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\*CORRESPONDENCE Bing Hao, ☑ Bing.Hao@ynau.edu.cn Wei Fan, □ fanwei1128@aliyun.com

<sup>†</sup>These authors have contributed equally to this work

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### Assessment and analysis of heavy metal pollution in key production areas of Gastrodia elata in Yunnan, China

Xixi Qian<sup>1,2,3†</sup>, Yu Luo<sup>4†</sup>, Honglei Yang<sup>1,2,3</sup>, Jinghui Wang<sup>1,2,3</sup>, Huimin Zhang<sup>1,2,3</sup>, Huineng Shi<sup>1,2,3</sup>, Qi Li<sup>1,2,3</sup>, Zhanhua Song<sup>1,2,3</sup>, Bing Hao<sup>2,3</sup>\* and Wei Fan<sup>1,2,3</sup>\*

<sup>1</sup>College of Resources and Environment, Yunnan Agricultural University, Kunming, China, <sup>2</sup>The Key Laboratory of Medicinal Plant Biology of Yunnan Province, Yunnan Agricultural University, Kunming, China, <sup>3</sup>National and Local Joint Engineering Research Center on Germplasm Innovation and Utilization of Chinese Medicinal Materials in Southwest China, Yunnan Agricultural University, Kunming, China, <sup>4</sup>College of Food Science and Technology, Yunnan Agricultural University, Kunming, China

Introduction: As a newly recognized medicinal and edible Chinese herbal medicine, the safety of Gastrodia elata has garnered significant attention. Yunnan Province is the main production area of G. elata in China, but there is a lack of systematic assessments of the distribution patterns of heavy metals in the soil-plant system and their associated human health risks.

Methods: This study evaluated the pollution status of five heavy metals—cadmium (Cd), arsenic (As), lead (Pb), mercury (Hg), and copper (Cu)-in the surface soil (0-20 cm) and G. elata tubers across four major planting areas: Kunming (KM), Zhaotong (ZT), Lijiang (LJ) and Tengchong (TC) in Yunnan Province. The concentrations of heavy metals in soil and G. elata tubers were determined using inductively coupled plasma mass spectrometry (ICP-MS) and atomic fluorescence spectrometry (AFS). The pollution status of heavy metals in soil was evaluated using the single pollution index (Pi), comprehensive pollution index (PN), potential ecological risk index (PERI), and enrichment factor (EF). The bioconcentration factor (BCF) was applied to assess the accumulation capacity of heavy metals in the plants, while the target hazard quotient (HQ) and total hazard index (HI) were used to evaluate the human health risks associated with heavy metals in G. elata tubers.

Results and discussion: The findings revealed that the surface soils in the sampling areas were acidic, with total nitrogen (TN), total potassium (TK), soil organic matter (SOM), and cation exchange capacity (CEC) reaching abundant levels, while total phosphorus (TP) was relatively low. The pollution levels of the five heavy metals were ranked as Hg > Cd > Cu > As > Pb, with Hg, Cd, and Cu identified as severely polluted. The potential ecological risk assessment indicated a moderate risk, with ZT showing the highest comprehensive pollution level and ecological risk. The enrichment capacity of heavy metals in soil was ranked as Hg > Cd > As > Pb > Cu, while in G. elata tubers it was Cd > Cu > Pb > As. However, there was no significant risk to human health. Correlation analysis indicated that soil Cd and Pb were significantly positively correlated with their respective heavy

metal content in G. elata (P < 0.05/P < 0.001). This study provides a scientific basis for controlling soil heavy metal pollution in G. elata planting areas and for assessing the safety of Chinese herbal medicines.

KEYWORDS

Gastrodia elata, heavy metal contamination, Yunnan Province, soil-plant system,, health risk assessment

#### 1 Introduction

Traditional Chinese medicine (TCM) herbs are fundamental components of ancient medical system, increasingly gaining global prominence due to their unique therapeutic effects and relatively mild side effects (Zhang et al., 2013; Ekor, 2014). In recent years, driven by a growing inclination towards natural remedies, the global market for TCM herbs has expanded significantly, with projections estimating it will surpass \$150 billion by 2025 (Hu et al., 2020; Zhang L. et al., 2021). As the leading producer and consumer of TCM herbs, China plays a crucial role in the global herbal medicine industry. However, this rising demand has brought about concerns related to the quality and safety of TCM herbs, particularly regarding heavy metal contamination (Yang et al., 2021; Liu J. J. et al., 2024). Research indicates that many TCM herb species possess a pronounced capacity for heavy metal accumulation due to their unique physiological characteristics. Coupled with their relatively long cultivation cycles, this has resulted in widespread instances of heavy metal contamination, often exceeding safety standards (Wang et al., 2019; Chen et al., 2020).

Heavy metal contamination not only diminishes the medicinal value of TCM herbs but also poses potential health risks. Common heavy metal contaminants found in TCM herbs include cadmium (Cd), arsenic (As), lead (Pb), mercury (Hg), and copper (Cu), which can lead to toxic effects such as neurotoxicity, liver and kidney damage, and carcinogenicity (Harris et al., 2011; Luan et al., 2015). Analyses of herbal samples from various regions in China have shown that at least one-third contain heavy metals at levels above the limits set by U.S. dietary supplement (NSF/ANSI Standard 173) (Harris et al., 2011). A study of 131 batches of herbal samples reported exceedance rates of 16.79% for Cd and 11.45% for Pb (Luan et al., 2015), while a more extensive examination of 2,427 batches highlighted severe Cd contamination with a 20.9% exceedance rate (Fei et al., 2021). Moreover, regulatory standards for heavy metals vary globally. For example, the limits for Cd, As, Pb, and Hg in China's "Green Industry Standards for the Import and Export of Medicinal Plants and Preparations" are lower than those in the United States and the European Union (Chen et al., 2020; Fei et al., 2021). Therefore, evaluating the safety of TCM herbs based on current Chinese standards may lead to heavy metal contamination issues becoming a significant bottleneck for the external development of the TCM herbs industry.

