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Scanning electron microscopy-energy dispersive X-ray spectrometer (SEM-EDX) detection of arsenic and cadmium in himematsutake mushroom

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The distribution of arsenic (As) and cadmium (Cd) in himematsutake was analyzed using scanning electron microscopy-energy dispersive X-ray spectrometer (SEM-EDX). The atomic percentage of the metals was confirmed by inductively coupled plasma-mass spectrometer (ICP-MS). Results show that the accumulation of As in pileus was higher than that in stipe by both SEM-EDX and ICP-MS analyses. The Cd level in pileus was higher than that in stipe by SEM-EDX analysis; whereas a higher level of Cd was found in stipe by ICP-MS analysis. Results of regression analysis showed that there was a positive correlation between the content of As and Cd in the piece samples by both SEM-EDX and ICP-MS methods. Results suggest that the SEM-EDX is one of the potential tools for rapid detection of metals, namely, As and Cd in himematsutake.

Key words: Arsenic (As), cadmium (Cd), scanning electron microscopy-energy dispersive X-ray spectrometer (SEM-EDX), coupled plasma-mass spectrometer (ICP-MS), himematsutake.

INTRODUCTION

Agaricus brasiliensis Wasser mushroom is a native species originated in the São Paulo State, Brazil (Wasser et al., 2002; Wasser, 2014). The name for *A. brasiliensis* mushroom include “Sun mushroom”, “God's mushroom” and “Almond Mushroom” in Brazil, “Himematsutake” and “Kawariharatake” in Japan, and Ji Song Rong in China (Largeteau et al., 2011; Wang, 2014; Wasser, 2014). Himematsutake is widely known as a medicinal mushroom in the world due to its abundant polysaccharide content, such as α-D-glucan, β-D-glucan, β-galactoglucan and xyloglucan (Largeteau et al., 2011; Firenzuoli et al., 2008; Mizuno and Nishitani, 2013).

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Abbreviations: SEM-EDX, Scanning electron microscopy-energy dispersive X-ray spectrometer; **As,** arsenic; **Cd,** cadmium; **ICP-MS,** inductively coupled plasma-mass spectrometer; **AAS,** atomic absorption spectrometry.

Clinical studies indicated that himematsutake has antitumor activity and hypocholesterolemic effect and can lower the diastolic and systolic blood pressure and improve the liver function (Firenzuoli et al., 2008; Wani et al., 2010; Wasser, 2011, Biedron et al., 2012). Because of these pharmacological activities, himematsutake has high market value with an annual production of 40 tons of dehydrated mushrooms in Brazil during 2006 to 2007 (Tomizawa et al., 2007). Previous studies indicated that certain mushrooms and macrofungi could easily accumulate heavy metals in its fruiting body (Falandysz et al., 2001; 2008; Soeroes et al., 2005; Tüzen, 2003; Tüzen et al., 1998). Kalač and Svoboda, reviewed the literatures on heavy metal accumulation in mushroms between 1970 and 2000 and indicated that *Agaricus campestris*, *Agaricus arvensis*, *Agaricus silvaticus*, *Agaricus silvicola* and other *Agaricus* spp., could accumulate cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu) and silver (Ag) (Kalač, 2010; Kalač and Svoboda, 2000, Sun et al., 2012; Wang et al., 2014). Moreover, the metals accumulated in wild macrofungi were higher than those in farm grown macrofungi (Kalač and Svoboda, 2000). Thus, the ability to accumulate heavy metals in these macrofungi are associated with *Agaricus* species (Alonso et al., 2003; Svoboda et al., 2000), polluted areas (Soeroes et al., 2005; Tüzen et al., 1998), age of mycelium (Kalač and Svoboda, 2000), composition and pH of soil and of type of heavy metal (Baldrian, 2003; 2010).

Current analysis methods for detection of heavy metals in mushroom include atomic absorption spectrometry (AAS) (Tüzen, 2003; Kojo and Lodeniu, 1989; Behbahani et al., 2014), and inductively coupled plasma mass spectrometry (ICP-MS) (Falandysz et al., 2001; 2008; Chudzyński and Falandysz, 2008; Rabinovich et al., 2007). However, sample preparation and operation of AAS and ICP-MS analyses are time-consuming and costly. Recently, scanning electron microscopy modified with energy dispersive x-ray spectrometer (SEM-EDX) has been used to investigate heavy metals in mushrooms, and found that Cd accumulates in *Pleurotus platypus* (Vimala and Das, 2011), the elements content in different strains of himematsutake (Jian et al., 2009), and the mechanism of cesium (Cs) accumulation in *Pleurotus ostreatus* (Sugiyama et al., 2000; 2008). Furthermore, Rumberger et al. (2005) used the SEM-EDX and ICP-MS to trace the metal elements in ectomycorrhiza from European beech (*Fagus sylvatica* L.) and Scots pine (*Pinus sylvestris* L.) forests in northern Brandenburg, and the results indicated that the amount of aluminum (Al), calcium (Ca), magnesium (Mg) and sulfur (S) analyzed by SEM-EDX were highly correlated with ICP-MS analysis. Thus, the SEM-EDX is one of the alternative equipment for the detection of the metal in bio-materials (Rumberger et al., 2005).

