

Distribution and Dispersion of Heavy Metals in the Rock–soil–moss System in Areas Covered by Black Shale in the Southeast of Guizhou Province, China

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Abstract

Black shales are easily exposed due to human activities such as mining, road construction, and shale gas development, which results in several environmental issues including heavy metals (HMs) pollution, soil erosion and the destruction of vegetation. Mosses are widely used to monitor metal pollution in the atmosphere, but few studies on the distribution and dispersion of HMs in the rock – soil – moss system are available. Here, mosses (*P. flexuosa* Harv), growing soils, and corresponding parent rocks were collected from black shale areas. After appropriate pretreatment, samples were analyzed for multiple elements concentration by ICP-AES and ICP-MS. The results show that black shales parent rocks have elevated HMs concentration, and act as a source of multiple metals. Soil significantly inherits and accumulates heavy metals released from black shale. Significant positive correlations between HMs in *P. flexuosa* Harv and the growing soils indicate that HMs are mainly originating from geological source rather than atmospheric deposition. Compared with other elements, only the transfer factor (TF) of Cd is greater than 1, the normal functioning of mineral elements (K and Zn) absorption and transportation may contribute to its high tolerance to Cd. Finally, both the BCF and TF for most HMs in *P. flexuosa* Harv are less than 1, indicating that it has a tolerance and exclusion mechanism for these metals. Therefore, the luxuriant and spontaneous growth of *P. flexuosa* Harv could be used as a phytostabilization pioneer plant in the black shale outcrop where vascular plants are rare.

1. Introduction

Research on black shales has increased in recent years since they are enriched in various harmful elements, including radionuclides, and may have adverse effects on the environment. It has been well documented that black shales are sedimentary rocks containing high concentrations of sulfur and organic carbon, and also host polymetallic deposits which have been mined for Cu, Ni, Zn, Mn, Mo, V, and U (Alloway, 2013; Parviainen and Loukola-Ruskeeniemi, 2019). Black shale is a natural source of heavy metal pollution because weathering, mining, and road construction can result in direct input of HMs to surrounding soils, and then lead to negative effects on soil environmental quality. Particularly, the weathering of HMs-enriched black shales may be one of the most important sources of environmental contamination in the black shales distributed area (Peng et al., 2004). The abundance of organic matter and sulphide minerals in black shale make it susceptible to chemical weathering, and produce acid rock drainage after exposed to oxic surface environment (Nordstrom, 2011; Ling et al., 2015; Fru et al., 2016), and subsequently resulted in acidification and elevated trace element concentrations in the soils which developed on top of black shales (Cappuyens et al., 2019). Further, the natural weathering processes are enhanced by anthropogenic activities that bring black shale to the surface, and consequently facilitate the release of HMs, transferring into the surrounding soils, water systems, and then translocation occurs to plants and agricultural products cultivated on black shale soils, leading to environmental pollution and human health threats (Liu et al., 2017; Duan et al., 2020). Therefore, it is worth giving more attention to the migration and dispersion of HMs liberated by black shales, as well as the phytoremediation of black shale-associated soils.

It is believed that mosses are primitive terrestrial higher plants and characterized by a simple structure and large surface area as well as no real root systems. Generally, the elements observed in moss depend on deposition and rainfall for nutrients, and subsequently they are used as biomonitors is a well-recognized technique in studies of atmospheric deposition. Several studies have been conducted on HMs present in mosses associated with gold, zinc, copper and mercury production (Smirnov et al., 2004; Cymerman et al., 2006; Bi et al., 2006; Yang et al., 2011). However, mosses uptake the mineral components partly from the soil substrate, and influenced by the soil composition. Thus, some authors suggest that mosses also can translocate the metal elements from the soil and conduct them internally (Kłos et al., 2012; Sabovljević et al., 2020), because they can also perform cushion-like growth, and being more in contact with the soil substrate. Therefore, the pollutants observed in moss samples may originate from geological, biological, and wet and dry deposition sources.

Many moss species play essential roles in water retention and pedogenesis (Jia et al., 2014), they absorb water bearing dissolved mineral elements but also other compounds over their entire surface (Sabovljević et al., 2020). When mosses appear in soil contamination, the great cation exchange capacity (CEC), absence of a cuticle, and one-cell-thick leaves enable them incapable of avoiding heavy metal adsorption, and as lack stomata, mosses are unable to screen airborne pollutants by closing stomata during stress (Glime, 2007). Hence, moss can efficiently enrich HMs from the polluted environment. Some aquatic bryophytes have been shown to be bioaccumulators of trace metals (Yang et al., 2011), and *Scopelophila ligulata* has been recognized as a Fe-hyperaccumulator because Fe concentration in *Scopelophila ligulata* was 10-61 times higher than that in normal mosses (Nakajima and Itoh, 2016). Thus, from the perspective of phytoremediation intention, moss species might be considered as a candidate for phytoremediation in heavy metal contaminated areas.

The Lower Cambrian black-rock-series is widely distributed in the Yangtze Platform of South China, and there is regionally developed a typical conformable polymetallic sulfide horizon. Guizhou province is one of the typical areas of its development, and it should be emphasized that the eastern Guizhou is an important area for the development of black rock series stratabound metal deposits. In this area, there was a set of black shale

rocks between the Dengying Formation and the Liuchapo Formation distributed in Sansui-Shibing-Tianzhu-Majiang region, which was characterized by large-scale vanadium ore and barite ore (Yang et al., 2013; Wang et al., 2016; Wei et al., 2017). In some weathering profiles formed by mining and road construction, few vascular plants can survive in the poor, acidified, and arid soil overlying black shales, but some moss species can grow normally and flourish in this challenging environment. However, few information is available on the distribution and migration of HMs, as well as the phytoremediation potential of moss species in the black shale areas.

In this study, the Yacha and Gaoqiao areas underlain by black shales were selected, (I) to investigate the dispersion and distribution characteristics of HMs in rock - soil - moss system; (II) to evaluate the

enrichment levels of HMs in soils overlying black shales; (III) to determine whether moss can be used to as a pioneer plant for phytoremediation of HMs in the black shales district.

2. Material And Methods

2.1. Research area

The research areas are located in Sansui County (26°47′-27°04′N, 108°32′-109°04′E) southeast of Guizhou Province, China (Fig. 1). They are typical black rock series vanadium mine that contained a variety of associated HMs (Ag, Ba, Co, Cu, Ga, Mo, Ni, and Pb), the concentrations of vanadium pentoxide (V_2O_5) in rock samples range from 0.07–1.28%, with an average of 0.40%, and part of the rock samples reach the industrial grade (Deng, 2014). The exposed stratum in the study area are mainly of the Niutitang Formation of the Lower Cambrian, and the lithology including black shale, mudstone, and siliceous rock. The average annual temperature is about 15 °C and an average annual precipitation is ~ 1147 mm, and the altitude ranges from 450 m to 1470 m. Moreover, the study areas have subtropical climate which is controlled by east Asian monsoon, and this warm and moist climate conditions are favorable for black shales weathering.

2.2. Sample collection and pretreatment

In August 2019, we collected a total of 39 samples of mosses, growing soil, and corresponding parent rocks from 13 different sampling sites in the two study areas (Fig. 1), and the information of sampling sites was provided by global positioning system (GPS). The sampling procedure of mosses in this study was referred to wang et al. (2015). Briefly, five 10 × 10 cm² quadrats were established in each site, and then a final sample (moss, soil, and rock, respectively) was composed of 5 subsamples were carefully packed into polyethylene bags to avoid cross-contamination. In particular, the entire surface layer of the mosses cover in each quadrat was removed down to the growing soil using a sampling knife, which was washed between samplings. After collection, samples were delivered to the laboratory to determine the species and the corresponding statistics.

Species of mosses were identified with classical morphological identification techniques, using an anatomical lens (HWG-1) and a microscope (XSZ-107), and referred to the atlas "Moss Flora of China". Mosses samples were classified into 7 species in 5 families. Among all the mosses, *Pohlia flexuosa* Harv was luxuriant and spontaneous growth throughout the study areas, accounting for approximately 70% of the total (Fig. 1). Therefore, the *P. flexuosa* Harv was selected as the main moss species to be studied in this study.

It was found that the biomass of mosses growing at some sites was relatively low (the total length of the moss was less than 1cm), and they were not easy to separate the rhizoid and shoot. Thus, four moss samples in each study area, which grew normally and the length of the moss was greater than 3cm, were selected to investigate the distribution of HMs in different mosses tissues (rhizoids and shoots).

