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## A Comparative Analysis of Mineral Elements in the Mycelia and the Fruiting Bodies of Shiitake Mushrooms

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#### ABSTRACT

Mineral elements in four strains of *Lentinula edodes* mycelia grown on a defined media and fruiting bodies of shiitake mushroom grown on sawdust along with three commercially available shiitake mushrooms were analyzed to determine major and minor element levels of Na, K, Ca, Mg, Al, Fe, Mn, Cu, Pb and Ni on a dry weight basis. Analyses indicated Na (1646-5322 mg kg<sup>-1</sup>),  $K (5379-10320 \text{ mg kg}^{-1})$  and  $P (3201-8184 \text{ mg kg}^{-1})$  as most abundant minerals in mycelia followed by other major elements. Trace/heavy metals in mycelia show Zn (19.0-49.7 mg kg<sup>-1</sup>), Fe  $(31.8-41.6 \text{ mg kg}^{-1})$ , Cu  $(9.2-12.7 \text{ mg kg}^{-1})$ , Mn  $(2.2-48.5 \text{ mg kg}^{-1})$ , Pb  $(0.02-2.5 \text{ mg kg}^{-1})$  and Ni (0.39-1.1 mg kg<sup>-1</sup>) with significantly higher accumulation of Zn in the mycelia of heat-resistant strain, LE 054 and significantly low levels of Mn in both heat-resistant strains relative to other strains (p<0.05). Major element ranged in fruiting bodies of mushroom collection suggest that  $K (13.2-62.2 \text{ g kg}^{-1}), P (7.8-54.5 \text{ g kg}^{-1}) \text{ and } Mg (3.4-6.5 \text{ g kg}^{-1}) \text{ as most abundant elements}$ followed by Ca  $(179.8-1698 \text{ mg kg}^{-1})$ , Na  $(191.3-3448 \text{ mg kg}^{-1})$  and Al  $(15.3-79.5 \text{ mg kg}^{-1})$ . The Al levels resemble the amounts reported for Al accumulating mushroom species. Most abundant trace/heavy metals in the mushroom collection were Zn (59.3-283.9 mg kg<sup>-1</sup>) followed by Fe  $(44.4-125.1 \text{ mg kg}^{-1})$ , Mn  $(18.2-73.2 \text{ mg kg}^{-1})$  and Cu  $(13.7-182.4 \text{ mg kg}^{-1})$ . Only trace amounts of toxic heavy metals, Cd (0.27-3.7 mg kg<sup>-1</sup>), Co (0-0.87 mg kg<sup>-1</sup>), Ni (0-0.54 mg kg<sup>-1</sup>) and Pb (0-2.2 mg kg<sup>-1</sup>) were found in mushroom collection. In some instances, preferential accumulation of certain elements was detected in caps relative to stems.

Key words: Nutrient elements, trace and heavy metals, shiitake mushrooms, Lentinula edodes

#### INTRODUCTION

The shiitake mushroom, Lentinula edodes, is an edible mushroom native to East Asia and is cultivated worldwide for its taste, nutritional value and health benefits (Tuzen et al., 1998). With the increasing popularity in the 1940's, various methods of cultivation including use of enriched hardwood sawdust and logs have been utilized to grow edible mushrooms. Mushrooms are acquired either fresh or dry and are consumed specially as delicacy because of their specific aroma and texture. Wild-grown edible mushrooms have been a very popular delicacy in several countries and an annual per capita consumption exceeds 10 kg per year (Manzi et al., 1999, 2001; Kalac and Svoboda, 2000). Mushrooms are enriched with minerals elements such as K, P, Zn, Fe, Cu, Mn, Mo, Se and Ni necessary for their growth and metabolism (Schroeder, 1973; Mattila et al., 2001; Baldrian, 2003). A 100 g serving of L. edodes provides: Ca (23 mg), Cu (1.23 mg), Fe (5.5 mg), K (2,700 mg), Na (18 mg) and Se (0.076 mg) (Stamets, 2005).

Mushrooms are considered as bioaccumulaters of heavy metals and efforts have been made to analyze the elemental content and subsequently evaluate the potential health risks associated with human consumption of contaminated mushrooms (Gast et al., 1988; Kalac and Svoboda, 2000; Isiloglu et al., 2001; Tuzen, 2003; Das, 2005; Ayodele and Odogbili, 2010; Radulescu et al., 2010; Zhu et al., 2011). Metals such as Fe, Cu, Zn, Cr, Se and Mn are considered as essential metals required in biological processes such as enzyme activators, whereas, As, Pb, Ni, Hg and Cd are non-essential metals, toxic even in trace quantities (Schroeder, 1973; Radulescu et al., 2010; Zhu et al., 2011). In some instances, growth rate in fungi has been affected by these heavy metal accumulations (Baldrian, 2003; Das, 2005). Many wild edible mushroom species are known to accumulate certain heavy metals such as Cr, Ni, Ag, Cd, Hg and Pb (Gadd, 1993; Gast et al., 1988; Kalac and Svoboda, 2000; Hatvani and Mecs, 2003; Radulescu et al., 2010). Uptake and accumulation of heavy metals by many cultivated and wild-grown mushrooms are documented (Bayramoglu et al., 2005; Ayodele and Odogbili, 2010; Zhu et al., 2011; Stihi et al., 2011; Damodaran et al., 2014).