In Taiwan, himematsutake is popular for its medicinal activities (Wu, 2001). More than 35 tons are imported from different countries and the production area in Taiwan has recently been increased (www.ir.tari.gov.tw:8080/bitstream/345210000/4825/2/ no155-all.pdf). However, the accumulation of Cd in himematsutake has also been reported in Taiwan and China, especially in the last few years (Hsu et al., 2009; Huang et al., 2008). For example, the Cd content exceeded the limit of 2 ppm based on the regulation set for the "Standard for the Tolerance of Heavy Metals in Edible Mushrooms" in Taiwan (Anonymous, 2007). Currently, a report showed that using sawdust bag-logs can reduce the heavy metals content (Wu, 2001). However, the safety is still doubtful due to the accumulation of heavy metals in edible parts of himematsutake, especially As and Cd (Chu, 2010; Chu et al., 2012). The high Cd content could damage the kidney and liver and cause diarrhea, nausea and vomit (Faroon et al., 2008; Das et al., 2010). The high As content could cause Blackfoot disease or cancer (Ng et al., 2003). In addition, the detection of heveay metals by AAS and ICP-MS is considerded a high-cost and time-consuming operation. The objectives of this study were to 1) analyze the distribution of As and Cd in pileus and stipe, 2) compare the detection efficacy of As and Cd with piece or powder specimens, and 3) evaluate whether SEM-EDX can be an althernative method for the detection of As and Cd in himematsutake.

MATERIALS AND METHODS

Collection of dried himematsutake fruiting body

The dried fruiting bodies of himematsutake were collected from three counties in Taiwan, namely Taichung, Changhua, Nantou, and China during 2010 to 2011. A total of six strains were evaluated in this study, including one USA strain collected from Taichung, two Japanese strains collected from Taichung and Changhua, one Taiwanese strain collected from Changhua, one unknown strain collected from Nantou and one strain collected from Guangdong in China (Table 1).

Analysis of arsenic (As) and cadmium (Cd) in piece and powder tissues of himematsutake by SEM-EDX

The SEM-EDX method was used to measure the percentage of As and Cd in pieces and powder of himematsutake. Each dried fruiting body was separated into pileus and stipe, and three fruiting bodies of each strain were selected for analysis. In the piece tissues analysis, three 15 × 3 × 2 mm^3 size of tissue samples were obtained from each pileus and stipe randomly, and then each tissue piece was cut into five sub-pieces (3 × 3 × 2 mm^3). The 45 tissue pieces were mounted on a cupper stub with carbon tape. On the other hand, the dried pileus and stipe tissues were milled, respectively by a sterile mortar, and each powder from the pileus or stipe was dusted moderately on the cupper stub with the carbon tape. The mounted pieces and powder samples were coated with platinum by a sputter coater (JEOL, JFC-1600, Japan) for 30 s at 20 mA and for 40 s at 10 mA, respectively. All samples were analyzed by SEM (JEOL, JFM-7401F, Japan) and EDX (Oxford, 7585, England) at 8 mm working distance with 15 kV and 500 X magnification. The atomic percentage of As and Cd was measured.

Table 1. Analysis of arsenic (As) and cadmium (Cd) in the piece of the pileus and the stipe of himematsutake strains collected from different areas by SEM-EDX method.

Himematsutake	Collected place	Tested part	Atomic percentage (%)[a]	
			As	Cd
USA strain	Taichung, Taiwan	Pileus	0.009±0.01*	0.013±0.02
		Stipe	0.008±0.01	0.014±0.02
Japanese strain	Taichung, Taiwan	Pileus	0.008±0.01*	0.009±0.02
		Stipe	0.006±0.01	0.010±0.02
Japanese strain	Changhua, Taiwan	Pileus	0.011±0.01	0.012±0.02
		Stipe	0.013±0.01*	0.015±0.02*
Taiwanese strain	Changhua, Taiwan	Pileus	0.013±0.02*	0.014±0.02
		Stipe	0.010±0.01	0.015±0.02
Unknown	Nantou, Taiwan	Pileus	0.015±0.02	0.015±0.02
		Stipe	0.013±0.02	0.025±0.03*
China	Guangdong	Pileus	0.015±0.02*	0.010±0.02
		Stipe	0.012±0.02	0.015±0.02*

[a]Three dried fruiting bodies were used for analysis. *Paired-Samples t test, P≤0.05

Analysis of As and Cd in himematsutake by ICP-MS

ICP-MS method was used to measure the concentrations of As and Cd in the pileus and the stipe of himematsutake. The milled sample of 0.25 g from pileus or stipe was put into polytetrafluoroethylene (PTFE) vessels (MarsX, CEM, Company, USA) followed by adding 6 ml of nitric acid (65%) as wetting material. The PTFE vessels were closed and micro-digested at 180°C for 15 min after pre-digested at 25°C to 28 for 30 min. Then, the mixture was cooled at 25 to 28°C for 20 min, and diluted with 25 ml of deionized water. The concetration of As and Cd was analyzed in the mixture by using ICP-MS (Agilent 7500 Series, Taiwan). The ICP-MS operation was accomplished by the following conditions (Radio frequency power: 1,500 W, RF matching: 1.75 V, Sample depth: 9 mm, Sample skimmer cones: Ni, Peristaltic pump: 0.10 rps, Argon flow rate Plasma: 15 L/min; Auxiliary: 0.32 L/min, Nebulizer: 0.87 L/min, Spray chamber temperature: 2°C, Integration time: 0.1 s, Mass monitored: As 75 m/z and Cd 111 m/z). There were three repeats for each treatment.

