



Bioaccumulation of heavy metals in the sporocarps of some wild mushrooms

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Abstract

The fruiting bodies of wild mushrooms show high concentrations of heavy metals due to their efficacious mechanism of accumulation of these elements from soil. In accordance with this ability, three wild macrofungi viz., *Macrolepiota procera* (edible species), *Amanita augusta* (non-edible species), *Boletus subvelutipes* (poisonous species) and their respective soil samples collected from different forest areas of Jammu Province were analysed for the presence of six heavy metals (Zn, Cu, Mn, Fe, Cd, Pb). The metallic content was established by Atomic absorption spectrophotometer (AAS) and Inductively coupled Plasma–Atomic Emission spectrometry method (ICP AES). The results of heavy metals concentration are given in mg of metal per kg of dry matter and demonstrate important variation between the level of concentration in edible, non edible and toxic species. Moreover, the mean element distribution varied depending upon the part of fruiting body of macrofungi. In *A. augusta* these elements were detected under permissible limits for consumption. On the contrary, high ratio of zinc, copper, manganese, iron, lead and cadmium were found in *A. augusta* and *B. subvelutipes* indicating that these elements are accumulated at much higher levels in these wild growing mushrooms. Thus, it is worthwhile to evaluate the metal content in the wild macrofungi to assess their contribution to the daily intake of several toxic elements so that it would adjudicate the mushrooms for its nutritive value in terms of minerals and also define the limits of safety. As mushrooms have been known to possess good nutraceutical and pharmaceutical potential, a detailed analysis of their sporocarps is desirable before their incorporation into routine diet, drugs and medicine.

Key words – Antioxidant – edibility – elements – macrofungi – pharmaceutical

Introduction

Wild growing mushrooms form an integral part of our ecosystem. Earlier, the fruiting bodies of these non green edibles were appreciated for their texture, aroma and flavour, but now their usage is undergoing a paradigm shift as a lot of work has been focussed on their biochemical characterization (Manzi et al. 2001, Keles et al. 2011, Yildiz et al. 2015). They are considered as a delicacy with high nutritional and functional value and are also accepted as nutraceutical foods, because of presence of many bioactive compounds and minerals like zinc, manganese, copper, selenium and iron. Also, keeping in view the fact that food security to the ever exploding world's population is going to be a major challenge of 21st century, mycologist world over are exploring ways to aware people regarding

the nutritive value of edible mushrooms and accelerate their transformation from consuming green to these non green edibles. However, research during the past three decades on nutritive aspects of wild macrofungi has exposed their negative facet too.

The sporocarps of wild mushrooms have been found to accumulate several heavy metals predominantly the iron, arsenic, mercury, cadmium, zinc and lead in concentration above threshold. Heavy metals are defined as metallic elements that have relatively high density compared to water. It has been reported that metals such as cobalt, copper, chromium, iron, magnesium, molybdenum, selenium and zinc are essential nutrients required for various physiological functions of the body. Other metals such as mercury, nickel, silver, gallium, cadmium, aluminium have no established biological functions and are considered as non essential metals. Also, for these metals, there is a very narrow range of concentration between beneficial and toxic effects and when present above threshold level may pose serious threat to human health leading to morphological abnormalities, reduced growth and increased mortality (Olumuyiwa et al. 2007, Elekes et al. 2010, Radulescu et al. 2010).

Macrofungi have been considered as natural soil component that have a great significance in reduction of soil pollution with heavy metals. The presence of rich network of hyphae in the upper horizon of the soil explains the fact that these vegetal organisms have specific genetically controlled ability to accumulate heavy metals from their substratum (Gadd, 1993). Soil may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, land application of fertilizers, coal combustion residues and spillage of petrochemicals. The presence of metals in the fruiting bodies of mushrooms directly reflects their amount in the soil and their bioaccumulation factor depicts the capacity of mushrooms to accumulate these elements from the soil. The concentration of heavy metals in the fruiting bodies of macrofungi is assumed to be species dependent but other edaphic factors like soil pH, organic matter and clay content also play a cardinal role in accumulation and translocation of these heavy metals in mushrooms (Kalac & Svoboda, 2000, Gursoy et al. 2009, Frankowska et al. 2010).

