)	0.13 ± 0.01b	0.94 ± 0.11a	0.50 ± 0.06a	0.72 ± 0.11a	0.18 ± 0.07b	0.110
Pb ($mg\cdot kg^{-1}$)	40.71 ± 5.64b	33.88 ± 1.87b	146.02 ± 18.57a	53.59 ± 6.47b	38.48 ± 3.15b	35.2
Different lowercase letters in a single row represent significant differences ($p < 0.05$, post LSD test)						

**indicates significant correlation at 0.01 level (bilateral); * indicates significant correlation at 0.05 level (bilateral).

3.3 Relationship between bryophyte communities and heavy metal contents in substrate

3.3.1 CCA analysis of bryophyte species and heavy metal elements

The 22 bryophyte species (total coverage > 1.0) selected for CCA analysis included five indicator species (Table A1). The significance of the contents of each heavy metal element in substrate and bryophyte species distribution was tested by envfit function in R software (999 simulation times were selected) and the heavy metal elements Mn, Cd, Hg, Pb with significant explanatory amount were screened out (Table 3) and tested by Monte Carlo test with the significance less than 0.05. This indicated that the sorting results could accept the interpretation of the selected heavy metal elements on species distribution. The sequencing results are shown in Fig. 4.

CCA results for heavy metals indicated that the first principal component axis and the second principal component axis explained 82.15% and 65.91% of the bryophyte species composition, respectively. The total explanatory amount of Mn, Cd, Hg, and Pb element was 23.9%, accounting for 5.3%, 5.2%, 6.8% and 7.6%, respectively. The distribution of bryophyte species on the ordination axis basically reflected the trend in variation of characteristics of plant spatial distribution with heavy metal contents. Most bryophytes were distributed in the negative direction of the second axis, while the species resistant to heavy metals were mainly distributed in the positive direction of the second axis. *Didymodon tectorus* (*Did.tec*), *Barbula ehrenbergii* (*Bar.ehr*), and *Barbula propagulifera* (*Bar.pro*) were distributed in regions with a high concentration of Pb and Mn in the substrate. Species distributed in the regions with high concentration of Pb and Cd included *Hydrogonium majusculum* (*Hyd.maj*), *Didymodon constrictus* (*Dym.con*), and *Anomobryum julaceum* (*Ano.jul*). The distribution of *Bryum pallescens* and *Ditrichum pallidum* were mainly affected by the concentration of Hg in the substrate. The distribution of bryophytes at the electrolytic plant was consistent with the variation direction of heavy metal elements, and the bryophytes at the electrolytic plant have a strong tolerance for heavy metals and were mainly affected by Pb and Mn. Bryophyte distribution in the vicinity of the mine and in the herb-shrubland were opposite to the direction of heavy metals, indicating that the distribution of bryophyte at the two locations was negatively correlated with the heavy metal contents in the substrate and that the bryophytes at the two locations showed weak tolerance to heavy metals.

Table 3
Results of significance test of heavy metals

Elements	CCA1	CCA2	r^2	Pr(>r)
Mn	-0.10085	0.99490	0.5126	0.001998 **
Cd	0.23639	0.97166	0.3021	0.027972 *
Hg	-0.67693	0.73604	0.3731	0.009990**
Pb	0.56690	0.82379	0.6139	0.000999 ***

Table A1
Occurrence, total coverage (TC), indicator values (IV) of bryophyte species in five locations. Species with indicator value > 0.5 are considered as the best indicators. Species with both high indicator value and a signifificant p value (p < 0.05) are shown in bold.

No.	Family name	Species name	Abbreviation	Location					TC	IV	*p*
				a	b	c	d	e			
1	Allisoniaceae (R.M.Schust.ex Grolle)Schljakov	*Calycularia crispula* Mitt*.	Cal. cri	0	0	0	0	+	0.096	-	-
2	Bartramiaceae Schwägr	*Philonotis hastata* (Duby) Wijk & Marg.	Phi.has	0	+	0	0	0	0.07	-	-
		Philonotis mollis (Dozy & Molk.) Mitt.	Phi.mol	+	+	0	+	0	0.9099	-	-
		Philonotis turneriana (Schwägr.) Mitt.	Phi.tur	0	+	+	0	0	0.7648	-	-
3	Brachytheciaceae Schimp.	*Brachythecium perminusculum* Müll. Hal.	Bra.per	0	0	0	0	+	0.1001	-	-
		Brachythecium reflexum (Stark.) Schimp.	Bra.ref	+	0	0	0	+	0.256	-	-
		Brachythecium rutabulum (Hedw.) Bruch & Schimp.	Bra.rut	+	0	0	0	0	0.172	-	-
		Eurhynchium laxirete Broth.	Eur.lax	+	0	0	0	0	0.434	-	-
		Rhynchostegium fauriei Cardot	Rhy.fau	0	0	+	0	0	0.084	-	-
		Rhynchostegium inclinatum (Mitt.) A.Jeag.	Rhy.inc	+	0	0	0	0	0.14	-	-
4	Bruchiaceae Schimp.	*Trematodon ambiguus(Hedw.)* Hornsch.	Tre.amb	0	+	0	0	0	0.1518	-	-
		Trematodon longicollis Michx.	Tre.lon	0	+	0	0	0	0.128	-	-
5	Bryaceae Schwägr.	*Anomobryum gemmigerum* Broth.	Ano.gem	0	0	0	0	+	0.063	-	-
		Anomobryum julaceum (Gärtn., Meyer & Scherb.) Schimp.	Ano.jul	0	0	+	+	0	3.0877	-	-
		Brachymenium exile (Dozy &. Molk.) Bosch & Sande Lac.	Bra.exi	0	0	+	+	0	0.5265	-	-
		Brachymenium leptophyllum (Müll. Hal.) A. Jaeger.	Bra.lep	0	0	0	+	0	0.354	-	-