Statistical method

All sample analyses were run in quadruplicate. Statistical analysis was done using the SAS package (version 10.0) developed by SAS Institute Inc (SPSS Inc., Chicago, USA). Correlation analysis between atomic percentage from SEM-EDX and concetration from ICP-MS was conducted.

RESULTS

Analysis of As and Cd in pieces of the pelius and the stipe of himematsutake by SEM-EDX

Results of SEM-EDX analyses showed that the percentage of As in the pileus was significantly higher than the stipe pieces among the most strains tested (p=0.05). The percentage of As in the pileus was higher than that in the stipe (Table 1). The percentage of As in the pileus was high (0.015%) in the China strain and low (0.008%) in the Japanese strain from Taichung. Similarly, in the stipe, the percentage of As was high (0.012%) in the China strain and low (0.006%) in the Japanese strain from Taichung. On the other hand, the percentage of Cd in the stipe was significantly higher than the pileus among the Japanese strains from Changhua, the Taiwanese strain from Nantou, and the China strain (p=0.05). The percentage of Cd in the stipe was higher than that in the pileus (Table 1). The percentage of Cd in the stipe was 0.015% for the Japanese strain from Changhua, 0.015% for the China strain, and 0.025% for the Taiwanese strain from Nantou, respectively. However, the percentage of Cd in the pileus was 0.010% for the China strain, 0.012% for the Japanese strain from Changhua, and 0.015% for the Taiwanese strain from Nantou, respectively.

Analysis of As and Cd in the powder of pelius and stipe of himematsutake by SEM-EDX

The percentge of As and Cd in the powder samples, made from the dried fruiting bodies of himematsutake were examined by SEM-EDX. The results showed that the percentage of As in the pileus and stipe were significantly different in Japanese strain from Taichung and the China strain (Table 2). The percentage of As in the pileus was 0.016% for the Japanese strain from

Table 2. Analysis of arsenic (As) and cadmium (Cd) in the powder of the pileus and the stipe of himematsutake strains collected from different areas by SEM-EDX method.

Himematsutake	Collected place	Tested part	Atomic percentage (%)[a]	
			As	Cd
USA strain	Taichung, Taiwan	Pileus	0.016±0.02	0.022±0.03
		Stipe	0.013±0.02	0.026±0.03
Japanese strain	Taichung, Taiwan	Pileus	0.016±0.02*	0.023±0.02
		Stipe	0.009±0.01	0.020±0.02
Japanese strain	Changhua, Taiwan	Pileus	0.019±0.02	0.035±0.04
		Stipe	0.020±0.03	0.050±0.07*
Taiwanese strain	Changhua, Taiwan	Pileus	0.019±0.03	0.031±0.03
		Stipe	0.018±0.02	0.037±0.04*
Unknown	Nantou, Taiwan	Pileus	0.013±0.01	0.019±0.03
		Stipe	0.012±0.01	0.017±0.02
China	Guangdong	Pileus	0.021±0.02*	0.023±0.03
		Stipe	0.014±0.02	0.023±0.03

[a]Three dried fruiting bodies were used for analysis *Paired-Samples t test, P≤0.05

Table 3. Analysis of arsenic (As) and cadmium (Cd) in the pileus and the stipe of himematsutake strains collected from different areas by ICP-MS method.

Himematsutake	Collected place	Tested part	Atomic concentration (mg/kg)[a]	
			As	Cd
USA strain	Taichung, Taiwan	Pileus	1.440±0.86*	6.158±2.84*
		Stipe	0.555±0.36	1.480±0.72
Japanese strain	Taichung, Taiwan	Pileus	0.450±0.17	0.657±0.35
		Stipe	0.170±0.09	0.193±0.10
Japanese strain	Changhua, Taiwan	Pileus	2.270±0.74*	2.393±1.54*
		Stipe	0.938±0.41	0.818±0.42
Taiwanese strain	Changhua, Taiwan	Pileus	1.418±0.26*	19.530±4.33*
		Stipe	0.735±0.18	9.550±2.94
Unknown	Nantou, Taiwan	Pileus	8.251±3.27	40.838±21.84*
		Stipe	6.020±2.56	24.823±10.89
China	Guangdong	Pileus	1.570±0.84	0.663±0.33
		Stipe	0.850±0.47	0.348±0.36

[a]Three dried fruiting bodies were used for analysis. *Paired-samples t test, P≤0.05.

Taichung and 0.021% for the China strain, respectively. The percentage of Cd in the pileus and the stipe was significantly different in the Japanese strain and the Taiwanese strain from Changhua (Table 2). The Japanese strain showed higher Cd percentage in the stipe (0.05%) than that in the pileus (0.035%). In the Taiwanese strain, the Cd percentage in the stipe was 0.037%; whereas in the pileus, it was 0.031%.

