Taiwania 69(2): 244–256, **2024**
DOI: 10.6165/tai.2024.69.244

Heavy metal monitoring by *Philonotis* (Bartramiaceae, Bryophyte) and *Spirogyra* (Algae) in a manganese slag discharge field wetland

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(Manuscript received 8 August 2023; Accepted 16 May 2024; Online published 5 June 2024)

ABSTRACT: Mining of manganese leads to significant heavy metal contamination, affecting the adjacent ecosystems. Therefore, it is crucial to monitor heavy metal levels in mining areas. This study examined heavy metal concentrations (Cr, Mn, Cu, Zn, Sr, Mo, Cd, and Pb) in vegetation and soil within the wetland area of the Zhaiying manganese slag discharge field in Songtao County, Guizhou Province, China. The assessment utilized the single-factor pollution index method (P_i), Nemerow comprehensive pollution index method (P_n), and correlation analysis. Two local plant species, *Philonotis* and *Spirogyra,* were used as sample materials. The findings revealed varying degrees of heavy metal contamination across all sites, with intensity increasing closer to the slag discharge area. Notably, *Philonotis* displayed a higher heavy metal accumulation capacity than *Spirogyra*. Statistical analysis revealed a significant positive correlation ($P < 0.05$) between *Philonotis* and soil heavy metals (Mn, Zn, and Cd) and between *Spirogyra* and specific soil heavy metals (Mn and Sr). Linear fit analysis indicated a strong association between *Philonotis* and soil manganese, suggesting that *Philonotis* is an effective bioindicator for monitoring environmental heavy metal pollution in slag discharge field wetlands. The manganese content in *Philonotis* exceeded 10,000 mg/kg, indicating potential as a hyperaccumulator, warranting further investigation. This research supports the use of *Philonotis* as a biological tool for monitoring heavy metal pollution in wetland ecosystems affected by manganese mining.

KEY WORDS: Enrichment capacity, Heavy metal contamination, Hygrophyte, Monitor, *Philonotis*, *Spirogyra*.

INTRODUCTION

Heavy metals represent a category of pollutants that have received considerable attention in environmental research. The toxicity of these metals and metalloids to living organisms varies based on both the duration and the intensity of exposure (Barukial and Hazarika, 2022). Heavy metals and residual substances emanating from activities like mining and smelting are prone to dispersal into soil, aquatic systems, and living organisms due to various external influences. This dispersal poses significant threats to the adjacent ecological environments (Nath *et al*., 2021) and could potentially jeopardize human health. Therefore, it is of paramount importance to address and rehabilitate areas heavily contaminated by mining activities.

The use of biological methods for ecological monitoring and restoration of mining sites has become a research hotspot. Studies have revealed that one of the effective methods for estimating the presence of heavy metals in the environment and their availability is the use of organisms, especially plants with high potential for the uptake and accumulation of heavy metals (Karadede-Akin and Unlu, 2007; Vicente-Martorell *et al*., 2009; Zinicovscaia *et al*., 2021). The presence of such bioindicators can effectively monitor potentially hazardous situations in the environment and ultimately prevent heavy metals from posing a risk to human health (Mahamood *et al*., 2021). *Spirogyra* (Hassett *et al*., 1981) are abundant aquatic species that are readily adapted to many different climates. *Spirogyras* can accumulate heavy metals in their organs and can be used as bioindicators of contaminated ecosystems (Goodyear and McNeill, 1999; Rajfur *et al*., 2011). Because of their roles as indicators of heavy metal concentration, *Spirogyra* have been extensively studied (Levkov and Krstic, 2002; O'Farrell *et al*., 2002; Rai *et al*., 2008; Rajfur *et al*., 2010). *Spirogyra* simultaneously reduces the amount of heavy metals present in an ecosystem (Gupta and Rastogi, 2008; Das and Ramanujam, 2011). Therefore, for assessing the presence or absence of metals in the environment, it is more effective to measure the concentrations of metals within the *Spirogyra's* body rather than conducting direct environmental measurements. (Trollope and Evans, 1976).

Wang and Zhang (2019) showed that higher plants have a high metal content and can absorb and accumulate large amounts of metal elements, suggesting their suitability as bioindicators of soil metal contamination caused by mining activities. Bryophytes, the most primitive terrestrial group of higher plants, have been used for decades as bioindicators of heavy metals from natural and anthropogenic sources due to their wide distribution and heavy metal accumulation capacity (Jiang *et al*., 2018; Patiño and Vanderpoorten, 2018; Wang *et al*., 2015; Hassel *et al*., 2013). Bryophyte leaves consist of a single layer or a few layers of cells that can provide a large surface area to absorb water and minerals, including heavy metals (Wu, 1998; Stankovic *et al*., 2018). Bryophytes have excellent stress resistance and are therefore commonly used to monitor environmental pollutants and changes in pollution levels (Wu *et al*., 2001; Huang and Zhang, 2006; Li and Zhang, 2008; Zuo *et al*., 2013). At present, mosses are widely used to monitor and manage arid mining areas, but rarely used in wetland habitats.

Manganese ore reserves in Guizhou Province account for about 60% of the national total, with the primary concentration located in Songtao County of Tongren City. Among these reserves, Zhaiying ancient town has the highest abundance of mineral resources (Lan, 2011; Luo, 2017). In addition to the extensive distribution of *Spirogyra* in wetlands near the Zhaiying Manganese mine, *Philonotis* was also found to exist widely in this habitat. *Philonotis* species have been reported to accumulate high concentrations of heavy metals such as Cd, Cr, Mn, and Pb (Shakya *et al*., 2004). The presence of papillae on the cells of *Philonoti* increases its surface area, enhancing its tolerance to heavy metal concentrations. *Philonotis* belongs to Acrocarpic mosses, which have very short pseudoroots that do not deeply penetrate the substrate (Nair, 2016). These pseudoroots might assist in stabilizing heavy metals in the surface soil (Taeprayoon, *et al*., 2023). A literature review in the Web of Science and similar platforms revealed a limited number of studies on *Philonotis,* primarily focusing on its classification and only a few studies on its role as a bio-indicator. However, reports on heavy metal accumulation in moss species, particularly in wetland habitats, are scarce. Therefore, employing *Philonotis* as a biomonitor for heavy metal pollution in wetlands represents a relatively novel approach. In this study, the wetland environment surrounding the Zhaiying manganese mine slag site in Songtao County was taken as the research object. As the “kidney” of the Earth, serving as a reservoir of species and climate regulator, wetland ecosystems play an irreplaceable role in protecting the ecological environment, maintaining biodiversity, and fostering economic and social development. This investigation included both field investigation and laboratory experiments. This study focused on examining the characteristics of local dominant plants - *Philonotis* and Spirogyra - as well as the soil's heavy metal composition. The study aimed to uncover the relationship between these plants and the soil's heavy metal levels.