Note: “*” represent liverworts

No.	Family name	Species name	Abbreviation	Location					TC	IV	*p*
				a	b	c	d	e			
		Bryum argenteum Hedw.	Bry.arg	+	0	+	+	0	0.6567	-	-
		Bryum atrovirens Brid.	Bry.atr	0	0	0	+	0	0.2747	-	-
		Bryum billarderi Schwägr.	Bry.bil	0	0	0	+	+	0.279	-	-
		Bryum blindii Bruch & Schimp.	Bry.bli	0	0	+	0	0	0.0512	-	-
		Bryum bornholmense Winkelm. & Ruthe.	Bry.bor	0	0	+	0	0	0.1961	-	-
		Bryum capillare Hedw.	Bry.cap	+	0	0	0	0	0.136	-	-
		Bryum coronatum Schwägr.	Bry.cor	0	0	+	0	0	0.3251	-	-
		Bryum dichotomum Hedw.	Bry.dic	0	0	+	+	0	0.7386	-	-
		Bryum leptocaulon Cardot.	Bry.lep	0	0	0	+	0	0.116	-	-
		Bryum pallescens Schleicher ex Schwägr.	Bry.pal	0	0	+	+	+	1.5712	-	-
		Bryum paradoxum Schwägr.	Bry.par	0	0	0	+	0	0.1931	-	-
		Bryum pseudotriquetrum (Hedw.) Gaertn.	Bry.pse	0	0	+	0	0	0.205	-	-
		Bryum thomsonii Mitt.	Bry.tho	0	+	0	+	0	0.108	-	-
		Bryum tuberosum Mohamed & Damanhuri	Bry.tub	0	+	0	0	0	0.018	-	-
		Philonotis falcata (Hook) Mitt.	Phi.fal	+	0	0	0	0	0.032	-	-
6	Dicranaceae Schimp.	*Microdus sinensis* Herz	Mic.sin	+	0	0	0	0	0.002	-	-
7	Dicranellaceae M.Stech	*Dicranella heteromalla* (Hedw.) Schimp.	Dic.het	0	+	0	0	0	0.06	-	-
	Dicranellaceae M.Stech	*Dicranella subulata* (Hedw.) Schimp.	Dic.sub	+	+	+	0	+	1.2176	-	-
	Dicranellaceae M.Stech	*Dicranella varia* (Hedw.) Schimp	Dic.var	0	0	0	+	0	0.0969	-	-

Note: “*” represent liverworts

No.	Family name	Species name	Abbreviation	Location					TC	IV	*p*
				a	b	c	d	e			
8	Ditrichaceae Limpr.	*Ditrichum pallidum* (Hedw.) Hampe	Dit.pal	+	+	0	0	0	2.8652	-	-
		Pleuridium subulatum (Hedw.)Rabenh.	Ple.sub	0	+	0	0	0	0.108	-	-
9	Fissidentaceae Schimp.	*Fissidens anomalus* Mont.	Fis.ano	0	0	0	0	+	0.246	-	-
		Fissidens dubius P. Beauv.	Fis.dub	0	0	0	0	+	0.7389	-	-
		Fissidens incognitus Gangulee.	Fis.inc	0	0	+	0	0	0.08	-	-
		Fissidens involutus Wilson ex Mitt.	Fis.inv	0	0	+	0	0	0.1122	-	-
		Fissidens taxifolius Hedw.	Fis.tax	+	0	0	0	+	0.039	-	-
10	Funariaceae Schwägr.	*Physcomitrium sphaericum* (Ludw.) Fürnr.	Phy.sph	+	0	+	0	0	0.1246	-	-
11	Hypnaceae Schimp.	*Ctenidium hastile* (Mitt.) Lindb.	Cte.has	0	+	0	0	0	0.16	-	-
		Gollania arisanensis Sakurai	Gol.ari	0	0	0	0	+	0.0375	-	-
		Gollania revoluta Higuchi	Gol.rev	0	0	0	0	+	0.045	-	-
		Gollania ruginosa (Mitt.) Broth.	Gol.rug	0	0	0	0	+	0.0693	-	-
		Hypnum calcicola Ando	Hyp.cal	+	0	0	0	+	3.8315	-	-
		Hypnum leptothallum (C.Muell.) Paris.	Hyp.lep	0	0	0	0	+	1.3028	-	-
		Hypnum oldhamii(Mitt.) A. Jaeger.	Hyp.old	+	0	0	0	+	2.8876	-	-
		Hypnum pallescens (Hedw.) P.Beauv.	Hyp.pal	+	0	0	0	+	0.9822	-	-
		Hypnum revolutum (Mitt.) Lindb.	Hyp.rev	0	+	0	0	+	1.2044	-	-
		Hypnum vaucheri Lesq.	Hyp.vau	0	0	0	0	+	0.184	-	-
		Pseudotaxiphyllum densum (Cardot) Z. Iwats.	Pse.den	+	+	0	0	0	0.112	-	-