Analysis of As and Cd in the pileus and the stipe of himematsutake by ICP-MS

The results indicated that As concentration in the pileus and the stipe were significantly different between the USA strain and the Taiwanese strain from Changhua. The concentration of As in the pileus was higher than that in the stipe (Table 3). The concentration of As in the pileus

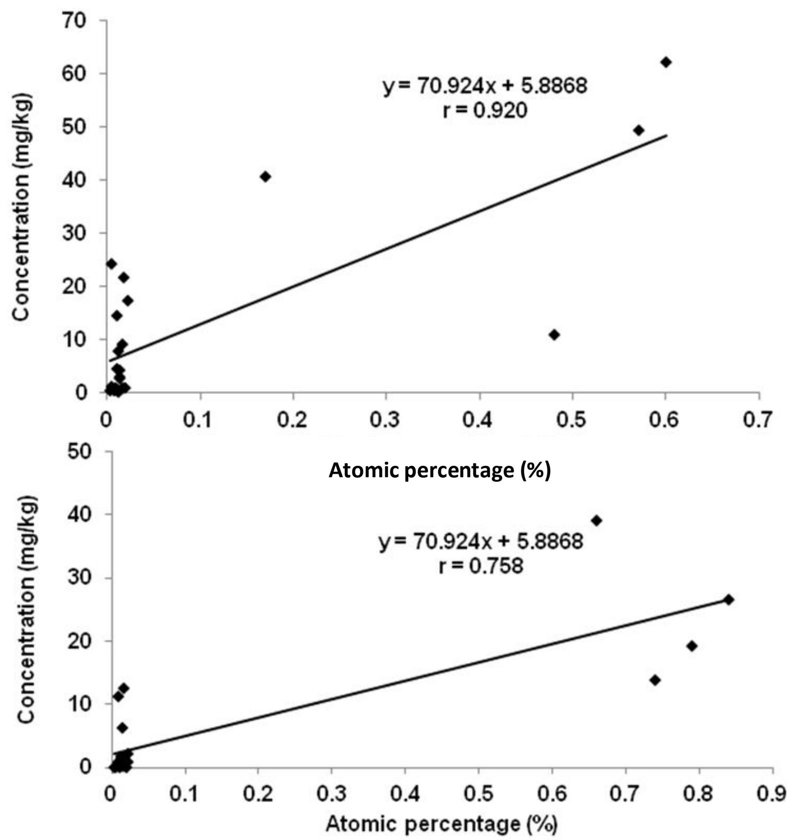


Figure 1. Correlation between the results of As in the pieces of the fruiting body obtained from the pileus (upper) and the stipe (down) examined by SEM-EDX and ICP-MS.

was 1.44 mg/kg for the USA strain and 0.450 mg/kg for the Japanese strain from Taichung. However, the As concentration in the stipe was 0.555 mg/kg for the USA strain and 0.170 mg/kg for the Japanese strain from Taichung. Similarly, the As concentration in the pileus (2.270 mg/kg) was higher than that in the stipe (0.938 mg/kg) for the Japanese strain from Changhua. In the Taiwan strain from Changhua, the As concentration in the pileus (1.418 mg/kg) was higher than that in the stipe (0.735 mg/kg). On the other hand, the Cd concentration in the pileus and the stipe were significantly different among the USA strain, the Taiwanese strains from Changhua and from Nantou. The Cd concentration in the pileus was higher than that in the stipe (Table 3). The Cd concentration in the pileus was 6.158 mg/kg for the USA strain and 2.393 mg/kg for the Japanese strain from Changhua. However, in the stipe, the Cd concentration was 1.480 mg/kg for the USA strain and 0.818 mg/kg for the Japanese strain from Changhua. The Taiwan strain from Changhua had higher Cd concentration in the pileus (19.530 mg/kg) than in the stipe (9.550 mg/kg). In the Taiwanese strain from Nantou, the Cd concentration in the pileus (40.838 mg/kg) was higher than that in the stipe (24.823 mg/kg).

The correlation between the results obtained by SEM-EDX and ICP-MS

In order to find the relationship between the results generated by SEM-EDX and ICP-MS, a correlation was prepared with STATISTICA software. For the piece samples, the results indicated that the atomic percentage of As and Cd in the pileus or the stipe samples by SEM-EDX analysis was positively related with the ICP-MS analysis. The correlation coefficient in terms of the atomic percentage of As and the concentration of As in the pileus and the stipe with pieces sampling were 0.920 and 0.758, respectively (Figure 1), and the correlation coefficient in terms of the atomic percentage of Cd and the concentration of Cd in the pileus and the stipe with pieces sampling were 0.771 and 0.835, respectively (Figure 2). However, when the powdery samples were examined, there was a low relationship between results generated from SEM-EDX and ICP-MS analyses. The correlation coefficient of the atomic percentage of As and the concentration of As in the pileus and the stipe were 0.277 and 0.205, respectively (Figure 3); meanwhile, the correlation coefficient of the atomic percentage of Cd and the concentration of Cd in the pileus and the stipe were