Gastrodia elata, a perennial herbaceous plant of the Orchidaceae family, is one of the most valued TCM herbs and features a unique symbiotic relationship with medicinal fungi (Zhan et al., 2016). Its primary active components constituent—gastrodin, phenolic compounds, polysaccharides, and organic acid—endow *G. elata* with remarkable pharmacological properties, particularly in treating neurological disorders (Zhu et al., 2019). Clinical studies

affirm its efficacy in managing headaches, epilepsy, and cardiovascular diseases (Zhan et al., 2016; Lu et al., 2022). Unlike many TCM herbs, *G. elata* is a heterotrophic, relying entirely on fungi such as *Armillaria* sp. for nutrient acquisition (Liu Y. et al., 2024). However, studies suggest that these fungi may also facilitate the absorption for certain heavy metals, raising concerns about potential contamination in *G. elata* (Ferrol et al., 2016; Yin et al., 2021; Goswami et al., 2023).

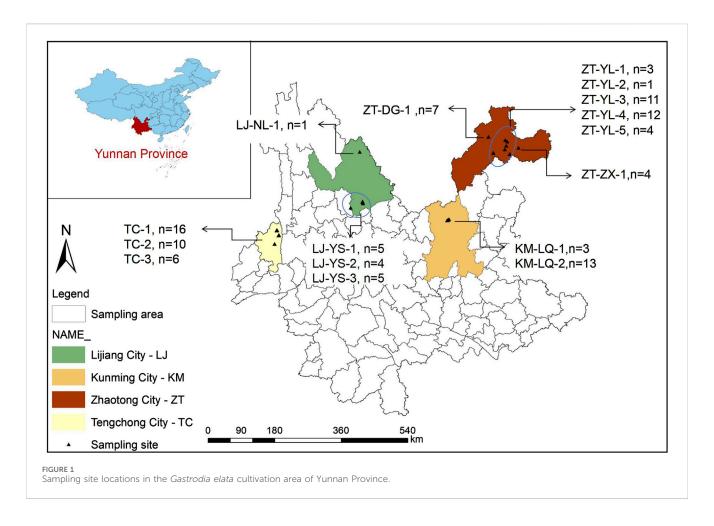
Yunnan Province is the main planting area of G. elata in China, with both its planting area and output ranking first nationwide (Wang et al., 2023). The unique geographical and climatic conditions in Yunnan create an excellent environment for G. elata cultivation, but they also pose potential risks of heavy metal pollution. Studies have shown that the background values of heavy metals in Yunnan's soil are generally high, with Cd, Pb, and Hg levels exceeding the national averages (Xu et al., 2024). Soil heavy metal pollution in G. elata planting areas displays both regional and composite characteristics, and environmental factors from different planting sites significantly influence the heavy metal content in G. elata (Jin et al., 2022; Chen and Deng, 2025; Huang et al., 2025). As one of the main planting regions, Zhaotong in Yunnan has been the subject of studies investigating the heavy metal content in soil and *G*. elata, but the scope of related research remains limited (Xu et al., 2018; Zhao et al., 2018). In contrast, research on emerging planting areas such as Lijiang, Kunming, and Tengchong remains relatively limited. Moreover, most existing studies have concentrated on the heavy metal content, pesticide residues, and sulfur dioxide residues in commercially available G. elata products (Zhang et al., 2018; Yan et al., 2021; Huang et al., 2024).

This study systematically investigated the heavy metal pollution status in the main *G. elata* planting areas of Yunnan Province. It aims to comprehensively analyze and assess the distribution characteristics of five key heavy metals in both soil and *G. elata* samples, and further identify the spatial distribution of soil heavy metals and the physicochemical properties of the soil in different *G. elata* planting regions. This research will not only provide a scientific foundation for sustainable development of Yunnan's *G. elata* industry but also offer significant theoretical and practical insights for ensuring the quality and safety of TCM herbs.

#### 2 Materials and methods

#### 2.1 Sample collection and processing

This study collected *G. elata* tubers and soil samples from major cultivation regions in Yunnan Province between December 2023 and January 2024 (Liu et al., 2015). The sampling locations included: Luquan County, Kunming City (KM-LQ, 16 sites); Yiliang



County (ZT-YL, 31 sites), Zhenxiong County (ZT-ZX, 4 sites), and Daguan County (ZT-DG, 7 sites) in Zhaotong City; Yongsheng County (LJ-YS, 14 sites) and Ninglang County (LJ-NL, 1 site) in Lijiang City; and Tengchong City (TC, 32 sites) (Figure 1). The sampling area encompassed latitudes N25 18'53"to N27 55'10", longitudes E98 34'52" to E104 30'48", and elevations ranging from 1,379.21 to 3,434.68 m (Supplementary Table S1). G. elata predominantly grew in environments characterized by Cyclobalanopsis glauca, Pinus yunnanensis, and Quercus dolicholepis, with soil types including red, brown, yellow-brown, and yellow soils (pH 4.10-6.10) (Tian et al., 2016; Zhang M. et al., 2021). To ensure the representativeness of the samples and the reliability of the data, this study employed a five-point sampling method at each site to collect surface soil (0-20 cm). The 1 kg of soil was collected from each of the five points and combined to form a single composite soil sample, resulting in a total of 105 mixed soil samples. The soil samples were air-dried indoors, and after drying, impurities such as dead branches, fallen leaves, and stones were removed. The soil was then ground using wooden tools and passed through a nylon sieve. The processed soil samples were preserved for future analysis. At each sampling site, 3 G. elata plants were also collected, yielding a total of 315 tuber samples, which were further grouped into 16 composite samples based on the variations in geographical names (Supplementary Table S1). The tuber samples were sequentially washed with tap water and deionized water, and excess surface moisture was removed with absorbent

paper. Subsequently, the tubers were steamed in a steamer for about 10 m to ensure there was no white core. After that, the tubers were sliced and dried in a 65°C oven for 72 h until they reached a constant weight. Finally, the samples were pulverized, sieved, and stored in polyethylene self-sealing bags for subsequent analysis (Sun et al., 2016; Huang et al., 2025).

