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Analytical Methods

Study of heavy metal concentrations in wild edible mushrooms in Yunnan Province, China

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ABSTRACT

Contamination with heavy metals in several species of edible mushrooms from the Yunnan Province in China was determined. Samples were collected from 16 locations in the Yunnan Province, and the contamination levels of Mn, Fe, Cu, Zn, As, Cd, and Pb were analyzed. The results demonstrated that the concentrations of essential elements (Mn, Fe, Cu, and Zn) in the mushrooms were at typical levels. The concentrations of potentially toxic metals (As, Pb and Cd) were higher than the national standard values of China (1.0 mg/kg for As, 0.2 mg/kg for Cd, and 2.0 mg/kg for Pb) in most cases. Bio-concentration factors suggested that it was easier for As and Cd to be accumulated in mushrooms than Pb, and a Health Risk Index assessment also suggested that As and Cd are greater risks to health than Pb. In conclusion, heavy metal pollution in wild edible mushrooms is a serious problem in the Yunnan Province. Among the toxic metals, As and Cd in the edible mushrooms in the area are the main sources of risk, as they may cause severe health problems. The local government needs to take measures in the form of concrete policies to protect the wild edible mushroom resources in the Yunnan Province.

1. Introduction

Mushrooms are ubiquitous in nature and play an important role in forest ecosystems. They are directly involved in recycling energy and nutrients, and they also affect plant communities through mycorrhizal symbiosis (Newbound, Mccarthy, & Lebel, 2010; Petkovšek & Pokorny, 2013). Some species of mushrooms are also popular foods throughout the world. Fruiting bodies of mushrooms are appreciated not only for their texture and flavor (Latiff, Daran, & Mohamed, 1996) but also for their nutritional properties. In general, the fruiting bodies contain approximately 39.9% carbohydrate, 17.5% protein, and 2.9% fat on a dry weight basis, with the remainder constituting minerals (Latiff et al., 1996). Mushrooms have also been reported to be therapeutic foods that are useful in preventing diseases such as hypertension (Talpur et al., 2002), hypercholesterolemia (Jeong et al., 2010) and several types of cancer (Lavi, Friesem, Geresh, Hadar, & Schwart, 2006; Sullivan, Smith, & Rowan, 1998).

Human activity including industry and the exploitation of raw materials induces the mobilization of large amounts of gas and silt contaminants. Entering the atmosphere, soil or water, these contaminants disturb the homeostasis of the ecosystem (Krupa & Kozdroj, 2004). Among all of the pollutants, heavy metals are one of the most important and hazardous types. Living organisms require trace amounts of some heavy metals, including iron (Fe), cobalt (Co), copper (Cu), manganese (Mn), chromium (Cr) and zinc (Zn). However, some other metal elements are considered to be harmful, such as arsenic (As), cadmium (Cd) and lead (Pb). It is well documented that the fruiting bodies of mushrooms have the ability to bioaccumulate metal ions, and the accumulation of heavy metals in macrofungi has been proven to be affected by environmental and fungal factors (Garcia, Alonso, Fernández, & Melgar, 1998). Heavy metal concentrations in mushrooms are considerably higher than those in agricultural crop plants, vegetables, and fruit (Zhu et al., 2011). Given their relative position in the food chain, the occurrence of high metal concentrations in mushrooms is considered important because of a potential toxicological hazard (Garcia et al., 1998; Zhu et al., 2011). Therefore, many studies have focused considerable attention on the accumulation of heavy metals in several mushroom species (Busuioc, Elekes, Stihi, Iordache, & Ciulei, 2011; Chen, Zhou, & Qiu, 2009; Cocchi, Vescovi, Petrini, & Petrini, 2006; Demirbaş, 2000; Zhang et al., 2010).

The Yunnan Province is located in the southwest region of China. The total area of the province is 0.394 million km^2, 80% of which consists of mountains (Yang, Yang, He, Liu, & Xu, 2013). There is an abundance of vegetation types, such as tropical

