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Using mosses and lichen to study roadside pollution and the correlation of toxic elements and elevation in a mountainous area of Guizhou, China

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ABSTRACT: In this study, three moss species (*Bryum argenteum*, *Rhynchostegium subspeciosum* and *Orthotrichum dasymitrium*) and one lichen species (*Parmelia saxatilis*) were sampled to monitor the levels of toxic elements along a section of a gorge road for assessing the enrichment ability of these species and the effects of toxic elements at different elevations. Hierarchical clustering was used to classify samples based on geographic data and ICP-MS to determine the contents of toxic elements in each plant. Based on the obtained levels of enrichment factors (EF), metal accumulation index (MAI) and geoaccumulation index (I_{geo}), *Bryum argenteum* (highest MAI and I_{geo}) was identified as the most suitable biomonitoring species. PMF source analysis showed that the main pollution sources were traffic dust (45%) and other traffic emissions (43%). Road pollution was alarming as high Pb, As and Cr levels were detected as the most important pollutants. The levels of toxic element depositions along the road of the Karst gorge area increased with rising elevation. The vertical distribution of pollutants was affected by temperature, topography, dominant winds and pollutant emissions. However, some variations in the distribution in similar areas were also observed.

KEY WORDS: Epiphytes, mosses and lichen, toxic elements, source analysis, atmospheric contamination.

INTRODUCTION

According to the World Health Organization (WHO), any kind of toxic element can seriously threaten human health and safety. For areas around roads, heavy metals can come from many different sources, of which automobile exhaust is one of the important sources of heavy metals. According to a recent report, the three main factors affecting the quality of soils are traffic, industry and material weathering. Further, studies have shown that road dust (Musa *et al*., 2019), mechanical wear (Fan *et al*., 2021), oil leakage (Bourliva *et al*., 2017), vehicle emissions (Timothy and Williams, 2019) and others were among the main sources of toxic elements pollution (Liu *et al*., 2018a). This has led scholars worldwide to pay close attention to road pollution (Heidari *et al*., 2021; Bownik and Wlodkowic, 2021). Typical traffic pollutants, such as Cd, Cr, Pb and Hg, are known to impact human health (Tchounwou, *et al*. 2012). According to the IARC (IARC, 1989; 2012), As, Cd, Ni and Cr (VI) are classified as carcinogenic, while Sb_2O_2 is potentially carcinogenic (Group 2B). These particulates are the source of toxic element pollution (Kumari *et al*., 2021). Surface soil and dust result from heavy metal pollution caused by atmospheric deposition. In China, despite their convenience, major roads such as national highways are also major contributors to air and soil pollution (Shi *et al*., 2020). Although leaded gasoline has been banned for decades, road traffic pollution remains a major source of greenhouse gases and toxic element pollution (Pariente *et al*., 2019). To monitor their potential effects on the surrounding, it is important to identify suitable biological monitoring species that respond quickly to changes in pollution levels to take timely measures and keep their levels within accepted ranges (Gallego-Cartagena *et al*., 2020). Monitoring road pollution using living organisms is a low-cost and environment-friendly strategy. However, in most studies, a single species of plant was selected as the biological monitoring material, which not only increased the difficulty of sample collection but also led to an increase in data deviation. To solve this issue, simultaneous use of various bryophytes and lichens to monitor pollutants could be implemented to improve the accuracy and screen out the most suitable model species for monitoring (Bajpai *et al*., 2014).

In general, the distribution of road pollution is influenced by the parent material properties and climate. Their relative mobility depends on soil parameters such as mineralogy, texture and soil classification (Ogundele *et al*., 2015). In this study, a mountainous road in the Duyun City of the Guizhou Province was selected for investigation based on the following considerations: (1) It is located in the largest karst area in China. The exposure of carbonate rocks caused by rocky desertification intensifies weathering. (2) The section is located in a mountainous area at a high altitude with large differences in heights. (3) It is surrounded by mountains on three sides, with only a valley connecting the north to the south, demonstrating the qualities of unique settlement law. Apart from its high altitude, the study area also has a large traffic flow and steep roads, which is representative of the Karst gorge road (Robinson *et al*., 2017). The road X922