#### 2.2 Indicator determination

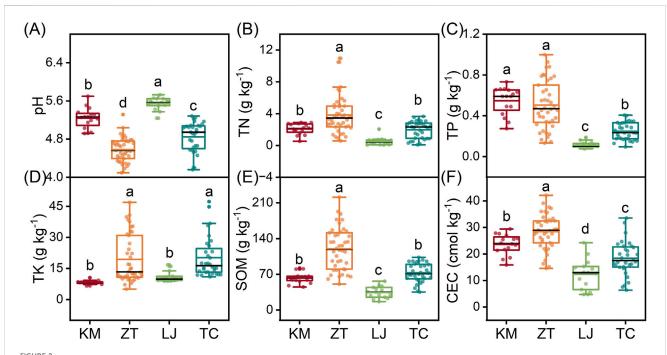
### 2.2.1 Determination of soil physicochemical properties

Soil samples sieved through 2 mm or 0.149 mm (100-mesh) sieves were measured to determine physicochemical properties: pH, total nitrogen (TN), total phosphorus (TP), total potassium (TK), soil organic matter (SOM), and cation exchange capacity (CEC). The pH was determined using the electrode method. Soil was mixed with deionized water at a water-to-soil ratio of 1:2.5, shaken, allowed to settle, and the supernatant was analyzed with a pH meter (Xu et al., 2021). TN was determined using the semi-micro Kjeldahl method; TP was measured using the NaOH fusion-molybdenum antimony anti-spectrophotometric method; TK was assessed using the NaOH fusion-flame photometric method; SOM was analyzed using the potassium dichromate volumetric method with external heating; CEC was measured employing the 1 mol/L ammonium acetate exchange method (Lu et al., 2024).

TABLE 1 Descriptive statistical analysis of heavy metal content in soil (mg·kg<sup>-1</sup>).

Statistics	Cd	As	Pb	Hg	Cu	рН
Range	0.07~1.83	6.78~48.45	3.60~122.27	0.02~0.68	2.49~205.78	4.10~6.10
Mean ± SD	0.48 ± 0.44	18.56 ± 9.57	30.21 ± 20.81	0.23 ± 0.12	54.58 ± 59.44	4.92 ± 0.44
Median	0.33	15.72	24.77	0.23	17.49	4.92
CV	0.93	0.52	0.69	0.53	1.09	0.09
Background value (K1)	0.22	18.40	40.60	0.06	46.30	5.70
Risk screening value (K2)	0.30	40.00	70.00	1.30	50.00	pH < 5.5
Risk control value (K3)	1.50	150.00	400.00	2.00	-	pH < 5.5
K1 (Exceedance percentage/%)	55.24	33.33	21.90	86.67	34.29	2.86
K2 (Exceedance Percentage/%)	52.38	3.81	3.81	-	34.29	-
K3 (Exceedance Percentage/%)	4.76	-	-	-	-	-

Note: "-" indicates that there is no relevant data or the standard is not exceeded, K1 indicates the soil background value of the surface layer (layer A) in Yunnan, China Province (China National Environmental Monitoring Center, 1990), K2 indicates the screening value of agricultural land soil pollution risk, and K3 indicates the control value of agricultural land soil pollution risk. For details, please refer to the environmental quality risk control standard of agricultural soil pollution in China (GB15618-2018).



Changes in soil pH, total nitrogen (TN), total phosphorus (TP), total potassium (TK), organic matter (SOM) and cation exchange capacity (CEC) in different regions. (Note: (A) pH, (B) TN, (C) TP, (D) TK, (E) SOM, (F) CEC, there are statistical differences between different lowercase letters, P < 0.05, black represents the median line and the same color represents the mean line.)

#### 2.2.2 Heavy metal content determination

Soil and plant samples that passed through a 100-mesh sieve were weighed at 0.2–1 g (accurate to 0.0001 g) using a precision balance with an accuracy of one ten-thousandth of a Gram (Mettler-Toledo ME204E). The concentrations of Cd, Pb, Cu, and Zr in the soil, as well as Cd, Pb, and Cu in the *G. elata*, were determined using an inductively coupled plasma mass spectrometer (Spectro SUPEC 7000), following the HJ1315–2023 and GB 5009.268–2016 methods, respectively. Ministry of Ecology and Environment of the People's Republic of China, 2023; Ministry of Environmental Protection of

People's Republic of China, 2013; National Health and Family Planning Commission of the People's Republic of China, 2014; National Health and Family Planning Commission of the People's Republic of China, China Food and Drug Administration, 2016; National Health and Family Planning Commission of the People's Republic of China, China Food and Drug Administration, 2021. The concentrations of As and Hg in the soil were measured using an atomic fluorescence spectrometer (Jitian AFS-820), according to the HJ 680–2013 method (Lu et al., 2024). The concentrations of Hg and As in *G. elata* were also measured using an atomic fluorescence

TABLE 2 Single factor pollution index and comprehensive pollution index of soil heavy metals.

Element	Single factor pollution index	Proportion of different pollution levels (%)				Comprehensive pollution index $(P_{Nave})$
	$(P_{iave})$	Unpolluted	Slightly polluted	Moderately polluted	Highly polluted	ITIUEX (FNave)
Cd	2.16	43.81	13.33	17.14	25.71	6.08
As	1.01	65.71	27.62	6.67	-	1.99
Pb	0.74	78.10	18.10	2.86	0.95	2.19
Hg	3.78	13.33	9.52	13.33	63.81	6.57
Cu	1.18	65.71	7.62	10.48	16.19	3.25

Note:  $P_{iave}$  represents the average single-factor index of each heavy metal at the sampling points (n = 105), while  $P_{Nave}$ , denotes the average comprehensive index of each heavy metal at the same sampling points (n = 105).

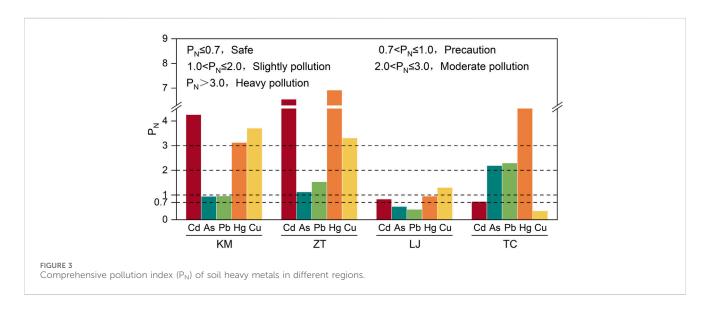


TABLE 3 Soil potential ecological risk index.