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rainforests, tropical monsoon forests, evergreen broad leaved forests, needle leaved forests, etc. (Brown & Davis, 2006; Parmesan & Yehe, 2003). In the Yunnan Province, the climate is mild and rainy in summer and autumn (Zhu et al., 2011). The geographic conditions in the Yunnan Province make it one of the main production areas of wild edible mushrooms in China. There are more than 850 species of wild edible mushrooms in the Yunnan Province, accounting for 91% of the edible mushroom species in China. In the Yunnan Province, the yield of wild edible mushrooms developed each year is approximately 0.1 metric tonnes (fresh weight), and the production value is approximately RMB 2,000,000,000 (Zhang, 2010). According to statistical data from the *Ministry of Commerce of the People's Republic of China*, in the year 2012, 8963 metric tonnes of wild edible mushrooms from the Yunnan Province were exported to other countries, 50% of which were exported to European countries. The total export amount reached USD 0.105 billion. (Zhang, 2010). It can be seen that the export of wild edible mushrooms has become an important part of the edible mushroom industry in the Yunnan Province. However, with the rapid urbanization and industrialization of this area (Luo, 2013), the wild edible mushrooms in the Yunnan Province are endangered with various pollutants, especially heavy metals. Accordingly, the quality of the wild edible mushrooms from the Yunnan Province is a very important issue of concern.

In this study, the heavy metal concentrations (Mn, Fe, Cu, Zn, As, Cd, Pb) in several wild edible mushroom species (*Tricholoma matsutake, Boletus edulis Bull, Morchella angusticeps, Morchella conica, Morchella elata, Russula vinosa Lindbl, Tuber indicum Cooke et Massee and Russula alutacea*) and the underlying soil samples collected from the Yunnan Province were investigated in order to provide information for a quality assessment as well as the protection of wild edible mushroom resources in the Yunnan Province.

2. Methods and materials

2.1. Soil and edible mushrooms sampling

The fruiting bodies of edible mushroom samples and the underlying soil samples were collected from 16 locations in Yunnan Province (eight species in total). The sampling locations, mushroom species and habitats are shown in Table 1. For the identification of specimens, the color, odor and other apparent properties of the mushrooms were noted. The mushrooms were identified using reference books (Mao, 2000, chap. 1–5). The study area included forests distant from sources of industrial pollution. During the sampling process, the edible mushroom samples were collected at the same growth stage, and old or damaged mushrooms were not included in the samples. The underlying soil samples were collected to include a depth of 0–20 cm.

After collection, the mushroom and soil samples were prepared for chemical analysis. The soil samples were first air-dried at room temperature until their weights were constant; the stones and plant materials were then removed, and each sample was sieved using 1-mm nylon mesh for further analysis. As for the mushroom samples, the fruiting bodies were carefully cleaned to remove any surface contamination and subsequently dried at 105 °C until a constant weight was obtained. All of the samples were then ready for further analysis.

2.2. Analytical procedure

To determine the concentrations of seven metals (Mn, Fe, Cu, Zn, As, Cd, Pb) in the edible mushroom and soil samples (on a dry weight basis), all samples were digested with concentrated acids using a Graphite Digestion System. Approximately 0.2 g of edible mushroom or soil sample was introduced into a polytetrafluoroethylene digestion tank; concentrated nitric acid (65%), perchloric acid (70%) and hydrofluoric acid (40%) (8:2:8 v/v) were also added to the tank. The tank was then heated with a digestion system for 2 h at 200 °C. The residue was then dissolved using diluted nitric acid (volume fraction of 0.5%) for subsequent determination. This process was conducted in triplicate. After the digestion process, the metal concentrations were determined following an international standard *ISO/TS 16965: 2013* by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The working conditions of ICP-MS were as follows:

Instrument: ICP-MS (Agilent 7500C)
RF power: 1.50 kW
Plasma gas flow rate: 15.00 L/min
Auxiliary gas flow rate: 1.00 L/min
Carrier gas flow rate: 1.00 L/min
Make up gas flow rate: 1.00 L/min
Sampling depth: 8.5 mm
Integration time: 0.3 s/point for As and Cd, 0.1 s/point for the other elements

During the analysis process, triplicate samples, blanks and a certified reference material (GSB04-1767-2004, provided by the National Centre of Analysis and Testing for Nonferrous Metals

Table 1
Sample locations, edible mushroom species and habitats.