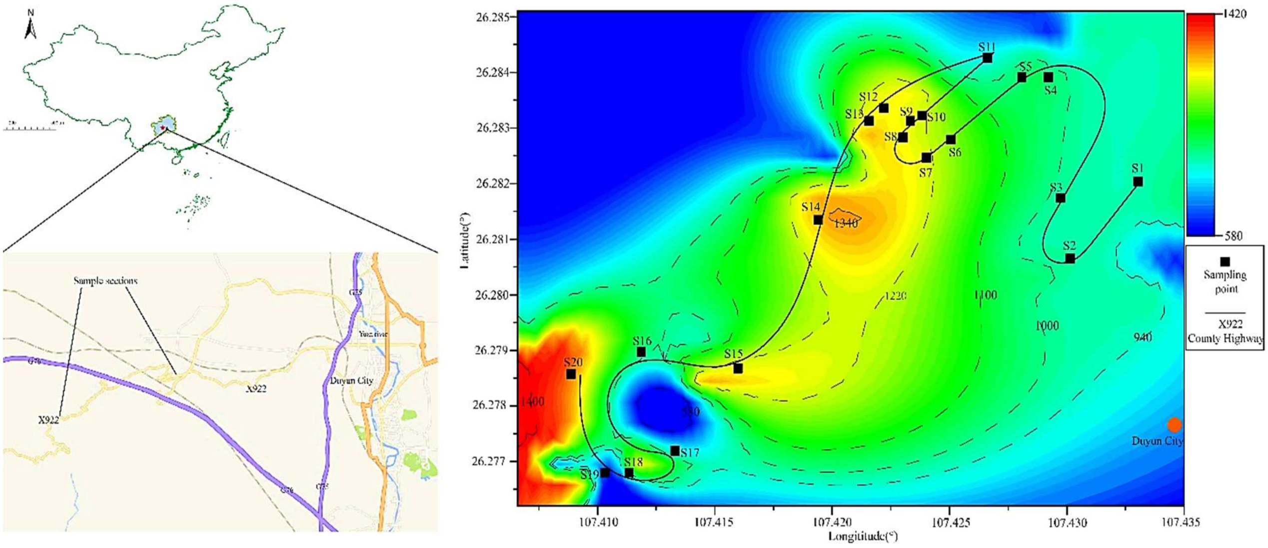

Fig 1. Sampling area and distribution of sample points.

passing through Duyun city was completed in 2010. In 2011, the number of motor vehicles in Duyun city alone was about 50,000. By 2020, the number had exceeded 250,000 motor vehicles, and it is still growing at a rate of about 20% every year. As there are a lot of mountains in this region and few roads, enormous pressure is imposed on the roads and the surrounding environment.

Bryophytes and lichens are considered as excellent biological monitoring plants due to their special physiological structure (Asakawa and Ludwiczuk, 2017). Also, in previous literature, mosses and lichens were widely used to assess the consequences of road pollution (Achotegui-Castells *et al*., 2013), while epiphytic plants were identified as excellent biomaterials for monitoring the atmosphere along the road (Czerepko *et al*., 2021). Parviainen *et al*. (2020) reported that the pollutant content in lichens could distinctively reflect traffic exhaust emissions. Compared with soil, lichens have a unique function of enrichment (Shoham-Frider *et al*., 2020) and differ from higher plants due to their unique structure, which makes them a more suitable model to reflect regional pollution status (Eldridge and Tozer, 2009; Niemelä *et al*., 2007). An ideal model species should have the following characteristics: (1) reduce the difficulty of monitoring, (2) sensitive to the concentration of pollutants at any given time (Bownik and Wlodkowic, 2021), and (3) provide enough data for the establishment of regional subsidence models (Chen and Lu, 2017). Bargagli (2016) found that mosses and lichens were the ideal indicators of heavy metal deposition caused by mineral deposits in mountainous areas. However, due to their innate characteristics, the adsorption of heavy metals is different, resulting in differences in the estimated concentration of pollutants in plants (Millhollen, 2006). In this regard, Godinho *et al*. (2009) proposed that the atmospheric pollutant deposition model based on suitable occult plants had high reliability. Therefore, this experiment attempted to identify species potentially suitable for monitoring atmospheric pollutant levels in mountainous areas.

In this study, *Bryum argenteum*, *Rhynchostegium subspeciosum*, *Orthotrichum dasymitrium* and *Parmelia saxatilis* were chosen because they are abundant at different elevations at the region of interest and could be ideal for screening. Based on a previous study concluding that the elevation gradient of mountainous roads affects pollutant settlement (Liu *et al*., 2018b), this study used pollution coefficient methods (EF, MAI, I_{geo}) to screen for the most appropriate species after dividing sample points by hierarchical clustering. Further, after confirming *Bryum argenteum* as the most suitable species, its corresponding EPA PMF model was adopted to analyze pollution sources.

MATERIALS AND METHODS

Description of the study area

The section investigated in this study was a national highway located in the southeast of Guizhou Province (China), which belonged to the Duyun City (Fig. 1). The region has a subtropical humid monsoon climate. Its dominant wind direction is northeast, followed by southeast. Its average annual sunshine hours are 1373 h, average annual temperature is 16.7°C, average annual precipitation is 1819 mm, and the frost-free period is about 298 d. The highest point of the study area was 1,961 m above sea level, the lowest point was 540 m above sea level, and the average elevation was 938 m.

Sample collection

Most sampling was conducted twice in November 2020 in Duyun city (Guizhou, China) after at least one consecutive week without precipitation to ensure that the precipitation did not wash away the adsorbed pollution particles on the sample surface. *Populus nigra* trees on both sides of the highway had been planted artificially, with roughly the same growth condition, similar age and about 10–15 m in height. Samples were taken from each tree trunk one meter above the ground, in sections of 20 cm each, until the tree trunk was two meters apart. Five samples, divided by height on each tree, were collected. During the collection, the coverage of epiphytes was recorded with a 10×10 cm square, and data such as elevation, light intensity, elevation from ground, longitude and latitude were also recorded. After collection, the samples were immediately put into a kraft envelope and sealed to prevent later contamination interference. The samples were identified and screened in a laboratory immediately after collection. A total of mixed samples of tree epiphytes were collected.