All the mosses samples were washed three times with deionized water (18.2 M Ω ·cm, 25 °C) until dust and foreign substance were removed, and each sample was rinsed with the deionized water after being cleaned for 15 min by the ultrasonic cleaner (power ratio: 100%; frequency: 25 kHz) in clean water. And all the samples were dried in a thermostatic air-blower-driven dryer at 45°C until constant weight. Then, these mosses samples were ground by the portable high speed universal grinder (50 g, AQ-180 E-X, Nail Machinery Ltd., Ningbo, Zhejiang Province, China). Subsequently, the mosses samples were sieved through an 80-mesh sieve (0.18 mm) and homogenized in the laboratory. In addition, rock and soil samples were disaggregated, sieved to - 10 mesh (2 mm), quartered and pulverized in a porcelain mortar to - 200 mesh (0.074 mm), rehomogenized, and repackaged in polyethylene bags for further analysis.

2.3 Sample analyses

The soil pH was measured in a 1:2.5 (soil: water w/v) mixture by a glass electrode pH meter (PHS-3E, Shanghai, China). OM was measured by the classic low-temperature external heat potassium dichromate oxidation-colorimetric method (Cao et al., 2020). In addition, all samples were sent to an accredited laboratory (ALS Minerals-ALS Chemex Co. Ltd. Guangzhou, China) to determine the concentrations of major and trace elements. The analysis procedure for the rock and soil samples was as follows (Peng et al., 2018; Zhang et al., 2020).

Each sample was digested in two methods. Firstly, 0.25 g of sample was accurately weighted and digested by a concentrated acid mixture (a ratio of 1 : 2.5 : 2 : 2.5 for the HClO_4 : HNO_3 : HF : HCl) in an oven at ~ 190°C for 48 h, cooled to room temperature, heated on a preheated hot plate (150°C) to get rid of excess acid until crystalline solid was formed, and diluted to a steady volume (12.5 mL) with 2% hydrochloric acid. The final solution was then analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES, America, Varian VISTA) and inductively coupled plasma mass spectrometry (ICP-MS, America, Agilent 7700x). The other sample (0.50 g) was dissolved in aqua regia (1:3, v/v: HNO_3 and HCl), digested slowly at ~ 190°C for 48 h in a graphite heating block, and then placed and heated on a preheated hot plate (150°C) under a fume hood until white fumes appeared and crystalline solid was formed. Afterwards, the resulting solution was diluted to volume with deionized water, mixed and analyzed by ICP-AES, followed by ICP-MS for the remaining suite of elements. According to the actual characteristics of the sample, the digestion effect and interelement spectral interferences, the comprehensive value was the final test result. As for the moss samples, 1.0 g moss powder was accurately weighted and added with 5 mL HNO_3 (ultra-pure grade) into a Teflon digestion vessel and digested ~ 8 h slowly at room temperature, and then heated for ~ 3 h on a preheated hot plate (150°C) under the fume hood. The remaining solid crystal was dissolved and transferred into a volumetric flask after cooling; the final volume was precisely adjusted to 25 mL using 2% hydrochloric acid. And then mixed thoroughly respectively and analyzed by ICP-MS.

2.4 Parameter analyses

2.4.1 Pollution assessment of HMs in soil substrates

The geo-accumulation index (I_{geo}) was first proposed by Muller, which has been widely used to assess the degree of HMs pollution in soil and sediment (Muller, 1969). Rahn presented a useful way to determine whether a particular element was found in greater abundance than what might be expected from crustal sources, introducing the concept of “enrichment factors” (Rahn, 1971). In this study, I_{geo} and EF are applied to determine the level of HMs contamination and the degree of HMs enrichment in soil, respectively. They are calculated using the following equations:

$$I_{geo} = \log_2 [C_n / (1.5 \times B_n)] \quad (1)$$

$$EF = (C_n / C_{ref})_{sample} / (C_n / C_{ref})_{background} \quad (2)$$

Where C_n is the concentration of metal element in soil, B_n is the geochemical background values of corresponding element and C_{ref} is the concentrations of reference element in soil. In this study, Al is used as a reference element due to its natural abundance and less interaction with other HMs. The Al and HMs concentrations of A layer soil (0–20 cm) in Guizhou Province are used as background values (Zhang et al., 2018).

2.4.2 The bioconcentration factor (BCF)

BCF was an important quantitative indicator of plant contamination and has commonly been used for estimating metal transfer from soil to plants. It represented the ratio of the element concentration in the plants to that in the soil (Fayiga et al., 2004). In this study, it is used to evaluate the accumulation capacity of HMs in moss and calculate through the following equation:

$$BCF = C_{plant} / C_{soil} \quad (3)$$

BCF is the bioconcentration factor of the HMs in mosses, C_{plant} is the metal element concentration in the moss, and C_{soil} is the elemental concentrations in the growing soil of the mosses.

2.4.3 The translocation factor (TF)

TF was defined as the ratio of element concentrations in the aerial parts to those of the roots (Zhang et al., 2012; Zou et al., 2012). In this study, it is calculated to assess the capability of mosses to accumulate the metal element, absorbed by rhizoid, into the shoot. It is calculated as follows:

$$TF = C_{ngt} / C_{rhi} \quad (4)$$

Where TF is the translocation factor of the metal element in moss, and C_{ngt} is the metal element contents in the shoots, and C_{rhi} is the metal element contents in the rhizoids.

2.5 QA/QC

Quality assurance and quality control for the HMs analyses were validated using duplicates, method blanks, and standard reference materials (SRM). Standard reference materials (SpL03 representing plant

samples, GBM908-10 representing soil samples, and MRGeo08 representing rock samples) were analyzed. The method blank was lower than the detection limits in all samples, and their HMs recoveries were 90–110%. The relative standard deviations of the HMs in the soil substrate and mosses samples for the duplicate analysis were both < 10%.

3. Results

3.1 HMs concentrations in black shales

Ranges and mean concentrations of HMs in black shales of the Yacha and the Gaoqiao areas are shown in Table 1. Cd, As, Pb, Cu, Cr, Ni, and Mo concentrations in the Yacha are 0.6–598.0 mg kg⁻¹, 12.3–88.3 mg kg⁻¹, 3.5–47.3 mg kg⁻¹, 8.1–1035.0 mg kg⁻¹, 60.0–1730.0 mg kg⁻¹, 17.3–513.0 mg kg⁻¹, and 11.1–146.5 mg kg⁻¹, respectively. While in the Gaoqiao are 3.2–97.7 mg kg⁻¹, 12.3–62.0 mg kg⁻¹, 11.6–105.0 mg kg⁻¹, 327.0–919.0 mg kg⁻¹, 100.0–4630.0 mg kg⁻¹, 7.2–98.2 mg kg⁻¹, and 4.3–78.8 mg kg⁻¹, respectively. The mean concentrations of all the elements are higher than those of average black shales. Also, the mean As concentration in black shales is higher than that in average shale. Such results indicating all the selected HMs are highly enriched in black shales in the study areas, especially Cd, Cr, and Cu. The average contents of HMs in the black shales of Yacha are in the order Cr > Cu > Ni > Cd > Mo > As > Pb, while in the Gaoqiao are in the order Cr > Cu > Pb > Mo > Ni > As > Cd. In addition, the concentration of most metal elements in black shale are characterized by a high variability, especially Cd, Cr, Ni, and Mo. This is good agreement with the results reported in previous studies, confirming an overall chemical heterogeneity within the black shale (Yu et al., 2012; Cappuyns et al., 2019).

It is well known that black shales are widely distributed in the world and many varieties of black shale have been recognized and studied on the basis of trace element concentrations. Compared to other black shales, the trace elements As, Pb, Ni, and Mo in black shales have been found to occur within the range of typical concentrations, but the concentration of Cd, Cu, and Cr are significantly higher than that in other studies (Lee et al., 1998; Pašava et al., 2003; Yu et al., 2008; Park et al., 2010), suggest that the black shales are mainly characterized by enrichment of Cd, Cr and Cu in the study areas.

Table 1
Concentrations of heavy metals in the black shale (mg kg⁻¹).