Element	Single factor potential ecological risk index ( $E_r^i$ ave)	Potential ecological risk index ( <i>RI</i> )	Proportion of ecological risk (%)				
	ecological risk index (E, ave)	Lov		Moderate	High	Slightly heavy	Heavy
Cd	64.81		47.67	20.95	23.81	8.57	-
As	10.09		100.00	-	-	-	-
Pb	3.72	235.88	100.00	-	-	-	-
Hg	151.37		13.33	9.52	28.57	46.67	1.90
Cu	5.89		100.00	-	-	-	-

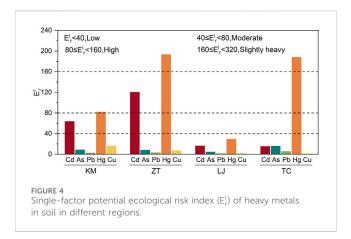
spectrometer, following the GB 5009.11–2014 and GB 5009.17–2021 methods, respectively. The detection limits for heavy metals in the soil (Cd, As, Pb, Hg, Cu, Zr) were 0.03, 0.01, 1, 0.002, 0.7, and 2 mg/kg, respectively, while those for plant samples (Cd, As, Pb, Hg, Cu) were 0.002, 0.01, 0.02, 0.0002, and 0.05 mg/kg, with quantification limits set at four times the detection limits. For additional information related to this experiment, please refer to the supplementary materials.

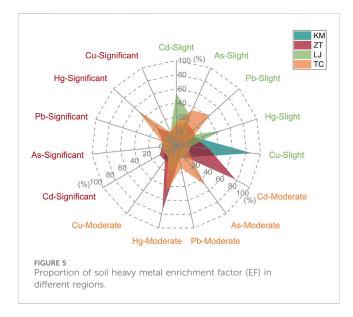
#### 2.3 Heavy metal pollution assessment

#### 2.3.1 Single factor pollution index (P<sub>i</sub>)

The pollution level of a specific heavy metal in soil was calculated using the formula:

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





where  $P_i$  is the pollution index of heavy metal i;  $C_i$  is the measured concentration of heavy metal i (mg·kg<sup>-1</sup>); and  $S_i$  is the evaluation standard value for heavy metal i (mg·kg<sup>-1</sup>). The background value of surface (A horizon) soil in Yunnan Province is selected as the standard for evaluating heavy metal pollution (Table 1) (Chen et al., 2021; China National Environmental Monitoring Center, 1990).

#### 2.3.2 Comprehensive pollution index (P<sub>N</sub>)

The comprehensive pollution index  $(P_{\rm N})$  incroporates both the average and maximum values of single-factor pollution indices, reflecting the overall pollution level of different contaminants in the soil. The calculation formula is as follows:

$$P_N = \sqrt{\frac{P_{imax}^2 + P_{iave}^2}{2}}$$

Where  $P_{imax}$  refers to the maximum value of the pollution index among the pollutants;  $P_{iave}$  refers to the average value of the pollution index. The grading standards are shown in Supplementary Table S2 (Ruan et al., 2023).

#### 2.3.3 Potential ecological risk index (RI)

Based on heavy metal toxicity and environmental behavior, the potential ecological risk index (RI) was employed to assess soil heavy metal pollution levels (Hakanson, 1980; Yan et al., 2024). The calculation formulas are as follows:

$$RI = \sum_{i}^{n} E_{r}^{i}$$
 
$$E_{r}^{i} = T_{r}^{i} \times C_{r}^{i} = T_{r}^{i} \times C_{n} / B_{n}$$

where  $B_n$  is the background value of heavy metal i,  $C_n$  is the concentration of heavy metal i in the sample,  $E_r^i$  is the single-factor potential ecological risk index, and  $T_r^i$  is the toxic response factor of the heavy metal (assigned values: Cd = 30, As = 10, Pb = 5, Hg = 40, Cu = 5).

#### 2.3.4 Enrichment factor (EF)

The enrichment factor (EF) serves as a critical indicator for evaluating pollutant sources and tracing the origins of elemental contamination. Zirconium (Zr) was selected as the reference element for calculating enrichment factors (Kuang et al., 2020), with the formula as follows:

$$EF_i = \frac{(C_i/Zr_i)}{(B_i/Zr_n)}$$

where EF is the enrichment factor of element i in soil;  $C_i$  and  $Zr_n$  represents the concentrations of element i and the reference element Zr in the sample, respectively;  $B_i$  and  $Zr_n$  refer to the background values of element i and Zr in the soil. The background values of

TABLE 4 Soil heavy metal enrichment factor.

Element	EF <sub>ave</sub>	Proportion of different enrichment levels (%)			
		Negligible	Slight	Moderate	Significant
Cd	1.70	50.48	14.29	32.38	2.86
As	1.09	64.76	20.00	15.24	-
Pb	0.80	77.14	18.10	3.81	0.95
Hg	3.74	15.24	14.29	47.62	22.86
Cu	0.73	72.38	23.81	3.81	-

Note:  $EF_{ave}$  represents the average enrichment factor of each heavy metal at the sampling points (n = 105).

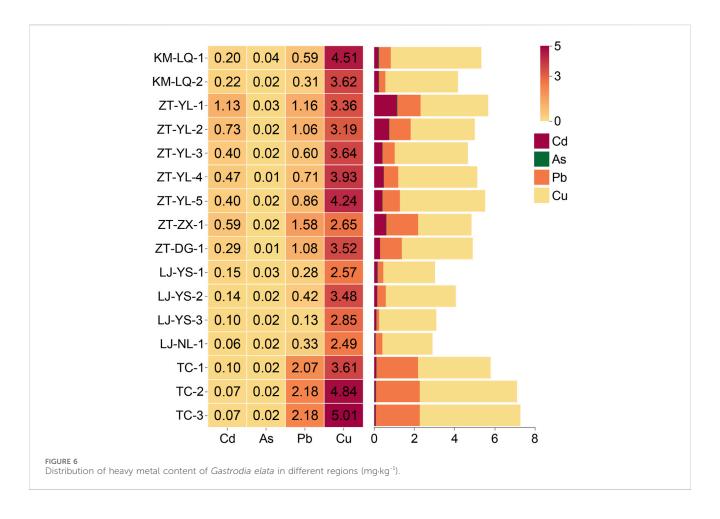


TABLE 5 Descriptive statistical analysis of heavy metal content in Gastrodia elata tubers (mg·kg<sup>-1</sup>).