Note: “*” represent liverworts

No.	Family name	Species name	Abbreviation	Location					TC	IV	*p*
				a	b	c	d	e			
		Pseudotaxiphyllum pohliaecarpum (Sull. & Lesq.) Z. Iwats.	Pse.poh	0	+	0	0	0	0.317	-	-
		Taxiphyllum aomoriense (Besch.) Z. Iwats.	Tax.aom	0	0	0	0	+	0.0316	-	-
		Taxiphyllum taxirameum (Mitt.) M. Fleisch.	Tax.tax	+	+	0	0	0	0.777	-	-
12	Jungermanniaceae Rchb.	*Solenostoma Parvitextum* (Amakawa) Váňa & D.G.Long*	Sol.Par	0	0	0	0	+	0.015	-	-
13	Lepidoziaceae Limpr.	*Bazzania albifolia* Horik.*	Baz.alb	0	+	0	0	0	0.3655	-	-
14	Leskeaceae Schimp.	*Claopodium rugulosifolium* S.Y.Zeng.	Cla.rug	0	+	+	0	0	0.56	-	-
		Lindbergia brachyptera (Mitt.) Kindb.	Lin.bra	0	0	+	0	0	0.1014	-	-
15	Leucobryaceae Schimp.	Campylopus coreensis **Card.**	**Cam.cor**	+	0	0	0	0	**1.5923**	**0.800**	**0.003**
		Campylopodiella himalayana (Broth.) J.P. Frahm	Cam.him	0	+	0	0	0	0.325	-	-
		Leucobryum bowringii Mitt.	Leu.bow	0	+	0	0	0	1.136	-	-
16	Lophocoleaceae Vanden Berghen	*Heteroscyphus planus* (Mitt.) Schiffn.*	Het.pla	+	0	0	0	+	0.14	-	-
		Chiloscyphus horikawanus (S. Hatt.) J. J. Engel & R. M. Schust.*	Chi.hor	+	0	0	0	0	0.033	-	-
17	Meteoriaceae Kindb.	*Meteorium polytrichum* Dozy & Molk.	Met.pol	0	0	0	0	+	0.2976	-	-
18	Mniaceae Schwägr.	*Pohlia hyaloperistoma* D. C. Zhang, X. J. Li & Higuchi.	Poh.hya	0	+	0	0	0	0.15	-	-
19	Monosolenium E.H.Wilson	*Monosolenium tenerum* Griff.*	Mon.ten	+	0	0	0	0	0.801	-	-
20	Polytrichaceae Schwägr	*Atrichum crispulum* Schimp. ex Besch.	Atr.cri	+	0	0	0	0	0.039	-	-

Note: “*” represent liverworts

No.	Family name	Species name	Abbreviation	Location					TC	IV	*p*
				a	b	c	d	e			
		Pogonatum neesii **(Müll. Hal) Dozy.**	**Pog.nee**	+	+	0	0	0	**7.0256**	**0.847**	**0.002**
21	Pottiaceae Schimp.	*Anoectangium euchloron* (Schwaegr.) Mitt	Ano.euc	0	0	+	+	0	0.3914	-	-
		Anoectangium fauriei Card	Ano.fau	0	0	+	0	0	0.5309	-	-
		Anoectangium thomsonii Mitt.	Ano.tho	+	+	0	0	0	1.462	-	-
		Barbula chenia Redf. & B. C. Tan.	Bar.che	0	0	0	+	0	0.6024	-	-
		Barbula ehrenbergii **(Lorentz) M.Fleisch.**	**Bar.ehr**	0	+	+	+	0	**3.9822**	**0.509**	**0.048**
		Barbula propagulifera (X. J. Li & M. X. Zhang) Redf. & B. C. Tan	Bar.pro	0	0	+	0	0	1.4938	-	-
		Bryoerythrophyllum brachystegium (Besch.) Saito	Bry.bra	+	0	+	0	0	0.1925	-	-
		Bryoerythrophyllum inaequalifolium	Bry.ina	0	0	0	0	+	0.0825	-	-
		Chenia leptophylla (Müll. Hal.) R. H. Zander.	Che.lep	0	+	0	0	0	0.306	-	-
		Didymodon subandreaeoides (Kindb.) R.H.Zander.	Did.sub	0	0	0	+	0	0.084	-	-
		Didymodon tectorus (Müll. Hal.) Saito	Did.tec	0	0	+	+	0	1.4756	-	-
		Dymodon constrictus (Mitt.) Saito.	Dym.con	+	0	+	+	+	4.5049	-	-
		Gymnostomum aeruginosum Smith.	Gym.aer	+	0	0	0	0	0.016	-	-
		Hydrogonium consanguineum (Thwait.et Mitt) Hilp	Hyd.con	+	0	0	+	0	0.188	-	-
		Hydrogonium laevifolium (Broth. et Yas) Chen	Hyd.lae	0	+	0	0	0	0.29	-	-
		Hydrogonium majusculum (C. Muell.)Chen	Hyd.maj	0	0	+	0	0	1.428	-	-

Note: “*” represent liverworts

No.	Family name	Species name	Abbreviation	Location					TC	IV	*p*
				a	b	c	d	e			
		Hyophila spathulata (Harv.) A. Jaeger	Hyo.spa	+	0	0	0	0	0.03	-	-
		Pleuroweisia schliephackei Limpr.	Ple.sch	0	0	0	0	+	0.316	-	-
		Pseudosymblepharis angustata **(Mitt.) Hilp.**	**Pse.ang**	0	0	0	+	+	**3.0538**	**0.768**	**0.004**
		Ptychomitrium sinense (Mitt.) A. Jaeger	Pty.sin	+	0	0	0	0	0.184	-	-
		Tortella humilis (Hedw.) Jenn.	Tor.hum	+	+	+	+	+	4.8824	-	-
		Tortula subulata Hedw.	Tor.sub	0	0	0	+	0	0.9838	-	-
		Trichostomum brachydontium **Bruch.**	**Tri.bra**	+	+	0	+	+	**4.1366**	**0.701**	**0.005**
		Trichostomum crispulum Bruch.	Tri.cri	0	0	0	0	+	0.8922	-	-
		Trichostomum hattorianum B. C. Tan & Z. Iwats.	Tri.hat	0	+	0	0	0	0.0792	-	-
		Trichostomum planifolium (Dixon) R. H. Zander	Tri.pla	0	+	0	+	0	0.8751	-	-
		Trichostomum tenuirostre (Hook. f. & Taylor) Lindb.	Tri.ten	0	0	0	+	0	0.315	-	-
		Weissia brachycarpa (Nees & Hornsch.) Jur.	Wei.bra	+	0	0	+	+	0.925	-	-
		Weissia controversa Hedw.	Wei.con	0	0	0	0	+	2.5123	-	-
		Weissia exserta (Broth.) P. C. Chen	Wei.exs	0	+	0	0	0	0.198	-	-
		Weissia planifolia Dix.	Wei.plan	0	0	0	+	+	1.1465	-	-
		Weisis platyphylloides Card.	Wei.plat	0	+	0	0	0	0.1386	-	-
22	Ptychomitriaceae Schimp.	*Ptychomitrium gardneri* Lesq.	Pty.gar	0	0	0	0	+	0.077	-	-
23	Racopilaceae Kindb.	*Racopilum orthocarpum* Wilson ex Mitt.	Rac.ort	+	0	0	0	0	0.584	-	-