Samples	Cd	As	Pb	Cu	Cr	Ni	Mo
YC-1	7.3	88.3	38.8	370.0	750.0	115.0	94.3
YC-2	131.5	72.2	47.3	751.0	1730.0	329.0	40.4
YC-3	3.6	51.0	34.9	456.0	1400.0	27.7	14.9
YC-4	598.0	39.8	15.0	1035.0	330.0	264.0	25.8
YC-5	12.1	12.3	3.5	16.5	60.0	29.9	11.1
YC-6	0.6	19.8	26.4	8.1	80.0	17.3	45.9
YC-7	2.8	58.0	21.2	159.0	110.0	172.0	53.8
YC-8	1.7	67.5	24.5	360.0	80.0	513.0	146.5
Mean	94.7	51.1	26.5	394.5	567.5	183.5	54.1
SD ⁽¹⁾	208.2	26.1	13.9	356.7	662.9	175.9	45.7
VC ⁽²⁾	219.9	51.0	52.5	90.4	116.8	95.9	84.5
GQ-1	22.2	62.0	102.0	795.0	1530.0	24.9	52.7
GQ-2	15.9	37.7	105.0	919.0	4630.0	29.9	36.0
GQ-3	97.9	31.8	76.5	664.0	100.0	98.2	78.8
GQ-4	15.0	12.3	11.6	375.0	450.0	7.2	4.3
GQ-5	3.2	27.9	43.1	327.0	1200.0	16.1	18.1
Mean	30.8	34.3	67.6	616.0	1582.0	35.3	38.0
SD	38.1	18.1	40.0	259.0	1797.0	36.2	29.2
VC	123.7	52.7	59.1	42.0	113.6	102.8	77.0
Average black shale ⁽³⁾	1.0	13.0 ⁽⁴⁾	20	70	100	50	10
(1) Standard Deviation; (2) Variation Coefficient (%); (3) Vine and Tourtelot, 1970; (4) Average shale (Turekian and Wedepohl, 1961).							

3.2. Geochemical characteristics of soils overlying black shales

3.2.1 Environmental parameters and heavy metals in soil substrates

As shown in Table 2, the pH values of soil range from 3.85 to 6.72 with an average of 4.97 in Yacha, and range from 4.24 to 5.31 with an average of 4.71 in Gaoqiao, indicating all sampling sites had highly acidity pH values. The result is very consistent with the previous study (Duan et al., 2020), indicating that the soils overlying black shales dominated by strongly acidic conditions. This can be likely attributed to the acid rock drainage produced by oxidation of sulphides in black shale. Moreover, compared to other soils derived from black shales (average 1.88% for Yanxi, average 2.12% for central Hunan, respectively) (Peng et al., 2009), the OM content in our study is significantly elevated (average 16.73% for Yacha, average 10.74% for Gaoqiao, respectively). The elevated OM content may be related to the formation and accumulation of Moss-dominated biological soil crusts. It has been well documented that biological soil crusts increased soil organic carbon, especially in the upper layers (0–10 cm) (Hashim et al., 2020; Kakeh et al., 2020). Overall, the soils developed from the black shales exhibit the acidic environment and high OM, which may have great impacts on the transport and fate of HMs in rock - soil - moss system.

Ranges and mean concentrations of trace elements in soils overlying black shales in the study areas are shown in Table 2. HMs concentrations of soil samples in Yacha contained 0.9–486.0 mg kg⁻¹ of Cd, 60.4–313.0 mg kg⁻¹ of As, 21.4–91.4 mg kg⁻¹ of Pb, 88.7–2220.0 mg kg⁻¹ of Cu, 90–2580 mg kg⁻¹ of Cr, 65.1–991.0 mg kg⁻¹ of Ni, and 36.4–366.0 mg kg⁻¹ of Mo, respectively. While contained 4.6–67.3 mg kg⁻¹ of Cd, 33.6–201.0 mg kg⁻¹ of As, 48.5–120.5 mg kg⁻¹ of Pb, 386.0–1060.0 mg kg⁻¹ of Cu, 910.0–2690 mg kg⁻¹ of Cr, 32.8–178.5 mg kg⁻¹ of Ni, and 13.5–163.0 mg kg⁻¹ of Mo in Gaoqiao, respectively, indicating HMs concentrations in soils overlying black shales are highly variable and show high variation coefficient (VC%), and Cd exhibits the maximum variation coefficient of all elements (163.4% in YC and 83.5% in GQ, respectively), this is very consistent with the black shale samples (Table 1). The average contents of HMs in the soils of YC are in the order Cr > Cu > Ni > As > Mo > Cd > Pb, while in the GQ are in the order Cr > Cu > As > Pb ≈ Ni > Mo > Cd.

Correlation coefficients are used to assess the correlations of element concentrations in the soil samples. Taking all sites into consideration, there are significant correlations among the investigated elements in the soils overlying black shale (Table 3), indicating that most metals in soils are derived from a similar pollution source. This is in good agreement with the previous findings that the heavy metal elements in black shale soil have similar sources, which is speculated to be related to the weathering of black shales (Lee et al., 1998; Park et al., 2010).

Table 2
Concentration of heavy metals in soil overlying black shales (mg kg⁻¹).

Samples	pH	OM(%)	Cd	As	Pb	Cu	Cr	Ni	Mo
YC-1	6.72	5.08	130.5	99.2	42.4	773.0	1040.0	991.0	36.4
YC-2	4.97	27.50	486.0	268.0	63.8	2220.0	1160.0	970.0	366.0
YC-3	4.04	14.95	44.3	313.0	91.4	1150.0	2580.0	628.0	175.0
YC-4	3.85	25.17	19.1	116.0	36.4	366.0	1040.0	422.0	165.0
YC-5	6.11	6.58	113.5	107.5	65.8	417.0	810.0	494.0	69.2
YC-6	4.42	32.86	0.9	66.4	41.3	88.7	160.0	65.1	75.7
YC-7	5.05	7.53	5.7	60.4	22.2	128.5	90.0	185.5	53.3
YC-8	4.60	14.16	2.5	67.4	21.4	342.0	110.0	211.0	95.7
Mean	4.97	16.73	100.3	137.2	48.1	685.7	873.8	495.8	129.5
SD ⁽¹⁾	0.99	10.56	163.9	97.5	24.0	710.8	824.6	349.7	107.8
VC ⁽²⁾	20.02	63.12	163.4	71.1	49.9	103.7	94.4	70.5	83.3
GQ-1	4.68	18.86	20.3	89.1	120.5	500.0	2170.0	32.8	75.3
GQ-2	4.40	11.52	54.6	201.0	82.6	750.0	2320.0	178.5	163.0
GQ-3	5.31	9.60	67.3	126.5	86.2	1060.0	2690.0	130.5	69.4
GQ-4	4.94	6.78	15.2	33.6	79.0	715.0	1910.0	39.7	13.5
GQ-5	4.24	6.96	4.6	78.7	48.5	386.0	910.0	34.4	73.9
Mean	4.71	10.74	32.4	105.8	83.4	682.2	2000.0	83.2	79.0
SD ⁽¹⁾	0.43	4.95	27.1	62.7	25.6	259.5	671.5	67.3	53.6
VC ⁽²⁾	9.07	46.03	83.5	59.3	30.7	38.0	33.6	80.9	67.8
Background values of soil in Guizhou province ⁽³⁾			0.66	20.0	35.2	32.0	95.9	39.1	2.4
(1) Standard deviation; (2) VC (Variation Coefficient (%)) = standard deviation*100/mean; (3) Wei et al., 1991									

Table 3
Correlation coefficients of heavy metals in soils from the study areas.

	Cd	As	Pb	Cu	Cr	Ni	Mo
Cd	1						
As	0.56*	1					
Pb	0.08	0.37	1				
Cu	0.86**	0.75**	0.37	1			
Cr	0.04	0.49	0.87**	0.46	1		
Ni	0.73**	0.57*	-0.12	0.62*	-0.03	1	
Mo	0.78**	0.79**	0.08	0.76**	0.12	0.54	1

⊠ Correlation is significant at the 0.05 level (2-tailed); ⊠⊠ Correlation is significant at the 0.01 level (2-tailed).

3.2.2 The results of soil heavy metals pollution evaluation

As shown in Fig. 2A, the I_{geo} values of Pb and Cr in GQ (0.60 and 3.71, respectively) are significantly higher than those in YC (-0.30 and 1.81, respectively), while the I_{geo} of Ni in YC (2.64) is significantly higher than that in GQ (0.12). Similarly, the EF values of Pb and Cr in GQ (2.93 and 25.56, respectively) are significantly higher than those in YC (1.48 and 9.83, respectively), as shown in Fig. 2B. Nevertheless, no significant differences are observed among other metal elements between the two study areas.