No.	Sample locations	Mushroom species	Habitats
M1	Lijiang County	*Tricholoma matsutake*	Pineland and mixed forest land
M2	Nanhua County, Chuxiong City		
M3	Zhongshan County, Chuxiong City		
M4	Deqin County		
M5	Deqin County	*Boletus edulis Bull*	Needle leaved forest land and mixed forest land
M6	Lameirong Natural Village, Ludian County	*Morchella angusticeps*	Spruce forest land and fir forest land
M7	Lameirong Natural Village, Ludian County		
M8	Lameirong Natural Village, Ludian County		
M9	Lameirong Natural Village, Ludian County		
M10	Lameirong Natural Village, Ludian County	*Morchella conica*	Forest edge space, weeds of broad leaved forest and mixed forest land
M11	Lameirong Natural Village, Ludian County	*Morchella elata*	Sandy soil of the needle leaved forest and mixed forest
M12	Zixishan Park, Chuxiong City	*Russula vinosa Lindbl*	Broad leaf forest land
M13	Chenggong County	*Tuber indicum Cooke et Massee*	Rhizosphere soils of *Pinus yunnanensis*, *Piuns armandii* and *Quercus acutissima*
M14	Pu'er City	*Russula alutacea*	Broad leaved forest land and evergreen forest land
M15	Pu'er City		
M16	Nanmei Township, Lincang Prefecture		

and Electronic Materials of China) were included for quality assurance.

2.3. Health Risk Index (HRI)

The Health Risk Index (HRI) was calculated as the ratio of estimated heavy metal exposure through test edible mushrooms to the oral reference dose (Cui et al., 2004). In the present study, the HRIs for As, Cd and Pb were calculated. According to the data in *Integrated Risk Information System* (IRIS) and the research article of Fang et al. (2014), oral reference doses for As, Cd and Pb are 0.0003, 0.001 and 0.004 mg kg^{-1} day^{-1}, respectively, whereas the estimated exposure was obtained by dividing the daily intake of heavy metals by their safe limits. Daily intake was calculated using the following equation: daily intake of metal (DIM) = $C_M \times D_{FI}/B_{AW}$. In this equation, C_M, D_{FI} and B_{AW} represent the heavy metal concentrations in mushrooms (mg/kg), daily intake of mushrooms and average body weight, respectively.

2.4. Statistical analysis

Statistical analysis was conducted with SPSS Statistics 20.0 software, including the analysis of variance (ANOVA) and correlation analysis (metal-to-metal correlation coefficient matrix for mushroom and soil samples).

3. Results and discussion

3.1. Concentrations of metals in mushroom samples

The concentrations of seven metals (Mn, Fe, Cu, Zn, As, Cd, Pb) in all of the edible mushroom samples are shown in Table 2. The metal-to-metal correlation matrix for edible mushroom samples is shown in Table 3. The highest determined Mn level was 110.50 mg/kg in Sample M13 (*T. indicum Cooke et Massee* from Chenggong County), whereas the lowest determined Mn level was 1.54 mg/kg in Sample M2 (*T. matsutake* from Nanhua County, Chuxiong City). The reported Mn concentrations in previous studies for wild-growing mushrooms were between 13.5 and 113 mg/kg dw (Zhu et al., 2011). The levels of Mn concentrations in this study were in agreement with those from previous studies.

In Table 2, it can be seen that the range of Fe concentrations was 2.00–826.50 mg/kg. The highest Fe level determined was obtained in Sample M13, while the lowest one was obtained in Sample M5

Table 3
Metal-to-metal correlation matrix of the edible mushroom samples.

	Mn	Fe	Cu	Zn	As	Cd	Pb
Mn	1						
Fe	0.793**	1					
Cu	0.743**	0.466	1				
Zn	−0.020	0.001	0.173	1			
As	0.673**	0.411	0.853**	−0.127	1		
Cd	−0.320	−0.370	0.225	0.312	−0.050	1	
Pb	−0.230	−0.162	0.213	0.617*	−0.151	0.839**	1

* $P < 0.05$.
** $P < 0.01$.

(*B. edulis Bull* from Deqin County). Several researchers have reported Fe concentrations in mushroom samples in several places, such as 11.7–135.8 mg/kg dw (island of Lesvos, Greece) (Aloupi, Koutrotsios, Koulousaris, & Kalogeropoulos, 2012), 17–150 mg/kg dw (the area located nearby to the town of Kościerzyna in the Pomorskie Voivodeship, Poland) (Zhang et al., 2010), 61.04–212.82 mg/kg dw (the forest which is 20 km from Targoviste City, Romania) (Busuioc et al., 2011) and 100–2277 mg/kg dw (Malaysia, samples were purchased from local market or collected from Pasoh Forest Reserve, Negeri Sembilan) (Latiff et al., 1996). Fe concentrations observed in this study were in agreement with those reported previously in the literature.