Note: “*” represent liverworts

No.	Family name	Species name	Abbreviation	Location					TC	IV	*p*
				a	b	c	d	e			
24	Sematophyllaceae Broth.	*Brotherella fauriei* (Card.) Broth.	Bro.fau	0	+	0	0	0	0.0693	-	-
25	Thuidiaceae Schimp	*Cyrto-hypnum gratum* (P.Beauv)Buck et Crum.	Cyr.gra	0	0	0	0	+	0.036	-	-
		Haplocladium discolor (Par.et Broth.) Broth.	Hap.dis	0	0	0	0	+	0.114	-	-
		Thuidium kanedae Sakurai.	Thu.kan	+	0	0	0	+	0.9839	-	-
		Thuidium pristocalyx (Müll. Hal.) A. Jaeger.	Thu.pri	+	0	0	0	0	0.392	-	-

Note: “*” represent liverworts

3.3.2 Correlation analysis between the bryophyte community index and the contents of heavy metals in substrate

The pearson correlation of bryophyte community Richness, Abundance index, Simpson diversity index, Shannon Wiener diversity index and Pielou evenness index with heavy metal content are shown in Fig. 5. The contents of Hg, Cu, Zn, Mn, Fe in the substrate were negatively correlated with the bryophyte communities index. The Shannon Wiener diversity index was positively correlated with Co and Cr but negatively correlated with other elements. With the exception of Cd, the Abundance index was negatively correlated with all other elements and the Richness was negatively correlated with all other elements besides Co. The Simpson diversity index was positively correlated with Ni and Cd, but negatively correlated with other elements, while the Pielou evenness index was positively correlated with Cd, Ni and Pb, but negatively correlated with other elements. Heavy metals can be classified into three groups according to the diversity index of the bryophyte community. The first group was Fe, Mn, Pb, Hg, Ni, Zn, Cu, the second group was Co and Cr and the third group was Cd.

4 Discussion

4.1 Differences between characteristics of bryophyte communities in different locations

Different characteristics of plant communities can reflect the environmental conditions of different regions (Pan et al., 2011; Dahwa et al., 2013; Liu Y et al., 2015), and environmental variations can also affect the distribution of plant communities (Qian et al., 2003; Pakeman et al., 2016; Fu et al., 2018; Liu et al., 2019). In mining areas, environmental damage resulting from open mining generates a reduction in bryophyte species diversity and leads to bryophyte communities dominated by short turf species that are better adapted to extreme and harsh environments (Zuo et al., 2013). In this study, the characteristics of the bryophyte communities in the five locations were quite different. In comparison to other mining areas, the number of bryophyte taxa associated with the manganese mining operation was quite large, while the diversity index was low. The higher species number may be due to limited disturbance of the bryophyte communities as the mining is carried out underground, although there are extensive damage and contamination of the environment surrounding the underground mining operation. The lower diversity index may be the result of lower evenness. The least number of bryophyte species and the lowest alpha diversity index were the result of human disturbance to the bryophyte community at the electrolytic plant. The electrolytic waste dump is located in the suburbs of Liaohu Town and here the alpha diversity index was higher than that of the electrolytic plant and of the mine, but lower than in the vicinity of the mine or herb-shrubland. The lower levels at these latter two sites are probably accounted for by the fact that there is only a medium level of disturbance that allows for the maintenance of relatively high diversity. The bryophyte communities at the electrolytic plant and at the electrolytic waste dump were dominated by species of Pottiaceae and Bryaceae. These species are widely distributed and most are short turfs, giving them strong adaptability to harsh environments (Zhang et al., 2012; Pang et al., 2018), consistent with bryophyte species found in other mining areas (Pan et al., 2011; Zuo et al.,2013; Wang et al., 2015). The leaves of most Pottiaceae are papillose, that is, roughened with minute protuberances, that enhance moisture uptake and storage and reflect solar radiation (Wagner et al., 2014), which may explain the dominance of Pottiaceae in the bryophyte communities at the two locations. The species composition in the vicinity of the mine and at the mine site were similar at family level, but the richness index and diversity index of bryophytes at the mine were significantly lower than in the vicinity of the mine, probably due to human activities such as mining and ore transportation which reduce species diversity.