While the biological effects of *Philonotis* and Spirogyra on manganese mining areas remained unclear, we hypothesized that *Philonotis* and *Spirogyra* in the study area would exhibit distinct capabilities in accumulating and monitoring soil heavy metals. By uncovering the relationship between these plants and the soil's heavy metal levels, this study would provide a scientific basis for ecological monitoring and managing soil heavy metal pollution in various mining regions.

MATERIALS AND METHODS

Overview of the study area

Songtao County, located at the junction of Guizhou Province, Hunan Province, and Chongqing City, contains rich manganese ore resources. It is one of the three major manganese ore mining and smelting bases in China. It is called the "Manganese Triangle", together with Huayuan County of Hunan and Xiushan County of Chongqing. Presently, Songtao County boasts an accumulation of approximately 712 million tons of manganese carbonate ore resources, with total reserves estimated at approximately 900 million tons.

The Zhaiying manganese deposit is in the transitional zone between the Yangtze block and the Cathaysia bloc, and the geotectonic position spans the Nanhua rift basin (Class I) between the Upper Yangtze block and the Jiangnan orogen (Wang *et al*., 2001). It is an acidic deposit with high iron and low phosphorus deposition dominated by carbonate manganese ore. Manganese minerals are mostly rhombite. The manganese ore in Songtao manganese mine is associated with a diverse range of geological mineral types, often exhibiting complex mineralogical characteristics (Xie *et al*., 2017; Zhou *et al*., 2021). This diversity has contributed to a plentiful supply of manganese for the Songtao manganese mine. Zhaiying Manganese mine is in the southwest of Songtao County, with a geographical position of 27°57'4.31"-27°57'6.73" N, 108°54'45.16 "-108°54'48.0" E, and an altitude of 392 – 407 meters. According to the field investigation, the manganese mine was mined in the 1970s and was abandoned in recent years. Currently, a portion of the land has been repurposed for agricultural use, another small section is occupied by manganese mining waste, and the remaining area is a largely unused wasteland.

Sample collection

In December 2021, during the winter dry period, ***Philonotis*** was observed growing abundantly, establishing itself as the dominant species. There were also large quantities of ***Spirogyra*** on the soil surface. Five sample plots, labeled S1 to S5, were established based on their proximity to the slag dump, ranging from distant to close and then back to a distant region, as illustrated in Fig.1. For each of these sample plots, five sampling points were set up using the five-point sampling method. The chosen areas were moist soil saturated with sewage. The ***Philonotis*** samples and soil samples from the top 0–5 cm layer were collected using a 10 × 10 cm sample box. Additionally, five ***Spirogyra*** samples were randomly gathered from each sample area. A total of 75 samples were collected and used to measure heavy metal content.

Sample digestion and heavy metal element determination

Abatement of *Philonotis* and *Spirogyra*: Plant samples were rinsed with distilled water and dried at a

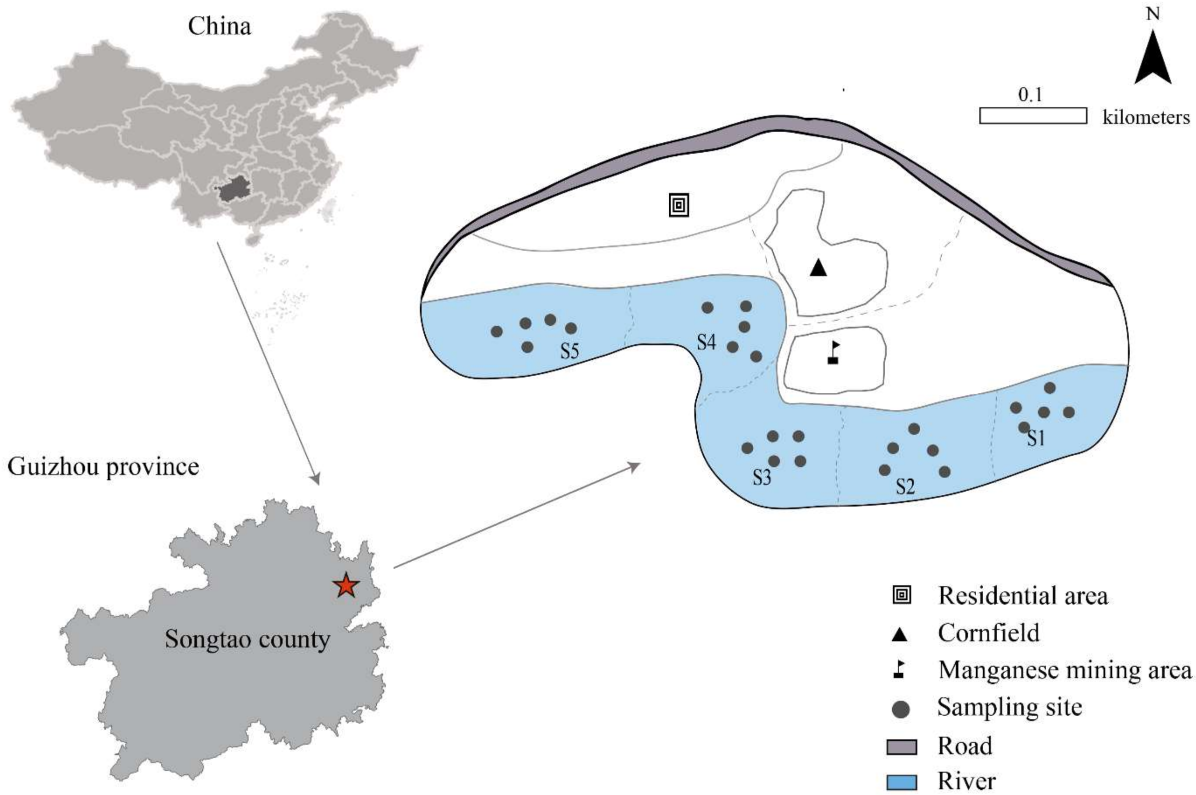

Fig. 1. Schematic diagram of sampling locations in the study area.

constant temperature of 60 °C for 24 h. Then, they were finely ground and sieved (Debén *et al*, 2016). A total of 0.2 g of the sample was weighed and placed in a Teflon digestion tube. The tube was then positioned in a Graphite digestion hole, and a blank test was conducted alongsidethe sample. Nitric acid (6 mL) was added to the digestion tube, followed by thorough shaking and secure tightening of the cap. For the digestion process, the digester was first heated to 60 ℃ for 4 h, then increased to 100 ℃ for reflow lasting 1 h, and finally raised to 120 ℃ for an additional hour of reflow. After cooling to room temperature, the tube cover was opened. The next step involved exhausting the digester, which was heated to 60 ℃ for 0.5 h to discharge red-brown nitrogen oxide gas. The final stage was volume fixation: the inner wall of the digestion tube was rinsed with a small amount of ultrapure water. Subsequently, 2% nitric acid was used to adjust the volume to 50 mL for measurement purposes.