Even though heavy metal adsorption can be better studied in liquid media, such an experimental system does not represent the natural fungal environment consisting of decomposing wood and acquisition of nutrients from the woody biomass (Baldrian, 2003). However, it is difficult to study the differential effects among the *L. edodes* strains grown on woody substrates. Therefore, a two component study was pursued here to determine the major and minor element accumulation of four strains of *L. edodes* mycelia, LE 6 (ATCC # 48855), LE 013 (ATCC # 38167), LE 015 (ATCC # 38169) and LE 054 (ATCC # 56002), grown on a defined media as well as field-grown and commercially available *L. edodes* fruiting bodies collection. We have also included a collection of button and oyster mushroom species for comparison purposes. Findings from this study are useful in providing information on nutrient and metal distribution in different *L. edodes* mycelial strains, as well as evaluating the quality of edible mushrooms for human consumption.

#### MATERIALS AND METHODS

Shiitake strains: Pure cultures of four strains of shiitake mycelia namely LE 6 (ATCC # 48855), LE 013 (ATCC # 38167), LE 015 (ATCC # 38169) and LE 054 (ATCC # 56002) acquired from the American Type Culture Collection (ATCC) were utilized to conduct the elemental analysis. The rationale for selection was based on temperature tolerance of shiitake mushrooms in culture; whereby, LE 6 and LE054 were found to be the strains that were able to withstand temperature of 30°C whereas, LE 015 and LE 013 strains grew better at 25°C.

Pure cultures of mycelia: Mycelia of the four strains of shiitake were grown on Potato Dextrose Agar (PDA). Four replicates of each of the strains were taken and inoculated in Petri plates. After three weeks of culture at 25°C, the mycelia were removed and freeze-dried overnight (Labconco Free Zone Freeze Dry System). Dried mycelia (0.25 g) were digested with concentrated HNO<sub>3</sub> acid (10 mL) using microwave assisted digestion (MARSX press, CEM Corporation, Matthews, NC). After digestion, samples were diluted with deionized water to a final volume of 15 mL. Extracts were analyzed using an inductively coupled plasma-optical emission spectrometer (ICP-OES) (Optima 2100 DV, PerkinElmer).

Metals and minerals in fruiting bodies of *L. edodes*: Fruiting bodies of shiitake mushroom were collected from oriental stores, retail grocery chain stores and from the mature fruiting bodies produced during a previous study completed at the Alabama A&M Agricultural Experiment

Table 1: Description of fruiting bodies of mushroom collection

Mushroom	Mushrooms type/source	Description
SCIC	Shiitake (caps)	Fresh shiitake from an oriental store (I)
SCIS	Shiitake (stems)	
SISC	Shiitake (caps)	Fresh shiitake from an oriental store (II)
SISS	Shiitake (stems)	
SBPC	Shiitake (caps)	Fresh shiitake purchased from a local grocery store
SBPS	Shiitake (stems)	
SWMC	Shiitake sawdust grown (caps)	Grown on hardwood bark, sawdust and Rye
SWMS	Shiitake sawdust-grown (stems)	
SRSC	Shiitake sawdust-grown (caps)	Grown on chestnut oak bark and sawdust (1:1)
SRSS	Shiitake sawdust-grown (stems)	
BUTC	Button (caps)	Grown on ½ straw (cooked) and ½ cotton hull
OGDC	Oyster 'Grey Dove' (caps)	mixed and autoclaved

Station, Hazel Green, Alabama (Dr. Catherine Sabota, personal communication). Sources and types of shiitake mushrooms studied are summarized in Table 1. Caps and stems of shiitake mushroom fruiting bodies obtained from oriental and grocery stores are designated as SCIC, SCIS, SISC, SISS, SBPC, SBPS (Table 1). The Button Mushroom Caps (BUTC) and Oyster 'Grey Dove' Caps (OGDC) were included in this study as comparisons. Caps and stems (SWMC and SWMS) of shiitake mushroom analyzed were obtained from mushroom grown on 33% hardwood bark (20.4 L), 67% sawdust (41.3 L), 1.45 kg of rye and 11.4 L of water in 2.3 kg polyethylene bags. The OGDC sample was obtained from oyster mushroom grown on ½ straw (cooked) and ½ cotton hull mixed and autoclaved. These shiitake and oyster mushrooms were grown at Alabama A&M Agricultural Experiment Station under controlled temperature and humidity. Shiitake stems and caps, SRSC and SRSS, used in this study was also grown at the Alabama A&M Agricultural Experimental Station on Chestnut Oak bark and sawdust (1:1) mixture.

All samples were freeze-dried and acid digested using microwave digestion prior to elemental analysis. Freeze dried mushrooms (0.25 g of caps or stems) were microwave digested as described in the previous sections using 10 mL of concentrated HNO<sub>3</sub> (trace metal grade) acid. Extracts were analyzed using ICP-OES for heavy metals (Pb, Cu, Zn, Mn, Ni, Fe, Co, As and Cd) and other minerals (Mg, Ca, Al, Na, K and P) levels.