4.2 Differences of heavy metal contents in moss growth matrix

There were some differences in the mean content of heavy metals. Contents of Mn, Fe, Cr, Co, Ni, Cu, Zn, Cd, Hg, Pb in the electrolytic plant substrate were 9.79, 16.53, 1.31, 1.97, 2.06, 2.39, 2.59, 1.062, 8.55 and 4.15 times higher respectively than the background values of those same soil elements in Guizhou Province. The high content of Mn, Cr, Cd and Fe probably derives from the dust and wastewater produced in the electrolytic process (Xu et al., 2011; Wang, 2012), while the excessive content of Cu, Zn, Ni and other elements mainly derives from road vehicle wear and traffic emissions. The contents of Mn, Fe and Cr in the manganese mine were 7.02, 13.38 and 1.13 times higher than the background values of those same soil elements in Guizhou Province; this may be due to leaching of slag and rock waste from both the mining operation and from transportation. Manganese carbonate ore contains substantial quantities of MnO and Fe_2O_3, and a small amount of Cr_2O_3 (He et al., 2013; Pan et al., 2016). Correlation analysis also shows that Mn, Fe, Zn, Cr, Cu, Ni, Co, Pb and other elements have a strong positive correlation, indicating a linked relationship. Of all the elements, Fe and Zn contents were relatively high at all five locations, the only difference was found at the electrolytic plant, probably because of the higher contents of Fe and Zn in the natural geological background of this area.

4.3 Effects of heavy metals in substrate on bryophyte communities

Plant growth is closely related to soil composition. Excessive content of heavy metal elements in the soil affects the composition of plant communities by modifying plant growth. At all five locations, contents of Hg, Mn, Pb, Cd in the substrate were the main factors affecting the distribution of bryophyte communities. This is because their content is high in most areas. Mn content in the vicinity of the mine, at the mine site, at the electrolytic plant and at the electrolytic waste dump were much higher than background values for soil in Guizhou Province; Hg content at the mine, at the electrolytic plant and at the electrolytic waste dump were much higher than Guizhou background value. Pb and Cd contents were much higher than Guizhou background values in the vicinity of the mine but were similar to Guizhou background values at the other four locations. As the concentration of Hg increased in the substrate, there was a corresponding increase in the number of bryophyte species with strong tolerance to Hg, but there was a decline in bryophyte species richness and diversity (Liu et al., 2011; Liu et al., 2018). Hg has a negative effect on the distribution of bryophyte communities (Fig. 5), consistent with previous research results (Liu et al., 2011; Liu et al., 2018). Mn is toxic to plants and this is one of the most concerning environmental problems generated by manganese mining and processing. Excessive content of Mn can interfere with plant metabolism and restrict plant growth and development (Boojar et al., 2008). Relatively few specially adapted plants have a tolerance to high levels of manganese (Boojar et al., 2008). Mn content in the substrate underlying bryophyte communities at both the manganese mine and electrolytic plant were found to be very high, resulting in a significant impact on bryophyte community distribution. Mn tolerant bryophyte species were mainly distributed around the electrolytic plant and Manganese mine, while those not Mn tolerant mainly occurred in the vicinity of the mine and in herb-shrubland. Pb and Cd appear not to have impacted the growth of bryophytes (Fig. 4), probably because of the low content of these metals. Most bryophyte species have poor tolerance to heavy metals, but some species such as *Didymodon tectorus*, *Barbula ehrenbergii* and *Barbula propagulifera* show strong tolerance and were well able to tolerate Mn and Pb; *Hydrogonium majusculum*, *Didymodon constrictus*, and *Anomobryum julaceum* were able to tolerate Pb and Cd; *Bryum pallescens* and *Ditrichum pallidum* were well able tolerate to Hg (Fig. 4). Bryophytes can be effective indicators of heavy metals in the environment: gold deposits have been discovered as a result of gold enrichment of aquatic bryophytes; and copper deposits have been located by the presence of *Mielichhoferia elongata* which is referred to as a *copper moss* because of its usual association with copper-rich environments. *Barbula propagulifera* showed the strongest tolerance to Pb and Mn amongst all the indicator species at each of the five locations, identifying the species as the most effective moss to utilize as an indicator of Mn and Pb pollution.

5 Conclusion

There were significant differences in the characteristics of bryophyte communities at each of the five locations. The richness index was in the order: vicinity of the mine > herb-shrubland > mine site > electrolytic waste dump > electrolytic plant; whereas the alpha diversity index was in the order: herb-shrubland > vicinity of the mine > electrolytic waste dump > mine site > electrolytic plant. The species composition of each location was quite different at the family level. The vicinity of the mine and the mine site were dominated by Hypnaceae and Polytrichaceae respectively, whereas Pottiaceae species dominated the electrolytic plant, electrolytic waste dump and the herb-shrubland. These differences are related to human activities, principally mining and ore processing, both of which will generate substantial quantities of heavy metal elements.

CCA analysis showed that the distribution of bryophyte communities was principally determined by the levels of Hg, Mn, Pb and Cd in the underlying substrates. Hg and Mn restrict the distribution and reduce the diversity of bryophyte communities. Bryophyte communities were not limited by levels of Pb and Cd, since the levels of those metals were relatively low and did not exceed the published background values for Guizhou. *Barbula propagulifera* was found to have the greatest tolerance to Mn and Pb concentration in the substrate and is practical to use as an indicator species of Mn and Pb pollution.

Declarations

- Ethics approval and consent to participate

Not applicable

- Consent for publication

Not applicable

- Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

- Competing interests

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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- Authors' contributions

He Chunmei: Conceptualization, Methodology, Software, Formal analysis, Data curation, Writing-original draft, Writing-review & editing, Visualization. **Zhang Zhaohui:** Methodology, Validation, Resources, Writing-review & editing, Supervision, Project administration, Funding acquisition. **Wang Zhihui:** Writing-review & editing, Supervision. **Shi Kuangzheng:** Software, Writing-review & editing. **Wu Qimei:** Project administration, Funding acquisition. **Wang Dengfu:** Software, Writing-review & editing.

