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Mineral constituents of a prized edible mushroom (*Tricholoma matsutake*) and soils beneath the fruiting bodies from the production areas across China

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Abstract: Fruiting bodies (500 g per site) of *Tricholoma matsutake* and the surface layer of soils collected from 20 spatially distant areas with pristine backgrounds across China were analyzed for potassium, magnesium, calcium, zinc, iron, copper, manganese and cadmium using inductively coupled plasma optical emission spectroscopy. In terms of the bioconcentration and bioexclusion concept, K, Mg, Zn, and Cu were highly bioconcentrated, and their bioconcentration factor values varied between 75-615, 2-107, 38-603 and 7-76, respectively, across the 20 sites. Fe, Mn and Cd were moderately bioconcentrated and their bioconcentration factors (BCFs) varied between 0.6-34.0, 0.4-37.0 and 0.9-7.0 respectively. However, Ca was excluded (BCF<1). *T. matsutake* is a species that is harvested in the wild as a valuable food and can contain a wide spectrum of both essential and hazardous mineral compounds that accumulate at elevated concentrations even if grown in pristine areas. The estimated intake rate of Cd in the fruiting bodies indicates cause for concern associated with this metal resulting from the daily consumption of between 200 and 400 g of fruiting bodies on a frequent basis during the mushrooming season.

Keywords: Fungi; Heavy metals; Mineral composition; Mushrooms; Wild food

Introduction

There are exceeds 300,000 species of fungi in

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this world which represent a highly group of biota. Fungi play an important role in metal circulation. They can dissolve, take-up, and introduce metals from organic and soil mineral substrates into the biosphere (Gadd et al. 2007; Falandysz and Borovicka 2013; Zhang et al. 2013). Among fungi, approximately 10,000 species are fleshy mushrooms. Around 2000 mushrooms growing in the wild from more than 30 genera are considered edible (Chang 1990). Edible wild mushrooms can contain a wide spectrum of mineral macro- and micronutrients, nonessential trace elements, and problematic heavy metals in their fruiting bodies. Mycorrhizal and saprobic mushrooms take part in the biogeochemical turnover of all mineral constituents contained in soil compartments, rotten wood or other substrates in which mycelia develop. Despite the fact that they play a key role, the biological mechanisms and environmental factors that drive and influence the metal bioconcentrations and contents of fruiting bodies are poorly known (Falandysz and Borovicka 2013). Mercury is an example of a metal that is considerably more enriched in the fruiting bodies of mushrooms than in plants or animals (Stijve and Besson 1976; Falandysz 2002; Falandysz and Brzostowski 2007; Melgar et al. 2009; Falandysz et al. 2014; Wiejak et al. 2014).

Tricholoma matsutake is a kind of fungi belonging to subgenus *Tricholoma* (Ding and Hou 2012). It is an ectomycorrhizal fungus that grows in a symbiotic relationship with the rootlets of *Pinus densiflora* (Gill et al. 1999; Guerin-Laguette et al. 2004; Kim et al. 2014). Its fruiting body is commercially important in Asia and northern Europe as a valuable food for its medicinal effects and attractive flavor (Ohnuma et al. 2000; Hoshi et al. 2005; Cho et al. 2006; Kim et al. 2008; Ding et al. 2010). In one recent study, data on occurrence of Ca, Cu, Fe, K, Mg, Mn, Na and Zn in whole carpophore of *T. matsutake* foraged from selected localizations in Yunnan have been provided, while no data on bioconcentration of those metallic elements by species (Li et al. 2013). Artificial cultivation of *T. matsutake* has not been successful because of its high environmental demand (Iwase 1997; Yamada et al. 2006; Yang et al. 2012; Murata et al. 2013; Vaario et al. 2013). China is one of the major production areas of *T. matsutake*, and this fungus is mainly distributed in the Hengduan (Southwestern China) and Changbai (Northeast China) mountains. The production of these two regions accounts for more than 95% of the *T. matsutake* yield in China. However, the soil conditions and bioconcentration factors (BCFs) of some metals in the *T. matsutake* production areas remains unknown (Liu et al. 2010; Liu et al. 2012; Li et al. 2013).