Element	Range	Mean ± standard deviation	Coefficient of variation	Import and export standards (K4)	K4 (exceedance percentage/%)
Cd	0.06~1.13	$0.32 \pm 0.30$	0.92	0.30	37.5
As	0.01~0.04	$0.02 \pm 0.01$	0.34	2.00	-
Pb	0.13~2.18	0.97 ± 0.70	0.72	5.00	-
Hg	-	-	-	0.20	-
Cu	2.49~5.01	3.59 ± 0.77	0.21	20.00	-

Note: The mean represents the average concentration of each heavy metal at the sampling points (n = 16). K4 indicates the limit standard of heavy metals as defined in the Green Industry Standard for Import and Export of Medicinal Plants and Preparations. "-" denotes no relevant data or no exceedance.

surface (A layer) soils in Yunnan Province were selected as the standard for each heavy metal: Cd 0.22 mg·kg<sup>-1</sup>, Pb 40.6 mg·kg<sup>-1</sup>, Cu 46.3 mg·kg<sup>-1</sup>, Hg 0.06 mg·kg<sup>-1</sup>, As 18.4 mg·kg<sup>-1</sup>, and Zr 229 mg·kg<sup>-1</sup> (China National Environmental Monitoring Center, 1990; Barbieri, 2016).

#### 2.3.5 Bioconcentration factor (BCF)

The bioconcentration factor (BCF) reflects the capacity of soil heavy metals to accumulate in plants (Tong et al., 2022). The calculation formula is as follows:

$$BCF = \frac{\text{Content of a specific heavy metal in plants}}{\text{Corresponding heavy metal content in soil}} \times 100\%$$

Based on BCF values, the uptake intensity of *G. elata* for soil heavy metals is classified into four levels: Low enrichment (0 < BCF  $\leq$ 1.5), Medium enrichment (1.5 < BCF  $\leq$ 4.5), and High enrichment (BCF >4.5) (Dong et al., 2023).

#### 2.3.6 Hazard quotient (HQ) and hazard index (HI)

The hazard quotient (HQ) is commonly used to assess non-carcinogenic risks posed by pollutants. The target hazard quotient is defined as the ratio of a pollutant's exposure dose to its reference dose (RFD), determined by the following formula:

$$HQ = \frac{C \times EF \times ED \times IRD \times t}{BW \times AT \times RFD}$$

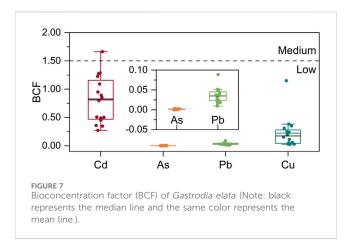


TABLE 6 Analysis of the hazard quotient and hazard index of Gastrodia elata

Element	HQ	НІ
Cd	2.00E-04~3.70E-03	
As	1.13E-04~5.75E-04	2.125.02
Pb	6.16E-05~1.02E-03	3.13E-02
Cu	1.02E-04~2.06E-04	

Note: the calculation range of HQ, is based on the recommended usage and dosage of Gastrodia elata in (Pharmacopoeia Standard)), Volume I, 2020 (10 g).

Where C (mg·kg<sup>-1</sup>) represents the detected concentration of each metal in G. elata; EF is the exposure frequency (set to 90 days/year); ED is the exposure duration (set to 20 years); IRD is the ingestion rate (set to  $0.01 \, \mathrm{kg \cdot d^{-1}}$ ) based on the maximum dosage in the Chinese Pharmacopoeia (National Pharmacopoeia Committee, 2020); the transfer rate (t) of heavy metals to the medicinal plant vary: 14% for Cd, Cu, and Pb; 35% for As; and 24% for Hg. The average body weight (BW) is set to 60 kg, following international standards; the averaging time for non-carcinogenic toxicity (AT) is calculated as 70 years × 365 days; and the RFD values are Cu  $0.04 \, \mu \mathrm{g \cdot g^{-1}}$ , As  $0.0003 \, \mu \mathrm{g \cdot g^{-1}}$ , Cd  $0.0005 \, \mu \mathrm{g \cdot g^{-1}}$ , Hg  $0.0003 \, \mu \mathrm{g \cdot g^{-1}}$ , and Pb  $0.0035 \, \mu \mathrm{g \cdot g^{-1}}$  (Luo et al., 2021).

When multiple heavy metals coexist in *G. elata*, the total target hazard index (HI) is calculated to assess the combined pollution risk:

$$HI = \Sigma HQ$$

Here, HQ represents the target hazard quotients of the five heavy metals. Higher HQ or HI values indicate greater health risks to humans, with an HI > 1 suggesting potential health risk.

#### 2.4 Data analysis

Descriptive statistics were conducted using Excel 2010. One-way ANOVA was performed with SPSS 26.0 software to evaluate differences in soil pH, TN, TP, TK, SOM, and CEC among different regions. Prior to ANOVA, Levene's test was used to assess the homogeneity of variances across groups. For variables with significant ANOVA results, Duncan's multiple range test was

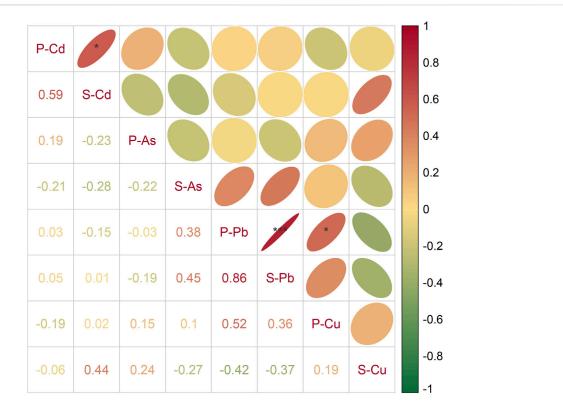
applied as a *post hoc* analysis to identify which regions differed significantly in means (P < 0.05). Pearson correlation coefficient analysis was used to examine the relationship between soil and plant heavy metals. Data visualization was carried out using Origin 2021. The sampling point map for the *G. elata* planting area in Yunnan Province was generated with ArcGIS 10.8.