**Statistical analysis:** All the strains and the fruiting bodies in these experiments were replicated four times. The samples for the experiments were arranged in a completely randomized design. Data were statistically analyzed using the Duncan's multiple range test using the Statistical Analysis System (SAS, 2010) (Version 9.2, Second Edition) and the p values less than or equal to 0.05 were considered to be not significantly different.

#### RESULTS AND DISCUSSION

Minerals and heavy metals in shiitake mycelium: The concentration of mineral elements Na, K, P, Mg and Al of the four shiitake strains showed that most abundant minerals found in all strains were Na, K and P (Table 2). Generally, regardless of the strain, the Na, K, P and Mg levels in all mycelia ranged from 1646-5322, 5379-10320, 3201-8184 and 295.4-1232 mg kg<sup>-1</sup>, respectively. The Al levels in mycelia strains amounts to 7.6-17.5 mg kg<sup>-1</sup>. Comparison of mineral

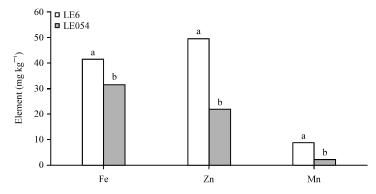


Fig. 1: Fe, Zn and Mn distribution in heat-resistant shiitake mycelia (LE 06 and LE 054). Means with the same letter within Fe, Zn or Mn distributions are not significantly different at p<0.05

Table 2: Mineral levels in the mycelium of shiitake strains

Strains		Mineral (mg kg <sup>-1</sup> )								
	 Mg	Ca	Al	Na	К	P				
LE 6	498.8 <sup>cb</sup>	440.5b	14.9 <sup>ba</sup>	4346 <sup>ba</sup>	5379b	3201 <sup>b</sup>				
LE 013	$1232^{a}$	1695.0 <sup>a†</sup>	17.5ª	5322ª	8810 <sup>ba</sup>	6011 <sup>ba</sup>				
LE 015	$726.5^{\rm b}$	1178.0ª	$11.2^{\mathrm{ba}}$	$1646^{\rm b}$	10320 <sup>a</sup>	8184ª				
LE 054	$295.4^{\circ}$	$284.0^{b}$	7.6 <sup>b</sup>	2379 <sup>ba</sup>	6859 <sup>ba</sup>	$3262^{\rm b}$				

 $<sup>^{\</sup>dagger}$ Means with the same letter within a column are not significantly different at p<0.05

Table 3: Heavy metal levels in the mycelium of shiitake strains

Strains	$Metal (mg kg^{-1})$								
	 Pb	Cu	Zn	Mn	Ni	Fe	Co	Cd	
LE 6	1.52 <sup>a†</sup>	11.5ª	21.9 <sup>b</sup>	8.9 <sup>b</sup>	0.90 <sup>ba</sup>	41.6ª	0.02ª	0.02a	
LE 013	2.50ª	10.4ª	$19.0^{\rm b}$	48.5ª	$1.10^{a}$	38.2ª	$\mathrm{ND}^{\ddagger}$	ND	
LE 015	0.17ª	9.2ª	$22.2^{b}$	39.7ª	$0.39^{b}$	35.6ª	ND	ND	
LE 054	$0.02^{a}$	12.7ª	49.7ª	$2.2^{b}$	$0.47^{\rm b}$	$31.8^{a}$	ND	ND	

<sup>†</sup>Means with the same letter within a column are not significantly different at p<0.05, †Not detected

content in mycelium of heat-tolerant strains LE 6 and LE 054 indicated that LE 054 accumulated significantly higher levels of Ca and Mg relative to LE 6 (p<0.05). The Ca and Mg levels in LE 6 were 36 and 41% higher than LE 054.

Trace/heavy metal content of the 4 shiitake strains studied is given in Table 3. The Zn levels in the shiitake strains varied between (19.2-49.7 mg kg<sup>-1</sup>) with a significantly high Zn level (49.7 mg kg<sup>-1</sup>) found in the heat-tolerant strain, LE 054, compared to the other 3 strains. Comparison of the Zn levels in two heat-resistance strains suggests the LE 6 having significantly higher Zn level (56%) relative to LE 054 (Fig. 1). Both Zn and Fe in growth media may act as stimulators for mycelial growth (Das, 2005). The Mn levels in the heat-resistant strains (LE 6 and LE 054) were significantly lower than the other two strains, LE 013 and LE 015. The amount of Mn in LE 013 and LE 015 were in the range of 39.7-48.5 mg kg<sup>-1</sup> while the Mn levels in the heat-resistant-strains were in the range of 2.2-8.9 mg kg<sup>-1</sup> with LE 6 containing 75% higher Mn level than LE 054 (Fig. 1).