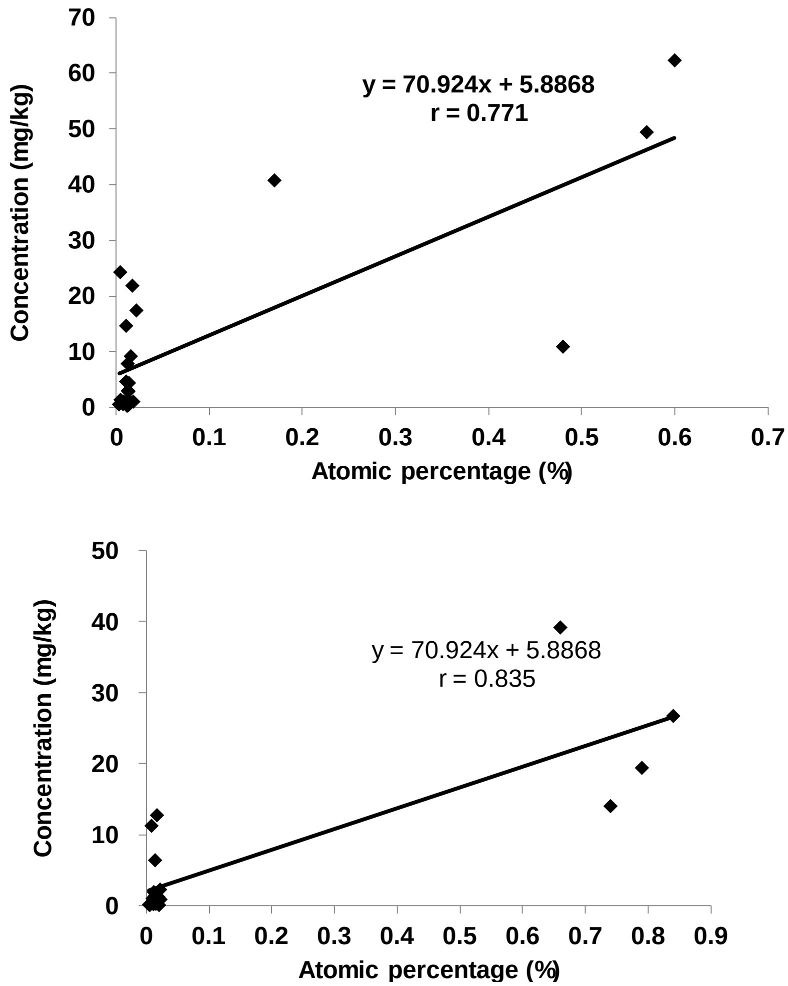


Figure 2. Correlation between the results of Cd in the pieces of the fruiting body obtained from the pileus (upper) and the stipe (down) examined by SEM-EDX and ICP-MS.

0.147 and -0.011, respectively (Figure 4).

DISCUSSION

The results indicate that the accumulation of As in the pileus was higher by SEM-EDX analysis while similar results were obtained by using ICP-MS analysis. Results of previous studies revealed that the process of metals accumulation started from the mycelial absorption of the substrates and then transitted to the stipe, the pileus or the gill (Kalač, 2010; Kalač and Svoboda, 2000; Thomet et al., 1999). Consequently, the distribution of the metals in different parts of the furiting body might show variations, particularly in the stipe and the pelius, the major accumulation sites (Chudzyński and Falandysz, 2008; Falandysz et al., 2003; Thomet et al., 1999). Our results demonstrated that the As more offent accumulated in the pelius of the himematsutake than the stipe did, which is in agreement with previous studies (Huang et al., 2007; Wang et al., 2009). Thus, the As can pass through the stipe easily and accumulate in the pileus after absorption from the substrate. In additon, the As content analyzed by ICP-MS showed similar result as did by SEM-EDX analysis. Brzostowski et al. (2011) indicated that the cultural substrate or environment could affect the accumulation of certain metals in the pileus or the stipe of *Paxillus involutus*, such as Ag (silver), Cd, Cr (chromium), Pb (lead) or Hg (mercury). In this study, the As content varied among different strains. We consider that culture substrates or production area could affect the As accumulation. However, the components and sources of the cultivate substrates used to culture the himematustake are commercial confidentiality among the growers, so the farmers would not provide them to us and the As content of the substrates could not be exmained.

In the Cd analysis, the results of SEM-EDX analysis were different from those of ICP-MS analysis. The results

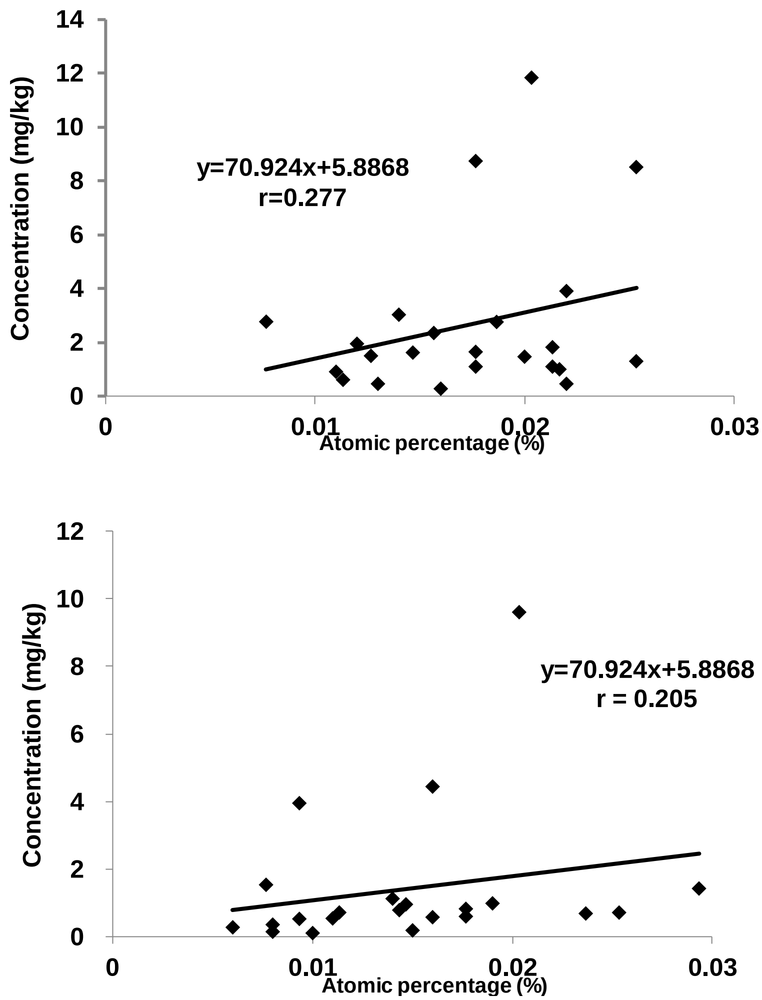
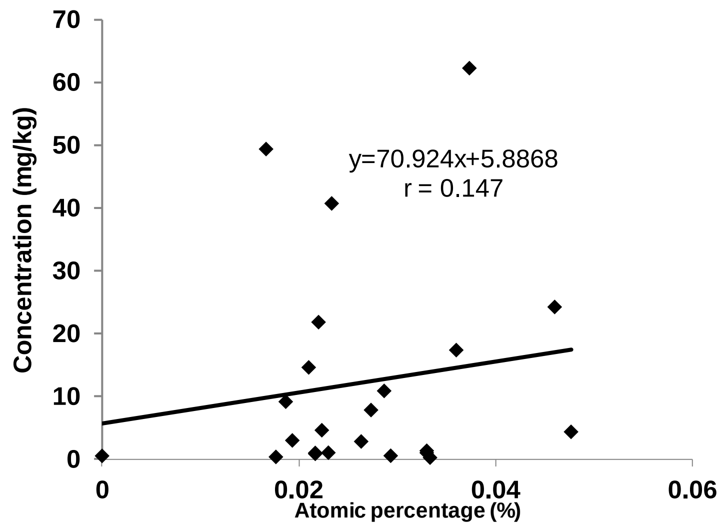