#### 3 Results and discussion

## 3.1 Analysis of soil physicochemical properties

The spatial distribution of heavy metals is influenced by multiple factors, including geochemical characteristics, microbial activity, human activities, and soil physicochemical properties (Lima et al., 2024; Korkanç et al., 2024; Zhong et al., 2011). In the study area, the surface soil exhibited a pH range of 4.10~6.10, with TN, TP, TK, SOM, and CEC values spanning g·kg<sup>-1</sup>, 0.08–1.00 g·kg<sup>-1</sup>, 5.05–47.33 g·kg<sup>-1</sup>, 0.08 - 10.9716.48-221.24 g·kg<sup>-1</sup>, and 4.74-42.16 cmol·kg<sup>-1</sup>, respectively (Figure 2). Among these, TN, TK, SOM, and CEC levels were found to be abundant, whereas TP was relatively low (National Soil Survey Center, 1988). Notably, the pH in ZT was significantly lower than in KM, LJ, and TC, with 97.62% of the samples exhibiting a pH below 4.5, indicating strong to extremely strong acidity (Figure 2A). Interestingly, the levels of TN, SOM, and CEC levels in ZT were significantly higher than those in other regions (Figures 2B-F). The soil in ZT predominantly consists of yellow soil, yellowbrown soil, and purple soil, among which yellow soil covers the largest area, accounting for 66.42% of ZT's total soil area. This soil type is characterized by relatively low pH and higher organic matter content compared to others (Zhaotong Municipal People's Government, 2023; Ji et al., 2023). The parent material of yellow soil originates from acidic rocks such as sandstone and shale, which release aluminum (Al3+) and proton (H+) during weathering, contributing to soil acidification (Hamer, 2024). At the same time, high TN levels can enhance the activity of ammoniaoxidizing bacteria, accelerating nitrification and releasing in further H+, which exacerbates soil acidity (Liu et al., 2023). The average annual temperature in ZT ranges from 11°C to 21°C, coupled with annual precipitation levels between 600 and 1,230 mm (Yuan et al., 2022). Such a humidity environment accelerates the leaching of base cations (Ca2+, Mg2+), while promoting the accumulation of organic acids, such as humic acid, which may further contribute to soil acidification (Wen et al., 2023). It is noteworthy that this study collected soil and G. elata samples during winter. The average temperature in the sampling areas ranged from 8°C to 15°C, while in the ZT region it was even lower, between 2°C and 9°C. During this period, frequent light rains occurred, with precipitation from December to January ranging from 66 to 272 mm. Winter rainfall may promote the migration and diffusion of heavy metals. Additionally, freeze-thaw cycles in the ZT region may alter the soil pore structure, further affecting the adsorption and migration of heavy metals (Tang et al., 2023; Gao et al., 2024).

Importantly, the average SOM content (84.95 g·kg<sup>-1</sup>) in the sampling areas was significantly higher than the cultivated soils in Yunnan Province, which averaged 9.15  $\pm$  29.01 g·kg<sup>-1</sup> (Figure 2E)



Heatmap of the correlation between heavy metals in soil and plants. (Note: S- represents heavy metals in soil and P- represents heavy metals in plants; \*, \*\* and \*\*\* indicate significant correlation at the levels of 0.05, 0.01 and 0.001 respectively.).

(Sun et al., 2022). This difference may be attributed to organic carbon inputs from understory vegetation, litterfall, microbial activity, and tree species diversity (Adamczyk et al., 2019; Augusto and Boča, 2022; Shen et al., 2024). The highly acidic environment also inhibits microbial decomposition of organic matter, promoting SOM retention (Li et al., 2024). This phenomenon may explain why ZT exhibits the lowest pH among all sampling areas, while concurrently having the highest SOM content (Figures 2A,E).

## 3.2 Risk assessment of soil heavy metal pollution

#### 3.2.1 Analysis of soil heavy metal content

As shown in Table 1, the heavy metal content in the soil of the *G. elata* planting area varied significantly. The concentrations of Cd, As, Pb, Hg, and Cu ranged from 0.07–1.83, 6.78–48.45, 3.60–122.27, 0.02–0.68, and 2.49–205.78 mg·kg<sup>-1</sup>, respectively, with average concentrations of 0.48, 18.56, 30.21, 0.23, and 54.58 mg·kg<sup>-1</sup>. Among the 105 sampling points, the exceedance rates of heavy metals compared to the background values of Yunnan soil followed the order of Hg > Cd > Cu > As > Pb, while the exceedance rate of Cd reached as high as 55.24%. Except for Pb, the average concentrations of the other four heavy metals exceeded their background values: 2.18 times (Cd), 1.01 times (As), 3.83 times (Cu), and 1.18 times (Hg) times the background levels, indicating

potential pollution point sources in the *G. elata* planting area. Specifically, the average concentrations of Cd and Cu exceeded the soil pollution risk screening values for agricultural land by 1.6 and 1.09 times, respectively, while As, Pb, and Hg did not exceed the risk screening or control values (GB 15618–2018). Overall, the issue of Cd exceedance is particularly pronounced. Compared to previous studies, the issue of soil Cd exceeding standards in *G. elata* planting areas appears to be more severe (Jin et al., 2022).

Coefficient of variation (CV) analysis revealed that the CVs for Cd, As, Pb, Hg, and Cu were 0.93, 0.52, 0.69, 0.53, and 1.09, respectively, indicating strong variability (CV > 0.2), whereas pH exhibited weak variability (CV < 0.2) (Table 1). This suggests that the soil heavy metals in the sampling area may be influenced by varying degrees of anthropogenic interference (Chen et al., 2021; Du et al., 2024), with higher CVs for Cd and Cu indicating that their distribution is significantly impacted by human activities.