The highest Cu concentration observed was 35.68 in Sample M13, while the lowest observed Cu concentration was 1.53 mg/kg in Sample M2. In previous studies, Cu concentrations in edible mushrooms were found to be between 100 and 300 mg/kg, which was not considered a health risk (Kalač & Svoboda, 2000). In the present study, the Cu concentrations detected in mushrooms were lower than previously reported in the literature. The Zn concentrations in the edible mushroom samples ranged from 8.71 to 59.53 mg/kg. The highest Zn level detected was in Sample M12 (*R. vinosa Lindbl* from Zixishan Park, Chuxiong City), whereas the lowest Zn level was measured in Sample M4 (*T. matsutake* from Deqin County). Previous studies determined that the concentrations of Zn in edible mushrooms ranged from 48.4 to 94.3 mg/kg (Zhu et al., 2011) and 35.8–410.0 mg/kg (Mykhailo, 2013). The range of Zn concentration obtained in the present study was lower than either of these studies.

The concentrations of As in the mushroom samples ranged from 0.76 to 11.86 mg/kg, and the highest concentration was detected in Sample M13. The highest Cd level detected was 2.88 mg/kg in

Table 2
Concentrations of metals in edible mushroom samples (mg/kg dw).[a]

No.	Mn	Fe	Cu	Zn	As	Cd	Pb
M1	29.39 ± 1.05	422.73 ± 19.98	12.61 ± 0.67	46.92 ± 2.33	7.12 ± 0.34	2.88 ± 0.12	8.63 ± 0.45
M2	1.54 ± 0.08	48.84 ± 1.79	1.53 ± 0.05	13.42 ± 0.65	1.89 ± 0.08	0.93 ± 0.04	2.91 ± 0.11
M3	4.67 ± 0.13	153.15 ± 6.75	3.37 ± 0.19	13.45 ± 0.58	2.15 ± 0.11	1.72 ± 0.08	3.59 ± 0.18
M4	8.82 ± 0.41	46.30 ± 2.03	2.47 ± 0.09	8.71 ± 0.29	2.38 ± 0.14	1.03 ± 0.05	2.69 ± 0.12
M5	7.15 ± 0.16	2.00 ± 0.09	11.97 ± 0.45	20.64 ± 1.32	5.38 ± 0.28	2.77 ± 0.09	5.63 ± 0.25
M6	26.69 ± 1.28	371.80 ± 15.78	11.17 ± 0.47	38.56 ± 1.87	1.10 ± 0.04	2.11 ± 0.12	4.47 ± 0.22
M7	25.39 ± 1.23	708.80 ± 29.35	4.86 ± 0.21	25.20 ± 1.08	1.39 ± 0.05	0.59 ± 0.02	4.21 ± 0.18
M8	50.28 ± 2.05	534.80 ± 25.32	3.97 ± 0.15	26.95 ± 1.21	1.13 ± 0.02	0.44 ± 0.02	4.25 ± 0.21
M9	36.61 ± 1.08	188.00 ± 8.93	4.13 ± 0.19	39.20 ± 1.99	1.09 ± 0.04	0.72 ± 0.04	2.38 ± 0.12
M10	13.26 ± 0.57	261.50 ± 12.34	6.19 ± 0.19	36.22 ± 0.98	1.10 ± 0.05	0.81 ± 0.03	2.88 ± 0.13
M11	17.44 ± 0.88	235.30 ± 10.11	4.24 ± 0.11	46.63 ± 2.10	0.76 ± 0.02	0.49 ± 0.03	2.86 ± 0.09
M12	18.43 ± 0.89	101.37 ± 4.58	20.51 ± 1.03	59.53 ± 2.86	3.34 ± 0.11	2.51 ± 0.18	10.18 ± 0.52
M13	110.50 ± 4.52	826.50 ± 29.06	35.68 ± 1.24	17.59 ± 0.56	11.86 ± 0.45	0.58 ± 0.03	1.60 ± 0.07
M14	/	/	/	/	2.68 ± 0.13	0.17 ± 0.01	0.48 ± 0.01
M15	/	/	/	/	8.52 ± 0.34	0.27 ± 0.01	0.66 ± 0.02
M16	/	/	/	/	11.69 ± 0.52	0.25 ± 0.02	1.37 ± 0.05

"/" Signifies that the metal was not analyzed.
[a] Values are the means and standard derivations.

