Bull Environ Contam Toxicol (2009) 83:280–285
DOI 10.1007/s00128-009-9767-8

Analysis of Several Heavy Metals in Wild Edible Mushrooms from Regions of China

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Received: 17 January 2009 / Accepted: 1 May 2009 / Published online: 19 May 2009

Abstract The metal (Cu, Ni, Cd, Hg, As, Pb) contents in wild edible mushrooms collected from three different sites in China were determined by atomic absorption spectrometry and atomic fluorescence spectrometry. All element concentrations were determined on a dry weight basis. A total of 11 species was studied, five being from the urban area and six from rural areas in China. The As content ranged from 0.44 to 1.48 mg/kg. The highest As content was seen in *Macrolepiota crustosa* from the urban area, and the lowest in *Russula virescens* from rural areas. A high Ni concentration (1.35 mg/kg) was found in *Calvatia craniiformis* from the urban area. The lowest Ni level was 0.11 mg/kg, for the species *R. virescens* and *Cantharellus cibarius*. The Cu content ranged from 39.0 to 181.5 mg/kg. The highest Cu content was seen in *Agaricus silvaticus* and the lowest in *C. cibarius*. The Pb content ranged from 1.9 to 10.8 mg/kg. The highest Pb value was found in *C. craniiformis*. The Cd content ranged from 0.4 to 91.8 mg/kg. The highest Cd value was found in *M. crustosa*. The Hg content ranged from 0.28 to 3.92 mg/kg. The highest Hg level was found in *Agaricus* species. The levels of the heavy metals Cd, Pb, and Hg in the studied mushroom species from urban area can be considered high. The metal-to-metal correlation analysis supported they were the same source of contamination. High automobile traffic was identified as the most likely source of the contamination. Based upon the present safety standards, consumption of those mushrooms that grow in the polluted urban area should be avoided.

Keywords Heavy metals · China · Wild edible mushrooms

The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic (Järup 2003). Many wild edible mushroom species are known to accumulate high levels of heavy metals and mainly cadmium, mercury and lead (Kalač and Svoboda 2000). Many investigations have dealt with the metal contents of mushrooms, especially edible ones (Demirbaş 2000; Gast et al. 1988; Lepsová and Mejestrík 1988) and numerous data have been published on the contents of several heavy metals in edible mushrooms, in particular arsenic, cadmium, copper, iron, lead, manganese, mercury, and zinc (Alonso et al. 2000; Blanusa et al. 2001; Lepsová and Král 1988; Svoboda and Kalač 2003; Vetter 1994). As some of these metals have significantly toxic and hazardous effects on human health at low concentrations, a great deal of effort has been made to evaluate the possible danger to human health from the ingestion of mushrooms (Gast et al. 1988).

The southern areas in China have a mild and rainy climate in summer and autumn, providing nearly ideal conditions for fungal growth, with temperatures ranging between 20 and 30°C. Wild-growing mushrooms are a favourite delicacy. In China, as in many other countries, fungi collection is very popular, particularly in summer and autumn. Thus, Some people collect fungi in such quantities that they can make a substantial contribution to food intake. Heavy metals may enter the food chain as a result of their uptake by edible mushrooms. However, there have not been any previous studies on the toxic metal contents of wild-growing edible mushrooms on the sampling sites in

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China. Therefore, it was considered necessary to be concerned about heavy metal contents of the most commonly collected fungi in China.

Two types of sample were of interest, on the one hand, fungi from densely populated area, where there is exposure to pollution, and on the other hand those from rural areas, where fungi are most commonly collected. The purpose of the present work was to evaluate the possibility of using mushrooms as bioindicators of environmental contamination, assess the contribution of mushrooms to the daily intake of several toxic elements in several species of edible wild mushrooms collected from China, and compare the results with the norms for these toxic elements in foodstuff.

Materials and Methods

The mushroom samples were collected from three forest sites in the southern areas in China during June–September of 2008. The first site chosen, in the vicinity of Cundong village, a rural commune in northeastern Meizhou (Guangdong province), is situated far away from the sources of industrial pollution. The samples, *Russula vinosa*, *Russula delica* and *Russula cyanoxantha* are selected because they are the most common mushrooms collected in the area and because members of the genus *Russula* have been identified as accumulators of metals (Byrne et al. 1976; Tyler 1980). The second sampling site was located in the Jindianhou Mountain, a rural village 8 km east of downtown Kunming city (Yunnan province). The chosen mushrooms species, *Lyophyllum decastses*, *Cantharellus cibarius* and *Russula virescens*, belong among the most widely consumed wild mushrooms in China. The third site, popular for mushroom picking by the local inhabitants, was the YueLu Mountain located about 1 km from the center of downtown Changsha (Hunan province), a city with approximately 2,200,000 inhabitants and a large flow of traffic. The dust resulting from this traffic causes a serious amount of suspended particle pollution in the air, much of which is deposited on the ground in the surrounding area. While many species of fungi were found, five species found in large abundance were the focus of this study: *Agaricus subrufescens*, *Russula albida*, *Calvatia craniiformis*, *Agaricus silvaticus* and *Macrolepiota crustosa*.