Sample preparation and analysis

Bryophytes were identified using a light microscope and Flora Bryophytarum Sinicorum Vol. 2 and Vols. 4–8 (Gao, 1996; Wu, 2002; Hu and Wan, 2005; Wu and Jia, 2011, 2017), based on which a total of 34 species, 22genera and 9 families of bryophytes were identified. The widely distributed *Rhynchostegium subspeciosum*, *Bryum argenteum*, *Orthotrichum dasymitrium* and *Parmelia saxatilis* were chosen as potential indicator species for biological monitoring.

Further, the collected moss samples were processed to remove excess debris in the laboratory. Before determination, the samples and blank controls were prepared. According to the standard (DB65/T 3974-2017), 0.2 g of the samples were weighed and placed in Polytetrafluoroethylene (PTFE) digestion tubes and graphite digestion holes. Blank tests were performed along with the samples. Digestion steps were completed using an automatic digestion apparatus (AutoDigiBlock automatic digestion apparatus, Beijing LabTech Instruments Co., Ltd., China).

After the digestion, elemental determination could be measured more accurately. According to the working conditions of the instrument, a mixed standard working solution was used, and the internal standard method was added online. The content of each element in the samples was measured based on the standard curve.

ICP-MS (NexION 300X, Perkin-Elmer, Thermo Fisher Scientific, USA) was used to determine the concentration of Cr (DL=0.003 µg/ml), Co (DL=0.00 5µg/ml), Ni (DL=0.009 µg/ml), Cu (DL=0.002 µg/ml), As (DL=0.03 µg/ml), Cd (DL=0.00 3µg/ml), Sb (DL=0.04 µg/ml) and Pb (DL=0.03 µg/ml).

Data processing methods

Pollution coefficient method

The Enrichment Factor (EF) is a suitable method for evaluating anthropogenic sources of atmospheric particulate pollutants (Sutherland, 2000). It was calculated using the following formula:

$$EF = \frac{(C_i/C_o)_{Moss}}{(C_i/C_o)_{Backgroound}}$$

Here, *Ci* represents the content of element i in the moss and background area, and C_0 represents the content of the reference element in the moss and soil.

The EF values were used to assess the pollution of toxic elements into the following classes: minimal enrichment (EF < 2), medium enrichment (2 < EF < 5), significant enrichment (5 < EF <20), high enrichment (20 < EF < 40) and most enrichment (EF > 40) classes (Barbieri, 2016).

The Metal Accumulation Index (MAI) was developed for plant toxic element enrichment based on the air quality index (Liu *et al*., 2007). It is helpful to identify the optimal monitoring species because it can remarkably reflect the ability to enrich toxic elements. The following equation was used to calculate MAI:

$$MAI = (1/N)\sum_{j=1}^{N} I_j$$

Here, N represents the number of metals needed to be analyzed. I_j is obtained by dividing the mean value of metal j by the standard deviation of metal j. Therefore, in this experiment, the MAI index of the toxic element content measured at each elevation was calculated.

The Ground Accumulation Index is a quantitative index widely used to evaluate the degree of pollution of heavy metals and other substances, using the following formula:

$$I_{geo} = log_2[C_s^i/(K \times C_n^i)]$$

Here, C_s^i represents the content of element *n* in living bodies. According to the changes in background values caused by the rocks in Guizhou, the value of *K* was 1.5. C_n^i was used as the background value of soil element content in Guizhou Province.

The geoaccumulation index was used to classify ecological pollution (Table 1).

Table 1. Classification of Muller geoaccumulation index.

Geoaccumulation index grade	Degree of contamination	Geoaccumulation index grade	Degree of contamination
0<I_{geo}<1	None	3<I_{geo}<4	Medium plus
1<I_{geo}<2	Slight	4<I_{geo}<5	Strong
2<I_{geo}<3	Medium	5<I_{geo}<6	Very strong

Positive Matrix Factorization (PMF) Analysis

The PMF model is a calculation method based on the conservation of mass between emission source and sample point. It uses the mass balance equation to assign the concentration of each element in each sample to different sources that influence that element. It can extract the optimal solution of the model according to different criteria such as residual distribution, G-space graph and

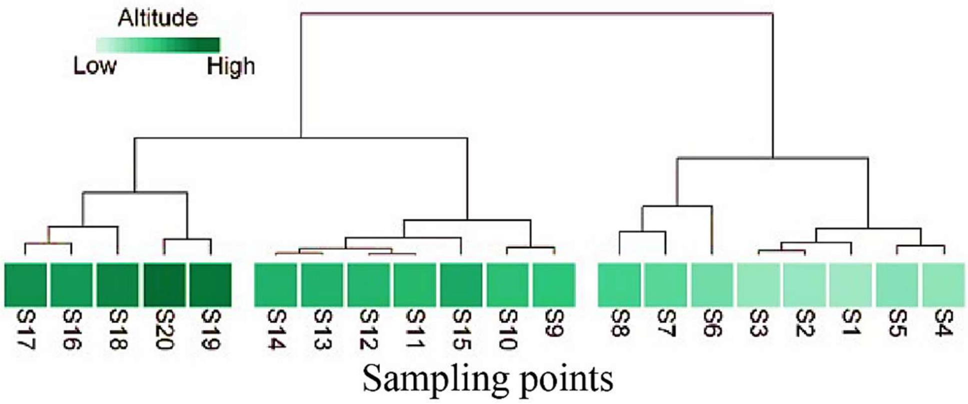

Fig 2. Cluster analysis of sample points. Low elevation (S1-S8, 899-1103 m); Medium elevation (S9-S15, 1103-1278 m); High elevation (S16-S20, 1278-1409 m).