Sample M1 (*T. matsutake* from Lijiang County), whereas the lowest Cd level was 0.17 mg/kg in Sample M14 (*R. alutacea* from Pu'er City). Reported Cd levels in edible mushrooms in a previous study were 0.06–0.58 mg/kg (Zhu et al., 2011). In the present study, the concentrations of Cd in the mushroom samples were higher (0.17–2.88 mg/kg). On the other hand, the highest obtained concentration of Pb in the edible mushroom samples was 10.18 mg/kg in Sample M12, while the lowest Pb level was 0.48 mg/kg in Sample M14. Concentrations of Pb in edible mushrooms in the previous study ranged from 0.1 to 40 mg/kg (Sesli & Tüzen, 1999). In the present study, the Pb levels were lower.

Among the seven metals, As, Cd and Pb are potentially hazardous. These elements in edible mushrooms may enter the food chain and potentially harm human health. Therefore, it is necessary to evaluate the health risks of these elements. According to *Maximum Levels of Contaminants in Foods* (GB2762-2005) and *Hygienic Standard for Edible Fungi* (GB7096-2003) proposed by the Ministry of Health of the People's Republic of China, the safe limits of various metals in edible mushrooms are 1.0 mg/kg for As, 0.2 mg/kg for Cd, and 2.0 mg/kg for Pb. According to our results, only As in M11 (*M. elata* from Lameirong Natural Village, Ludian County) was below acceptable safe levels. The level of Cd contamination in mushrooms was similar, and only the concentration in Sample M14 was lower than the safe limit. The Pb concentrations in Samples M14 and M15 (*R. alutacea* from Pu'er City) were lower than the safe limit, while those in the other samples exceeded the safe limit. It can be determined that the wild edible mushrooms in the Yunnan Province collected for this study were contaminated with heavy metals (As, Cd and Pb), which may pose serious health risks and, thus, deserve increased attention.

3.2. Concentrations of metals in underlying soil samples and bioconcentration factors

The concentrations of seven heavy metals in underlying soil samples from the 16 locations were shown in Table 4. The metal-to-metal correlation matrix for the underlying soil samples is shown in Table 5. In this work, the bioconcentration factor (BCF, or the quotient between the metal concentration in the fruiting body and the metal concentration in the substrate dry matter) (Zhang, Gao, Ma, Luo, & Su, 2008) was applied to evaluate the accumulation of heavy metals, which can demonstrate if the mushroom was able to bioconcentrate (BCF > 1) or exclude (BCF < 1) specific metal ions (García, Alonso, & Melgar, 2013). The BCFs from the study are also shown in Table 4.

According to the *Environmental Quality Standard for Soils* (GB15618-2008) proposed by the Ministry of Environmental Protection of the People's Republic of China, safe limits for heavy metals in soils are 35 mg/kg for As, 0.6 mg/kg for Cd, and 50 mg/kg for Pb. It can be seen from Table 4 that As and Pb concentrations in all of the soil samples were below the safe limits. However, there were eight samples in which the Cd concentrations exceeded the safe limit (Samples M6–M11, and Samples M15–M16), indicating that the soil in the study area where the edible mushrooms grew had been significantly contaminated by Cd. The cause of the contamination might be the industries in the southern region of China (Fang et al., 2014).

The bioconcentration levels of metals in mushrooms depended on the properties of the metals, as well as the mushroom species (Giannaccini et al., 2012). According to the BCFs shown in Table 4, among the three hazardous metals (As, Cd and Pb), As and Cd are more readily accumulated in edible mushrooms, suggesting these might pose an increased health risk among those consuming wild mushrooms. Pb is not easily bioconcentrated by any specie of edible mushrooms in the study, so it is likely not as dangerous as the other two metals. These results were in

Table 4
Concentrations of metals in underlying soil samples and BCFs.