The habitat, edibility and the families of mushroom species are given in Table 1. The collected samples were washed with distilled water and placed on trays. Each sample was oven-dried at 60°C to constant weight and then powdered to pass through a 40 mesh sieve.

For the identification of specimens, the color, odour and other apparent properties of the mushrooms and vegetation were noted. The mushrooms were identified using the reference books (Mao 2000).

One gram of sample was placed in a porcelain crucible and ashed in an oven at 420–450°C for 15–24 h. Ashed material was dissolved in 2 mL concentrated HNO_3, evaporated to dryness, heated again to 450°C for 3 h, dissolved in 1 mL concentrated H_2SO_4 and 2 mL concentrated HNO_3, and diluted with distilled water up to 25 mL. A blank digest was carried out in the same way. For the element analyses, a Hitachi Z-8000 atomic absorption spectrometer was used in this study. Pb, Cd and Ni in mushroom samples were determined by a HGA graphite furnace, using argon as inert gas. Determination of Cu contents was carried out in an air/acetylene flame. As and Hg levels in the mushroom samples were determined using an AFS-230a atomic fluorescence spectrometry.

In order to validate the method for accuracy and precision, certified reference materials, namely spinach CRM 10015 (National Institute of Standards and Technology) were analyzed for corresponding elements. A control sample was digested and analyzed with each analytical batch of samples to check the effectiveness of our digestion procedure. Each time its concentration deviated more than

Table 1 Families, habitat and edibility of mushroom species

Class, family and species of mushrooms	Habitat	Edibility
Russula vinosa Lindbl.	Under beech	Edible
Russula delica Fr.	In coniferous and mixed woodland	Edible
Russula cyanoxantha (Schaeff.: Fr.)	Under broad leafed trees	Edible
Russula virescens (Schaeff. ex Zanted) Fr.	Amongst grass under broad leafed trees	Edible
Lyophyllum decastses (Fr.) Sing	Under broad leafed trees or conifers	Edible
Cantharellus cibarius Fr.	In mixed woodland	Edible
Russula albida Peck	In woodland	Edible
Agaricus subrufescens Peck	In woodland	Edible
Calvatia craniiformis (Schw.) Fr.	In woodland	Edible (only when young)
Agaricus silvaticus Schaeff.: Fr.	Under broad leafed trees or conifers	Edible
Macrolepiota crustosa Shao et Siang	Under deciduous trees	Edible

10% from the certified value the calibration curve was reconstructed.

Results and Discussion

The result of the analysis of the CRM showed good agreement with the certified levels, as shown in Table 2. The average heavy metal concentrations of wild-growing edible mushroom species are given in Table 3. All metal concentrations were determined on a dry weight basis.

Kalač and Svoboda (2000) reported that Cu levels in the accumulating species are usually 100–300 mg/kg dry matter, which is not considered a health risk. In the present study, Cu contents for the different species ranged from 39.0 to 181.5 mg/kg.

The Cu concentrations were higher in *A. silvaticus*, and *M. crustosa* from urban area. The lowest Cu content was found in *C. cibarius* from rural areas. The difference in the concentrations of Cu was significant between the urban and rural areas. Cu levels in mushrooms are higher than those reported earlier (Demirbaş 2000; Ouzouni et al. 2007). Cu contents in mushrooms higher than those in vegetables should be considered as a nutritional source of the element. Nevertheless, for people, bioavailability from mushrooms was reported to be low, due to limited absorption from the small intestine (Schellmann et al. 1980). Also, it is known that copper may be toxic to both humans and animals when its concentration exceeds the safe limits and its concentration in some human tissues such as thyroid can be change dependent on the tissue state including cancerous or non-cancerous (Yaman and Akdeniz 2004; Yang et al. 2002).