Q value. The PMF of pollution sources was performed using the receptor model. After the data was imported, the final solution was based on 100 runs, and the interpretation was evaluated using different Fpeak values (in the range of -1.0 to 1.5). In this study, Fpeak = -0.5 was used as the best solution.

The PMF model was also used to solve the pollutants for a user-specified number of source factors (k = n) (Norris *et al.*, 2014). The chemically speciated IMPROVE data provided the input, x_{ij}. The number of samples, a time series of daily averages, corresponded to i. The number of chemical species used in the analysis is represented by j. g represents the mass of each factor (k) contributing to each sample (i), and f is the chemical fingerprint of each factor. e_{ij} is the residual of each species for a given sample.

$$x_{ij} = \sum_{k=1}^{n} g_{ik} f_{kj} + e_{ij}$$

The model solved for 'g' and 'f' using multivariate analysis and conditions wherein no source factor may be significantly negative for any given sample. Factor sources were determined by examining each factor's chemical fingerprint, as calculated by the model.

The sampling area overview was performed using ArcMap 10.7. The normality of the data was assessed using Shapiro-Wilk test. The sample plot elevation map and bar chart were drawn using Origin 2021. Further, clustering analysis was performed using R 4.0.4, image processing and beautification were conducted using Adobe illustrator 2021, and EPA PMF was employed to analyze the source of contaminants.

RESULTS

Cluster analysis of sampling points

Hierarchical clustering was used to divide sampling points based on their corresponding elevation gradient. As shown in Fig. 2, where S1-S20 represents the sample points, and darker colors represent higher elevation. The clustering results showed that the sample points could be divided into three parts based on their geographical coordinates, which were labeled as: low elevation (S1-S8, 899-1103 m), medium elevation (S9-S15, 1103-1278 m) and high elevation (S16-S20, 1278-1409 m) sample areas.

Concentrations of toxic elements in mosses and lichens

In this experiment, the contents of 8 typical traffic toxic elements in the samples were determined. As shown in Fig. 3, the concentration of Cr and Co tended to decrease as the elevation increased, while that of Pb was reversed. In the other elements, no obvious change was observed with elevation. In addition, we noted that the concentration of Pb was highest in each gradient and each species, followed by Cu.

The background value of pollutants required for calculation was the content of toxic elements in the A-layer soil of Guizhou Province (Liu, 2018). The specific values are shown in Table 2.

Table 2. Background values of soil elements in layer A of Guizhou Province.

Element	Cr	Co	Ni	Cu	As	Cd	Sb	Pb
Concentration (mg/kg)	109	23	51	61	25	0.3	1.5	37

Pollution coefficient evaluation

EF of different species

According to Fig. 4, the Cd coefficient of plants in the selected area was very high, and its enrichment effect was the most severely affected. On the contrary, the enrichment coefficients of Co and Ni were very low, with almost no enrichment. According to the enrichment factor of the elements, their enrichment ability could be arranged as follows:

Low elevation: *Parmelia saxatilis* > *Orthotrichum dasymitrium* > *Bryum argenteum* > *Rhynchostegium subspeciosum.*

Medium elevation: *Parmelia saxatilis* > *Orthotrichum dasymitrium* ≈ *Rhynchostegium subspeciosum* > *Bryum argenteum.*

High elevation: *Rhynchostegium subspeciosum* > *Orthotrichum dasymitrium* > *Parmelia saxatilis* > *Bryum argenteum.*