Soil digestion: Soil samples were naturally dried indoors, from which plant roots and other debris were removed. They were then ground in a mortar and passed through a 100-mesh nylon sieve. Each sample, weighing 0.2 g, was placed in a Teflon digestion tube. The tubes were then positioned in Graphite digestion holes, and a blank test was performed with the samples. Liquids were added as follows: 6 mL of nitric acid, 1.5 mL of hydrofluoric acid, and 1 mL of perchloric acid were poured into the digestion tubes. After thorough shaking, the caps were securely tightened. The digestion process involved gradually heating the digester: initially to 60 ℃ for 0.5 h as a preheat, then to 100 ℃ for 1 h for heat reflux, followed by an increase to 140 ℃ for another hour of heat reflux, and finally to 165 ℃ for an additional hour. Once the process was completed, the tubes were cooled to room temperature, and the covers were opened. The next step was the acid rush, where the digester was heated to 140 ℃ until the acid was reduced to a non-flowing liquid bead. The final stage was volume fixation: the inner walls of the digestion tubes were rinsed with a small amount of ultrapure water, and then 2% nitric acid was used to adjust the volume to 50 mL, preparing the samples for measurement.

Determination of element content: Samples were tested for Cr, Mn, Co, Cu, Zn, Sr, Mo, Cd, and Hg using an inductively coupled plasma mass spectrometer (ICP-MS, NexION 300X model, USA). A blank was added during the test, parallel samples were used, and the determination was repeated three times with the relative standard deviation controlled at less than %.

1. Operating conditions of the instrument: RF power 1150 W, cooling gas 18 L/min, auxiliary gas 1.2 L/min, nebulizing gas 0.81 L/min, sampling depth 11 mm, peristaltic pump 20 r/min, KED measurement mode, and internal standard element 103 Rh.
2. Determination: According to the working conditions of the instrument, the mixed standard working solution was used, and the standard curve of each element was drawn

by adding the internal standard method online, with the mass concentration as the horizontal coordinate and the measured signal intensity as the vertical coordinate. The content of each element in the sample was determined according to the standard curve.

Statistical analysis

To have a comprehensive understanding of the state of soil heavy metal pollution in the study area, the soil pollution assessment methods used were the single factor pollution index method and the Nemerow integrated pollution index method.

Index of Single Factor pollution (P_i) :

$$P_i = \frac{C_i}{S_i}$$

Nemerow integrated pollution index (P_n):

$$P_n = \sqrt{\frac{(\overline{P_i})^2 + (P_{i\max})^2}{2}}$$

Where: C_i is the measured mass concentration of heavy metal i in soil (mg/kg); Si is the standard background value of heavy metal i (mg/kg); $P_{i\max}$ is the maximum value of the single factor pollution index in soil heavy metal; $\overline{P_i}$ is the average value of the single factor pollution index in soil heavy metal. The pollution situation was divided into five levels, and the division criteria are shown in Table 1 and Table 2 (Lu *et al*,2017).

The enrichment factor (BCF) characterized the enrichment capacity of plants for heavy metals and is calculated as follows:

$$BCF = \frac{C_i}{C_n}$$

where C_i is the heavy metal content in the plant; C_n is the heavy metal content in the soil. The ratio reflects the relative enrichment and depletion of an element in the plant and its matrix: when BCF < 0.1, strongly depleted; when BCF <0.5, relatively depleted; when 0.5 < BCF<1.5, both are at the same level; when BCF ≥ 1.5, relatively enriched; when BCF > 3, strongly enriched.

The coefficient of variation (CV) was used to reflect the spatial variability of the data.

$$CV = \frac{SD}{MN} \times 100\%$$

where CV < 15% means low variability, 15% ≤ CV ≤ 35% means medium variability, CV > 35% means high variability, SD is the standard deviation of heavy metal i in soil, and MN is the mean value of heavy metal i in soil (Jiang *et al*.,2018).

Data Processing

Excel was used for data statistics, and SPSS 26.0 software and Origin 2021 software were used for correlation analysis. The ArcGIS software was used to complete statistical analysis of the study area, and R Studio was used for mapping.

RESULTS

Soil heavy metal content and pollution level

The contents of Cr, Mn, Cu, Zn, Sr, Mo, Cd, and Pb in the soil samples of the study area were statistically analyzed, as shown in Table 3. The contents of the same heavy metal elements varied significantly among the sampling sites. The CV of heavy metals reached medium variation and above except for Cr element. The contents of eight elements mostly exceeded the background values of China's A-layer soil, among which Mn was the most serious, which was 19.28–67.31 times the background values of China's A-layer soil. The second was Cd, which was 6.94–13.59 times higher than the background value of soil layer A in China.

Table 1. Grading standard of Single-factor pollution index (P_i) method

Grades	P_i	pollution assessment
I	P_i≤1	Non-pollution
II	1< P_i≤2	Minor pollution
III	2< P_i≤3	Light pollution
IV	3< P_i≤5	Moderate pollution
V	P_i >5	Heavy pollution

Table 2. Grading standard of Nemerow composite index (P_n) method

Grades	P_n	Pollution level
I	P_n < 0.7	Security level
II	0.7< P_n≤1.0	Alert level
III	1.0< P_n≤2.0	Light pollution level
IV	2.0< P_n≤3.0	Moderate pollution level
V	P_n > 3.0	Heavy pollution level

To understand the degree of soil heavy metal pollution in the wetland of the manganese mine site, Nemerow pollution index method was used to evaluate soil heavy metal pollution in the wetland of the manganese mine site with reference to the background value of China soil elements and the pollution classification standard. The single-factor pollution index showed that the pollution degree of each heavy metal at the sample site was different. Except for Cr and Sr, other heavy metals caused various degrees of soil pollution (P_i≥ 1). In all sampling sites, the contents of Mn and Cd reached the severely polluted level (Level V- Highest level), the contents of Cu, Mo, and Pb were unpolluted to slightly polluted level (Level I–III), the contents of Zn were slightly polluted (Level II), and the contents of Cr and Sr were at unpolluted level (Level I). The contents of all heavy metals were at the lowest value in S1, Mn, Cu, and Zn reached extreme values in S3, and Mo, Cd, and Pb reached extreme values in S4. Based on the change of Nemerow comprehensive pollution index (P_n), the pollution degree of each region could be ranked as S3 (49.41) > S4 (30.14) > S5 (18.60) > S2 (17.31) > S1 (14.41). The pollution level of S1–S5 increased first and then decreased.