The As content for the different species ranged from 0.44 to 1.48 mg/kg. The highest As content was seen in *M. crustosa* from the urban area, and the lowest in *R. virescens* from rural areas. There are no great differences among the species both areas. As concentrations have been reported to be up to 1 mg/kg dry matter in most of the species (Slekovec and Irgolic 1996; Stijve and Bourqui 1991; Vetter 1994), which is in agreement with our results. Inorganic arsenic is acutely toxic and intake of large quantities leads to gastrointestinal symptoms, severe disturbances of the cardiovascular and central nervous systems, and eventually death (Järup 2003).

The highest Ni concentration (1.35 mg/kg) was found in *C. craniiformis* from the urban area. The lowest Ni level was 0.11 mg/kg, for the species *R. virescens* and *C. cibarius*. The variation in the concentrations of Ni within two studied areas was fairly low except for *C. craniiformis*. The range of Ni obtained in this study was lower than 0.05–5 mg/kg reported for plant foods (National Academy of Sciences 1975) and reported values for macrofungi species (Demirbaş 2001a; Isildak et al. 2004; Ouzouni et al. 2007). Although, one may be tempted to indicate that the samples are safe from Ni point of view, the tolerable upper intake level of 1 mg/day reported for this toxic element [Food and Nutrition Board 2001] is also low. Ni has been linked to lung cancer (Yen 1999).

Table 2 Values determined for the measured metals using certified reference material (spinach) as sample

Metals	Certified value (mg/kg)	Determined (mg/kg)[a]	Recovery (%)
Cu	8.9 ± 0.4	8.7 ± 0.14	97
Ni	0.92 ± 0.12	0.93 ± 0.11	101
As	0.23 ± 0.03	0.22 ± 0.05	96
Pb	11.1 ± 0.9	11.0 ± 1.13	99
Hg	0.02 ± 0.003	0.02 ± 0.004	100
Cd	0.15 ± 0.025	0.14 ± 0.021	93

[a] Average of three separate digestions

Table 3 Metal contents (mg/kg, dry weight) in the fruiting bodies of eleven mushroom species from regions of China

Mushroom samples	Cu	Ni	Pb	Cd	As	Hg
Russula vinosa	47.5 ± 3.1	0.42 ± 0.05	2.7 ± 0.7	0.9 ± 0.1	0.87 ± 0.09	0.28 ± 0.04
Russula delica	61.9 ± 4.7	0.24 ± 0.05	1.9 ± 0.3	1.1 ± 0.1	0.66 ± 0.06	1.01 ± 0.13
Russula cyanoxantha	51.1 ± 4.4	0.33 ± 0.04	2.1 ± 0.3	1.3 ± 0.2	0.97 ± 0.09	0.53 ± 0.05
Russula virescens	43.8 ± 3.4	0.11 ± 0.04	2.0 ± 0.3	0.4 ± 0.1	0.44 ± 0.08	0.99 ± 0.09
Lyophyllum decastses	69.5 ± 5.3	0.34 ± 0.08	3.0 ± 0.4	1.6 ± 0.2	1.46 ± 0.19	2.81 ± 0.14
Cantharellus cibarius	39.0 ± 3.8	0.11 ± 0.04	4.6 ± 0.4	0.9 ± 0.2	0.52 ± 0.06	0.32 ± 0.05
Russula albida	65.0 ± 3.2	0.71 ± 0.07	9.6 ± 0.8	3.0 ± 0.5	1.23 ± 0.05	3.88 ± 0.13
Agaricus subrufescens	86.7 ± 11.5	0.56 ± 0.11	4.4 ± 0.8	2.5 ± 0.9	0.92 ± 0.02	3.92 ± 0.10
Calvatia craniiformis	154.0 ± 27.6	1.35 ± 0.28	10.8 ± 1.4	9.1 ± 0.8	0.88 ± 0.09	2.18 ± 0.11
Agaricus silvaticus	181.5 ± 28.6	0.13 ± 0.05	9.3 ± 0.7	51.9 ± 4.1	1.18 ± 0.17	3.84 ± 0.23
Macrolepiota crustosa	174.4 ± 29.5	0.69 ± 0.14	7.4 ± 0.9	91.8 ± 9.0	1.48 ± 0.19	2.20 ± 0.23

Results represent means of three replicates ± SD

Pb concentration in samples ranged from 1.9 to 10.8 mg/kg. We found the higher concentrations of Pb in *Agaricus* species and *C. craniiformis* from urban area, and the lowest Pb content in *R. delica* from rural areas. The Pb concentrations of previous studies were between 0.1 and 40 mg/kg (Kalač et al. 1989; Sesli and Tüzen 1999). Lead is especially toxic to the growing brain and can affect the behavioral development of youngsters, even at low concentrations. Organic lead compounds are fat soluble and are more toxic than other forms. Such forms of lead can pass through the placenta and thus affect a growing fetus (Demirbaş 2001a).