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Figures

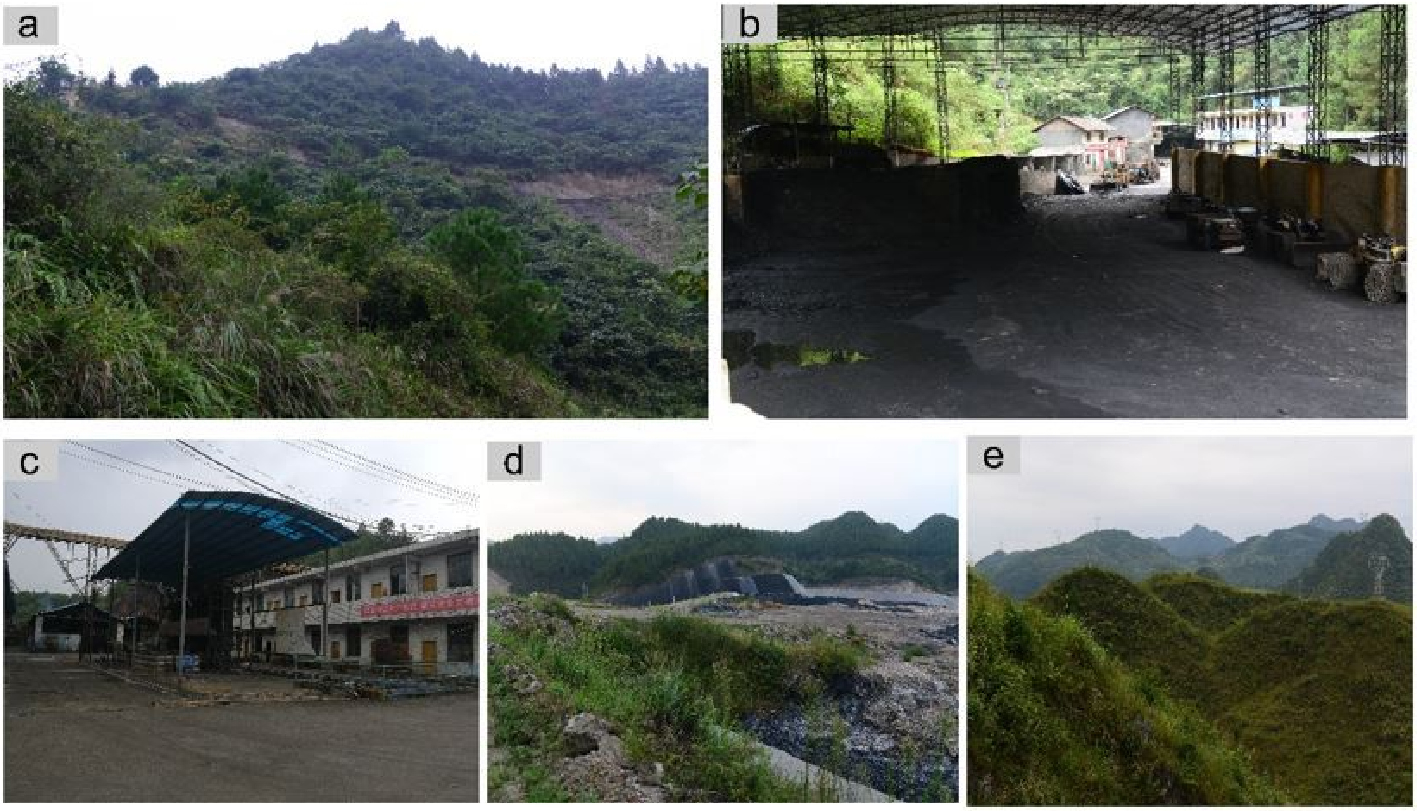

Figure 1

a: Vicinity of the mine (a1-a5); b: Mine site (b1-b5); c: Electrolytic plant (c1-c5); d: Electrolytic waste dump (d1-d5); e: Herb-shrubland (e1-e5).