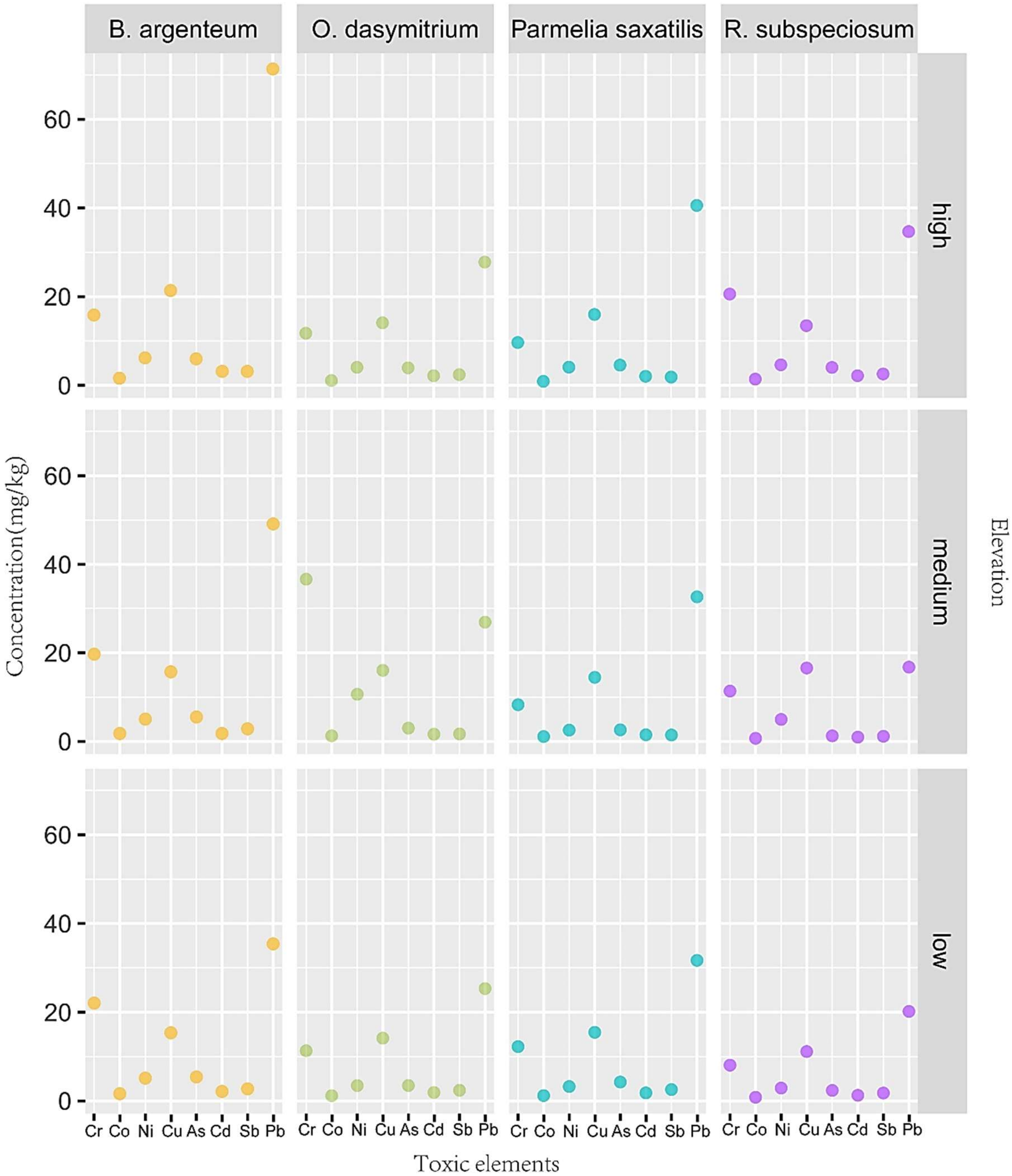

Fig 3. Concentrations of Cr, Co, Ni, Cu, As, Cd, Sb and Pb of three mosses ***Bryum argenteum***, ***Orthotrichum dasymitrium***, ***Rhynchostegium subspeciosum*** and the lichen ***Parmelia saxatilis*** (Determined by ICP-MS)

Parmelia saxatilis and *Orthotrichum dasymitrium* had stronger enrichment capacity, while the enrichment capacity of *Bryum argenteum* was poor. In addition, based on the horizontal contrast, the EF of the elements increased with elevation, except for Cr, Cu and Ni, suggesting that elevation affected the deposition of pollutants.

MAI of different species

MAI provides an overview of a plant's ability to absorb various toxic elements (Fig. 5). For this study, a bryophyte was selected as the common species at all elevations, so the influence of elevation change on the bryophyte species was not considered in the MAI index (Fig. 5). Among these four plants, the MAI index of *Bryum argenteum* was the highest ($P = 0.012$). It also had the strongest metal accumulation ability at different elevations. The other three species demonstrated similar accumulation capacity for different metals.