Value of Cd concentration was ranged from 0.4 to 91.8 mg/kg for samples. The Cd contents of the fungi were much higher in the urban area than in the rural areas. The higher levels of Cd were found in *Macrolepiota crustosa* (91.8 mg/kg) and *A. silvaticus* (51.9 mg/kg) from urban area, and the lowest level of Cd was found in *R. virescens* (0.4 mg/kg) from rural areas. Very high concentrations of Cd have been found in the genus *Agaricus* (Kojo and Lodenius 1989; Lodenius et al. 1981; Schmitt and Meisch 1985; Quinche 1987; Vetter 1994), which is in agreement with our present study. Cadmium is known as a principal toxic metal, since excessive cadmium exposure may give rise to renal, pulmonary, hepatic, skeletal, reproductive effects and cancer. The major effects of this metal poisoning are experienced in the lungs, kidneys, bones and overexposure. However, skeletal and reproductive effects are also discussed as possible critical effects (Nordberg 2003). It can be taken up directly from water and via food and it has a tendency to accumulate in plants and animals (Demirbaş 2001b; Schmitt and Meisch 1985).

The Hg content for the different species ranged from 0.28 to 3.92 mg/kg. In most cases the Hg contents of the fungi were higher in the urban area than in the rural areas. The higher Hg level was found in *A. subrufescens* (3.92 mg/kg) and *A. silvaticus* (3.84 mg/kg) from urban area, and the lowest level of Hg was found in *Russula vinosa* (0.28 mg/kg) from rural areas. High Hg levels are characteristic for genera *Agaricus*, *Macrolepiota* and *Boletus* (Kalač and Svoboda 2000), which is in agreement with our present values. The mean Hg level in macrofungi surpasses, by two orders of magnitude, those in green plants (green plants: 0.015 mg/kg; macrofungi: 1–1.5 mg/kg) and varies according to the type of fungi, since litter-decomposing species (*Agaricus*, *Marasmius*) have higher mercury concentrations (0.1–72 mg/kg) than the wood-destroying species and genera (1.5–2.0 mg/kg) (Laaksovirta and Lodenius 1979). Acute mercury exposure may give rise to lung damage. Chronic poisoning is characterized by neurological and psychological symptoms, such as tremor, changes in personality, restlessness, anxiety, sleep disturbance and depression. The symptoms are reversible after cessation of exposure (Järup 2003).

It is known that high metal levels (Pb, Cd, Hg, Cu) have been observed in mushrooms growing in heavily contaminated areas, such as those in the close vicinity of highways with heavy traffic (García et al. 1998), emission areas of metal smelters (Lepsová and Mejestrík 1988), domestic heating and long-range transport (Grigalaviciene et al. 2005; Viard et al. 2004). In the present study, the samples collected in YueLu Mountain (urban area), showed the maximum Pb, Cd, Hg and Cu levels. As observed, the vehicle loads around the mountain are uncovered which will lead to pollution of the air and surrounding soil. There isn't any metal smelter in the vicinity of the mountain. The metal contamination of other factors is assumed to be negligible. Therefore, high automobile traffic becomes the most likely source of the contamination in the mushrooms. As reviewed by Wondratschek and Röder (1993), no mushroom species can be considered as a precise indicator of environmental pollution with heavy metals but fruiting bodies can be useful for distinguishing between polluted and unpolluted areas.

The whole data was subjected to statistical analysis and correlation coefficients were determined. Metal-to-metal correlation coefficients were given in Table 4. Significant correlations were found between Cu–Pb, Cu–Cd and Cu–Hg pairs, suggesting that the greatest amount of contamination in the mushroom samples is associated with Cd, Pb, Hg, Cu.

The fruiting bodies tested in the presented paper all originated from areas greatly favored by mushroom pickers. Every year during harvesting in summer and autumn, the three forest sites in China, are visited by thousands of mushroom pickers. Therefore, the knowledge of the content of toxic metal, such as Pb, Cd, and Hg, in fruiting bodies of edible fungi from these regions is of particular importance.