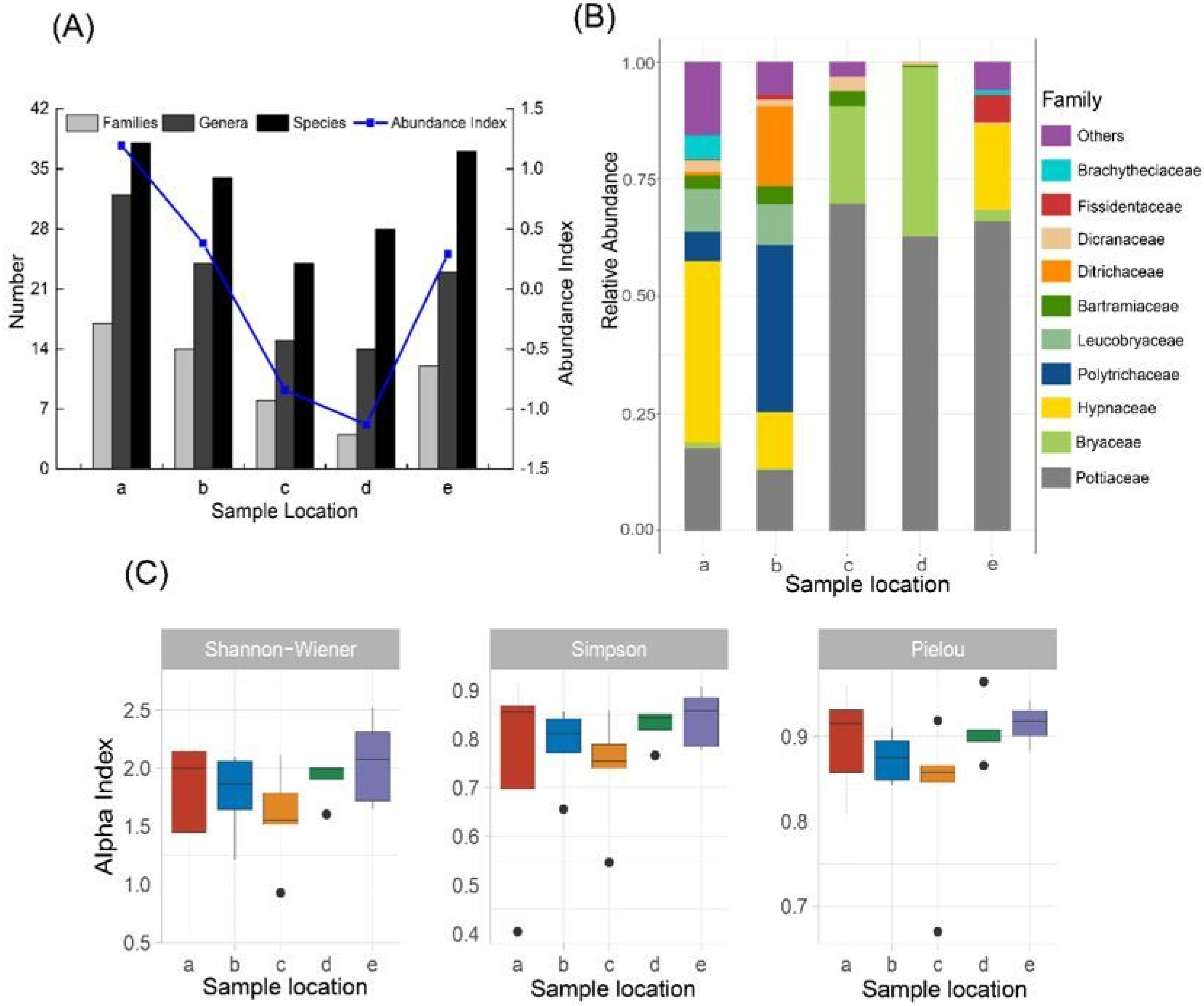

Figure 2

(A) Number of bryophyte families, genera and species together with abundance index for each of the five locations. (B) Relative abundance of bryophytes species at the family level at each location. (C) Alpha diversity of bryophyte communities at the five locations: a. Vicinity of the mine; b. Mine site; c. Electrolytic plant; d. Electrolytic waste dump; e. Herb-shrubland

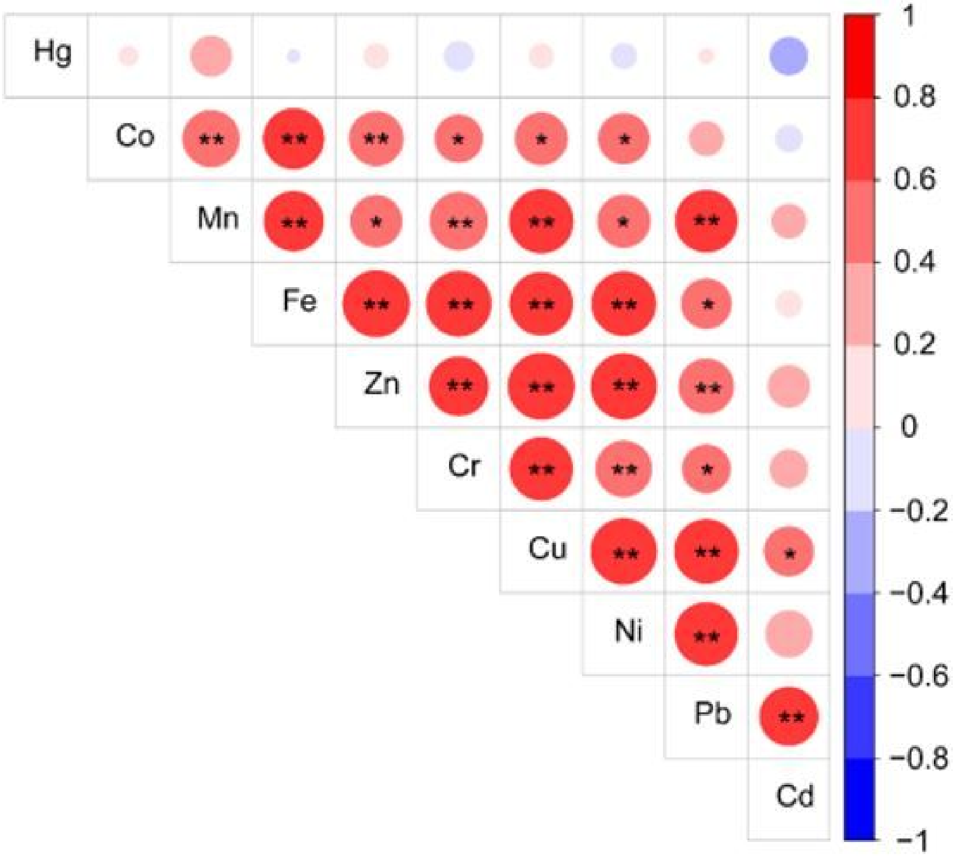

Figure 3

Correlation of heavy metals content in substrate **indicates significant correlation at 0.01 level (bilateral); * indicates significant correlation at 0.05 level (bilateral).