The correlation between heavy metal elements was shown using Pearson correlation analysis (Fig. 3). The results indicated that the heavy metals except Sr interacted with each other, with significant positive correlations (P < 0.01, P < 0.05) between Cr, Mn, and Cu,

Table 3. Heavy metals in soil samples from different sample sites - One-Way Analysis of Variance (ANOVA) mg·kg^{-1}

Element	Cr	Mn	Cu	Zn	Sr	Mo	Cd	Pb
S1	38.75±0.77d	11244.88±217.67c	21.29±1.18c	80.78±1.14e	47.24±0.98d	1.76±0.19d	0.63±0.02d	18.85±0.88d
S2	40.71±1.69cd	13409.22±449.60d	22.19±1.07c	103.44±1.59d	57.35±1.63c	2.14±0.06d	0.78±0.04cd	25.68±1.43c
S3	51.50±0.90a	39241.87±637.58a	38.87±0.89a	130.29±1.45a	65.95±0.89b	3.23±0.31c	0.91±0.01bc	32.23±0.48b
S4	45.11±1.40b	23645.96±360.63b	29.37±0.55d	118.71±0.99b	88.65±1.45a	13.85±0.53a	1.32±0.14a	70.31±1.13a
S5	43.51±1.27bc	14721.92±171.96c	22.86±0.93c	109.60±1.36c	49.66±1.04d	4.69±0.22b	1.04±0.03b	26.49±2.23c
***P* value**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**
CV	0.10	0.50	0.25	0.15	0.24	0.87	0.25	0.53
Background values	61	583	22.6	74.2	167	2	0.097	26

Note: Numbers in the table are means ± standard deviation, followed by different lowercase letters representing significant differences (P < 0.05) in soil heavy metals among the five sampling sites, and significant *P*-values in one-way ANOVA are in bold.

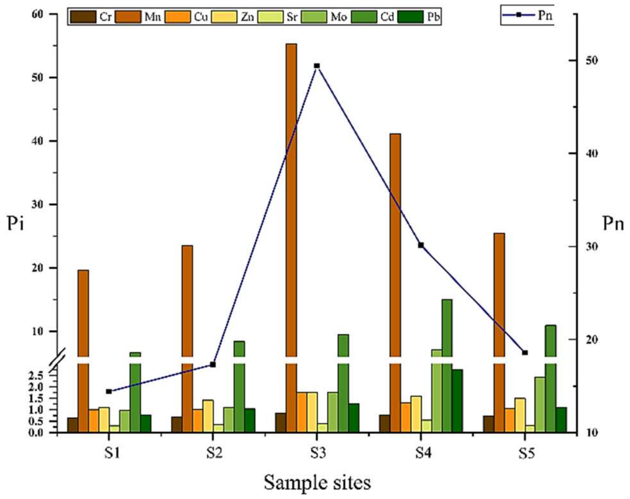

Fig. 2. Nemerow contamination index of surface soil at different sampling sites. **Notes**: P_i ≤1 Non-pollution、1< P_i ≤2 Minor pollution、2<P_i≤3 Light pollution、3<P_i≤5 Moderate pollution、P_i> 5 Heavy pollution；P_n<0.7 Security level、0.7<P_n≤1.0 Alert level、1.0<P_n≤2.0 Light pollution level、2.0<P_n≤3.0 Moderate pollution level、P_n> 3.0 Heavy pollution level.

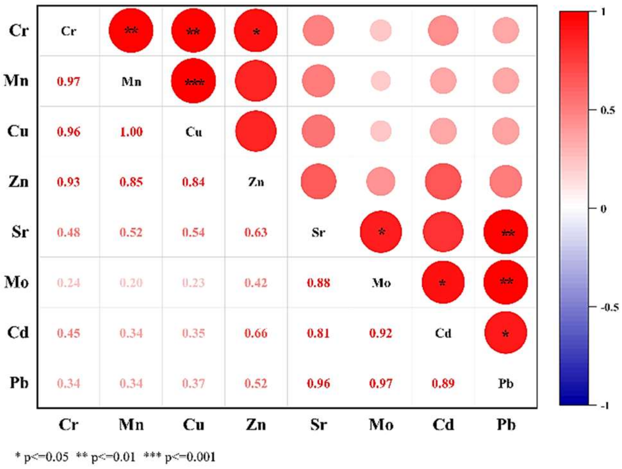

Fig.3. Pearson correlation analysis of soil heavy metal content at sampling sites. Notes: *P<0.05; **P<0.01.

and between Mo, Cd, and Pb. These findings suggest that Cr-Mn-Cu and Mo-Cd-Pb have similar origins and that synergism exists among the other heavy metals.

Plant heavy metal content and correlation analysis

The heavy metal content of the *Philonotis* and *Spirogyra* samples was analyzed, as shown in Table 4. There was a significant difference between the heavy metal content of the two plants. The heavy metal content of *Philonotis* was higher than that of Spirogyra, except Sr. The heavy metal content of *Philonotis* was Mn > Zn > Sr > Cr > Pb > Cu > Mo > Cd. The heavy metal content of *Spirogyra* was Mn > Sr > Zn > Cu > Cr > Pb > Mo > Cd.

The enrichment factor (BCF) is the ratio of heavy metal content in the above-ground part of the plant to heavy metal content in the root-soil. This value can be used to evaluate the plant's heavy metal uptake capacity (Dun *et al*., 2020; Gou *et al*., 2021). Both *Philonotis* and *Spirogyra* have some enrichment capacity for heavy metals in the soil, as shown in Fig. 4. Both have the largest enrichment factor for Sr and the smallest enrichment factor for Mn. Among them, the enrichment capacity of *Philonotis* exceeded that of *Spirogyra* for all soil heavy metals except Sr. The enrichment capacity of *Philonotis* for eight heavy metals was in the order of Sr > Pb > Zn > Cd > Cu > Cr > Mo > Mn, while the enrichment capacity of *Spirogyra* for the nine heavy metals was in the order of Sr > Zn > Cd > Cu > Mo > Pb > Cr > Mn.