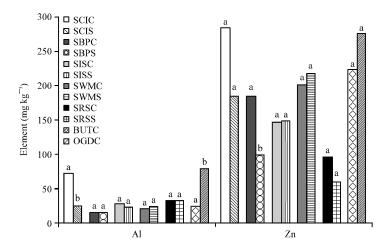


Fig. 2: Distribution of Al and Zn in caps and stems of shiitake mushrooms, button and oyster mushroom<sup>†</sup>. <sup>†</sup>Means with the same letter within distribution of Al or Zn in SCIC/SCIS, SBPC/SBPS, SISC/SISS, SWMC/SWMS, SRSC/SRSS or BUTC/OGDC are not significantly different at p<0.05

The Fe levels in the four shiitake strains varied between 31.8-41.6 mg kg<sup>-1</sup> with no significant differences between the strains. However, comparison of the Fe levels in heat-resistant strains indicate that LE 6 having higher Fe content (24%) relative to LE 054 (Fig. 1). The Cu (9.2-12.7 mg kg<sup>-1</sup>) and Pb (0.02-2.5 mg kg<sup>-1</sup>) levels were not significantly different among the strains. Very low levels of Ni (0.39-1.1 mg kg<sup>-1</sup>) was detected in all four strains. The two remaining heavy metals Co and Cd were only detected in the strain LE 6 at a low concentration of 0.02 mg kg<sup>-1</sup>. Little information is available on actual uptake of nutrients and heavy metals by mycelia itself. In addition, only limited information exists on mechanism of metal translocation from the mycelia to the fruiting body (Das, 2005). Previous studies on trace element (Cu, Zn and Fe) accumulation in both shiitake and oyster mushroom mycelia have indicated that accumulation of certain elements depends on the pH of the media with a decrease in accumulation of metals at acidic pH (Regula and Siwulski, 2007). The heavy metal sorption capacity of mushroom may depend on several other factors. Effect of temperature on the sorption capacity of Hg, Cd and Zn has been studied using live and heat inactivated L. edodes pellets (Bayramoglu and Arica, 2008). Higher sorption capacity for these metals has been found in the heat inactivated fungus compared to the native fungus (Bayramoglu and Arica, 2008). Heat inactivation is suggested to create additional sorption sites through denaturation of proteins in the cell wall structure.

Minerals in mushroom collection: Mineral contents of mushrooms obtained from oriental grocery stores, as well as mushrooms grown locally (Table 1) were analyzed and are given in Table 4. Six mineral elements, Mg, K, P, Ca, Al and Na were analyzed and detected in the fruiting bodies of these mushrooms. Distribution of minerals in caps and stems of shiitake mushrooms, showed that K (13.2-62.2 g kg<sup>-1</sup>), P (7.8-54.5 g kg<sup>-1</sup>) and Mg (3.4-6.5 g kg<sup>-1</sup>) as the most abundant elements followed by Na (191.3-3448 mg kg<sup>-1</sup>), Ca (179.8-1699 mg kg<sup>-1</sup>) and Al (15.3-79.5 mg kg<sup>-1</sup>). In addition, comparison across the mushroom collection also showed that the caps of button mushroom (BUTC) grown at the experimental station contain significantly higher amounts of P (54.5 g kg<sup>-1</sup>), Na (3348 mg kg<sup>-1</sup>) and K (62.2 g kg<sup>-1</sup>), relative to other

Table 4: Mineral distribution of mushroom collections<sup>†</sup>

Mushroom	$\mathrm{Mg}~(\mathrm{g~kg^{-1}})$	$\mathrm{Ca}(\mathrm{mg}\mathrm{kg}^{-1})$	$\mathrm{Al}\ (\mathrm{mg}\ \mathrm{kg}^{-1})$	Na (mg kg <sup>-1</sup> )	$\mathrm{K}~(\mathrm{g~kg^{-1}})$	P (g kg <sup>-1</sup> )
SCIC	4.7 <sup>ba</sup>	260.7°	71.8ª	279.3 <sup>cd</sup>	$23.1^{ m cbd}$	34.50 <sup>cbd</sup>
SCIS	5.8 <sup>ba</sup>	949.2 <sup>bac</sup>	$25.6^{\rm cb}$	$357.4^{ m cbd}$	$18.4^{ m cd}$	$33.80^{\mathrm{cbd}}$
SBPC	$6.5^{a\dagger}$	$285.4^{ m bc}$	$15.3^{\circ}$	592.5°bd	$29.1^{\rm cb}$	39.60 <sup>b</sup>
SBPS	5.6 <sup>ba</sup>	$304.7^{\mathrm{bc}}$	$15.3^{\circ}$	$194.6^{d}$	13.3 <sup>d</sup>	$25.40^{ m cebd}$
SISC	6.1ª	$220.8^{\circ}$	$28.3^{\mathrm{cb}}$	$247.4^{ m cd}$	$21.2^{\mathrm{cd}}$	$35.50^{\mathrm{cbd}}$
SISS	$4.3^{\text{ba}}$	$220.1^{\circ}$	$22.7^{\mathrm{cb}}$	$191.3^{d}$	$22.5^{ m cbd}$	$19.40^{ m efd}$
SWMC	4.8 <sup>ba</sup>	$179.8^{\circ}$	$20.6^{\rm cb}$	$611.9^{\text{cb}}$	36.8 <sup>b</sup>	$38.40^{\rm cb}$
SWMS	4.2 <sup>ba</sup>	$286.3^{bc}$	$24.7^{ m cb}$	$709.4^{b}$	$31.6^{\rm cb}$	$35.40^{\mathrm{cbd}}$
SRSC	$4.3^{\mathrm{ba}}$	$184.3^{\circ}$	$33.2^{b}$	$359.1^{ m obd}$	$19.5^{\rm cd}$	$14.10^{ m ef}$
SRSS	$3.4^{b}$	$1092.0^{ba}$	32.8 <sup>b</sup>	$294.7^{\rm cd}$	$13.2^{d}$	7.80 <sup>f</sup>
BUTC	6.1ª	1699.0ª	$24.3^{ m cb}$	$3448.0^{a}$	62.2ª	54.50 <sup>a</sup>
OGDC	5.2 <sup>ba</sup>	$460.9^{bc}$	79.5ª	339.5°bd	$21.6^{\rm cd}$	$22.54^{ m cefd}$