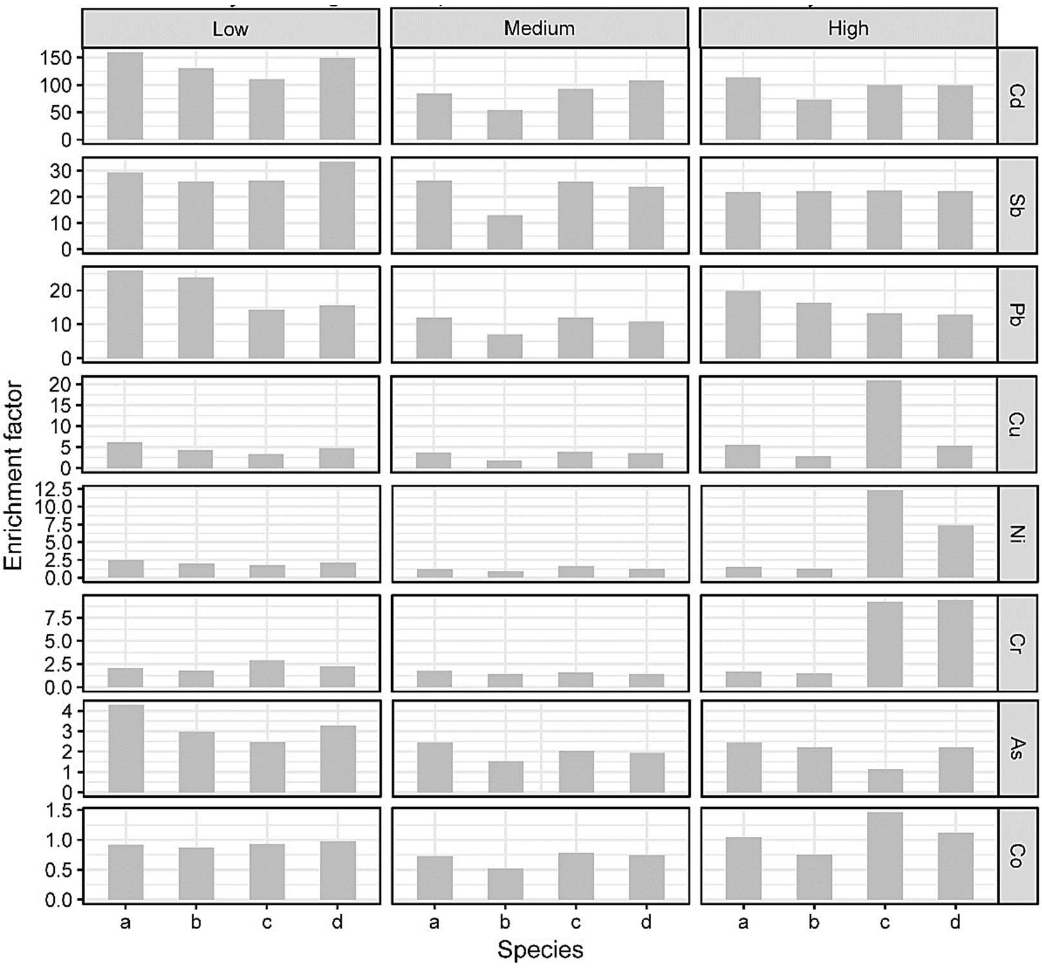

Fig 4. Enrichment factor of different species at different elevations. (a: ***Parmelia saxatilis***, b: ***Bryum argenteum***, c: ***Rhynchostegium subspeciosum***, d: ***Orthotrichum dasymitrium***)

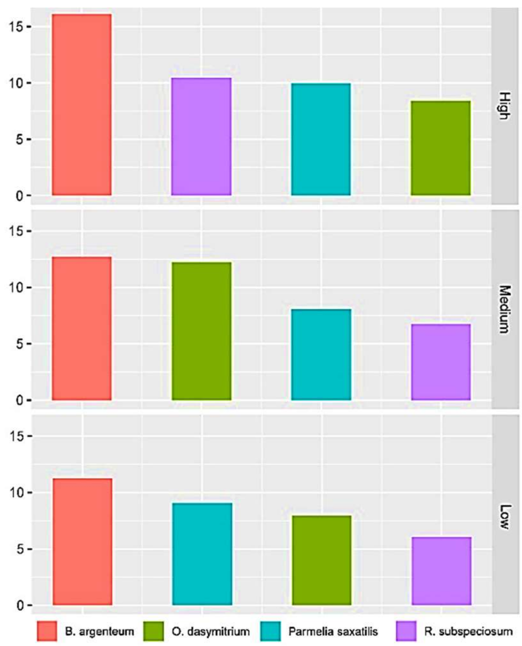

Fig 5. Metal accumulation index of different species.

Geoaccumulation index of different species

Fig. 6 illustrates the Geoaccumulation index of four species at different elevations. In the selected study area, apart from Cd, Pb and Sb, the other elements demonstrated no obvious enrichment of exogenous pollution. Cd had the highest enrichment degree at different elevations, while *Bryum argenteum* had the highest land accumulation index at the same elevation. Similar rankings were observed in three different altitudinal groupings: *Bryum argenteum* > *Orthotrichum dasymitrium* > *Parmelia saxatilis* > *Rhynchostegium subspeciosum*.

Further, in this study, *Bryum argenteum* was the most suitable indicator species for assessing the degree of regional pollution. In addition, all species showed that the regional risk level was better reflected at higher elevations.

Pollutant source analysis

Here, *Bryum argenteum* was chosen as the ideal indicator species. In terms of EF, our data showed that *B. argenteum* did not perform optimally and may not be a