Enrichment and contamination level can be classified based on I_{geo} and EF and values (Zhang et al., 2018; Rasool and Xiao, 2018). The average values of I_{geo} for all the investigated metals in soil are higher than 1 (except for Ni in GQ, Pb in YC and GQ, respectively), indicating that the soils are contaminated by multiple metals to varying degrees. Particularly, both Mo and Cd in the two study areas show higher I_{geo} (> 4) and EF (> 40), suggesting that the soil is extremely polluted and high enrichment by Mo and Cd. Such a result is very consistent with previous reports. For example, the notable enrichment of Mo and Cd in soils associated with a weathering profile of black shale in Hunan, China (Yu et al., 2012); soils surrounding in the black shale U mine were enriched in Mo, Cd, and U (Peng et al., 2009). Overall, it is possible that the soil overlying black shale is prone to multiple metals contamination characterized primarily by Cd and Mo in this study.

3.3. Geochemical characteristics of *P. flexuosa* Harv overlying soil substrate

3.3.1 Heavy metals in *P. flexuosa* Harv

The mean and range of HMs concentrations in the study areas are shown for *P. flexuosa* Harv samples in Table 4. Metal concentrations in the investigated mosses samples are highly variable. HMs concentrations of moss samples in Yacha contained 1.1–345.0 mg kg⁻¹ of Cd, 1.3–62.5 mg kg⁻¹ of As, 4.5–26.8 mg kg⁻¹ of Pb, 21.6–2180.0 mg kg⁻¹ of Cu, 45.4–387.0 mg kg⁻¹ of Cr, 32.8–742.0 mg kg⁻¹ of Ni, and 1.1–119.5 mg kg⁻¹ of Mo, respectively. While in Gaoqiao contained 6.2–43.4 mg kg⁻¹ of Cd, 0.6–13.0 mg kg⁻¹ of As, 3.8–18.2 mg kg⁻¹ of Pb, 356.0–1025.0 mg kg⁻¹ of Cu, 5.6–220.0 mg kg⁻¹ of Cr, 35.0–206.0 mg kg⁻¹ of Ni, and 3.2–31.3 mg kg⁻¹ of Mo, respectively. The average HMs concentrations in mosses were in the order Cu > Ni > Cr > Cd > Mo > As > Pb, while in the GQ were in the order Cu > Cr > Ni > Cd > Mo > Pb > As. Likewise, Cd exhibits the relatively high variation coefficient of all elements (163.1% in YC and 84.1% in GQ, respectively), this is very consistent with the rock and soil samples. In addition, the average concentration of Cu was significantly higher than that of other HMs ($P < 0.05$), and part of moss samples have a Cu content greater than 1000 mg kg⁻¹, which is probably due to the high copper content in the corresponding soil (Table 2). However, the average concentration of Cu (847.4 mg kg⁻¹ in YC, 688.6 mg kg⁻¹ in GQ, respectively) in all moss samples is lower than 1000 mg kg⁻¹, indicating that *P. flexuosa* Harv is not a hyperaccumulator for the investigated elements.

Table 4
Heavy metal concentrations of *P. flexuosa Harv* (mg kg⁻¹).

Samples	Cd	As	Pb	Cu	Cr	Ni	Mo
YC-1	94.1	7.3	6.4	567.0	92.4	382.0	10.7
YC-2	345.0	41.6	8.3	2080.0	45.4	742.0	119.5
YC-3	23.0	62.5	8.6	1025.0	387.0	65.6	34.8
YC-4	34.3	21.4	4.5	2180.0	231.0	51.0	31.2
YC-5	60.2	10.3	7.0	296.0	113.0	692.0	30.6
YC-6	1.1	1.3	26.8	21.6	60.2	32.8	1.1
YC-7	3.6	20.1	6.8	41.8	68.3	97.1	18.7
YC-8	4.8	41.0	11.3	568.0	135.5	70.3	27.5
Mean	70.8	25.7	10.0	847.4	141.6	266.6	34.3
SD ⁽¹⁾	115.4	20.9	7.1	855.1	115.1	299.8	36.3
VC ⁽²⁾	163.1	81.4	71.1	100.9	81.3	112.4	106.0
GQ-1	6.2	13.0	18.2	356.0	217.0	35.5	17.0
GQ-2	43.4	11.7	4.1	1025.0	134.0	206.0	31.3
GQ-3	28.7	3.5	7.2	579.0	107.5	200.0	6.1
GQ-4	10.0	3.6	13.8	864.0	220.0	66.1	3.2
GQ-5	8.1	0.6	3.8	619.0	5.6	35.0	8.5
Mean	19.3	6.5	9.4	688.6	136.8	108.5	13.2
SD	16.2	5.5	6.3	260.5	88.6	87.2	11.3
VC	84.1	85.1	67.2	37.8	64.8	80.3	85.8
(1) Standard deviation; (2) VC (Variation Coefficient (%)) = standard deviation*100/mean.							

3.3.2 Bioconcentration factors and transfer factors of heavy metals in *P. flexuosa Harv*

A plant's ability to accumulate metals from soils can be estimated using the BCF, according to Table 1 and Table 2, the BCF average value in all the sites of Cd, As, Pb, Cu, Cr, Ni, and Mo are 0.92, 0.16, 0.20, 0.91, 0.26, 0.81, and 0.23, respectively; only *P. flexuosa Harv* growing at site YC-4 exhibited the abnormal BCF (5.98), this is probably due to the relatively low copper content in the soil, at 366.0 mg kg⁻¹.

1. As shown in Fig. 3A, the average BCFs of the HMs in *P. flexuosa* Harv are all lower than 1, which means limited ability of HMs accumulation by the mosses. Moreover, it is worth noting that there are significant differences in the BCF values of metal elements in *P. flexuosa* Harv samples, the BCFs of Ni, Cu, and Cd are significantly higher than other elements ($p < 0.01$), and the nonessential Cd exhibited the largest BCF value compared to other elements.

A plant's ability to translocate metals from roots to shoots is measured using the TF, which is defined as the ratio of metal concentration in the shoots to the roots. In this study, *P. flexuosa* Harv is not an accumulator of most HMs in shoots, and their transfer to its shoots parts from its rhizoids is restricted. As shown in Table 5, the content of all the investigated metals in shoots of *P. flexuosa* Harv are lower than rhizoids except for Cd, and the average TFs in all the sites of Cd, As, Pb, Cu, Cr, Ni, and Mo are 1.49, 0.29, 0.48, 0.56, 0.28, 0.90, and 0.59, respectively, (Fig. 3B). Similarly, Cd exhibit the maximum TF value compared to other metal elements, which is consistent with the BCF of Cd.

Table 5
Range and mean of heavy metal concentrations in *P. flexuosa* Harv tissues (mg kg^{-1}).

Area	Organ		Cd	As	Pb	Cu	Cr	Ni	Mo
YC	Shoots (n = 4)	Rang	2.3– 238.0	3.0– 51.7	5.5– 8.4	637.0– 1350.0	26.6– 238.0	17.6– 430.0	7.0– 158.0
		Mean	101.6	19.0	6.7	876.3	84.2	155.8	63.3
	Rhizoids (n = 4)	Rang	2.9– 266.0	12.1– 141.0	10.2– 15.6	1050.0– 2060.0	85.5– 552.0	29.9– 562.0	10.4– 162.5
		Mean	91.9	57.0	14.0	1362.5	216.0	200.6	85.6
GQ	Shoots (n = 4)	Rang	6.6– 82.1	0.4–5.0	2.2– 7.3	170.0– 871.0	3.7– 54.9	21.4– 275.0	2.6– 18.3
		Mean	32.8	2.1	4.1	438.8	32.0	114.8	8.2
	Rhizoids (n = 4)	Rang	2.5– 45.3	1.5– 17.6	3.8– 25.8	464.0– 1175.0	25.8– 395.0	19.5– 232.0	4.9– 27.9
		Mean	20.5	8.2	11.3	893.3	171.2	124.9	16.4

Essential mineral elements are important components regulating various physiological functions and playing important roles in plant growth. However, HMs may indirectly affect plant growth by influencing the absorption of mineral elements (Peng et al., 2018). In this study, the concentration of typical mineral elements in *P. flexuosa* Harv tissues (rhizoids and shoots) are also given in Table 5. The average content of Mg and P in rhizoids are higher than that in shoots, whereas the average content of K and Zn in rhizoids are lower than that in shoots in both study areas. As shown in Fig. 4, the average TF in all the sites of Mg, P, K, and Zn are 0.92, 0.64, 2.35, and 1.04, respectively. Interestingly, the TFs of K and Zn are great than 1, and significantly higher than that of Mg, this trend is well in agreement with the found TF of Cd, imply that the translocation of Cd in shoots may have a synergistic effect with K and Zn.