<sup>†</sup>Means with the same letter within a column are not significantly different at p<0.05

mushroom. Among the mushroom collection, preferential accumulation of some elements can be seen in either caps or stems of certain mushrooms. For instance, mushrooms obtained from grocery stores have higher K content in caps relative to stems. The K content in caps (SCIC and SBPC) was 21-54% higher relative to stems (SCIS and SBPS). In addition, preferential accumulation of Na can be observed in caps (SBPC and SISC) relative to stems (SBPS and SISS) with 23 and 67% higher Na levels in SISC and SBPC, respectively. Comparison of the mineral content in button and oyster mushrooms shows significant variation for Ca, Na and K, in which the Na and K levels were 90% and 65% higher in oyster mushroom than button mushroom; whereas, the Ca levels in button mushroom was 72% higher than oyster mushroom. No significant differences were found in Mg levels of button and oyster mushroom caps. The Ca, Na, K and P levels in caps of button mushroom (BUTC) were 73, 90, 65 and 59% higher than oyster mushroom (OGDC), respectively, indicating higher nutritional value in these locally grown button mushrooms relative to oyster mushroom. Mineral elements and trace elements that are essential for the growth of mushrooms are generally supplied by the media or the substrate. Variations in mineral content of mushrooms may depend on the type of mushroom or the substrate in which they were grown, as well as other factors including species and age of mushrooms and pilei/substratum diameter. Accumulation of greater amounts of elements in pileus than in the stipe has been observed with several mushroom species (Demirbas, 2001; Falandysz et al., 2001; Mattila et al., 2001; Bernas et al., 2006).

As shown in Fig. 2, significantly higher accumulation of Al was seen in oyster mushroom caps (OGDC) relative to button mushroom caps (BUTC). The Al level in BUTC and OGDC were 24.3 and 79.5 mg kg<sup>-1</sup>, respectively, indicating that Al content in BUTC is 70% higher than OGDC. In addition, significant variation in distribution of Al was also found in shiitake mushroom caps (SCIC) and stems (SCIS) obtained from a grocery store, in which the caps contained 69% more Al than the stems. The Al content in other shiitake mushroom locally grown on hardwood bark contained Al ranging from 20.6-33.2 mg kg<sup>-1</sup> distributed in caps and stems with no preferential accumulation in caps. In previous studies, species such as *Amanita rubescens*, *Leccinum scabrum*, *Xerocomus chrysenteron* were reported as Al accumulators grown in unpolluted areas and the Al levels in the fruiting bodies of these mushrooms were in the range of 20-150 mg kg<sup>-1</sup> (Kalac, 2013). Therefore, some of the mushrooms analyzed in our study can be considered as potential Al accumulators for phytoremediation of Al.

Table 5: Heavy metal levels of mushroom collections<sup>†</sup>

Mushroom	Heavy me	Heavy metals (mg kg <sup>-1</sup> )								
	Pb	Cu	Zn	Mn	Ni	Fe	Co	Cd		
SCIC	1.8 <sup>ba</sup>	55.7b	283.9ª	44.8 <sup>bc</sup>	0.33ª	97.6 <sup>ba</sup>	$0.05^{b}$	3.70ª		
SCIS	0.86 <sup>ba</sup>	25.3 <sup>cd</sup>	$184.7^{ m bc}$	60.2 <sup>ba</sup>	$\mathrm{ND}^{\ddagger}$	$60.7^{\circ}$	ND	$0.66^{\rm cbd}$		
SBPC	$1.2^{\mathrm{ba}}$	$44.1^{\rm cb}$	$184.8^{\mathrm{bc}}$	$45.7^{\rm bc}$	$0.18^{a}$	55.0°	ND	$1.70^{\mathrm{cb}}$		
SBPS	0.66 <sup>ba</sup>	$26.5^{ m cd}$	$99.4^{\mathrm{de}}$	25.9 <sup>cd</sup>	$0.24^{a}$	$44.4^{\circ}$	ND	$0.69^{\rm cbd}$		
SISC	$1.2^{\mathrm{ba}}$	$19.2^{ m cd}$	$146.7^{ m dc}$	$42.7^{\mathrm{bc}}$	$0.06^{a}$	50.5°	$0.02^{b}$	$1.40^{ m cbd}$		
SISS	ND	$13.7^{d}$	$148.9^{\mathrm{dc}}$	$41.9^{\mathrm{bc}}$	0.02ª	58.2°	ND	$1.70^{ m cbd}$		
SWMC	0.86 <sup>ba</sup>	$40.4^{ m cbd}$	$200.3^{bc}$	$18.2^{d}$	$0.54^{a}$	55.1°	$0.30^{b}$	$0.71^{\rm cbd}$		
SWMS	ND	$37.4^{ m cbd}$	218.1 <sup>ba</sup>	38.3 <sup>bc d</sup>	0.23ª	$53.4^{\circ}$	0.69ª	$1.3^{ m cbd}$		
SRSC	$0.14^{b}$	$24.2^{ m cd}$	$96.2^{de}$	66.8ª	$0.50^{a}$	52.5°	$0.71^{a}$	$2.00^{b}$		
SRSS	ND	$19.8^{ m cd}$	59.3°	73.2ª	$0.32^{a}$	57.4°	$0.87^{a}$	$0.48^{\rm cd}$		
BUTC	$0.42^{\mathrm{ba}}$	182.4ª	223.9 <sup>ba</sup>	$18.7^{ m d}$	$0.11^{a}$	69.9 <sup>bc</sup>	ND	$0.27^{\rm d}$		
OGDC	$2.2^{a}$	$21.9^{ m cd}$	275.8ª	31.9°d	0.38ª	$125.1^{a}$	$0.09^{b}$	$1.50^{ m cbd}$		