Figure 3. Correlation between the results of As in the powder of the fruiting bodies obtained from the pileus (upper) and the stipe (down) examined by SEM-EDX and ICP-MS.

of SEM-EDX analyses showed that the percentage of Cd in the stipe was higher than that in the pileus, while the Cd content in the pileus was higher than that in the stipe based on ICP-MS analysis. Results of previous studies indicated that the accumulation of Cd in the pileus of himematsutake was higher than that in the stipe (Huang et al., 2007; Wang et al., 2009). Moreover, Thomet et al. (1999) examined the Cd accumulation in the pileus, the stipe, and the gills of *A. macrosporus* and reported that the accumulation of Cd was the least in the stipe. In this study, the results of ICP-MS analyses showed that Cd could accumulate in the stipe of mushroom as reported in previous studies (Huang et al., 2007; Wang et al., 2009; Brzostowski et a., 2011; Thomet et al., 1999). Thus, the site of Cd accumulation in himematsutake is similar to As accumulation. We consider that Cd might not be distributed evenly over the pileus or the stipe and the random piece or powder samples could not represent the Cd content completely in himematsutake. Moreover, SEM-EDX is always used for qualitative analysis of bio- or nonbio-materials and not for quantitative analysis due to sampling limitation (Jian et al., 2009; Rumberger et al., 2005; Sugiyama et al., 2008; Vimala and Das, 2011).

The results of the analysis of the piece and the powder samples demonstrated that the atomic percentage in the piece samples using SEM-EDX was positively related to that with ICP-MS analysis. Results of previous studies indicated that the thickness of the specimen might interfere with the penetration depth of the electron beam and the surface electrochemistry of the specimens, and the standard sample size for quantitative analysis by EDX should be less than 0.1 μm (Goldstein et al., 2003). Although, the powder specimen was smaller than the piece specimen, the size of each powdery specimen used in this study was greater than 50 μm. Thus, the size of the powdery specimen is still too big for quantification analysis by SEM-EDX completely. Moreover, it is difficult to analyze each powdery specimen by SEM-EDX, and



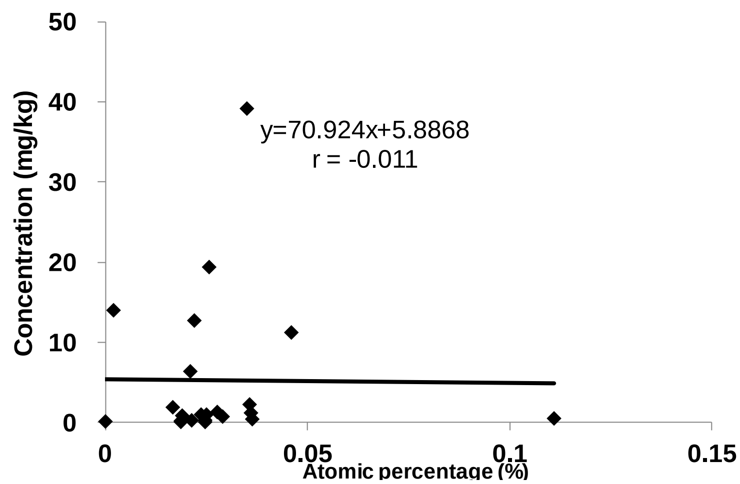


Figure 4. Correlation between the results of Cd in the powder of the fruiting bodies obtained from the pileus (upper) and the stipe (down) examined by SEM-EDX and ICP-MS.