#### 3.2.2 Analysis of heavy metal pollution index

Pollution indices,  $P_i$  and  $P_N$ , are essential for assessing soil heavy metal pollution risk. By calculating the ratio of predicted no-effect concentration to exposure concentration, the risk level of heavy metals in soil can be determined (Zhang et al., 2023). According to Table 2, the pollution levels of five heavy metals were ranked as Hg > Cd > Cu > As > Pb. Notably, the  $P_i$  values for Hg and Cd were 3.78 and 2.16, respectively, while the  $P_i$  values for the remaining heavy metals were less than 2, indicating that Hg pollution is the

most severe, followed by Cd. Specifically, the proportion of unpolluted from Hg was 13.33%, slightly polluted accounted for 9.52%, moderately polluted was 13.33%, and highly polluted was 63.81%; for Cd, the proportion of unpolluted was 43.81%, slightly polluted was 13.33%, moderately polluted was 17.14%, and highly polluted was 25.71%; both Cu and As had the same proportion of unpolluted (65.71%), but Cu had a 16.19% highly polluted, whereas As had highly polluted; the proportion of unpolluted for Pb was 78.10%, indicating a lower pollution risk. Thus, it is evident that Hg and Cd have a wider range of pollution, followed by Cu, while As and Pb have a smaller range of pollution.

From the perspective P<sub>N</sub> value, heavy metal pollution levels were ranked as Hg > Cd > Cu > Pb > As. Among these, Hg, Cd and Cu were classified as severely polluted, Pb as moderately polluted, and As as mildly polluted. Comparative analysis of the P<sub>N</sub> values across the four regions showed that Hg exhibited high P<sub>N</sub> levels, ranked as ZT > TC > KM > LJ, where ZT, TC and KM reflected heavy pollution, while LJ remained at a precautionary level (Figure 3). Cd pollution levels followed, with rankings of ZT > KM > LJ > TC, where both ZT and KM also indicated heavy pollution, while LJ and TC were categorized as precautionary (Figure 3). Cu and Cd displayed similar regional pollution patterns, with both ZT and KM experiencing heavy pollution (Figure 3). Notably, the nonferrous metal smelting industry is a primary sources of heavy metal pollution, with approximately 45.6% of smelting enterprises in China located in Jiangxi, Yunnan, Henan, Guangdong, and Hunan provinces. Research indicates that ZT and its vicinity including KM are crucial zones for non-ferrous metal smelting within Yunnan province. Cd and Hg from smelting emissions can contaminate soil through atmospheric deposition (Han et al., 2023; Shao et al., 2013). Moreover, Cu from tailings in the ZT leadzinc mining area is dispersed via runoff, increasing mobilization in acidic soils (Yu et al., 2025), contributing to elevated levels of Cd and Cu contamination.

### 3.2.3 Potential ecological risk assessment of heavy metals

RI is an important tool for quantitatively assessing the ecological risks of toxic metals in soil and sediments (Wang et al., 2024). As shown in Table 3, the RI values of the five heavy metals ranged from 150 to 300, indicating a moderate potential ecological risk. The values of  $E_r^i$  followed the order of Hg > Cd > As > Cu > Pb, with Hg recording an  $E_r^i$  of 151.37, where the proportion of heavy risk reached 46.67%. Cd had an  $E_r^i$  of 64.81, indicating a slight heavy risk (8.57%), while As, Pb, and Cu exhibited low risk levels. To reduce the influence of sampling point differences,  $E_r^i$  values of the five heavy metals were calculated across the four regions. Results showed that Hg risk was particularly pronounced in the order of ZT > TC > KM > LJ, with ZT and TC reaching slightly heavy risk, while KM was categorized as high risk. The risk for Cd ranked second to Hg, in the order of ZT > KM > LJ > TC, with ZT reaching high risk levels; low risk was observed for As, Pb, and Cu across the four regions (Figure 4). Notably, the toxicity coefficient of Hg (40) is significantly higher than that of other elements (e.g., Cd = 30), meaning that even low concentrations can significantly elevate the RI value (Hakanson, 1980). Thus, the pollution level and potential ecological risk of Cd in ZT warrant heightened attention.

#### 3.2.4 Heavy metal enrichment factor analysis

The EF for the five heavy metals in the soil were as follows: Cd 0.24-6.90, As 0.17-4.75, Pb 0.06-6.47, Hg 0.28-12.95, and Cu 0.05-3.55, with EF levels ranked as Hg > Cd > As > Pb > Cu (Table 4). Among these, the proportion deemed negligible for Cd, As, Pb, and Cu was the highest (50.48%-72.38%), while moderate enrichment for Hg accounted for the largest proportion (47.62%). A comparison of EF levels across the four regions revealed notable Cd and Hg enrichment in ZT, with significant Hg and As enrichment in TC (Figure 5). The study suggests that Hg enrichment in TC may be linked to transboundary pollutants, such as emissions from coal combustion carried by the South Asian monsoon (Tripathee et al., 2019). ZT, located in the Pb-Zn polymetallic mining belt of Sichuan, Yunnan, and Guizhou, has a high baseline value of native Cd, further exacerbated by smelting emissions (Li et al., 2013). Additionally, the high SOM in ZT can adsorb Hg<sup>2+</sup> through functional groups, such as carboxyl and phenolic hydroxyl, preventing vertical migration and resulting in surface enrichment (Li et al., 2012).