†Means with the same letter within a column are not significantly different at p<0.05, ‡Not detected

To some degree, the major element levels in the mushroom collection in our study followed the pattern observed by other researches (Mattila et al., 2001; Gulati et al., 2011; Manjunathan and Kaviyarasan, 2011; Liu et al., 2012; Mallikarjuna et al., 2013). Major element content in mushrooms varies and the ranges reported in literature as mg per 100 g dry weight basis are K (2500-1400), P (120-2000), Ca (1.8-59.0), Na (6.0-92), Mg (60-250) and Mg (60-250) (Mallikarjuna et al., 2013). Normally, most prevalent nutrient elements in fruiting bodies of mushrooms were reported as K, Ca, P and Mg with low availability of Na (Mallikarjuna et al., Fruiting bodies of L. edodes grown on sawdust were reported to contain K (1302 mg 100 g<sup>-1</sup>  $\pm$ 101), P (769.9 mg 100 g<sup>-1</sup>  $\pm$ 64.1), Ca (174.9 mg 100 g<sup>-1</sup>  $\pm$ 34.3), Na  $(327.4 \text{ mg } 100 \text{ g}^{-1} \pm 51.6)$  and Mg  $(40.7 \text{ mg } 100 \text{ g}^{-1} \pm 1.2)$  levels. On the other hand, the mineral content of wild grown shiitake mushroom L. cadopus was reported to contain much lower concentrations of these elements (Mallikarjuna et al., 2013). High levels of K (7.53 mg g<sup>-1</sup>) followed by Ca  $(2.66 \text{ mg g}^{-1})$ , Mg  $(2.45 \text{ mg g}^{-1})$  and Na  $(1.30 \text{ mg g}^{-1})$  have been detected in cultivated edible oyster mushroom L. tuberregium grown in India (Manjunathan and Kaviyarasan, 2011). In addition, wild grown Lentinus species from India appear to have mineral contents that vary with the species in which L. conatus contained high Ca and Mg; L. squarrosulus, high Na and L. cladopus, high K levels (Gulati et al., 2011). These studies, along with our study, provide information that variation of nutritional elements in fruiting bodies of mushroom may vary with the species and composition of substrate.

Heavy metals in mushroom collection: The heavy metal concentrations in the mushroom collection are given in Table 5. The trace element distribution shows Zn (59.3-283.9 mg kg<sup>-1</sup>) as the most abundant metal found in the mushrooms studied here followed by Fe (44.4-125.1 mg kg<sup>-1</sup>), Mn (18.2-73.2 mg kg<sup>-1</sup>) and Cu (13.7-182.4 mg kg<sup>-1</sup>). Only trace amounts of toxic heavy metals, Cd (0.48-3.7 mg kg<sup>-1</sup>), Co (0-0.87 mg kg<sup>-1</sup>), Ni (0-0.54 mg kg<sup>-1</sup>) and Pb (0-1.8 mg kg<sup>-1</sup>) were found in our mushroom collection. Comparison of metal contents across the whole mushroom collection in our study does not show any statistically significant differences for preferential accumulation of metals in any particular mushroom. Mushrooms are considered Zn accumulators and high Zn concentrations have been observed with several mushroom species (Bano *et al.*, 1981; Kalac and

Svoboda, 2000; Isildak et al., 2004). Copper concentrations ranging from 100-300 mg kg<sup>-1</sup> per dry weight has been reported for Cu accumulating mushroom species and these levels are not considered as levels for health risks (Kalac and Svoboda, 2000; Soylak et al., 2005). Zhu et al. (2011) report Fe as the most abundant metal found in a wild edible mushroom collection from China. Accumulation of Zn, Cu and Sn in fruiting bodies of wild grown mushrooms has been shown to depend on the metal content of soil (Elekes et al., 2010).