the powdery specimens were selected randomly in this study. Previous study indicated that the element distribution in biological materials is not uniform (McCully et al., 2010), and the random selection of powdery specimens might cause the low correlation with ICP-MS analysis. In the other hand, each piece specimen could be observed in this study and the metals of As and Cd could be detected with high reproducibility. Thus, the piece samples will provide higher detection efficacy than the powdery samples for SEM-EDX examination. In this study, the results demonstrate that the accumulation of both As and Cd could be detected by SEM-EDS with the piece samples and the results represent the possible content in himematsutake. Thus, SEM-EDX can be a useful tool for the primary detection of metal in himematsutake rapidly. For increasing the accuracy and sensitivity of SEM-EDX, Tylko et al. (2004) suggested using the standard specimen with similar matrix to general specimen for the calibration of SEM-EDX. However, the preparation of the standard specimen, the stability and the accuracy of SEM-EDX technique warrant further study.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

Alonso J, García MA, Pérez-López M, Melgar MJ (2003). The concentrations and bioconcentration factors of copper and zinc in edible mushrooms. Arch. Environ. Con. Tox. 44:180-188.

Anonymous (2007). Standard for the Tolerance of Heavy Metals in Edible Mushrooms. Department of Health, Executive Yuan, Taipei, Taiwan ROC.

Baldrian P (2003). Interactions of heavy metals with white-rot fungi. Enzyme Microb. Technol. 32:78-91.

Baldrian P (2010). Effect of heavy metals on saprotrophic soil fungi. Soil Heavy Metals 19:263-279.

Behbahani M, Tapeh NA, Mahyari M, Pourali AR, Amin BG, Shaabani A (2014). Monitoring of trace amounts of heavy metals in different food and water samples by flame atomic absorption spectrophotometer after preconcentration by amine-functionalized graphene nanosheet. Environ. Monit. Assess. 186:7245-7257.

Biedron R, Tangen JM, Maresz K, Hetland G (2012). *Agaricus blazei* Murill – immunomodulatory properties and health benefits. Funct. Foods Health Dis. 2:428-447.

Brzostowski A, Falandysz J, Jarzyńska G, Zhang D (2011). Bioconcentration potential of metallic elements by Poison Pax (*Paxillus involutus*) mushroom. J. Environ. Sci. Health 46:378-393.

Chu CY (2010). Selection of beneficial microorganisms and their effects on fruiting body development of *Agaricus blazei* Murrill. Taichung: Doctoral Dissertation of National Chung Hsing University.

Chu JN, Young CC, Tan CC, Wu SP, Young LS (2012). Improvement of productivity and polysaccharide-protein complex in *Agaricus blazei*. Pesqui. Agropecu. Bras. 47:96-102.

Chudzyński K, Falandysz J (2008). Multivariate analysis of elements content of Larch Bolete (*Suillus grevillei*) mushroom. Chemosphere 73:1230-1239.

Das KK, Das SN, Dhundasi SA (2010). Nickel, its adverse health effects and oxidative stress. Indian J. Med. Res. 128:412-425.

Falandysz J, Brzostowski A, Kawano M, Kannan K, Puzyn T, Lipka K (2003). Concentrations of mercury in wild growing higher fungi and underlying substrate near Lake Wdzydze, Poland. Water Air Soil Pollut. 148:127-137.

Falandysz J, Kunito T, Kubota R, Gucia M, Mazur A, Falandysz JJ, Tanabe S (2008). Some mineral constituents of Parasol Mushroom (*Macrolepiota procera*). J. Environ. Sci. Health B. 43:187-192.

Falandysz J, Szymczyk K, Ichihashi H, Bielawski L, Gucia M, Frankowska A, Yamasaki S (2001). ICP/MS and ICP/AES elemental analysis (38 elements) of edible wild mushrooms growing in Poland. Food Addit. Contam. 18:503-513.

Faroon O, Ashizawa A, Wright S, Tucker P, Jenkins K, Ingerman L, Rudisill C (2008). Toxicological Profile for Cadimium, ed. Agency for Toxic Substrances and Disease Registry, Ed. Atlanta: Centers for Disease Control press.

Firenzuoli F, Gori L, Lombardo G (2008). The medicinal mushroom *Agaricus blazei* Murrill: review of literature and pharmaco-toxicological problems. Evid. Based Complement. Alternat. Med. 5:3-15.

Goldstein J, Newbury D, Joy D, Lyman C, Echlin P, Lifshin E, Sawyer L, Michael J (2003). Scanning electron microscopy and X-ray microanalysis. New York: Kluwer Academic Publishers.

Hsu CL, Chen SS, Chu CM, Lin YW, Kao YM (2009). Investigation of heavy metals (lead and cadmium) in *Agaricus blazei* Murill. Annual Report Bureau Food and Drug Administration. Taiwan R.O.C. 27:216-223.

Huang J, Ying Z, Yu Y, Li K (2007). Accumulation rule of heavy metal and the controlling technique by *Agaricus blazei* Murrill. Chin. Agric. Sci. Bull. 23:406-409.

Huang JC, Li KB, Yu YR, Wu H, Liu DL (2008). Cadmium accumulation in *Agaricus blazei* Murrill. J. Sci. Food Agric. 88:1369-1375.

Jian ZH, Weng BQ, Lei JG, Xiao SX, Wang YX, Tang XQ (2009). Cell biology identification and analysis on trace elements contents in different parts of pileus of *Agaricus blazei* Murril. J. Chin. Electron Microsc. Soc. 28:462-466.