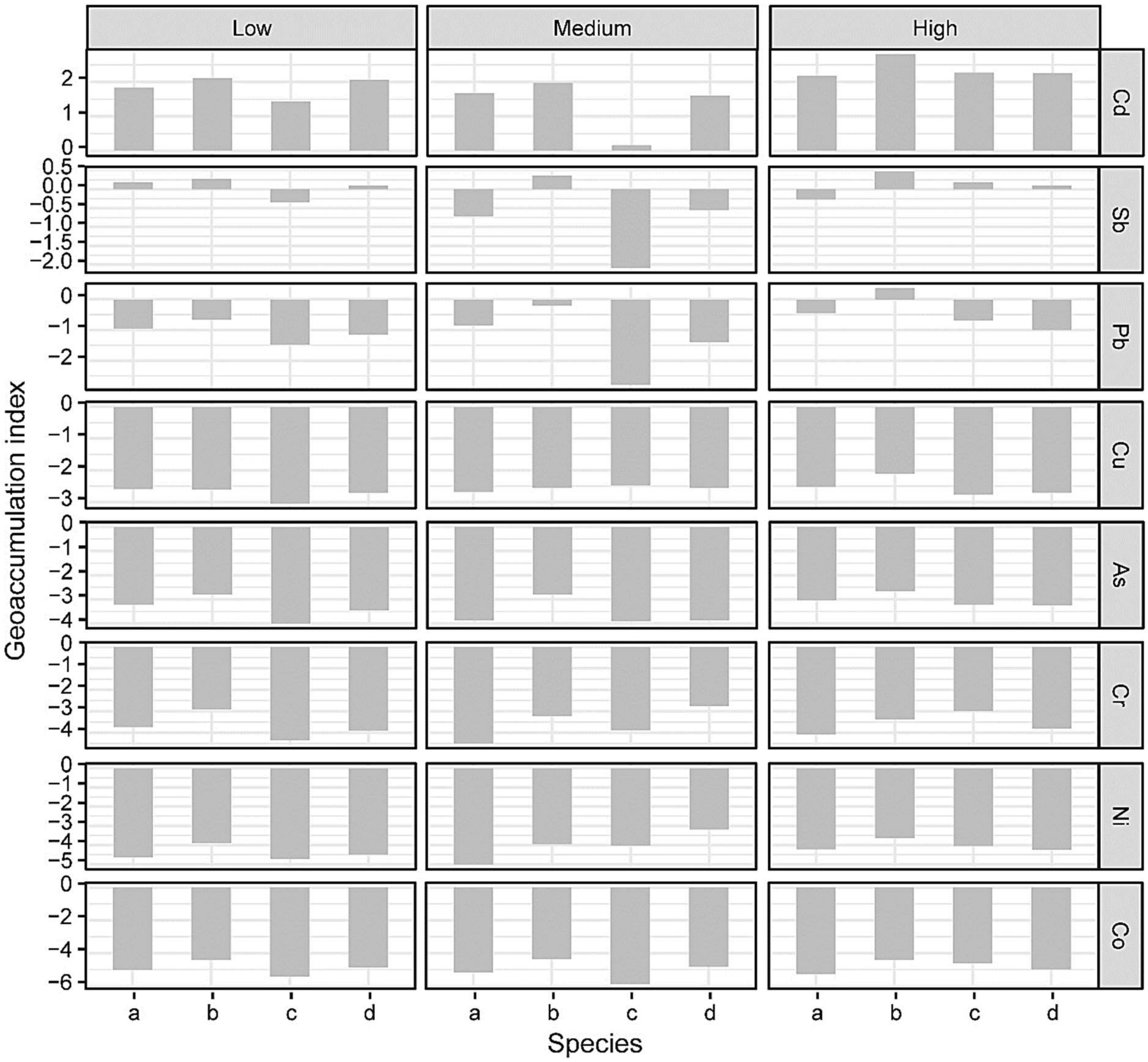

Fig 6. Geoaccumulation index of different species at different elevations. (**a:** ***Parmelia saxatilis*****, b:** ***Bryum argenteum*****, c:** ***Rhynchostegium subspeciosum*****, d:** ***Orthotrichum dasymitrium***)

suitable species for environmental remediation through enrichment. However, its adsorption capacity for different pollutants, based on MAI, and its evaluation value for ecological conditions, based on I_{geo}, indicated that it was a suitable species for monitoring. Therefore, *B. argenteum* was selected for source analysis using EPA PMF5.0 to assess specific sources of pollution in the area (Fig. 7). The background values of toxic elements in Guizhou Province were selected as the standard. Three different sources were found in this region. Factor 1 (12%) was the element absorbed from the growth matrix and other sources and contributed less. Factor 2 (45%) was dust deposition. High Sb, As, Cr and Co in the background value caused excessive accumulation in plants. Factor 3 (43%) was other traffic emissions, in which Pb, As and Cr were still the most important pollutants.

DISCUSSION

Pollution coefficient difference analysis

In this study, the EF of the lichen was not significantly higher than that of the mosses (Fig. 4). Many studies showed that lichens were several times more likely to accumulate contaminants than mosses (Koroleva and Revunkov, 2017). This could be because the plants were not subjected to multiple deionized water washing before elemental content determination. After washing, most pollutant particles adsorbed on the surface of plants could be eluted. Thus, the structure differed from bryophytes and lichens, and their fixation capacity for particulate matter was different (Abas and Din, 2020). In many aspects, it was shown that the measured element content of bryophytes was higher than in lichens (Culicov and Yurkova, 2006). In this study, we observed that *Bryum*