Literature reveals that heavy metal contamination through wild edible macrofungi to be one of the prime contributors to various disorders that are posing serious problems to human health (Das, 2005, Radulescu et al. 2010). Moreover, the inability of human system to excrete them through homeostatic filtration mechanism further leads to their accumulation in the body that eventually complicates the situation. Worldwide several workers have investigated the heavy metal concentration in many wild growing mushrooms (Isildak et al. 2004, Elekes et al. 2010, Magdziak et al. 2013, Mazurkiewicz & Podlasinska, 2014, Dulay et al. 2015). However, from India, few scanty studies have been carried out on heavy metal accumulation by wild macrofungi (Das, 2005, Mallikarjuna et al. 2013) and as far as our literature survey could ascertain no work has been done on heavy metal estimation from the state so far.

Thus, the present investigation was undertaken for analysis of six heavy metals (Zn, Cu, Mn, Fe, Cd, Pb) in three wild mushrooms viz., *Macrolepiota procera* (Scop.) Singer (edible species), *Amanita augusta* Bojantchev & R. M. Davis (non-edible species), *Boletus subvelutipes* Peck (poisonous species) collected from different regions of Jammu Province. In the perspective that wild macrofungi significantly contribute to the minerals in the diet, the data would serve as an useful basis to define the nutritive value of mushrooms along with its potent toxicity in certain species.

Materials & Methods

Biological Material

Three species of wild mushrooms collected from different forest areas of Jammu province (Udhampur and Patnitop) were identified as *Macrolepiota procera* (edible species), *Amanita augusta* (non-edible species), *Boletus subvelutipes* (poisonous species) (Table 1). The fruiting bodies of these mushroom species were divided into cap and stipe portion and ashed at 100°C in muffle furnace in porcelain dish. In addition to the mushroom collection, soil samples of the upper forest horizon (0-10 cm) after removing the surface layer of the organic detritus were also collected from appropriate sampling places.

Determination of Mineral Contents

Total ash was taken for the analysis of mineral contents. Briefly, 2 mL of concentrated HNO₃ was added to the ash and heated for 2 minutes. One drop of hydrogen peroxide was added into the solution to remove turbidity. The solution was then transferred into a volumetric flask and total volume was made 50 mL by adding deionised water. This was then used to analyze the concentration of zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), cadmium (Cd) and lead (Pb) by flame photometry with atomic absorption spectrophotometer (Perkin Elmer A Analyst 700) and Inductively Coupled Atomic Emission Spectrophotometer (ACTIVIA - M). For estimation of heavy metal in soil samples, hot extractions with nitric acid (1:1) were done.

All the experiments were performed in triplicate and data analysis was done by mean \pm SD of three parallel measurements.

Table 1 Families, habitat and edibility of mushroom species.

Sample	Mushroom species	Family	Habitat	Edibility
1	<i>Macrolepiota procera</i> (Scop.) Singer 1948	Agaricaceae	In open ground	Edible
2	<i>Amanita augusta</i> Bojantchev & R. M. Davis 2013	Amanitaceae	In forest	Non-edible
3	<i>Boletus subvelutipes</i> Peck 1889	Boletaceae	In forest	Poisonous

Results and Discussion

In the present investigation, three wild mushrooms namely *Macrolepiota procera* (edible mushroom), *Amanita augusta* (non-edible mushroom) and *Boletus subvelutipes* (poisonous mushroom) were investigated for the presence of six heavy metals (Zn, Cu, Mn, Fe, Cd, Pb). The results of the heavy metals investigated in the collected wild mushrooms and their soil samples have been illustrated in Table 2.

The concentration of zinc ranged from 87.2 \pm 1.18 mg/kg to 299.0 \pm 0.86 mg/kg being lowest in *M. procera* and highest in *B. subvelutipes*. The concentration of this metal was found to be 211.1 \pm 0.86 mg/kg in *A. augusta* (Table 2 and Fig 1). Zinc was found to be accumulating more in the cap region than stipe portion depicting its higher rate of translocation in the sporocarps of wild mushrooms. Furthermore, as evident from the Table 1, a steep intensification in the bioaccumulation of this metal from the soil was observed in the toxic species as compared to edible and non edible species. Permissible range of Zn in mushrooms has been limited between 28.6 to 179.0 mg/kg (Tuzen et al. 2003). Zinc is one of the exigent trace elements and the chronology of events in the research of zinc biology is revealing its ever increasing variety of functions. Zinc forms an integral part of many enzymes like alcohol dehydrogenase, carboxypeptidases and other metalloproteins. It is also found to support a healthy immune system. Nevertheless, the concentration above threshold may pose serious threat to human health as excess of zinc can affect cardiac function, disturb copper metabolism and reduced immune function (Zhu et al. 2011).