Table 5

Ranges and means of mineral element concentrations in *P. flexuosa* Harv tissues (mg kg^{-1}).

Area	Organ		Mg	P	K	Zn
Yacha	Shoots (n = 4)	Rang	830–1170	1890–3830	2100–6300	51.0–3140
		Mean	1020	2848	3400	1151
	Rhizoids (n = 4)	Rang	790–1300	2890–8880	620–2700	94.1–4200
		Mean	1045	5533	1880	1377
Gaoqiao	Shoots (n = 4)	Rang	1830–2140	2020–5010	2100–6900	104.5–790
		Mean	1985	2915	3500	435.9
	Rhizoids (n = 4)	Rang	2000–2740	1790–7250	1300–3300	125.5–554
		Mean	2430	4370	2175	353.9

4. Discussion

4.1. Parent rock - soil - moss relationships

As shown in Fig. 5, all the investigated elements have the similar distribution trend in the rock-soil-moss system. That is, the black shales parent rocks have elevated HMs concentrations and act as a source of HMs. It is widely accepted that extreme acidity and sulfide minerals were usually related to high solubility of HMs, and therefore increased concentration in the soil (Sabovljević et al., 2020). Here, we noticed that the soil inherited and enriched metal elements obviously which released from black shale, leading the investigated HMs concentrations in soil are higher than that in the corresponding black shales parent rocks. It was believed that heavy metal elements released from black shales (such as sulfides, silicates, and carbonates) may migrate as ions under acidic conditions, and soil OM played a key role in capturing and retaining these metal ions (Pašava et al., 2003; Perkins et al., 2015). In this study, higher OM contents (average 16.73% for YC, average 10.74% for GQ, respectively) determine more metal sorption sites and more metal chelators, and facilitate the increased accumulation and retention of HMs (Shu et al., 2016). Therefore, the released HMs from the weathering of black shale parent materials contribute considerably to their accumulation and pollution in soils.

A hot issue is whether the HMs in mosses mainly originate from atmospheric deposition or from the soil substrate. Some moss species were used to monitor the atmospheric deposition of trace elements (Smirnov et al., 2004; Cymerman et al., 2006). However, the geochemical properties of hosting soils have been shown to have a significant effect on the elemental concentrations in plants. We also find from Fig. 5 that the gradient diffusion of all the investigated HMs decreasing from soil substrate to rhizoids, and from rhizoids to upper shoots (except for Cd) is obviously. This finding is similar to the results of

Sabovljević et al. (2020). Moreover, taking all sites into consideration, significant linear relationship correlations (except for Pb) are found between the concentration data of the same elements in *P. flexuosa* Harv and corresponding soil (Fig. 6), confirming that HMs are mostly originating from soils substrate that *P. flexuosa* Harv lives on and less from the atmospheric deposition. Mosses are plants with relatively simple morphology, but unistratose leaves and uniseriate rhizoids provide a large surface area for cation exchange, allowing the free uptake of dust particles and droplets of moisture (Wang et al., 2015). Thus, the metals-containing dust particles derived from the black shale parent materials are absorbed and utilized by the *P. flexuosa* Harv. This is well in agreement with the reported, confirming that the uplifting of the pollutant-containing dusts from the soil or substrate were absorbed and translocated by mosses (Kłos et al., 2012; Sabovljević et al., 2020). Therefore, as mentioned above, black shale parent rocks act as the major source of heavy metal pollutants, and then cause geogenic pollutants input into soil and *P. flexuosa* Harv in the black shale distributed areas.

4.2. Tolerance mechanisms for heavy metals in *P. flexuosa* Harv

A tolerant plant has specific physiological mechanisms that collectively enable it to function normally even in the presence of high concentrations of HMs. In this study, few plants can survive in soil overlying black shales that is highly acidic and rich in multi-metals, but *P. flexuosa* Harv are capable of growing and flourishing in this challenging environment. More specifically, *P. flexuosa* Harv grow normally and function well in soil contaminated heavily (up to 486.0 mg kg^{-1} Cd and 2220 mg kg^{-1} Cu in YC-2 site), suggesting that moss growing on the soil are tolerant of these metals. Thus, *P. flexuosa* Harv exhibit a high tolerance to multiple metals, and might be a promising candidate for remediation in contaminated soils derived from black shales.

In this study, the average BCFs for all the HMs of *P. flexuosa* Harv are lower than 1, indicating that *P. flexuosa* Harv can insulate HMs in contaminated growth soil to reduce their effect on its tissues (Fig. 2A). Likewise, all the HMs concentrations of the shoots are lower than those in rhizoid (TF < 1) except for Cd (Fig. 2B), indicated that *P. flexuosa* Harv tend to accumulate and retain HMs in their rhizoids. Uniseriate rhizoids of *P. flexuosa* Harv provide a large surface area for cation exchange, allowing the free uptake of dust particles that were derived from the black shale soil. Thus, it is believe that *P. flexuosa* Harv can immobilize and retain HMs through absorption and accumulation by rhizoids, adsorption onto rhizoids, or precipitation within the rhizosphere (Yoon et al., 2006). A similar result was found in the previous study conducted in indigenous zinc smelting area that a tolerant plant (*Juncus effusus*) can immobilize HMs through absorption and accumulation by root, and caused BCF and TF lower than 1 for most HMs (Peng et al., 2018). This might be a protective behavior for the mosses to accumulate HMs in the rhizoids vacuoles and limit their transportation to the upper shoot tissues, and some studies have found similar results (Shanker et al., 2005; Huang and Wang, 2010). Overall, *P. flexuosa* Harv is a plant that is tolerant of high concentration of HMs and restricts transferring these metals from the soil to its rhizoids and from

the rhizoids to the shoots. Restriction of upward movement from rhizoids into shoots can be considered as an important tolerance mechanism for *P. flexuosa Harv.*

Plants were defined as hyperaccumulators when they accumulate more than 100 mg kg^{-1} of Cd or more than 1000 mg kg^{-1} of As, Cr, Cu and Pb (Baker et al., 1994). The average content of all the investigated metal elements in *P. flexuosa Harv* is not greater than 1000 mg kg^{-1} (Table 3), i.e. *P. flexuosa Harv* is not a hyperaccumulator for HMs in this study. However, the ability of *P. flexuosa Harv* to tolerate HMs may be useful for phytostabilization, which plays an important role in situ site stabilization technique that uses plants as a preventative barrier. Both BCF and TF can be used to estimate a plant's potential for phytoremediation purpose, i.e. BCF and TF are both less than 1 for HMs are considered most suitable for the phytostabilization purposes (Marchiol et al., 2013; Lu et al., 2017). In our study, both BCF and TF are less than 1 for all metals except for Cd, together with *P. flexuosa Harv* cover in the black shale distributed areas, whereas few vascular plants can survive in the poor soil conditions. Thus, the luxuriant and spontaneous growth of *P. flexuosa Harv* could be used for phytostabilization in the black shale outcrop. In general, mosses have lower biomass compared with vascular plants, which might limit their potential for broad applications. However, the lower biomass might not be a problem, because the main functions of Moss-dominated biological soil crusts including resistance to erosion and increasing soil OM content (Belnap et al., 2004; Lange and Belnap, 2016), which facilitates the accumulation and retention of HMs instead of migrating to other environmental medias (e.g., paddy soil, surface water, and sediment, etc). Moreover, mosses are less likely to be preyed by animals, thus reducing the risk of HMs into the high trophic level via food chain. Therefore, as mentioned above, we suggest that *P. flexuosa Harv* may be considered as an excellent candidate for phytostabilization in the black shale distributed areas where vascular plants are rare.

4.3. Influences of Cd on the absorption of mineral elements in *P. flexuosa Harv*

Compared with other heavy metal elements, only Cd has a transfer factor greater than 1, indicating that *P. flexuosa Harv* growing on the soil contaminated by multiple metals are most efficient in translocating Cd and accumulating significant levels of Cd in upper shoots. This may be attributed to inefficient immobilisation of Cd by the rhizoids of *P. flexuosa Harv*, resulting in their efficient translocation and accumulation to shoots, as evidenced by the acidified soil (Table 2) and increase the potential for Cd uptake at the lower pH. Thus, *P. flexuosa Harv* growing at the black shale soils exhibited increased Cd levels in their shoots compared to their rhizoids, which may represent a strategy for Cd toxicity reduction, by which *P. flexuosa Harv* take up harmful Cd to non-photosynthetically active organs such as shoots to alleviate Cd toxicity by biomass diffusion. Moreover, it is worth noticing that the nonessential Cd can indirectly affect plant growth by influencing the absorption of mineral nutrients during this process (Feng et al., 2018). Significant correlation between the TFs of metal elements indicated that plants had a synergistic effect on the absorption of these metals (Yoon et al., 2006). As shown in Fig. 7, there are

significant correlations between the TF of Cd and K, Zn in *P. flexuosa Harv* samples ($p < 0.05$), this means the *P. flexuosa Harv*, which is effective in translocating Cd, is also effective in translocating K and Zn and vice versa. Indeed, it has been reported the increasing Zn^{2+} activities plays an important role in the increasing transfer ratio of Cd from root to shoot (Cai et al., 2019), confirming that rhizoids-to-shoots mineral elements (K and Zn) transport is stimulated by nonessential Cd.