No.	Metal concentrations (mg/kg)[a]							BCFs						
	Mn	Fe	Cu	Zn	As	Cd	Pb	Mn	Fe	Cu	Zn	As	Cd	Pb
M1	710.56 ± 29.09	3065.00 ± 32.50	51.85 ± 2.34	84.55 ± 4.21	5.44 ± 0.24	0.05 ± 0.01	18.30 ± 0.87	0.041	0.138	0.243	0.555	1.309	57.600	0.472
M2	383.95 ± 18.15	573.75 ± 24.31	31.34 ± 1.25	101.04 ± 4.55	5.81 ± 0.28	0.21 ± 0.01	24.25 ± 1.21	0.004	0.085	0.049	0.133	0.325	4.429	0.120
M3	98.00 ± 4.37	290.00 ± 14.98	31.00 ± 1.76	70.81 ± 3.45	5.61 ± 0.18	0.03 ± 0.00	20.34 ± 1.03	0.048	0.528	0.109	0.190	0.383	57.333	0.176
M4	616.55 ± 29.88	665.83 ± 31.24	26.49 ± 1.23	95.68 ± 4.59	8.26 ± 0.37	0.11 ± 0.02	24.03 ± 1.19	0.014	0.070	0.093	0.091	0.288	9.364	0.112
M5	434.71 ± 19.97	463.75 ± 21.47	103.78 ± 3.57	90.04 ± 3.94	5.31 ± 0.23	0.18 ± 0.08	24.95 ± 0.98	0.016	0.004	0.115	0.229	1.013	15.389	0.226
M6	2014.00 ± 99.62	486.23 ± 19.76	21.54 ± 1.59	76.35 ± 3.98	3.21 ± 0.11	5.23 ± 0.21	5.21 ± 0.17	0.013	0.765	0.519	0.505	0.343	0.403	0.858
M7	1822.00 ± 89.07	1034.34 ± 47.75	8.32 ± 0.35	52.11 ± 2.54	3.75 ± 0.09	3.21 ± 0.16	5.01 ± 0.25	0.014	0.685	0.584	0.484	0.371	0.184	0.840
M8	2744.00 ± 93.42	892.38 ± 29.46	9.32 ± 0.54	70.21 ± 4.02	3.98 ± 0.23	2.11 ± 0.10	4.90 ± 0.21	0.018	0.599	0.426	0.384	0.284	0.209	0.867
M9	2628.00 ± 89.86	403.21 ± 19.76	7.49 ± 0.35	80.43 ± 4.03	3.79 ± 0.11	3.54 ± 0.14	3.21 ± 0.11	0.014	0.466	0.551	0.487	0.288	0.203	0.741
M10	1904.00 ± 98.76	429.39 ± 20.18	12.82 ± 0.56	78.22 ± 3.45	4.32 ± 0.24	4.21 ± 0.21	3.40 ± 0.14	0.007	0.609	0.483	0.463	0.255	0.192	0.847
M11	1877.00 ± 87.65	398.21 ± 13.92	6.98 ± 0.29	93.24 ± 4.68	2.10 ± 0.08	1.23 ± 0.05	4.80 ± 0.23	0.009	0.591	0.607	0.500	0.362	0.398	0.596
M12	153.36 ± 7.64	2117.50 ± 87.65	10.45 ± 0.49	80.70 ± 4.91	4.64 ± 0.22	0.00 ± 0.00	18.05 ± 0.79	0.120	0.048	1.963	0.738	0.720	/	0.564
M13	1091.00 ± 43.57	4620.00 ± 11.72	32.88 ± 1.56	77.40 ± 3.84	7.48 ± 0.25	0.18 ± 0.02	20.73 ± 0.99	0.101	0.179	1.085	0.227	1.586	3.222	0.077
M14	/	/	/	/	2.34 ± 0.14	0.18 ± 0.04	8.15 ± 0.28	/	/	/	/	1.145	0.944	0.059
M15	/	/	/	/	5.25 ± 0.21	1.55 ± 0.08	18.65 ± 0.68	/	/	/	/	1.623	0.174	0.035
M16	/	/	/	/	8.61 ± 0.39	0.96 ± 0.05	12.66 ± 0.56	/	/	/	/	1.358	0.260	0.108

"/" Means signifies that the metal was not analyzed or the BCF was unable to be calculated;
[a] Values are the means and standard derivations.

Table 5
Metal-to-metal correlation matrix for the underlying soil samples.

	Mn	Fe	Cu	Zn	As	Cd	Pb
Mn	1						
Fe	−0.219	1					
Cu	−0.524*	0.104	1				
Zn	−0.379	−0.097	0.334	1			
As	−0.604*	0.435	0.374	0.292	1		
Cd	0.784**	−0.385	−0.445	−0.446	−0.440*	1	
Pb	−0.900**	0.306	0.654**	0.499*	0.669**	−0.767**	1

* $P < 0.05$.
** $P < 0.01$.

agreement with those of previous studies (Demirbaş, 2000; Gucia et al., 2012).