Pearson's analysis showed a significant positive correlation between plants and soil heavy metals (Fig. 5): Individual heavy metals between *Philonotis* and soil interacted with each other and showed a significant positive correlation (P < 0.05), where ***Philonotis*** showed a significant positive correlation (P < 0.01) with soil heavy metals (Mn, Zn, and Cd). A positive correlation (P < 0.05) was also found between each heavy metal in *Spirogyra* and soil except Zn. Among them, *Spirogyra* correlated significantly (P < 0.05) with soil heavy metals (Mn and Sr).

Since there was a correlation between Mn, Zn, Cd, and Sr content in *Philonotis*, *Spirogyra,* and soil, they were analyzed by linear fitting (Fig. 6). The results

Table 4. Heavy metals in ***Philonotis*** and ***Spirogyra*** samples from different sample sites - Independent-Samples T test mg·kg-1

	Element	Cr	Mn	Cu	Zn	Sr	Mo	Cd	Pb
	Philonotis	16.78±0.52a	924.85±7.38a	10.80±0.98a	50.48±1.61a	39.67±1.34b	0.55±0.06a	0.31±0.02a	15.45±0.79a
S1	***Spirogyra***	2.71±0.21b	422.87±12.33b	3.38±0.33b	20.33±1.09b	60.39±0.55a	0.36±0.03b	0.14±0.02b	2.13±0.02b
	***P* value**	**＜0.001**	**＜0.001**	**0.002**	**＜0.001**	**＜0.001**	**0.045**	**0.003**	**＜0.001**
	Philonotis	21.64±1.32a	2580.16±95.76a	13.73±0.89a	71.06±1.80a	62.02±1.79b	1.05±0.17a	0.46±0.05a	24.08±1.56a
S2	***Spirogyra***	3.07±0.09b	787.80±8.18b	5.53±0.32b	33.05±1.44b	78.83±0.80a	0.40±0.02b	0.25±0.02b	3.66±0.19b
	***P* value**	**0.005**	**0.003**	**0.001**	**＜0.001**	**0.005**	**0.061**	**0.019**	**0.005**
	Philonotis	26.20±2.13a	11799.08±454.56a	20.10±2.21a	83.17±1.55a	68.97±1.78b	1.33±0.12a	0.58±0.05a	33.39±2.25a
S3	***Spirogyra***	4.29±0.01b	1247.29±33.16b	7.55±0.46b	33.71±1.08b	92.58±0.85a	0.46±0.04b	0.31±0.01b	5.45±0.31b
	***P* value**	**0.001**	**＜0.001**	**0.005**	**＜0.001**	**＜0.001**	**0.003**	**0.007**	**＜0.001**
	Philonotis	28.11±1.15a	6916.06±64.02a	20.26±2.15a	83.59±1.92a	69.12±1.73b	1.72±0.15a	1.13±0.01a	27.49±1.50a
S4	***Spirogyra***	6.00±0.18b	847.03±10.80b	7.12±0.07b	36.79±1.94b	100.87±1.52a	0.42±0.05b	0.26±0.02b	3.70±0.19b
	***P* value**	**＜0.001**	**＜0.001**	**0.004**	**＜0.001**	**＜0.001**	**0.001**	**＜0.001**	**0.003**
	Philonotis	26.74±0.86a	3061.05±91.46a	16.24±1.06a	76.56±1.92a	66.21±2.22a	0.84±0.09a	0.50±0.06a	17.72±0.64a
S5	***Spirogyra***	3.53±0.11b	790.64±8.72b	4.39±0.38b	24.79±1.50b	60.59±1.37b	0.37±0.01b	0.21±0.03b	2.29±0.12b
	***P* value**	**0.001**	**0.001**	**＜0.001**	**＜0.001**	**0.098**	**0.029**	**0.015**	**0.001**

Note: Numbers in the table are means ± standard deviation, followed by different lowercase letters representing significant differences ($P < 0.05$) between mosses and sponges in the five sampling sites, and significant *P*-values in the Independent-Samples T test are in bold.

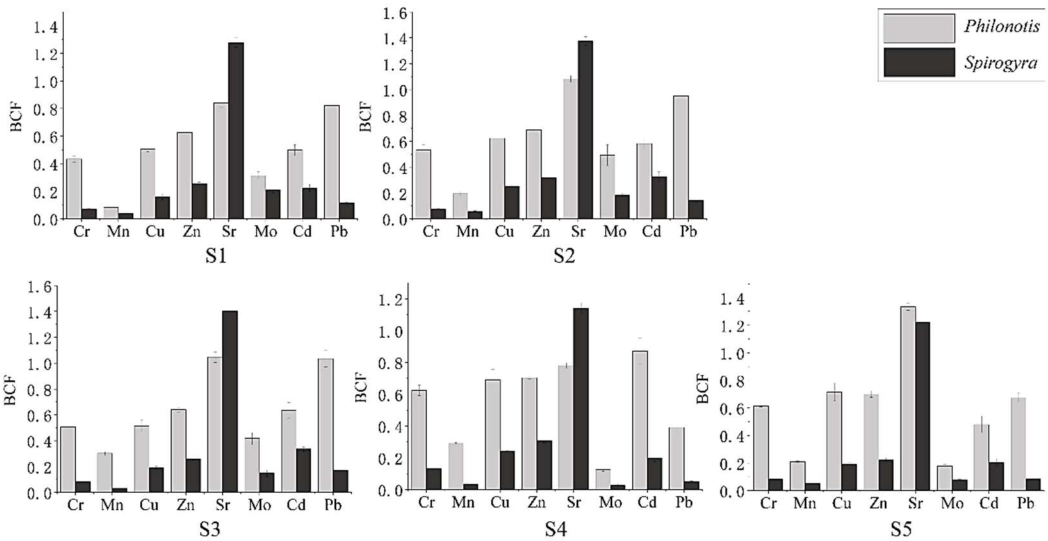

Fig.4. Comparison of heavy metal enrichment coefficients between ***Philonotis*** and ***Spirogyra***. Notes：when BCF<0.1, strongly depleted; when BCF<0.5, relatively depleted; when 0.5<BCF<1.5, both are at the same level; when BCF≥1.5, relatively enriched; when BCF>3, strongly enriched.