The concentration of copper was found to be highest in poisonous species of *B. subvelutipes* (290.0 \pm 1.08 mg/kg) followed by *A. augusta* (218.0 \pm 1.65 mg/kg) and least in *M. procera* (83.6 \pm 0.20 mg/kg) (Table 2 and Fig 1). Cu was detected to be accumulating more in the cap region for *A. augusta* and *B. subvelutipes* and in the stipe portion for *M. procera*. In comparison to the edible species, the copper concentration showed a gradual increase from non edible to poisonous species. Copper concentration in the wild macrofungi has been reported to be in the range of 100-300 mg/kg of dry matter which is not considered to be a risk for human consumption (Kalac & Svoboda, 2000). Under permissible range, this metal is required for normal metabolism as it is a component of haemocyanin, the blue, oxygen carrying blood pigment and is a prerequisite for the synthesis of haemoglobin, the red, oxygen carrying pigment found in the blood. However, elevated levels of copper may lead to copper toxicity that progenerate many disorders like hypoglycaemia, arthritis and low blood pressure (Zahorcova et al. 2016).

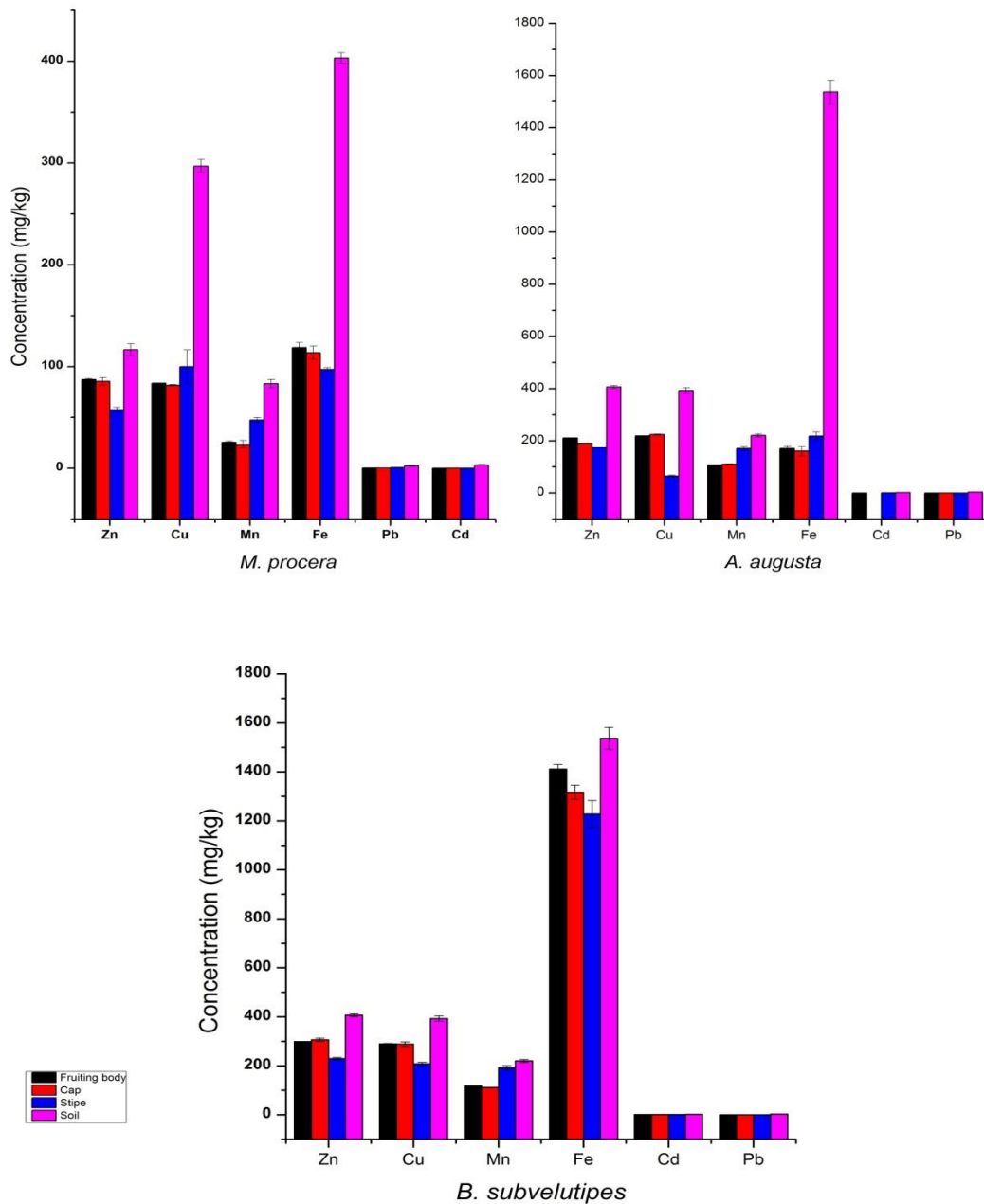


Fig. 1 – Graph showing concentration of minerals in three wild macrofungi (Zn, Cu, Mn, Fe, Cd, Pb)

Similarly, the concentration of manganese ranged between 25.50 ± 1.20 mg/kg in *M. procera* to 118.2 ± 0.11 mg/kg in *B. subvelutipes* (Table 2 and Fig 1). Also, the level of this element was established to be assembling more in the stipe portion than pileus region depicting its lower range of translocation in the fruiting body of investigated mushrooms. Of the three studied macrofungi, *A. augusta* and *B. subvelutipes* showed higher bioaccumulation efficiency for this element