Knowledge on the trace element composition of wild mushrooms is important, both to know the amount and intake rates as well as their time trends due to anthropogenic emission and environmental pollution (Falandysz et al. 2011; Gucia et al. 2012; Wang et al. 2014). The main point of this study is concerted to an ongoing wild mushroom survey aimed to investigate the mineral content and composition and the bioconcentration of the mineral constituents of the highly prized edible mushroom (*Tricholoma matsutake*) growing in the main production areas of China. The contamination status of this mushroom was assessed as well as its mineral absorption rates, nutritional significance, and risk to consumers.

In this article, the concentrations of 8 metals in soils and in the edible *T. matsutake* mushroom from 20 distant sites (counties) across China are reported. The trace element composition of the soil was investigated to determine possible differences between the various locations as well as the BCFs of this prized mushroom. To our knowledge, this is the first study to present the soil conditions and bioconcentration factors (BCFs) of the highly prized mushroom (*T. matsutake*) in main production areas across China.

1 Materials and Methods

Approximately 500 g (about 10 fruiting bodies per sample) of wild *Tricholoma matsutake* fruiting bodies and 500 g soil beneath the fruiting bodies (<15 cm) were collected from each site. Altogether, we collected fruiting bodies and soil from 20 geographically distant sites (counties) across China (Appendix 1, Figure 1), which represent the major *T. matsutake* production areas in China (over 90% of the total output in China). The fruiting bodies were air-dried for several days after a plastic knife was used to remove any visible plant vegetation and soil substrate debris. Then, the fruiting bodies were dried at 65°C to a constant weight. The dried mushrooms were pulverized using an agate mortar and were stored in brand new, sealed polyethylene bags in a dry condition.

The pulverized subsamples (400 mg) of the fruiting bodies were weighted into pressure-resistant and analytical-quality prodigestive vessels made of polytetrafluoroethylene. Then, they were predigested for 24 h with concentrated nitric acid at room temperature and further digested under pressure in an automatic microwave digestion system (Shantou Keyi instrument & Equipment co.ltd, China). The digest was diluted to 25 mL using deionized water and was subjected to instrumental analysis (Brzostowski et al. 2009, 2011a and 2011b).

Figure 1 Location of the Tricholoma matsutake sampling sites. Antu (1), Hunchun (2), Longjin (3), Wangqing (4), Dechang (5), Daofu (6), Jiulong (7), Kangding (8), Lixian (9), Maerkang (10), Mianning (11), Xiaojin (12), Yajiang (13), Bomê (14), Gongbo (15), Nyingchi (16), Mainling (17), Lufeng (18), Mouding (19) and Yaoan (20). These sites (counties) are the main production areas of *T. matsutake* in China.

Soil samples were air-dried at room temperature under clean conditions for several weeks and were sieved through a pore size of 2 mm and further dried in an electric oven at 40°C to a constant weight. Next, the soil subsamples (5 g) were placed in quartz vessels and were cold-treated with nitric acid (20% HNO_3; 20 mL) and allowed to stand for 24 h. The obtained extract ac measuring vessel (50 mL), and after adding an internal stanac measuring vessel (50 mL), and after adding an internal standard solution (yttrium; 20 mg L^{-1}), a 50 mL mixture was made using deionized water and was subjected to instrumental analysis as outlined above for the fruiting bodies. Two blank digests were run with every set of 15 soil samples.

K, Mg, Ca, Cd, Cu, Fe, Mn, and Zn were determined by inductively coupled plasma optical emission spectroscopy (Optima 2000 DV, PerkinElmer, USA), and the yttrium was used as an internal standard (Brzostowski et al. 2009, 2011a and 2011b).

The differences between the certified values and the measured concentrations were less than 10%. Duplicate and blank samples were included following every set of twenty mushroom or soil samples examined. No major interferences were found for the blank samples for the elements quantified. The limits of detection for K, Mg, Ca, Cd, Cu, Fe, Mn, and Zn were between 0.01 and 0.10 µg g^{-1} dry weight (dw). The coefficients of variation for these measurements were well below 10% for routine runs.