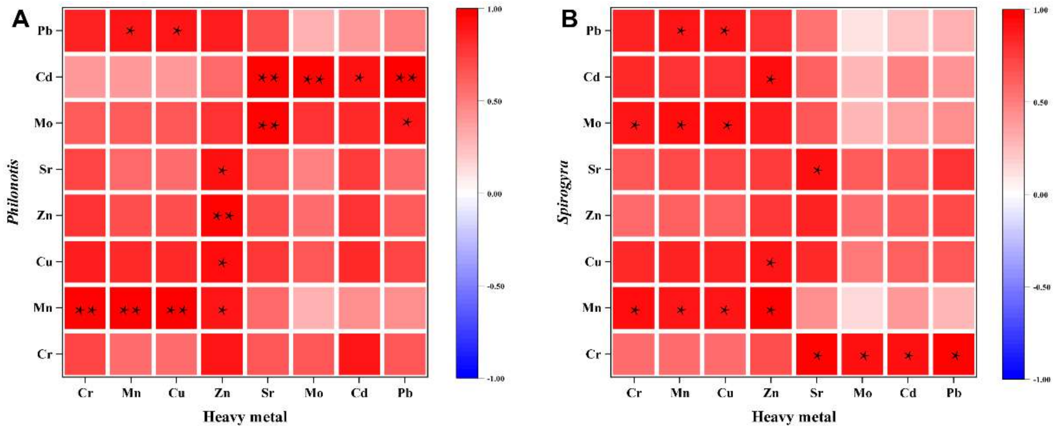

Fig.5. Pearson correlation coefficient of heavy metal content in ***Philonotis*** and ***Spirogyra*** with soil. Note: Panel a shows Pearson correlation analysis of ***Philonotis*** and soil; Panel b shows Pearson correlation analysis of ***Spirogyra*** and soil. *P<0.05; **P<0.01.

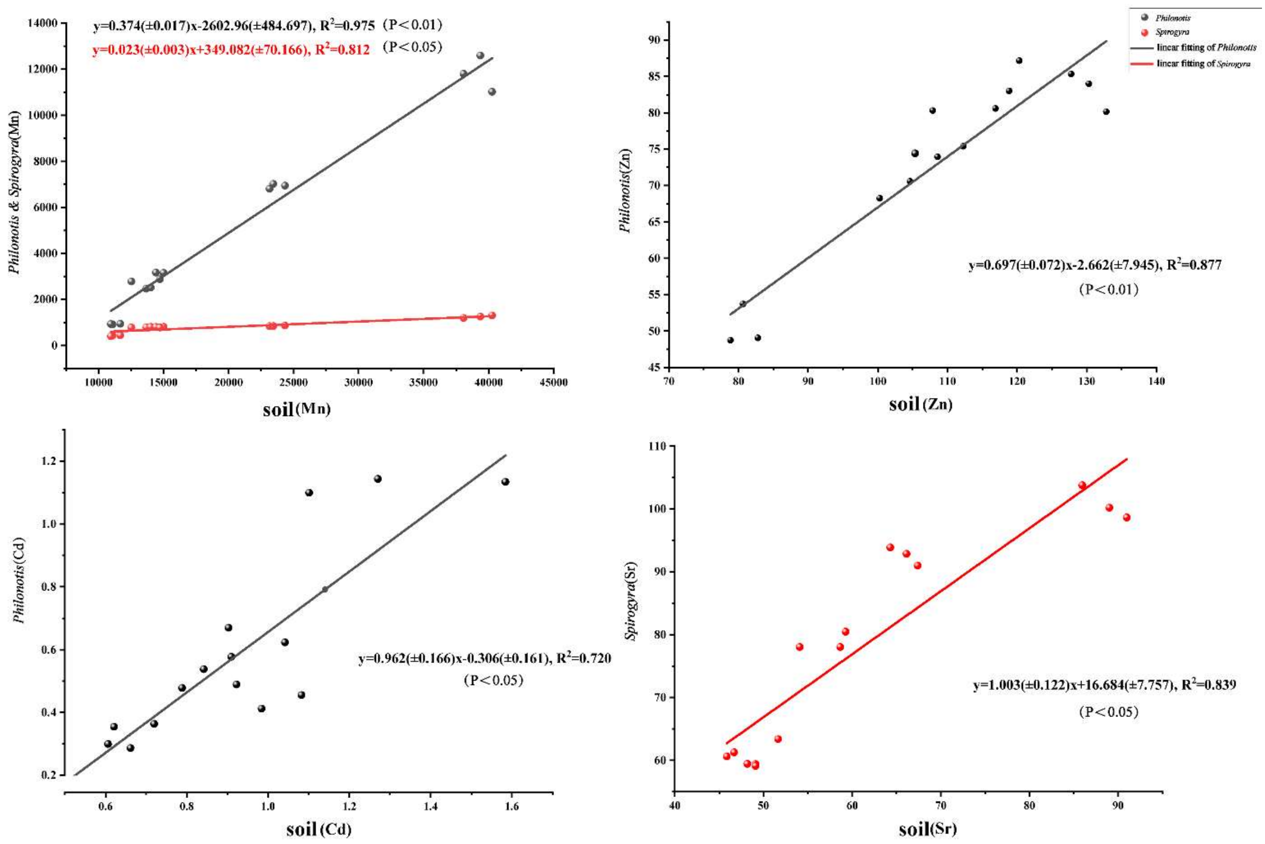

Fig.6. Linear fitting of heavy metal content in ***Philonotis*** and ***Spirogyra*** with soil

revealed that heavy metal content in *Philonotis*, *Spirogyra,* and soil varied similarly, showing a general increasing trend and linear relationship. As the heavy metal content in the soil rose, the heavy metal content in the plant also increased. *Philonotis* had the best fit to soil Mn with a positive correlation ($P < 0.01$) and a fitting equation of $y = 0.374(\pm 0.017)\ x\text{-}2602.96\ (\pm 484.697)$, $R^2 = 0.975$, followed by Zn ($R^2 = 0.877$); and *Spirogyra* had a good linear fit to soil Sr ($R^2 = 0.839$).

DISCUSSION

Analysis of heavy metal elements in soil

Due to the utilization of additives during manganese production, as well as the presence of associated elements in the ore itself, enterprises involved in manganese electroplating are primarily concerned with extracting Mn while other heavy metals remain in waste slag (Zhang and Lin, 2020). As this residue is stored and undergoes physical, chemical, and biological weathering processes, these heavy metals may be released into the surrounding environment causing changes in their location, concentration and composition. The soil heavy metal contents at different sampling points were found to be significantly different, among which the coefficients of variation of Mn, Mo, and Pb reached high variations. These findings indicate that the spatial distribution of these three heavy metals was not uniform, which could be the result of the combination of natural processes and human activities (Li *et al*., 2017). The regulation of precipitation-dissolution, adsorption-desorption, chelation-complexation, oxidation- reduction, and other geochemical processes (Li *et al*., 2019) play a crucial role. Such as clay minerals, which are widely present in mining soils and constitute a major component of them, exhibit significant adsorption capacity for Pb. Consequently, the migration ability of Pb within mining soil is limited and hindered, resulting in its high concentration near pollution sources (S4) and leading to an uneven distribution phenomenon (Han *et al*., 2023). Similar circumstances can be observed for other heavy metals. The migration of metal ions can be influenced to varying degrees by changes in soil pH and other factors (Zhang and Lin, 2020). Moreover, soil microorganisms play a significant role in the movement and transformation of heavy metals within the soil. Different sampling sites possess distinct microenvironments and species structures that can either immobilize heavy metals (Jia *et al*., 2020) or convert them into activated states, facilitating their migration (Adeyemi, *et al*., 2021).