According to Table 4, the BCFs were different between various mushroom species. It can be seen that As was bioconcentrated in Samples M1, M5 and M13–M16, while more Cd was present in Samples M1–M5 and M13. However, the BCF_{As} and BCF_{Cd} of Sample M6–M11 were less than 1. It can be seen that the mushrooms *T. matsutake, B. edulis Bull* and *T. indicum Cooke et Massee* were bioconcentrated both As and Cd in the fruiting bodies, while *R. alutacea* was bioconcentrated only As. This might result from differing biological features. According to Mao (1988), Yamaç, Yıldız, Sarıkürkcü, Çelikkollu, and Solak (2007) and Yang et al. (2013), *B. edulis Bull*, *T. indicum Cooke et Massee* and *T. matsutake* all belong to mycorrhizal fungi. According to several studies (Schützendübel & Polle, 2002; Tam, 1995; Turnau, Kottke, & Dexheimer, 1996; Turnau, Przybyłowicz, & Mesjasz-Przybyłowicz, 2001), the root adsorbing area of mycorrhizal fungi is relatively larger compared with other species, resulting in greater ability to adsorb and fix elements from soil. Although *R. vinosa Lindbl* and *R. alutacea* (M12, M14–16) are also mycorrhizal fungi, it was proven in a previous study that their capacities to adsorb Cd from soil were much less (Kalač & Svoboda, 2000), and our result were in agreement with this. According to Mao (1988), the *Morchella* (M6–M11) belongs to *Geophilous* (nonmycorrhizal fungi), which is much less able to adsorb metals from underlying soil than mycorrhizal fungi. Therefore, in the present study, the BCF_{As} and BCF_{Cd} of Sample M6–M11 were less than 1. In conclusion, the metal bioconcentration levels in edible mushrooms in the study area were different, and depended on the properties of the metals as well as the biological features of the mushrooms.

3.3. Health risk assessment

3.3.1. Provisional tolerable weekly intakes (PTWI)

Among the metals measured in the study, As, Cd and Pb present a potential health hazardous for humans. The Joint FAO/WHO Expert Committee on Food Additives (JEFCA) recommended that provisional tolerable intakes of As, Cd and Pb be 15, 7 and 25 μg per kg body weight weekly, respectively (Fang et al., 2014; Yang et al., 2005). A body weight of 60 kg and an intake quantity of fresh mushrooms per day of 300 g (containing 30 g of dry matter) were assumed (Kalač & Svoboda, 2000; Svoboda, Zimmermannova, & Kalač, 2000). Based on these data, actual weekly intake of the three metals can be calculated and the results are shown in Table 6.

The data in Table 6 suggest intake of As through consumption of wild edible mushrooms in the five sample locations are higher than recommended levels, including Samples M1 (*T. matsutake* from Lijiang County), M5 (*B. edulis Bull* from Deqin County), M13 (*T. indicum Cooke et Massee* from Chenggong County), M15 (*R. alutacea* from Pu'er City) and M16 (*R. alutacea* from Nanmei Township, Lincang Prefecture). Similarly, intakes of Cd from four sample locations are higher than recommended levels including Samples M1, M5, M6 (*M. angusticeps* from Lameirong Natural Village, Ludian County) and M12 (*R. vinosa Lindbl* from Zixishan Park, Chuxiong City). There were only two mushroom samples consumption would mean weekly intakes of Pb exceeded the safe limit, specifically Samples M1 and M12. This illustrates that the intakes of Pb through consumption of wild mushrooms in the area might pose a relatively low health risk compared with As and Cd intakes. In conclusion, if an adult weighing 60 kg ate 300 g of fresh mushrooms each day, from the study areas, intakes of As, Cd and Pb would be in the safe range, but in some cases values might exceed national guidelines. However, intakes of As and Cd may be more of a serious health risk than Pb.

3.3.2. Health Risk Index (HRI)

The HRI calculation was also based on the above assumption (a body weight of 60 kg and an intake quantity of dried mushrooms per day of 30 g). An index of more than 1 is considered unsafe for human health. The HRIs were calculated, and the results are shown in Table 6. There were only five mushroom samples the HRI_{As} of which were less than 1, including Samples M6, M8, M9 (*M. angusticeps* from Lameirong Natural Village, Ludian County), M10 (*M. conica* from Lameirong Natural Village, Ludian County) and M11 (*M. elata* from Lameirong Natural Village, Ludian County), suggesting As in the mushroom samples collected might pose a health risk to humans. The HRI_{Cd} of four edible mushroom samples were greater than 1, including Samples M1 (*T. matsutake* from Lijiang County), M5 (*B. edulis Bull* from Deqin County), M6 and M12 (*R. vinosa Lindbl* from Zixishan Park, Chuxiong City). Thus, Cd in mushrooms in the study area poses a potential health risk. Conversely, the HRI_{Pb} of all of the edible mushroom samples were less than 1, indicating that Pb in mushrooms in the study area does not pose a risk to human health. In conclusion, As in wild edible mushrooms from the study area had the highest health risk among the three toxic metals examined. While Cd also posed a potential human health risk, Pb did not.