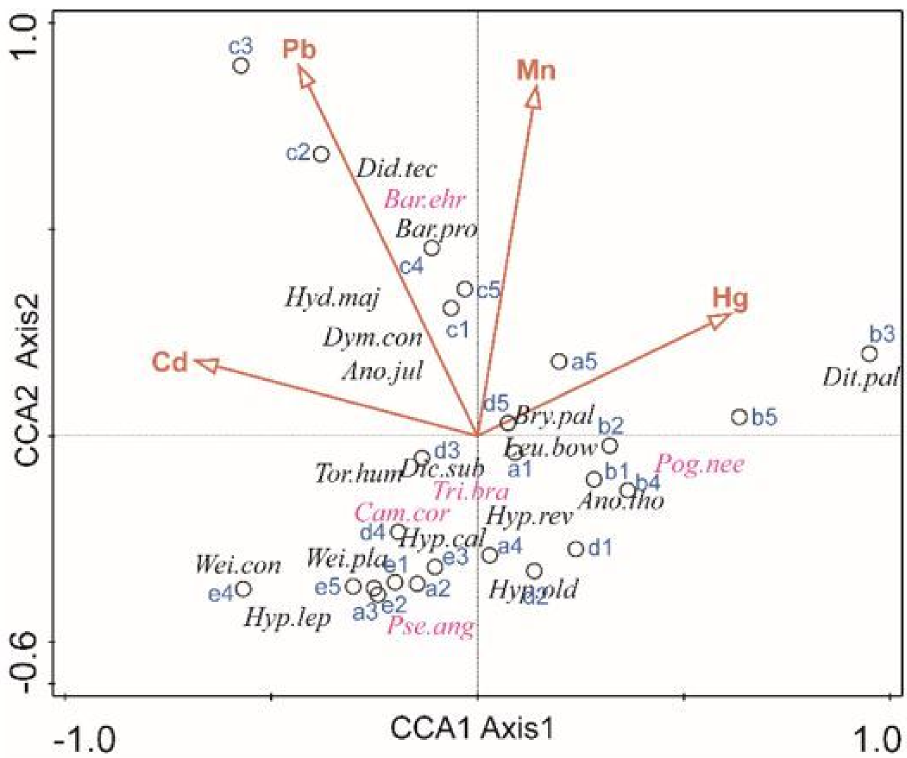

Figure 4

Relationship between heavy metal content in the substrate and distribution of bryophyte communities at the five locations. a1-a5: Vicinity of the mine; b1-b5: Mine site; c1-c5: Electrolytic plant; d1-d5: Electrolytic waste dump; e1-e5: Herb-shrubland.

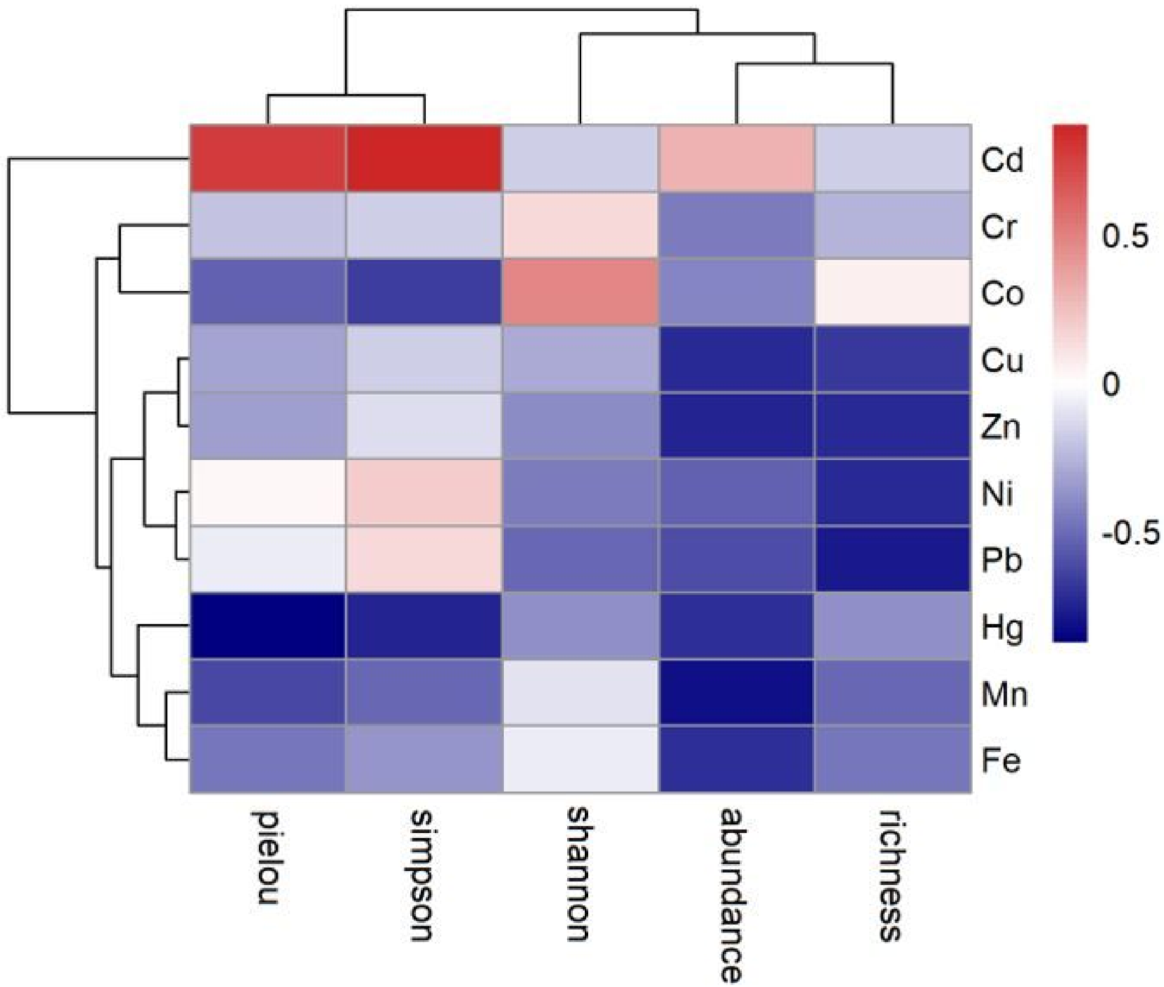

Figure 5

Heatmap of correlation between bryophyte communities and heavy metals

Supplementary Files

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