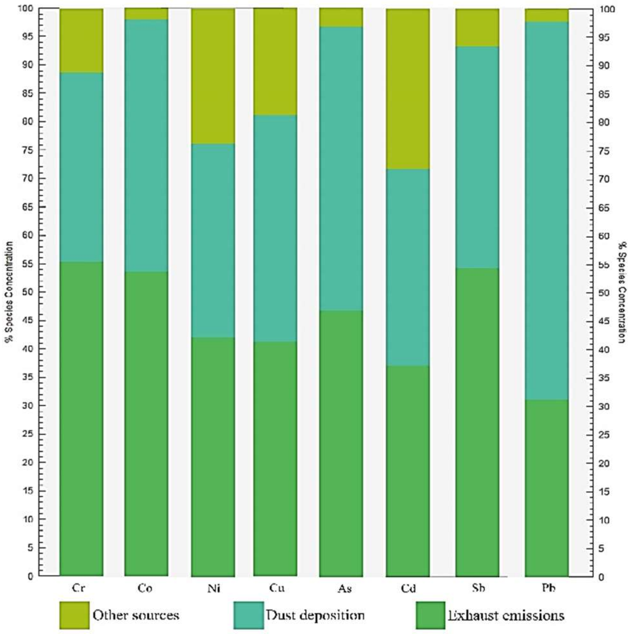

Fig. 7. Source analysis of ***Bryum argenteum*** by US EPA PMF5.0

argenteum had the highest MAI, suggesting that this kind of moss has a stronger ability to accumulate various kinds of toxic elements from the atmosphere than other species in this study (Nogami *et al*., 1987).

Potential correlation of sedimentation of pollutions with elevation

Conventional research suggests that the content of toxic elements in high elevation areas would be significantly lower than in low elevation areas due to the natural precipitation of toxic elements in the atmosphere (Liu *et al*., 2018a). In this experiment, based on the values of EF (Fig. 4) and MAI and I_{geo} (Fig. 5), we observed that the deposition of toxic elements tended to increase with increasing elevation. This could be related to the different geographical locations of the selected researched area. Liu *et al*. (2018b) performed a study in a collapsed Karst mountain region, with low terrain on both sides, high road slope, high ratio of vertical distance to distance, and an obvious trend of subsidence from high to low under the influence of natural factors such as mountain wind. In contrast, the area chosen for this study was a valley with long mountain ranges in the north and south, which may have blocked the region's prevailing northeast wind and southeast wind. The transportability of mountain and valley breezes also has certain effects on pollution (Lang *et al*., 2015). Generally, valley winds change direction twice a day. Rising thermals allow pollutants to mix well with the air above them. In addition, considering the average annual foggy weather in Guizhou Province was more than 30 days (Chen *et al*., 2013), especially in its mountainous areas, this might have intensified the scale of valley wind movement to higher elevations (Zardi and Whiteman, 2013). As a result, the natural subsidence effect of the atmospheric movement was weakened, and road pollution was concentrated nearby. The road section selected in this study was comparatively long, had a small slope, and a large range of different elevations. Further, the "cold trapping effect" (Zhang *et al*., 2013) of high mountains was also one of the reasons for this rule. The atmospheric precipitation and cloud water interception increased as the temperature decreased, leading to a significant increase in the content of pollutants in high-elevation areas (Bing *et al*., 2016). In addition, at high elevations, cooler temperatures can reduce the amount of oxygen in the air, causing vehicles to spend more fuel to drive, resulting in a direct increase in pollutant emissions (Bishop *et al*., 2001). In the later stage, the coefficient of the pollutant settlement model in similar areas can be adjusted according to the terrain to make it more consistent with the actual situation (Xu *et al*., 2021).

Possible sources of contaminants

The PMF 5.0 model analysis showed that the fallout was associated with serious lead pollution in this study. Guizhou Province is extremely rich in mineral resources and has a high content of Pb and other metals in the bedrock of its parent material, coupled with serious road dust and automobile exhaust emissions, which can synergistically lead to the high content of Pb, high enrichment coefficient, and separate classification of Pb in taxonomy. Pb mainly comes from automobile exhaust emission and tire wear. Although leaded gasoline has been completely banned in China, Pb in the environment around roads cannot be disgraded for a long time. It will still float into the atmosphere with the dust caused by vehicles or atmospheric movement (Zhao *et al*., 2019) and can still be absorbed by various plants.

Previous studies showed that tire wear and diesel and lubricating oil leaks released large amounts of Cd. Thus, automobile tire and brake wear, diesel oil and lubricating oil leakage can be considered the main contributors to excessive contents of Zn, Cu and Cd (Singh *et al*., 2019). According to previous studies, their sources may mainly be tire and brake wear of motor vehicles (Evangeliou *et al*., 2020), diesel oil and lubricating oil leakage (Hanfi *et al*., 2019), tire and bearing wear, and others.

CONCLUSION

In this study, based on our observations from the mountainous roads of the Yunnan-Guizhou Plateau, *Bryum argenteum* was identified as the most suitable species to monitor toxic elements along roads. Although plants of *Bryum argenteum* had poor enrichment ability, they were highly sensitive and widely distributed, making them an ideal plant for establishing ecological monitoring networks. Combined with the pollution coefficient interpretation of our source analysis results, the influence of excessive background values on the pollutants in trees' epiphytic bryophytes was small. Traffic emissions were the main source of air pollution. Thus, this study suggests that local environmental quality can be quickly understood by spreading moss in the area.

AUTHOR CONTRIBUTION

Ding, H,F, was responsible for conducting experimental work and writing papers. Dr. Zhang, Z.H. and Dr. Wu, Q.M. provided experimental funds and research platform for this study. Dr. Wang, Z.H. and Dr. Wang, D.F. provided help for the experimental thinking and writing of this research.

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