### 3.3 Heavy metal evaluation of *Gastrodia elata*

#### 3.3.1 Analysis of heavy metal content

In the sampled G. elata tubers, except for Hg, which was not detected, the content ranges of Cd, As, Pb, and Cu were found to be 0.06-1.13, 0.01-0.04, 0.13-2.18, and 2.49-5.01 mg·kg<sup>-1</sup>, respectively, with average contents of 0.32, 0.02, 0.97, and 3.59 mg·kg<sup>-1</sup> (Figure 6). It is worth noting that, compared to the study by Lv et al. (2016), this research found relatively high levels of Cd and Pb in the ZT area, whereas As and Cu concentrations were relatively low. According to the "Green Industry Standards for the Import and Export of Medicinal Plants and Preparations," the exceedance rate of Cd in G. elata tubers was 37.5%, while As, Pb, and Cu levels did not exceed the limits (Table 5). According to the same standards, Jin et al. (2022) investigated the planting areas of G. elata in Yunnan (region not specified) and found that the soil exhibited slight Cd pollution, with a Cd exceedance rate of 3.1% in tubers. Our study showed that the median Cd content in tubers from the ZT area was 2.3, 3.9, and 6.6 times higher than that in the KM, LJ, and TC regions, respectively (Figure 6). ZT is primarily involved in building materials, chemical manufacturing, lignite chemical production, and mineral processing, boasting the largest coal and sulfur reserves in the province and the top three non-ferrous metal bases in Yunnan, as well as possesses the second-largest lignite field in southern China (Pang et al., 2022; Wang et al., 2022; Shao et al., 2024). These factors are likely contributed to the elevated levels of Cd pollution observed.

#### 3.3.2 Heavy metal enrichment capacity

Previous studies highlight that ZT has the highest enrichment coefficient for Cd in the edible parts of crops among 11 cities in Yunnan Province (Dong et al., 2022). Analysis of the heavy metal enrichment capacity in *G. elata* tubers revealed that these tubers exhibit the strongest capacity for Cd, with an average enrichment coefficient of 0.8100, while As exhibited the weakest, with an average enrichment coefficient of 0.0015. Except for non-detectable Hg, the average enrichment coefficients for heavy metals in *G. elata* tubers

followed the order: Cd > Cu > Pb > As (Figure 7). Notably, despite the relatively high soil concentrations of Hg and Cu where G. elata is cultivated (Table 2), excessive accumulation of these metals in the tubers was not observed. Conversely, the accumulation of Cd in G. elata appears more influenced by total Cd content. One explanation is that acidic soils (pH < 5.5) enhance Cd availability by facilitating its desorption from soil colloids, thereby promoting absorbed by plants (Li et al., 2021). In contrast, Hg2+ tends to form stable complexes with SOM, such as Hg-SOM, which limits its bioavailability (Xu et al., 2014). Moreover, G. elata is a mycoheterotrophic plant reliant on Armillaria spp. for nutrient absorption (Liu J. J. et al., 2024). Fungal hyphae can absorb and accumulate Cd (Vinichuk et al., 2019), selectively enriching Cd2+ through membrane transport proteins (e.g., ZIP family) and transferring it to G. elata tubers via the hyphal network. In contrast, Hg and Cu may be excluded or sequestered within vacuoles by the hyphae, thus limiting their transfer to the host plant (Long et al., 2025).

### 3.3.3 Health risk assessment of heavy metals in humans

Using a health risk assessment model, the HQ and HI values for heavy metal intake through G. elata tubers consumed by residents near the sampling points were calculated. The results indicated that the HQ and HI values for Cd, As, Pb, and Cu were all below 1 (Table 6). According to the National Center for Food Safety Risk Assessment's publication, "Opinions on Managing 9 Substances as Both Food and Traditional Medicinal Materials," the recommended daily intake of G. elata is  $\leq 3$  g (not suitable for pregnant women, breastfeeding women, or infants) (Zhao et al., 2018). This indicates that the risk of heavy metal contamination in G. elata is within acceptable limits. Although current health risks from heavy metals in G. elata are low, further research is needed on the long-term accumulation of heavy metals in the human body and their potential health effects.

#### 3.4 Soil and plant correlation analysis

Certain elements may exhibit synergistic or antagonistic effects, with variations in soil heavy metal content significantly influencing their accumulation in plants (Gong et al., 2024). Correlation analysis revealed an extremely significant positive correlation of Pb (P < 0.001,  $R^2 = 0.86$ ) and a significant correlation of Cd (P < 0.05,  $R^2 = 0.56$ ) between soil and G. elata (Figure 8). Cd2+ and Pb2+ compete for ion channels in plant roots (such as Ca2+ channels) due to their similar charge; however, in acidic soils, the bioavailable forms of Cd are more readily absorbed by plants, resulting in a significantly higher Cd accumulation coefficient compared to Pb (Figure 7) (Kanwal et al., 2024). Conversely, As and Cu levels in both G. elata and the soil did not exhibit significant correlation (Figure 8). In the soil, As primarily exists in the forms such as As5+ or As3+, with its absorption by plants highly dependent on its redox state and adsorption by rhizosphere iron oxides (Chen et al., 2022). Cu tends to forms stable complexes with SOM, particularly in soils rich in high organic matter (Bradl, 2004).

#### 4 Conclusion

In Yunnan Province, the degree of soil heavy metal pollution in the main *G. elata* planting areas follows the order: Hg > Cd > Cu > As > Pb, with a  $P_N$  values ranging from 1 to 7, indicating slight to heavy pollution and a moderate potential ecological risk. The EF for the five heavy metals are ranked as Hg > Cd > As > Pb > Cu, with EF indices between 0 and 4, suggesting no enrichment to moderate enrichment. Regionally, the P<sub>N</sub> and P<sub>i</sub> values for soil Hg and Cd are highest in the ZT area, indicating a more serious pollution risk compared to the KM, LJ and TC areas. The exceedance rate of Cd in G. elata is 37.5%, while other heavy metals remain within standard limits and do not pose a health risk. The accumulation ability of G. elata for Cd shows a significant positive correlation with soil Cd, but there is no accumulation of Hg. When selecting areas for G. elata cultivation, special attention should be given to soil levels of Hg, Cd, and Cu, and monitoring and control of Cd pollution in the ZT area should be strengthened.

### Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

#### **Author contributions**

XQ: Writing – review and editing, Methodology, Writing – original draft, Visualization, Data curation. YL: Methodology, Data curation, Visualization, Writing – review and editing, Writing – original draft. HY: Methodology, Writing – review and editing, Data curation. JW: Methodology, Writing – review and editing, Data curation. HZ: Data curation, Methodology, Writing – review and editing. HS: Methodology, Writing – review and editing, Data curation. QL: Writing – review and editing. ZS: Writing – review and editing. BH: Writing – review and editing, Supervision, Writing – original draft. WF: Supervision, Writing – original draft, Writing – review and editing.

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# Supplementary material

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