According to the EU Scientific Committee for Food Adult Weight parameter, 60 kg of body weight was used for intake calculations as the weight of an average consumer. In addition, for intake calculations, usually a 300 g portion of fresh mushrooms per meal is assumed, which

Table 4 Metal-to-metal correlation coefficient matrix for mushroom samples

	Cu	Ni	Pb	Cd	As	Hg
Cu	1.000					
Ni	0.497	1.000				
Pb	0.890**	0.615	1.000			
Cd	0.806**	0.156	0.566	1.000		
As	0.593	0.257	0.344	0.624	1.000	
Hg	0.658*	0.223	0.526	0.378	0.559	1.000

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Table 5 Daily metal intakes by a normal, 60 kg consumer in mg/serving

Mushroom samples	Cu	Ni	Pb	Cd	As	Hg
Russula vinosa	1.425 ± 0.09	0.013 ± 0.00	0.081 ± 0.02	0.027 ± 0.00	0.026 ± 0.00	0.008 ± 0.00
Russula delica	1.857 ± 0.14	0.007 ± 0.00	0.057 ± 0.01	0.033 ± 0.00	0.020 ± 0.00	0.030 ± 0.00
Russula cyanoxantha	1.533 ± 0.13	0.010 ± 0.00	0.063 ± 0.01	0.039 ± 0.01	0.029 ± 0.00	0.016 ± 0.00
Russula virescens	1.314 ± 0.10	0.003 ± 0.00	0.060 ± 0.01	0.012 ± 0.00	0.013 ± 0.00	0.030 ± 0.00
Lyophyllum decastses	2.085 ± 0.16	0.010 ± 0.00	0.090 ± 0.01	0.048 ± 0.01	0.044 ± 0.01	0.084 ± 0.00
Cantharellus cibarius	1.170 ± 0.11	0.003 ± 0.00	0.138 ± 0.01	0.027 ± 0.01	0.016 ± 0.00	0.010 ± 0.00
Russula albida	1.950 ± 0.10	0.021 ± 0.00	0.288 ± 0.02	0.090 ± 0.02	0.037 ± 0.00	0.116 ± 0.00
Agaricus subrufescens	2.601 ± 0.35	0.017 ± 0.00	0.132 ± 0.02	0.075 ± 0.03	0.028 ± 0.00	0.118 ± 0.00
Calvatia craniiformis	4.620 ± 0.83	0.041 ± 0.01	0.324 ± 0.04	0.273 ± 0.02	0.026 ± 0.00	0.065 ± 0.00
Agaricus silvaticus	5.445 ± 0.86	0.004 ± 0.00	0.279 ± 0.02	1.557 ± 0.12	0.035 ± 0.01	0.115 ± 0.01
Macrolepiota crustosa	5.232 ± 0.89	0.021 ± 0.00	0.222 ± 0.03	2.754 ± 0.27	0.044 ± 0.01	0.066 ± 0.01

contains 30 g of dry matter (Kalač and Svoboda 2000; Svoboda et al. 2000). Table 5 are presented the metal intakes by a normal (60 kg) consumer in mg/serving for all studied Chinese mushrooms.

Present results (Table 5) conform to EU Scientific Committee (2001) standards for Pb and Cd (toxic metals). Provisional tolerable weekly intake values for Pb and Cd for adults (of 60 kg) are 1.50 and 0.4 mg, respectively, (Council of Europe 2001). These values correspond to 0.21 and 0.06 mg of Pb and Cd, respectively, on a daily basis. According to FAO/WHO (1976), the acceptable daily intake of Hg for an adult is 0.04 mg, of which a maximum of 0.03 mg can be in the form of the toxic methylmercury. Therefore, the intake of heavy metals (Pb, Cd, Hg) by consumption of 30 g dry weight of wild mushrooms daily from the urban area poses risk for the consumer.

The two sampling sites (rural areas) were not polluted. The levels of the heavy metals Cd, Pb, and Hg in the mushroom species, *R. vinosa*, *R. delica*, *R. cyanoxantha*, *R. virescens*, *L. decastses*, and *C. cibarius* from the two sites can be considered low and therefore, pose no health risk.

The mushroom species in YueLu Mountain (urban area) were polluted. The five mushroom samples, *A. subrufescens*, *R. albida*, *A. silvaticus*, *C. craniiformis* and *M. crustosa* from the area were polluted by Cd, Pb, and Hg at levels much higher than in the rural areas. The maximum Pb, Cd and Hg contents of the five mushroom samples were nearly 11, 92 and 8 fold higher than the Chinese limit value (NY/T 749-2003), respectively. Consumption of these species by the local dwellers which do accumulate these metals should thus be restricted.

The source of the pollution in the urban area likely comes from the vehicular transportation. Analysis indicated that there was a significant correlation between Cd, Hg, Pb and Cu, suggesting that they were the same source of contamination. Better management of local traffic would relieve or eliminate pollution.

Acknowledgments This work has been supported under the projects by the National Basic Research Program of China (No. 2004CB619204) and the National Natural Science Foundation of China (No. 50621063).

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