Generally, Mn is a common element in the lithosphere (Zhu *et al*., 2004; Gilbert *et al*., 2006; Lv *et al*., 2014).

The abundance of Mn is high, and their concentrations are not easily affected by anthropogenic factors (Delplace *et al*., 2020; Sun *et al*., 2018). However, the high concentration and CV of Mn in the study area indicate that it is mainly derived from manganese mining and accumulation. Furthermore, Mo and Pb elements reached the extreme values in S4 (P_i-Mo: Level V, P_i-Pb: Level III). Considering the actual situation of the sampling site, S4 was close to the slag discharge site and the farmland planting maize and other crops. Mo elements are essential trace elements for plants and play an important role in improving the yield and quality of agricultural products and enhancing crop resistance (Xu, 2018; Li, 2019; Syaifudin, 2020; Zhang, *et al*., 2021). Related studies have demonstrated that spraying Mo fertilizer has the effect of delaying crop leaf senescence and improving leaf photosynthetic properties, which can improve water retention of crop (maize) leaves, improve crop drought resistance, and reduce drought damage (Sun *et al*., 2016). Under the influence of rainwater runoff and agricultural fertilization, it is speculated that Mo may have come from farmland fertilization activities in the study area. Simultaneously, relevant studies also showed that Pb came from farmland (Luo *et al*., 2015). The CV of Cr, Cu, Zn, Sr, and Cd revealed low to medium variation, indicating that the spatial distribution was relatively uniform, which could be the result of natural sources (Zhao *et al*., 2010; Li *et al*., 2017; Wu *et al*., 2020). The concentration of various heavy metals in the soil of the study area varied, and these metals may have had antagonistic or inhibitory effects on each other. Consequently, the interactions between each heavy metal were analyzed using the Pearson correlation coefficient method. This analysis aimed to identify similar pollution sources or associated pollution phenomena between the heavy metals, thereby determining the primary factors influencing the soil's heavy metal content in the study area. In the correlation analysis, Cr-Mn-Cu and Mo-Cd-Pb were significantly correlated ($p < 0.05$), indicating that these metals may have derived from a common source (Xu *et al*., 2014). Therefore, it is assumed that Cr-Mn-Cu originated from manganese ore activity and Mo-Cd-Pb from nearby farmland activity.

In terms of average values, except for Cr and Sr, the six heavy metals in the study area exceeded the background values of soil elements in layer A of China and were generally polluted by Mn, Zn, and Cd. The single factor pollution index exceeded 1, and specifically for Mn and Cd, this index was greater than 5, indicating severe pollution. This finding aligned with the results presented by Zheng *et al*. (2020) and Li *et al*. (2018). Therefore, Mn and Cd pollution should be given priority in soil management. According to the Nemerow Comprehensive Pollution Index (P_n), the study area has reached a serious pollution level. Based on the index changes, the pollution levels in the study area followed a pattern of S3 > S4 > S5 > S2 > S1, exhibiting an initial increase and then a decrease. This trend was attributed to the distance from the manganese mine area, with S1 and S2 being farther away while S3 and S4 were closer. The downward migration of heavy metal elements, influenced by rain leaching, likely resulted in higher pollution levels at S3 and S4 compared to S1 and S2. This pattern indicated that the degree of wetland soil pollution was associated with the proximity to the manganese mine pile. The nearer to the manganese ore pile, the greater the severity of heavy metal pollution (Liu *et al*., 2011b). Several studies also reported that the highest accumulation of heavy metals was found near the mining activity area (Niane *et al*., 2014; Resongles *et al*., 2014; Chen *et al*., 2019; Adewumi and Laniyan, 2020).

Analysis of heavy metal elements in plants

When heavy metals accumulate in plants beyond a specific level, they degrade macromolecules such as proteins, lipids, and nucleic acids, induce the production of reactive oxygen species, disrupt cell metabolic activities, and lead to cell death (Ouelhadj *et al*., 2006, Chen *et al*., 2020). These events have toxic effects on plants. Although heavy metals have adverse effects on plant growth and development and various physiological and biochemical activities, most plants in nature can maintain growth, development, and reproduction in high concentrations of heavy metals. This indicates that plants have gradually formed a relatively complete mechanism for tolerance to heavy metals during long-term evolution (Chen *et al*., 2020). Accordingly, *Philonotis* and *Spirogyra,* which were able to grow in the study area, were more adaptable and tolerant to heavy metals.

The general Mn content in plants is Mn 20–500 mg/kg (Liu *et al*., 2009; Liu *et al*., 2011a). Table 4 indicates that the maximum Mn contents of *Philonotis* and *Spirogyra* are 11799 and 1247 mg/kg, respectively, which exceed the normal values of Mn contents of general plants. The concept of hyperaccumulator was introduced by Brooks in 1977 (Brooks, 1977). Most current definitions of hyperaccumulators are based on Baker and Brooks' 1989 proposal that hyperaccumulators can take up and accumulate more than 10000 mg/kg Mn from contaminated soils (Baker and Brooks, 1989). Therefore, the Mn content of *Philonotis* has reached the standard of hyperenrichment. The unique structure of Philonotis endows it with a robust capacity for cation exchange (Lou, 2013). The leaves are composed of a single layer or a few layers of cells. The physiological and metabolic characteristics of *Philonotis*, such as the high ratio of plant surface area to biomass, low degree of differentiation, and relatively vigorous cell growth potential energy, are beneficial to the enrichment of heavy metals in the environment due to the hairy branching structure of *Philonotis*, which have a strong ability to adsorb metal ions (Bleuel *et al*., 2004; Li, 2006).

Further research is required to ascertain if these plants are hyperaccumulators.