The uptake rate of the mineral constituents from the soil to the fruiting bodies was assessed using the common concept of a transfer factor. This is commonly expressed as an enrichment factor or BCF, which is simply the constituent concentration of the mushroom divided by the mineral constituent concentration of the soil (or other substratum media).

All data were statistically analysed using parametric tests to determine whether there were significant differences between the variables.

2 Results and Discussion

2.1 Macroelements (K, Mg, Ca)

Potassium was consistently abundant in the fruiting bodies of *T. matsutake* collected from 20 different sites (Appendix 2). The mean concentrations varied between 12.4 and 35.4 mg g^{-1} dw for the sites surveyed, and these values show a certain degree of variability. Compared with the K content of the fruiting body, the K content of the soil was relatively low. The mean values for the 20 sites varied between 0.045 and 0.32 mg g^{-1} dw (Appendix 3). The mean BCF values of K for the fruiting bodies in this study ranged between 75 and 615 (Appendix 4). Compared with K content in other mushrooms from China and Europe, the K content in the fruiting bodies of *T. matsutake* was at an average level, and its BCF differs largely between different sites (Kalač 2009; Kalač 2012; Falandysz et al. 2012; Wang et al. 2014).

Soil magnesium was present at average concentrations, varying between 0.019 and 0.34 mg g^{-1} dw, and the values measured in most stands were typically <0.50 mg g^{-1} dw. The mean values of Mg in the fruiting bodies varied between 0.71 and 1.57 mg g^{-1} dw. This metal element, which is essential for fungi and animals, is well taken up by *T. matsutake* and its mean BCF values across the twenty sites varied between 2.1 and 107.2, suggesting that the sampling site affects the BCF of Mg than in other mushrooms (Zhang et al. 2013).

The soil calcium content varied more than the other macroelements determined. The mean values of the Ca concentrations varied between 0.016 and 1.27 mg g^{-1} dw soil across the twenty sites. The Ca content of the fruiting bodies of this species are also varied significantly, the mean concentration values varied between 0.048 and 0.819 mg g^{-1} dw. For most of the sites surveyed, Ca is excluded in *T. matsutake*. The mean BCF values of this element in the fruiting bodies at sixteen sites surveyed varied between 0.09 and 0.87, and the maximum value in a single specimen was 20.55. Like in many other mushrooms (Chudzynski and Falandysz 2008; Zhang et al. 2013; Kuldo et al. 2014), the *T. matsutake* BCF of Ca is less than 1 showing that Ca is not accumulated in *T. matsutake*.

Previous studies found that calcium sensitizers isolated from *T. matsutake* can significantly increase the calcium ion concentration in myocytes, which is greatly dependent on the influx of extracellular Ca^{2+} (Hou et al. 2013). If it does keep active in *T. matsutake* remains to be studied.

2.2 Essential trace metals (Cu, Fe, Mn, Zn)

The soil collected from the various sites varied significantly with respect to the copper content, with a mean value between 0.44 and 4.35 mg kg^{-1} dw. *T. matsutake* fruiting bodies are rich in Cu with mean values being between 13.09 and 40.52 mg kg^{-1} dw. The mean Cu BCF values at the sites with relatively high soil Cu concentrations were between 6.8 and 34.1, while 75.8 was recorded for one site where the soil was relatively low in this element. These differences suggest more active regulation of Cu uptake and sequestration in maturating fruiting bodies than other mushrooms (Wang et al. 2015).