Plants need to prevent damage from non-essential metals and ensure the proper homeostasis of essential elements. Previous studies showed that Zn-Cd interaction was important because of their chemical properties were similar and sharing transporters, and synergistic interactions were observed in many plant species under field conditions (Liu et al., 2007; Takahashi et al., 2012; Zare et al., 2018). The application of Zn was considered an effective measure to reduce Cd uptake and toxicity in Cd-contaminated soils for many plant species (Cai et al., 2019). Moreover, the supplementation of K in culture medium could improve the growth under Cd stress and improve Cd in halophytes, and K can alleviate the toxic effects of Cd on plants by reducing the bioavailability of Cd (Ghnaya et al., 2005; Liu et al., 2012). Thus, potential synergistic absorption of Cd on K and Zn in *P. flexuosa Harv* is realizable in this study, confirming that the mechanism underlying the regulation of mineral element absorption might exist in *P. flexuosa Harv* to alleviate Cd toxicity. Overall, the normal functioning of K and Zn absorption and transportation might contribute to its high tolerance to Cd.

5. Conclusions

The black shale rock samples are highly enriched in HMs and may cause geological pollution for the surrounding environment. Also, the concentration of HMs in black shales are characterized by a high variability for the trace elements, especially Cd, Cr, and Ni, indicating an overall chemical heterogeneity within the black shales parent rocks. Also, black shale acts as a release source of metal elements, leading to significant inheritance and enrichment of these metals in the soil. Significant correlation of HMs between *P. flexuosa Harv* and growing soil indicating HMs in *P. flexuosa Harv* are primarily originating from growing soils and less from the atmospheric deposition. *P. flexuosa Harv* can naturally grow in and cover the black shale areas where vascular plants are rare, and the BCFs and TFs of all metal elements are not higher than 1 (except for Cd), indicating that moss might be considered as a promising pioneer plant for phytoremediation in the black shale areas.

Our study show that Cd affects the absorption and transport of K and Zn by *P. flexuosa Harv* obviously, and a mechanisms underlying the regulation of K and Zn absorption might exist in *P. flexuosa Harv* to resist Cd toxicity. However, more case studies on the detoxification of Cd by the absorption and metabolism of K and Zn in *P. flexuosa Harv* are needed.

Declarations

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed mainly by Yiyuan Xu, Ruidong Yang, and Jian Zhang. Supervision and discussions of data collection and analysis were performed by Lei Gao and Xinran Ni. The first draft of the manuscript was written by Yiyuan Xu, and Ruidong Yang, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

All data generated or analysed during this study are included in this article.

Compliance with ethical standards

Ethical approval and consent to participate: Not applicable.

Consent to publish:

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

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Figures

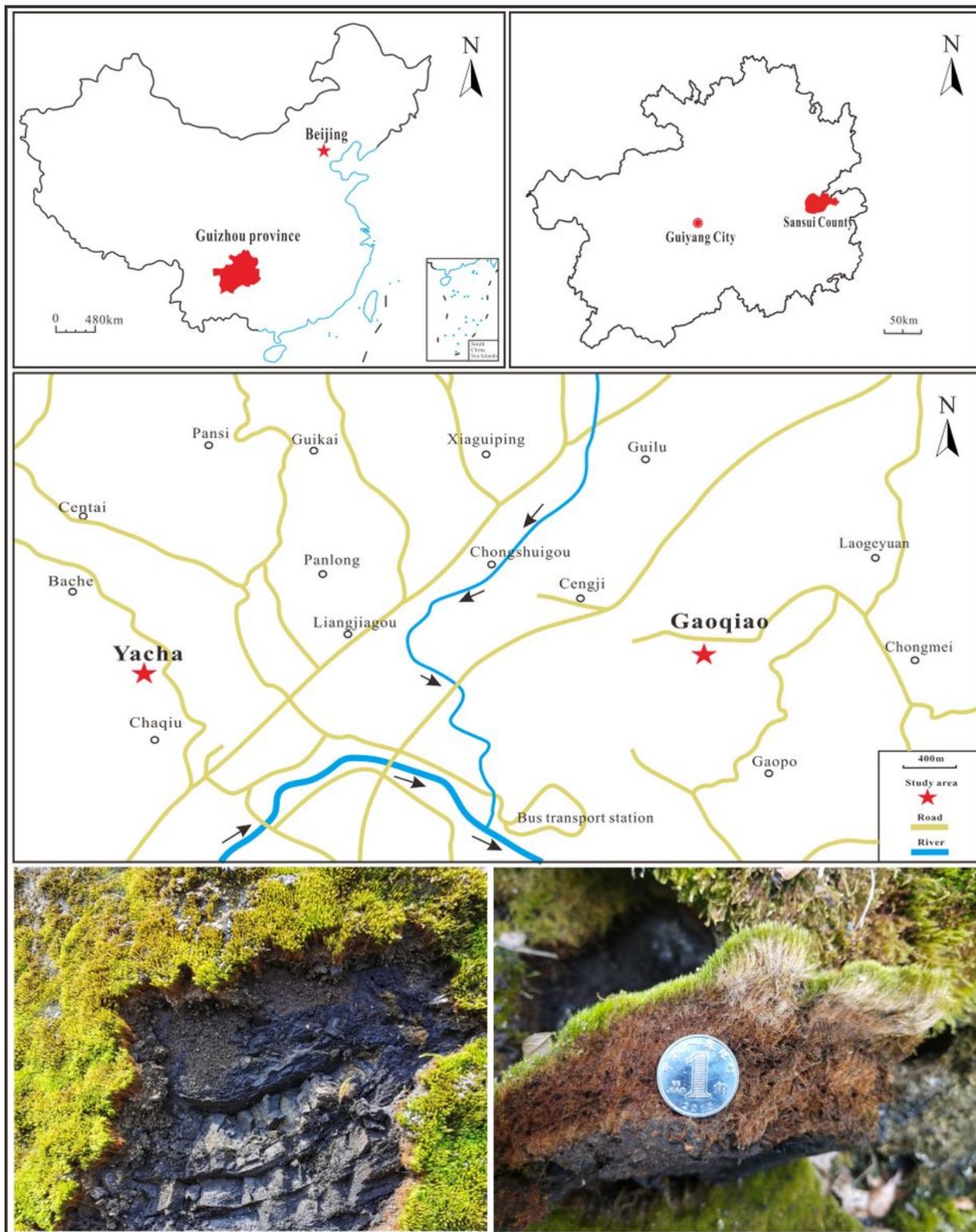


Figure 1

Sampling location in the research area. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