Table 6
Actual weekly intake and Health Risk Indexes of As, Cd and Pb in edible mushroom samples.

No.	Weekly intake (μg/kg body weight)			Health Risk Indexes			No.	Weekly intake (μg/kg body weight)			Health Risk Indexes		
	As	Cd	Pb	As	Cd	Pb		As	Cd	Pb	As	Cd	Pb
M1	24.92	10.08	30.21	5.54	1.44	0.30	M9	3.82	2.52	8.33	0.85	0.36	0.08
M2	6.62	3.26	10.19	1.47	0.47	0.10	M10	3.85	2.84	10.08	0.86	0.41	0.10
M3	7.53	6.02	12.57	1.67	0.86	0.13	M11	2.66	1.72	10.01	0.59	0.25	0.10
M4	8.33	3.61	9.42	1.85	0.52	0.09	M12	11.69	8.79	35.63	2.60	1.26	0.36
M5	18.83	9.70	19.71	4.18	1.39	0.20	M13	41.51	2.03	5.60	9.22	0.29	0.06
M6	3.85	7.39	15.65	0.86	1.06	0.16	M14	9.38	0.60	1.68	2.08	0.09	0.02
M7	4.87	2.07	14.74	1.08	0.30	0.15	M15	29.82	0.95	2.31	6.63	0.14	0.02
M8	3.96	1.54	14.88	0.88	0.22	0.15	M16	40.92	0.88	4.80	9.09	0.13	0.05

3.4. Countermeasures of heavy metal pollution in wild edible mushrooms

Given the pollution conditions and health risks of heavy metals in wild edible mushrooms in the Yunnan Province, measures need to be taken to improve the quality of the edible mushrooms. The heavy metal pollution in wild edible mushrooms might have something to do with industrialization in the Yunnan Province. According to Luo (2013) and *Yunnan Statistical Yearbook 2014*, the industrial production value accounted for a large percentage of the total production value in the Yunnan Province (for example, the percentage was 32% in 2013), indicating rapid industrialization. The main industries in the Yunnan Province are the energy and raw material industries, including coal, electricity, petrochemical, natural gas, nonferrous metals, salt chemical and phosphate fertilizer industries. Manufacturing may lead to heavy metal soil pollution in this area. Although the sampling locations were distant from sources of industrial pollution, it can still be presumed that toxic metals could transfer within the entire environmental system, which may have resulted in the heavy metal pollution observed in wild edible mushroom samples used in the study. To improve the conditions and protect the wild edible mushroom resources, the governments need to take action in the form of concrete policies, such as providing financial and technical support for the remediation of contaminated soil, closing the most polluting enterprise, carrying out clearer production technology, etc.

In addition, and in agreement with Huang, Li, Guo, Wang, and Gui (2014), during the wild edible mushroom production process, environmental monitoring and supervision should be strengthened in the producing area to make sure that the surrounding soil, water and air are environmentally safe. Exogenous contamination in the manufacturing and transportation of edible mushroom products should also be avoided.

4. Conclusions

Heavy metal pollution in wild edible mushrooms from the Yunnan Province in China has become a serious problem. The essential element concentrations (Mn, Fe, Cu, and Zn) in the mushrooms were determined to be at typical levels. However, the potentially toxic metals (As, Cd, Pb) concentrations in nearly all of the mushroom samples exceeded safe limits. Thus, it can be determined the wild edible mushrooms in the study area have been contaminated with heavy metals that also pose a threat to human health.

Among the toxic heavy metals, As and Cd were bioconcentrated by the mushrooms, while Pb was excluded, indicating that As and Cd in wild edible mushrooms may be a higher health risk than Pb. Intakes of As and Cd by consuming wild edible mushrooms from the study area may cause serious health problems. The HRI calculation also suggested As and Cd in wild edible mushrooms from the study area may pose a greater health risk than Pb.

Overall, heavy metal pollution in wild edible mushrooms from the Yunnan Province in China may constitute a serious problem for human health. As and Cd were the main risk to human health and should demand increased attention. The local government needs to take action in the form of concrete policies to protect the wild edible mushroom resources in the Yunnan Province.

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