Although the Mn content of *Spirogyra* exceeded the normal range of plant Mn content, it did not meet the ultra-hyperenriched standard. Related studies have shown that *Spirogyra* can accumulate toxic elements (Rai *et al*., 2008), which may involve changes in the chemical formula of toxic metal ions (Folsom *et al*., 1986). This phenomenon is related to the ability of *Spirogyra* to aggregate these metals from soil. These are similar to the observations of Rajfur *et al*. (2011) for copper, zinc, iron, manganese, and lead. The low concentration of heavy metals in *Spirogyra* suggests that the uptake of these metals by *Spirogyra* is regulated (Trollope and Evans., 1976). *Spirogyra* can adsorb heavy metals at low concentrations, but its adsorption capacity is reduced at higher concentrations (Mane *et al*., 2012). *Spirogyra* can protect itself from heavy metal-induced toxicity through multiple mechanisms and is able to exclude these heavy metals, thus limiting heavy metal accumulation in *Spirogyra* (Abd-el-Monem *et al*., 1998; Yu and Kaewsarn, 1999; Omar, 2002; Hassler *et al*., 2005). Therefore, *Spirogyra* was less able to enrich heavy metals than *Philonotis*.

Both *Philonotis* and *Spirogyra* have a specific ability to enrich heavy metals. Among the eight heavy metals, both *Philonotis* and *Spirogyra* had the largest enrichment coefficient of Sr elements, indicating that plants have the most substantial enrichment ability and high demand for Sr, which is consistent with previous studies (Boyer *et al*., 2018). Although the contents of Mn in the two species are extensive, the enrichment coefficient of Mn in the two species is the lowest. The main reason for the small enrichment coefficient may be related to the leaching, content, and existence form of Mn in the soil (Fang *et al*., 1998). On the other hand, for the enrichment coefficients of the same element, *Philonotis* and *Spirogyra* showed similar characteristics. This "convergence" could be the result of long-term selection and adaptation between plants and soil in a specific natural environment (Fang *et al*., 1998). Generally, plants differ in their enrichment of different elements in the environment, which is not only influenced by the degree of accumulation of heavy metals in the growing substrate but is also closely related to their absorption and accumulation characteristics of these elements and their growth requirements (Olajire, 1998; Salemaa *et al*., 2004).

Correlation analysis between soil and plants

Analysis of the correlation between heavy metals in *Philonotis* and *Spirogyra* in the study area and heavy metal content in soil showed that there was a significant positive correlation between *Philonotis* and Mn, Zn, and Cd in soil, and *Spirogyra* and Mn and Sr elements in soil. This indicates that the heavy metals in the plant body come mainly from the soil. Furthermore, the increase of these heavy metals in the plant can be attributed to the content of heavy metal elements in the soil. These results are consistent with Long and Zhang (2016) and Bargagli *et al*. (1995). Due to synergistic or antagonistic effects, different elements in plants may promote or inhibit the uptake of elements (Zeng *et al*., 2002; Woods *et al*., 2021). A significant positive correlation between two elements implies a synergistic effect between them, while a significant negative correlation suggests an antagonistic effect (Yuan *et al*., 2006; Jiang and Zhang, 2013). Except for Sr, there were highly significant correlations between the different elements in *Philonotis*. Therefore, it is assumed that these elements are of similar origin and that there is a synergistic effect when the elements are absorbed, as is the case in *Spirogyra*.

A linear fitting model was used to reflect the enrichment effect of plants on heavy metals; the closer the R2 value is to 1, the more it reflects a linear relationship. In the linear model, the best fit was obtained for *Philonotis* to soil Mn with an R^2 value of 0.975. This was followed by the fit of *Philonotis* to soil Zn with $R^2 = 0.877$; *Spirogyra* was not as well adapted to soil Mn as *Philonotis* ($R^2 = 0.812$). Compared to *Spirogyra*, *Philonotis* is more suitable for use as a biomonitor to reflect the heavy metal content in the soil and to indicate the level of heavy metal contamination from manganese ore. Several studies have shown that moss is a good indicator of soil heavy metals (Shaw, 1987; Fu and Zhang, 2011). The study by Xu *et al*. (2021) showed that bryophytes have an excellent indicator role in the beneficiation area, storage area, waste rock area, and mine area of manganese mine. Han reported that bryophytes had an optimal monitoring effect on soil heavy metal pollution at different stages of ecological succession in the mining area (Han *et al*., 2022). Other studies have reported that moss is suitable as a biodetector to characterize wetland environmental conditions (Gecheva *et al*., 2011; Gecheva *et al*., 2015). Among them, Taeprayoon's research results indicated that *Philonotis* had an excellent indicator effect on Cd elements in the wetland environment of a mining area in Thailand (Taeprayoon *et al*., 2023).

Compared to the direct use of soil heavy metal content to reflect regional pollution, the use of mosses is more stable and reliable. Most mosses are perennial plants, which are more suitable for long-term monitoring of heavy metal pollution (Galsomiès *et al*., 1999). Furthermore, Bryophytes as indicators of environmental quality have several other distinct advantages: the ability to obtain bryophyte material year-round and at low cost, the speed and convenience of sampling, and the ability to be used in different types of study environments. Because of its wide distribution, the authors believe that *Philonotis* is suitable not only for monitoring the study area but also for monitoring heavy metal pollution in similar mining areas.

CONCLUSION

This study investigated the content of heavy metals (Cr, Mn, Cu, Zn, Sr, Mo, Cd, and Pb) in soil, *Philonotis*, and *Spirogyra* samples in the wetland of Zhaiying Manganese Mine slag discharge field, as well as the monitoring and accumulation of heavy metals. The results of this study offer valuable insights into other wetlands contaminated with heavy metals and aid in monitoring and managing heavy metal pollution. The main findings of this study are as follows:

1、There are different degrees of heavy metal accumulation in the wetland soil of the Zhaiying manganese mine dumping area, and the study area was polluted by Mn and Cd, which reached a heavy pollution level. The closer to the manganese ore pile, the more serious the heavy metal pollution.

2、The ability of *Philonotis* to accumulate and monitor heavy metals was found to be superior to that of *Spirogyra*. Therefore, *Philonotis* could be used as an indicator plant for monitoring soil heavy metals in manganese mine wetlands. The Mn content of *Philonotis* was more than 10000 mg/kg, which reached the standard of hyperenriched plants. However, further studies are needed to validate these findings.

ACKNOWLEDGMENTS

The authors thank the National Nature Science Foundation of China (No.31960044.) and the Department of Science and Technology Foundation of Guizhou Province China [DSTFGC (2019)] for financial support. We thank Zhu Di, Chen Pengpeng, Ma Yijing for their help with fieldwork.

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