Kalač P (2010). Trace element contents in European species of wild growing edible mushrooms: a review for the period 2000–2009. Food Chem. 122:2-15.

Kalač P, Svoboda L (2000). A review of trace element concentrations in edible mushrooms. Food Chem. 69:273-281.

Kojo MR, Lodeniu M (1989). Cadmium and mercury in macrofungi–mechanisms of transport and accumulation. Angew. Bot. 63:279-292.

Largeteau ML, Llarena-Hernández RC, Regnault-Roger C, Savoie JM (2011). The medicinal *Agaricus* mushroom cultivated in Brazil: biology, cultivation and non-medicinal valorisation. Appl. Microbiol. Biotechnol. 92:897-907.

McCully ME, Canny MJ, Huang CX, Miller C, Brink F (2010). Cryo-scanning electron microscopy (CSEM) in the advancement of functional plant biology: energy dispersive X-ray microanalysis (CEDX) applications. Funct. Plant Biol. 37:1011-1040.

Mizuno M, Nishitani Y (2013). Immunomodulating compounds in Basidiomycetes. J. Clin. Biochem. Nutr. 52: 202-207

Ng JC, Wang J, Shraim A (2003). A global health problem caused by arsenic from natural sources. Chemosphere 52:1353-1359.

Rabinovich M, Figlas D, Delmastro S, Curvetto N (2007). Copper- and zinc-enriched mycelium of *Agaricus blazei* Murrill: bioaccumulation and bioavailability. J. Med. Food 10:175-183.

Rumberger MD, Lentzsch P, Munzenberger B, Huttl RF (2005). Nutrient amounts of ectomycorrhizae analysed by EDX using ESEM and ICP. Mycorrhiza 15:307-312.

Soeroes CS, Kienzl N, Ipolyi I, Dernovics M, Fodor P, Kuehnelt D (2005). Arsenic uptake and arsenic compounds in cultivated *Agaricus bisporus*. Food Control 16:459-464.

Sugiyama H, Takahashi MN, Terada H, Kuwahara C, Maeda C, Anzai Y, Kato F (2008). Accumulation and localization of cesium in edible mushroom (*Pleurotus ostreatus*) mycelia. J. Agric. Food Chem. 56:9641-9646.

Sugiyama H, Terada H, Shibata H, Morita Y, Kato F (2000). Radiocesium concentrations in wild mushrooms and characteristics of cesium accumulation by the edible mushroom (*Pleurotus ostreatus*). J. Health Sci. 46:370-375.

Sun L, Liu G, Yang M, Zhuang Y (2012). Bioaccessibility of cadmium in fresh and cooked *Agaricus blazei* Murill assessed by in vitro biomimetic digestion system. Food Chem. Toxicol. 50:1729-1733.

Svoboda L, Zimmermannová K, Kalač P (2000). Concentrations of mercury, cadmium, lead and copper in fruiting bodies of edible mushrooms in an emission area of a copper smelter and a mercury smelter. Sci. Total Environ. 246:61-67.

Thomet U, Vogel E, Krähenbühl U (1999). The uptake of cadmium and zinc by mycelia and their accumulation in mycelia and fruiting bodies of edible mushrooms. Eur. Food Res. Technol. 209:317-324.

Tomizawa MM, Dias ES, Assis LJ, Gomide PHO, Santos JB (2007). Genetic variability of mushroom isolates *Agaricus blazei* using markers RAPD. Ciênc. Agrotec. 31:1242-1249.

Tüzen M (2003). Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry. Microchem. J. 74:289-297.

Tüzen M, Özdemir M, Demirbaş A (1998). Heavy metal bioaccumulation by cultivated *Agaricus bisporus* from artificially enriched substrates. *Z. Lebensm.* Unters.–F. A 206:417-419.

Tylko G, Banach Z, Kilarski W (2004). PROZA and calibration curves for quantitative X-ray microanalysis of biological samples. Microchim. Acta 144:271-276

Vimala R, Das N (2011). Mechanism of Cd (II) adsorption by macrofungus *Pleurotus platypus*. J. Environ. Sci. 23:288-293.

Wang LL, Li HB, Wei HL, Wu XQ, Ke LQ (2014). Identification of cadmium-induced Agaricus blazei genes through suppression subtractive hybridization. Food Chem. Toxicol. 63:84-90.

Wang X, Li T, Li B (2009). A preliminary study on accumulation

characteristics of copper, zinc, silver, cadmium and mercury in *Agaricus blazei* Murill. Environ. Chem. 28:94-98.
Wani BA, Bodha RH, Wani AH (2010). Nutritional and medicinal importance of mushrooms. J. Med. Plants Res. 4:2598-2604.
Wasser SP (2011). Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Appl. Microbiol. Biotechnol. 89:1323-1332.
Wasser SP (2014). Medicinal mushroom science: Current perspectives, advances, evidences, and challenges. Biomed. J. 37:345-356.
Wasser SP, Didukh MY, de A. Amazonas MAL, Nevo E, Stamets P, da Eria AF (2002). Is a Widely Cultivated Culinary-Medicinal Royal Sun Agaricus (the Himematsutake Mushroom) Indeed *Agaricus blazei* Murrill? Int. J. Med. Mushrooms 4:267-290.
Wu KT (2001). Himematsutake (*Agaricus blazei*) cultivation on sawdust. J. Tech. Service 45:18-20.