Specific accumulation of heavy metals in pileus than in the stipe has been reported for metals such as Cu, Mn, Fe, Ni and Cd in edible mushrooms (Latiff et al., 1996; Elekes et al., 2010; Ayodele and Odogbili, 2010). Elekes et al. (2010) observed higher concentration of Cu accumulated in stipe; whereas, Zn accumulation was higher in the caps of some wild growing edible mushrooms. In our study, as with the mineral distribution, preferential accumulation of certain elements in caps can be seen with some mushrooms (Fig. 2). For instance, significantly high levels of Zn, Cu and Pb were found in caps (SBPC) relative to stems (SBPS) in mushrooms obtained from a grocery store (Table 5). The Zn and Cu levels in the caps of this mushroom were 46 and 40% higher relative to the stems. In addition, caps of one of the shiitake mushrooms (SISC) grown on sawdust also showed significantly higher accumulation of Cu with 29% higher Cu level in caps relative to stems (SISS). However, majority of the mushroom collection does not show selective accumulation of metals in caps relative to the stems.

Comparison between button and oyster mushroom caps shows 82% higher concentration of Cu in button mushroom relative to oyster mushroom. In addition, the Mn level in oyster mushroom was 41% higher than the button mushroom. Comparison between button and oyster mushroom caps shows 88% higher concentration of Cu in button mushroom relative to oyster mushroom.

Heavy metals have been detected in many wild edible mushroom species (Latiff et al., 1996; Mattila et al., 2001; Zhu et al., 2011; Radulescu et al., 2010; Ayodele and Odogbili, 2010; Stihi et al., 2011). Previous study on heavy metal content in L. edodes grown on sawdust provides data that demonstrate Fe, Zn, Cu, Mn and Ni levels of 14.8±2.3, 9.44±0.24 and 1.48±0.03 1.00±0.32 mg per 100 g dry weight basis, respectively (Mallikarjuna et al., 2013). Higher Fe content (35.3 mg 100 g<sup>-1</sup>) and much lower concentration for other elements were also reported by Mallikarjuna et al. (2013) for the wild grown L. cadopus. In addition, significantly lower levels of toxic metals had been found in cultivated mushrooms relative to wild-grown mushrooms of similar species or taxonomically related mushrooms (Kalac and Svoboda, 2000). Comparison of our findings with similar studies reported agrees with the facts that heavy metal absorbing capacity of mushrooms may depend on the substrate, age of mycelium, selective uptake and capability of storage of such elements in tissues and mushroom species (Latiff et al., 1996; Demirbas, 2001; Kalac and Svoboda, 2000; Mattila et al., 2001; Isiloglu et al., 2001; Zhu et al., 2011; Ayodele and Odogbili, 2010; Gulati et al., 2011; Stihi et al., 2011).

#### CONCLUSION

This study provides information on nutritional values of an edible shiitake, button and oyster mushroom collection and selective accumulation of certain nutrients, trace elements/heavy metals in caps relative to stems of these mushrooms. Information is also provided on nutrient and metal accumulation of *L. edodes* mycelia strains grown under laboratory conditions on a defined media. The Al and Zn accumulating ability of shiitake mushrooms should be further investigated to evaluate them as potential Al and Zn accumulators, especially if the substrates utilized have and anthropomorphic origin. In addition, health effects associated with relatively high levels of Al in certain edible mushrooms will be of concern. Our study as well as findings by several other

researchers suggests that *L. edodes* can be considered safe when they are grown in defined culture media or in log and sawdust growing environments. At the same time, we must remain vigilant for toxic heavy metal accumulation in edible species should always be our concern due to health risks.

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#### REFERENCES

- Ayodele, S.M. and O.D. Odogbili, 2010. Metal impurities in three edible mushrooms collected in Abraka, Delta State, Nigeria. Micologia Aplicada Internacional, 22: 27-30.
- Baldrian, P., 2003. Interactions of heavy metals with white-rot fungi. Enzyme Microb. Technol., 32: 78-91.
- Bano, Z., K. Nagaraja, S. Vibhakar and O.P. Kapur, 1981. Mineral and the heavy metal contents in the sporophores of pleurotus species. Mushroom Newslett. Trop., 2: 3-7.
- Bayramoglu, G., G. Celik, E. Yalcin, M. Yilmaz and M.Y. Arica, 2005. Modification of surface properties of Lentinus sajor-caju mycelia by physical and chemical methods: Evaluation of their Cr<sup>6+</sup> removal efficiencies from aqueous medium. J. Hazard. Mater., 119: 219-229.
- Bayramoglu, G. and M.Y. Arica, 2008. Removal of heavy mercury (II), cadmium (II) and zinc (II) metal ions by live and heat inactivated *Lentinus edodes* pellets. Chem. Eng. J., 143: 133-140.
- Bernas, E., G. Jaworska and Z. Lisiewska, 2006. Edible mushrooms as a source of valuable nutritive constituents. Acta Sci. Pol. Technol. Aliment., 5: 5-20.
- Damodaran, D., K.V. Shetty and B.R. Mohan, 2014. Uptake of certain heavy metals from contaminated soil by mushroom-*Galerina vittiformis*. Ecotoxicol. Environ. Saf., 104: 414-422.
- Das, N., 2005. Heavy metals biosorption by mushrooms. Nat. Prod. Radiance, 4: 454-459.
- Demirbas, A., 2001. Concentrations of 21 metals in 18 species of mushrooms growing in the East Black Sea region. Food Chem., 75: 453-457.
- Elekes, C.C., G. Busuioc and G. Ionita, 2010. The bioaccumulation of some heavy metals in the fruiting body of wild growing mushrooms. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38: 147-151.
- Falandysz, J., K. Szymczyk, H. Ichihashi, L. Bielawski, M. Gucia, A. Frankowska and S. Yamasaki, 2001. ICP/MS and ICP/AES elemental analysis (38 elements) of edible wild mushrooms growing in Poland. Food Addit. Contamin., 18: 503-513.
- Gadd, G.M., 1993. Interactions of fungi with toxic metals. New Phytol., 124: 25-60.
- Gast, C.H., E. Jansen, J. Bierling and L. Haanstra, 1988. Heavy metals in mushrooms and their relationship with soil characteristics. Chemosphere, 17: 789-799.
- Gulati, A., N.S. Atri, S.K. Sharma and B.M. Sharma, 2011. Nutritional studies on five wild *Lentinus* species from North-West India. World J. Dairy Food Sci., 6: 140-145.
- Hatvani, N. and I. Mecs, 2003. Effects of certain heavy metals on the growth, dye decolorization and enzyme activity of *Lentinula edodes*. Ecotoxicol. Environ. Safety, 55: 199-203.
- Isildak, O., I. Turkekul, M. Elmastas and M. Tuzen, 2004. Analysis of heavy metals in some wild-grown edible mushrooms from the middle black sea region, Turkey. Food Chem., 86: 547-552.