from the soil. The reported manganese values in the literature for wild edible mushrooms are in the range of 5.54-135 mg/kg (Genccelep et al. 2009). Manganese plays pivotal role in carbohydrate metabolism, calcium absorption and blood sugar regulation. As a component of the antioxidant enzyme such as superoxide dismutase (SOD), it helps fighting free radicals. However, higher Mn concentration may lead to various serious and ultimately disabling neurological effects in human (Mallikarjuna et al. 2013, Zahorcova et al. 2016).

Table 2 The results of heavy metal analysis of three wild mushrooms collected from different forest areas in Jammu province.

Mushroom species	Sample/soil analysed	Zn	Cu	Mn	Fe	Cd	Pb
<i>Macrolepiota procera</i>	Fruiting body	87.2±1.18	83.6±0.20	25.5±1.20	118.6±5.01	0.23±0.01	0.046±0.05
	Cap	85.5±3.59	81.7±0.60	23.6±3.80	113.7±6.40	0.24±0.03	0.097±0.00
	Stipe	57.6±2.30	100.0±16.40	47.4±2.55	97.2±1.92	0.65±0.29	0.043±0.01
	Soil *	116.6±5.90	297.0±6.37	83.2±4.27	403.3±5.11	2.49±0.53	3.5±0.36
<i>Amanita augusta</i>	Fruiting body	211.1±0.86	218.6±1.65	108±0.11	171.1±11.21	0.61±0.11	0.41±0.03
	Cap	190.8±0.91	223.9±2.55	111.2±1.16	160.9±19.28	Nd	0.36±0.05
	Stipe	176.3±0.88	64.8±4.16	170.9±9.27	218.2±15.99	0.75±0.17	0.47±0.05
	Soil**	406.6±5.77	393.0±11.26	220.6±6.11	1536.8±44.83	1.67±0.11	3.27±0.53
<i>Boletus subvelutipes</i>	Fruiting body	299±0.86	290±1.08	118.2±0.11	1411.1±18.6	0.71±0.21	0.46±0.05
	Cap	306.5±6.53	288.8±9.23	111.2±1.16	1316.6±28.86	0.76±0.02	0.36±0.05
	Stipe	229.5±4.68	207.7±6.72	190.9±9.27	1228.0±54.83	0.98±0.02	0.48±0.05
	Soil**	406.6±5.77	393.0±11.26	220.6±6.11	1536.8±44.83	1.67±0.11	3.27±0.53

All the values are expressed as mean ±SE; * study area 1 (Udhampur); ** study area 2 (Patnitop).