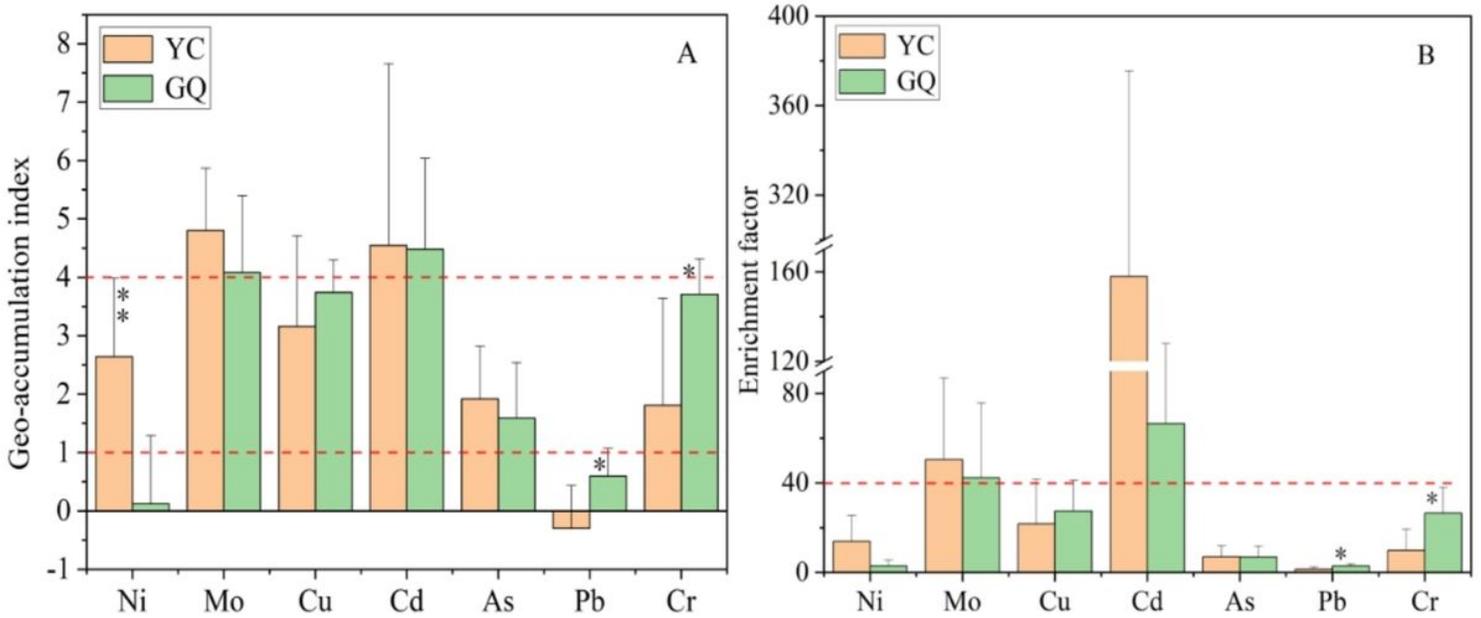


Figure 2

The Geo-accumulation index (A) and enrichment factor (B) of heavy metals in soils.

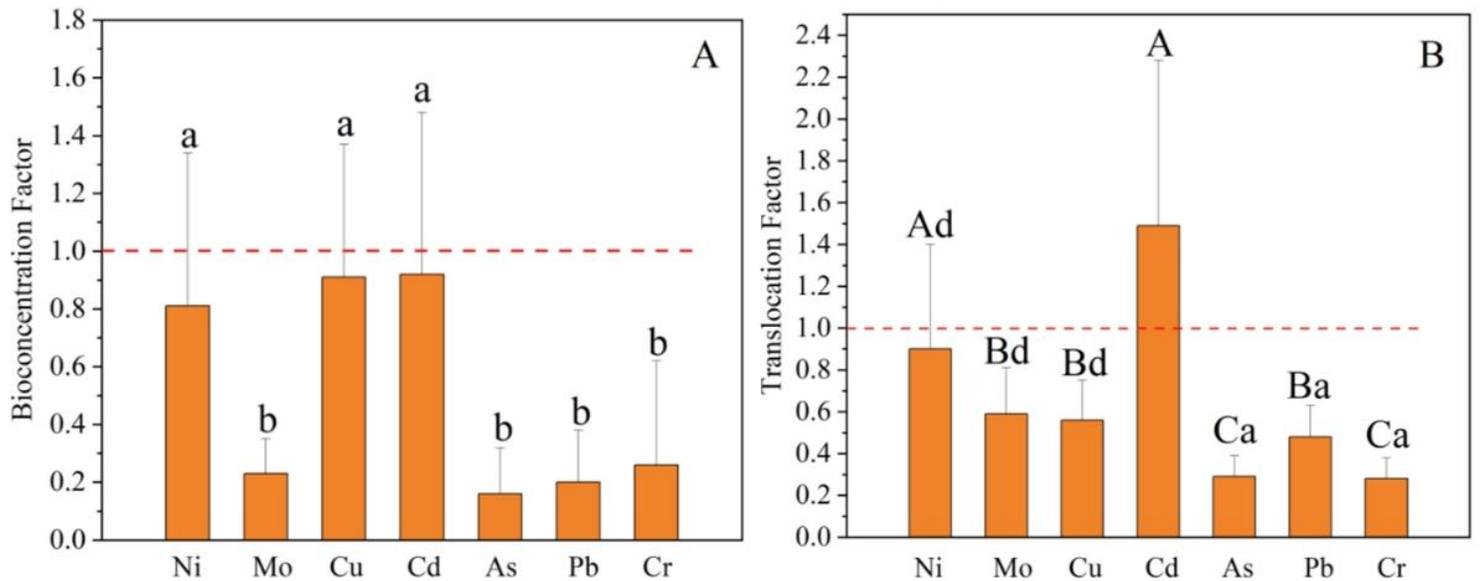


Figure 3

The bioconcentration factor (A) and the translocation factor (B) of heavy metals in *P. flexuosa* Harv collected from the contaminated soils overlying black shales. In Fig. 3(A), the different lower case letters at the top of error bars represent statistical difference at a level of $p < 0.01$; In Fig. 3(B), the different upper case letters at the top of error bars represent statistical difference at a level of $p < 0.01$; The different lower case letters at the top of error bars represent statistical difference at a level of $p < 0.01$; The same lower case letter represent statistical difference at a level of $p < 0.05$, and with respect to the same letter do not differ significantly ($p > 0.05$).

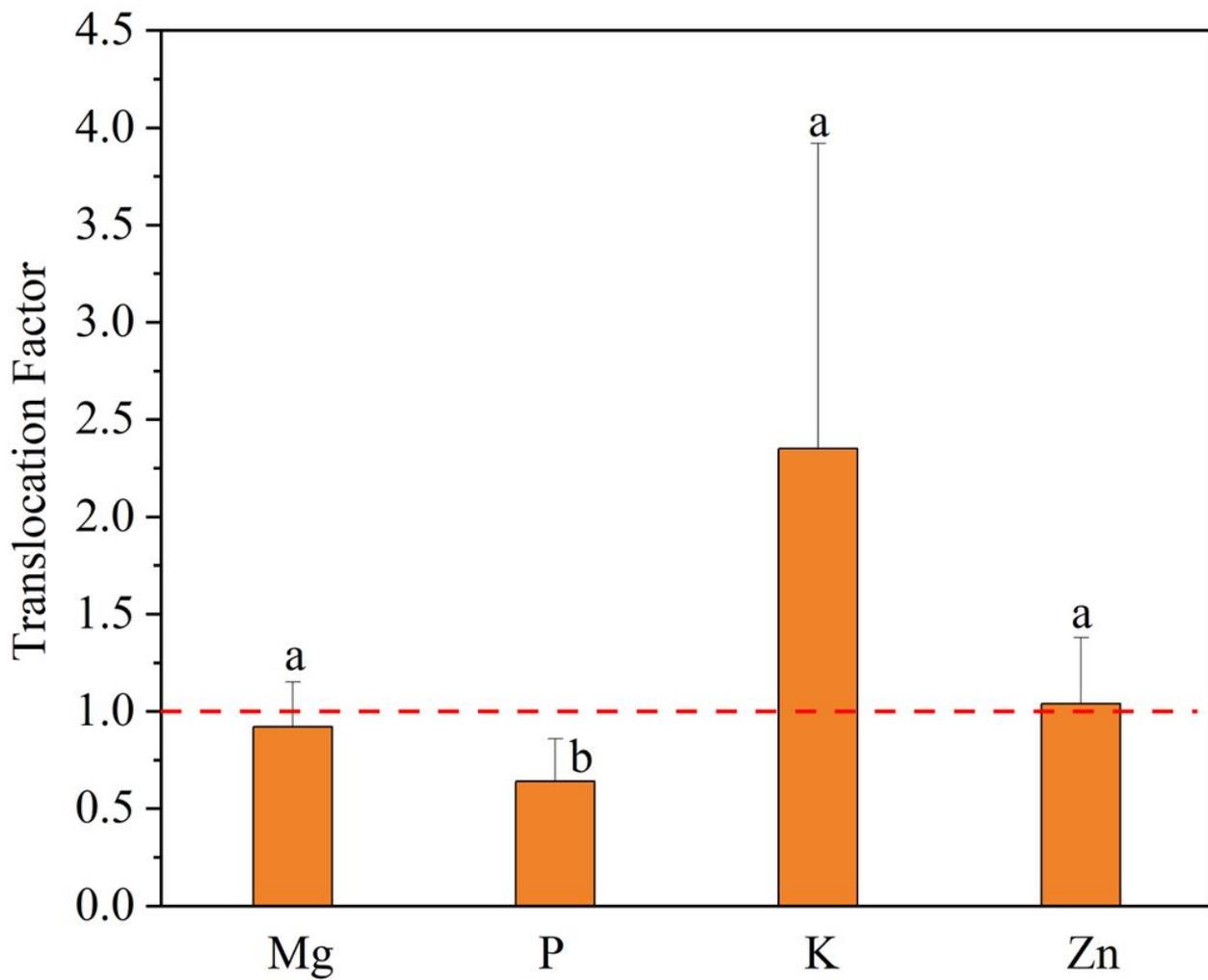


Figure 4

The translocation factor (B) of mineral elements in *P. flexuosa* Harv collected from the contaminated soils overlying black shales. The different lower case letters at the top of error bars represent statistical difference at a level of $p < 0.05$.