- Isiloglu, M., F. Yilmaz and M. Merdivan, 2001. Concentrations of trace elements in wild edible mushrooms. Food Chem., 73: 169-175.
- Kalac, P. and L. Svoboda, 2000. A review of trace element concentrations in edible mushrooms. Food Chem., 69: 273-281.
- Kalac, P., 2013. A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. J. Sci. Food Agric., 93: 209-218.
- Latiff, L.A., A.B.M. Daran and B.M. Mohamed, 1996. Relative distribution of minerals in the pileus and stalk of some selected edible mushrooms. Food Chem., 56: 115-121.
- Liu, Y.T., J. Sun, Z.Y. Luo, S.Q. Rao, Y.J. Su, R.R. Xu and Y.J. Yang, 2012. Chemical composition of five wild edible mushrooms collected from Southwest China and their antihyperglycemic and antioxidant activity. Food. Chem. Toxicol., 50: 1238-1244.
- Mallikarjuna, S.E., A. Ranjini, D.J. Haware, M.R. Vijayalakshmi, M.N. Shashirekha and S. Rajarathnam, 2013. Mineral composition of four edible mushrooms. J. Chem. 10.1155/2013/805284
- Manjunathan, J. and V. Kaviyarasan, 2011. Nutrient composition in wild and cultivated edible mushroom, *Lentinus tuberregium* (Fr.) Tamil Nadu., India. Int. Food Res. J., 18: 809-811.
- Manzi, P., L. Gambelli, S. Marconi, V. Vivanti and L. Pizzoferrato, 1999. Nutrients in edible mushrooms: An inter-species comparative study. Food Chem., 65: 477-482.
- Manzi, P., A. Aguzzi and L. Pizzoferrato, 2001. Nutritional value of mushrooms widely consumed in Italy. Food Chem., 73: 321-325.
- Mattila, P., K. Konko, M. Eurola, J.M. Pihlava and J. Astola *et al.*, 2001. Contents of vitamins, mineral elements and some phenolic compounds in cultivated mushrooms. J. Agric. Food Chem., 49: 2343-2348.
- Radulescu, C., C. Stihi, G. Busuioc, A.I. Gheboianu and I.V. Popescu, 2010. Studies concerning heavy metals bioaccumulation of wild edible mushrooms from industrial area by using spectrometric techniques Bull. Environ. Contam. Toxicol., 84: 641-646.
- Regula, J. and M. Siwulski, 2007. Dried shiitake (*Lentinulla edodes*) and oyster (*Pleurotus ostreatus*) mushrooms as a good source of nutrient. Acta Sci. Pol. Technol. Aliment., 6: 135-142.
- SAS., 2010. Statistical analysis system software version 9.2, second edition. SAS Institute Inc., Cary, NC., USA.
- Schroeder, H.A., 1973. The Trace Elements and Nutrition. 1st Edn., Faber and Faber, London, pp: 13-17.
- Soylak, M., S. Saracoglu, M. Tuzen and D. Mendil, 2005. Determination of trace metals in mushroom samples from Kayseri, Turkey. Food Chem., 92: 649-652.
- Stamets, P., 2005. Mycelium Running: How Mushrooms can Help Save the world. 1st Edn., Ten Speed Press, Berkeley, CA., ISBN-13: 978-1580085793, Pages: 356.
- Stihi, C., C. Radulescu, G. Busuioc, I.V. Popescu, A. Gheboianu and A. Ene, 2011. Studies on accumulation of heavy metals from substrate to edible wild mushrooms. Rom J. Phys., 56: 257-264.
- Tuzen, M., M. Ozdemir and A. Demirbas, 1998. Study of heavy metals in some cultivated and uncultivated mushrooms of Turkish origin. Food Chem., 63: 247-251.
- Tuzen, M., 2003. Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry. Microchem. J., 74: 289-297.
- Zhu, F., L. Qu, W. Fan, M. Qiao, H. Hao and X. Wang, 2011. Assessment of heavy metals in some wild edible mushrooms collected from Yunnan Province, China. Environ. Monitior. Assess., 179: 191-199.