The value of iron ranged 118.6±5.01 mg/kg in *M. procera* to 1411.6±18.60 mg/kg in *B. subvelutipes* respectively. Pileus of fruiting bodies of *M. procera* and *B. subvelutipes* possessed maximum level of Fe than stipe region. As evident from the Table 2 and Fig 1, the poisonous species of *B. subvelutipes* showed highest bioaccumulation capacity for this element. Iron is vital for normal functioning of R.B.C's and oxygen transport. Also, adequate iron in a diet is very important in order to decrease the incidence of anaemia. On the contrary, the concentration of iron in the body should be tightly regulated as it can form free radical and may cause several neurodegenerative disorders (Nakalembe et al. 2015).

The concentration of cadmium in the investigated mushrooms ranged between 0.23±0.01 mg/kg to 0.71±0.21 mg/kg (Table 2 and Fig 1). Moreover, the cap portion of *A. augusta* showed no results for the presence of cadmium in the cap region. Higher concentration for this element was detected in the stipe portion than pileus region in all the three studied macrofungi. Of the three macrofungi studied, *A. augusta* and *B. subvelutipes* exhibited high accumulation rate for this metal. Cadmium is one of the heavy metals found in trace amount in the fruiting bodies of mushrooms and its higher amounts renders wild macrofungi unfit for human consumption as elevated levels of cadmium may pose a damage to immune system and lead to various psychological disorders (Soylak et al. 2005, Soyлак & Topalak, 2015, Zahorcova et al. 2016).

Another toxic heavy metal namely lead showed maximum concentration in poisonous species of *B. subvelutipes* (0.46 ± 0.05 mg/kg), while as minimum level was observed in the edible species of *M. procera* (0.046 ± 0.05 mg/kg) (Table 2 and Fig 1). Comparatively, the stipe portion of *A. augusta* and *B. subvelutipes* showed higher efficiency for accumulation of this element than the cap region. Lead is a heavy metal that should be present in negligible amount in the cells as it interferes with a variety of body processes and is toxic to many tissues including the heart, bones, kidneys and reproductive systems (Das, 2005, Zahorcova et al. 2016).

Statistically the correlation between the metal content in soil and fruiting bodies of investigated mushroom species were estimated to be lower for Pd and were significant for Cu and Fe i.e. more than 1, which means that these analysed mushrooms are accumulators and hyperaccumulators for these elements. The highest value of Cu and Fe were recorded in *B. subvelutipes*.

The Pearson coefficient between metal content in soil and metal content in sample indicates that metal concentration is influenced by species, morphological part of the fruiting body and soil characteristics. The correlation has significance at level for Zn and Mn at 0.1% ($p < 0.001$) and 5% ($p < 0.005$) for Cu, Cd, Fe, Pb.

Conclusion

Three wild mushrooms viz., *Macrolepiota procera* (edible species), *Amanita augusta* (non-edible species), *Boletus subvelutipes* (poisonous species) were investigated for the presence of six heavy metals (Zn, Cu, Mn, Fe, Cd, Pb) that revealed a significant variation in the capacity for the accumulation of these elements from the soil depending upon the type of mushroom and its part i.e stipe and pileus. The analysed metals were under permissible limit for *M. procera*, thus making it an easily accessible source of proteins and minerals for consumers. However, the other two analysed wild mushrooms (*A. augusta* and *B. subvelutipes*) reflected the presence of heavy metals in excess amounts than threshold concentration. This can be attributed to the fact that these two macrofungi undergo ectomycorrhizal association that in turn increase their efficiency to uptake metals from the soil (Krupa & Kozdroj, 2004). Thus, further extensive assays on these two mushrooms are recommended to scrutinize their level of potent toxicity before dispensing them to pharmaceutical industry for incorporation into drugs and medicine. Our study comprises first report on the heavy metal estimation in these wild mushrooms and, therefore, this data can act as baseline for further research on heavy metal estimation in economically important wild growing mushrooms.

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