Iron was abundant in the soil substratum of *T. matsutake*. The mean concentrations across the sites varied between 123 and 1924 mg kg^{-1} dw, and the mean concentrations in the fruiting bodies ranged between 330 and 4179 mg kg^{-1} dw. These concentrations varied largely between the sites. Fe was enriched in this species at most sites. The mean BCF value for most of the sites ranged from 1.16 to 34.49 and values of 0.64 and 0.84 were recorded for two of the sites. In some mushrooms, Fe did not accumulate to fruiting bodies (Chudzynski and Falandysz 2008; Zhang et al. 2013; Kuldo et al. 2014). However, *T. matsutake* bioconcentrated Fe from soil at most sites surveyed, which led to higher content of Fe in *T. matsutake* fruiting bodies than in other mushrooms (Kalač 2009; Kalač 2012; Wang et al. 2014).

The soil substratum manganese concentrations varied between 1.78 and 37.04 mg kg^{-1} dw. This mushroom is moderately abundant in Mn. The mean values of Mn in *T. matsutake* from the sites across China varied between 12.33 and 122.87 mg kg^{-1} dw. The mean BCF values of the fruiting bodies from most of the sites surveyed in this study were between 0.45 and 8.38, and the value for an individual sample was as high as 37.00. Like Fe, in general Mn in *T. matsutake* fruiting bodies was also more abundant than in other mushrooms (Kalač 2009; Kalač 2012; Wang et al. 2014).

The soil zinc concentrations varied significantly between some of the sites. The mean values of Zn in the soil across the twenty sites varied between 0.28 and 1.65 mg kg^{-1} dw. *T. matsutake* has a higher Zn concentration compared with that contained in the soil. The mean *T. matsutake* concentration values generally ranged between 40.72 and 114.70 mg kg^{-1}

dw; however, 361.87 mg kg^{-1} dw was recorded in Mainling county, Tibet Autonomous Region, which is significantly higher than the other values measured in this survey. This metal is highly bioconcentrated in the fruiting bodies; the mean BCF values were between 37.8 and 299.7 at most sites, and the value for an individual sample was as high as 603.1. Due to the low content of Zn in soil, Zn in *T. matsutake* fruiting bodies is in the average level among other mushrooms, although its BCF is relatively high (Kalač 2009; Kalač 2012; Wang et al. 2014).

2.3 Toxic metals (Cd)

The mean cadmium concentrations in the soil substrate of *T. matsutake* at the sites surveyed varied between 0.28 and 0.57 mg kg^{-1} dw. This metal is moderately bioconcentrated in *T. matsutake* fruiting bodies. The BCF ranged between 1.4 and 7.0, and a value of 0.9 was determined for one individual site. The mean Cd concentrations in the fruiting bodies were between 0.46 and 3.54 mg kg^{-1} dw.

Cadmium in the fruiting bodies of edible wild mushrooms is among the toxic substances of concern. In the European Union, the maximum level of cadmium in cultivated mushrooms (Oyster Mushroom *Pleurotus ostreatus*, Champignon Mushroom *Agaricus bisporus*, and Shiitake *Lentinus edodes*) is 0.20 μg g^{-1} fresh weight (fw) (equivalent to 2.0 μg g^{-1} dw, assuming 90% moisture) and for other fungi is 1.0 μg g^{-1} fw (10 μg g^{-1} dw) (EU 2008). In comparison with the European Commission regulations on Cd, the mean values of the cadmium concentrations in *T. matsutake* were well below 10 μg g^{-1} dw for all the sites examined.

2.4 Cluster analysis of the data

To evaluate the similarities in the accumulation patterns in *T. matsutake* between the