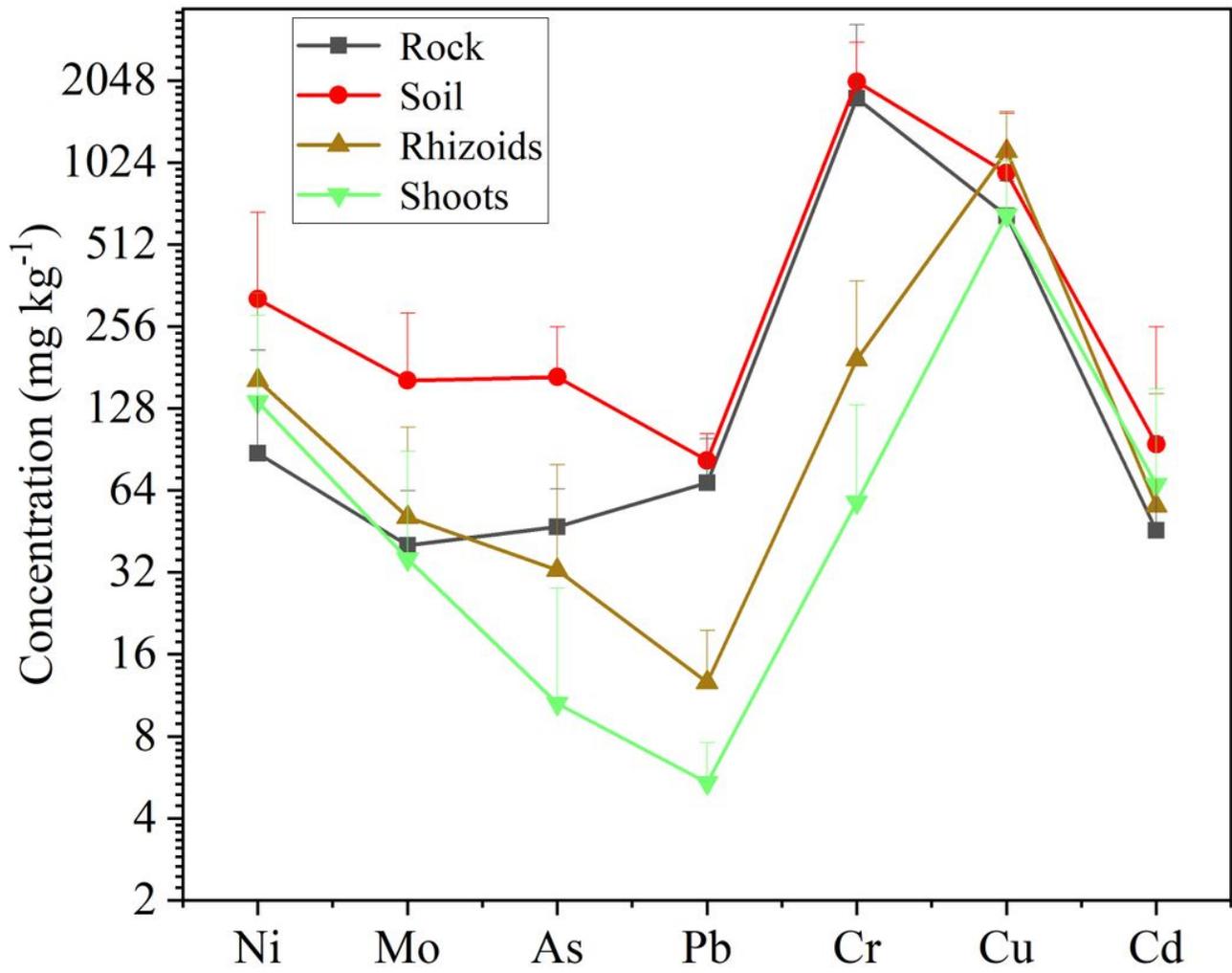


Figure 5

Comparison of heavy metal concentrations in rock - soil - moss system in the study areas (n=8).

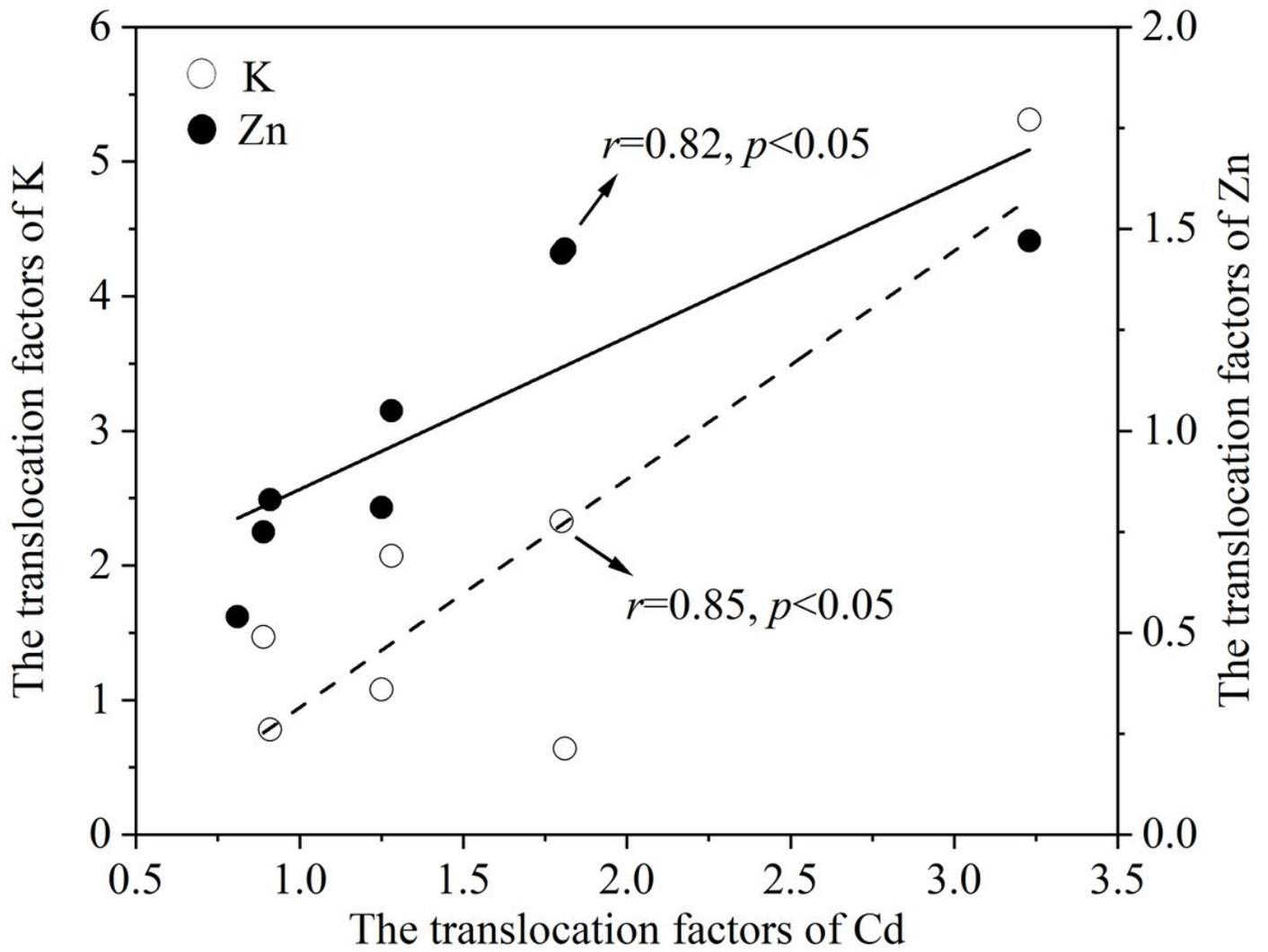


Figure 7

Correlation between Cd and K, Zn transfer factors in *P. flexuosa* Harv.