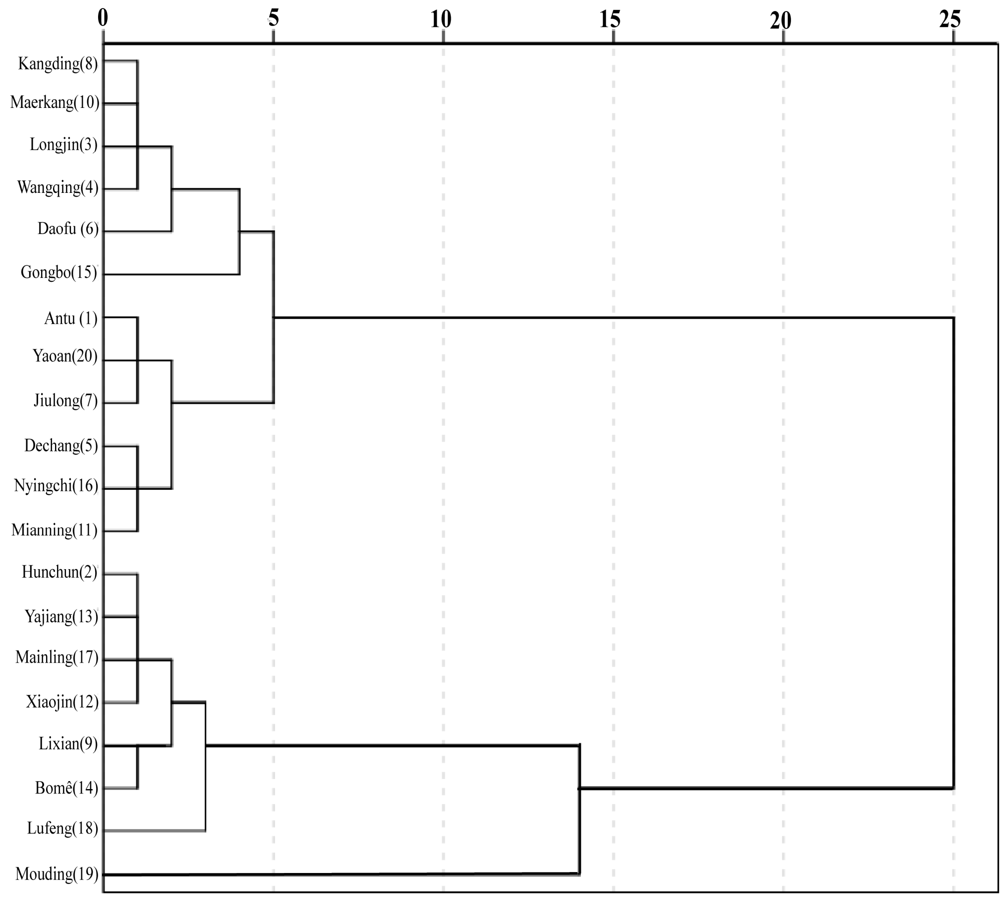

Figure 2 Hierarchical cluster analysis of the similarities in the accumulation patterns in *Tricholoma matsutake* among the sampling sites using the SPSS package and the between-groups linkage method.

sampling sites, hierarchical cluster analysis was applied to the data set using the SPSS package and the between-groups linkage method. The results of the hierarchical cluster analysis are shown in Figure 2. A cluster analysis dendrogram of the element accumulation similarities between the sampling sites show two main clusters. The first cluster contains Kangding (8), Maerkang (10), Longjin (3), Wangqing (4), Daofu (6), Gongbo (15), Antu (1), Yaoan (20), Jiulong (7), Dechang (5), Nyingchi (16) and Mianning (11). The second cluster contains Hunchun (2), Yajiang (13), Mainling (17), Xiaojin (12), Lixian (9), Bomê (14), Lufeng (18) and Mouding (19). The sites in the first cluster accumulated more K, Mg, Ca, Fe, and Cd. In the second cluster, up to two subfractions could be recognized, which described the high concentrations of Zn, Cu, and Mn.

3 Conclusions

The potential of *T. matsutake* to bioconcentrate soil compounds in the fruiting bodies can be very high. However, whether elements not analyzed here, for example mercury, bioconcentrate in *T. matsutake* requires further study. *T. matsutake* is a species that is harvested in the wild as a valuable food, and can contain a wide spectrum of essential and hazardous mineral compounds that accumulate at elevated concentrations even if grown in pristine areas. The estimated intake rates of Cd in the fruiting bodies shows cause for concern associated with these metals resulting from the daily consumption of between 200 and 400 g of fresh fruiting bodies on a frequent basis during the harvest season for *T. matsutake*. Further study on the soil condition and BCFs of other highly prized mushrooms in China is needed to standardize the levels of the active ingredients.

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