Elemental Analysis of Mushroom Flora from Budgam District of Kashmir , India

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Abstract
Three mushroom species *Ganoderma applanatum, Fomes fomentarius* and *Pleurotus ostreatus* from two different sites of the Budgam district were analyzed for the variation in elemental composition. Much variation was not found in the macronutrient content of N, H, S, C and crude protein in the samples from two sites. There was a significant variation in the concentration of micronutrients such as; Cu, Zn, Fe, Mn, Cd and Cr.
Keywords: Trace metals, mushrooms, atomic absorption spectrometry.

Introduction

Mushrooms are mycorrhizal, parasites and saprophyte. They include members from both basidiomycota and ascomycota. Mushrooms have been popular food supplement in many countries and are being cultivated artificially world wide for their edibility and delicacy. Wild growing edible mushroom collection has become a hobby in many cultures for instance, Czech Republic 72% of families collect mushrooms with a mean yearly level of 7 kg per household (Kalac and Svoboda 2000). They fall between the best vegetables and animal protein source. Mushrooms are valuable health foods, low in calories, high in vegetable proteins, iron, zinc, chitin, fibre, vitamins, amino acids and minerals. They also have a long history of use in traditional Chinese medicine (Demirbas, 2001; Mendil *et al.* 2004; Racz *et al.* 1996). Mushrooms are rich sources of essential amino acids, water-soluble vitamins (riboflavin, biotin and thiamine) and essential minerals (Chang and Buswell, 1996; Buigut, 2002). In general, their fruiting bodies on dry weight basis contain about 39.9% carbohydrate 17.5% protein and 2.9% fats, with the rest constituted of minerals (Demirbas, 2001; Latiff *et al.* 1996; Mendil *et al.* 2004). It has been reported recently that compared to plants certain metals like Cd, Hg, Pb, As, Cu, Ni, Ag, Cr, Hg accumulate in fungal fruiting bodies (Malivewska *et al.* 2004; Meistrik and Lepsova, 1993; Schimitt and Sticher, 1991; Wondratschek and Roder, 1993), consequently effort has been made to evaluate the possible hazardous effects to human health from the ingestion of mushrooms (Gast *et al.* 1988).

The essential metals can also produce toxic effects when the metal intake is excessively elevated. Recently, studies have drawn attention to the metal pollution of soil and plant samples (Tuzen, 2003). The contents of metals are related to species of mushroom, collecting site of the sample, age of fruiting bodies and mycelium and distance from the site that is polluted. Metals such as Zn and Se are essential metals since they play important role in biological systems, where as Cd ad Cr are non- essential metals as they are toxic, even in traces (Schroeden, 1973). The concentration of 4 metals in samples of mushroom fruiting bodies representing three species, one edible fleshy fungi and two inedible bracket fungi, has been determined by atomic absorption spectroscopy.

Kashmir is located between Jammu and Ladakh region of JandK state in India. Complex geography and vegetation as well as diverse climatic conditions provide a variety of natural habitats for a rich resource of mushrooms. The seasons are normally wet with mild temperatures; especially spring and autumn are suitable for mushroom growth. People who live in Kashmir widely consume wild edible mushrooms because of their delicacy and abundance. Kashmir is important exporter of wild mushrooms like *Morchella* and *Pleurotus* hence has a large edible mushroom potential. Studies on the mushroom samples for the levels of metals determination are scarce or equal to nothing in Kashmir. In this study, the

levels of metals in mushroom samples collected from Rawalpora, Kashmir were determined by graphite furnace atomic absorption spectroscopy (GFAAS) after acid digestion and by elementar.

Material and Methods

Three different fully matured mushroom species were collected from two different sites in Rawalpora area of Kashmir region during August 2005. Samples were uprooted from its substratum with the aid of a scalpel and after complete cleaning samples were oven dried at 40–105^{o}C for 2-24 hrs. Dried samples were homogenized and stored in polyethylene Ziplock bags prior to analysis. The samples of mushroom include edible fleshy fungi *Pleurotus ostreatus* and inedible, hard fungi *Fomes fomentarius* and *Ganoderma appanatum*. All the plastic ware and glassware were cleaned by soaking overnight in a 10% nitric acid solution and then rinsed with deionized water.

Sample preparation and analysis by GFAAS: 0.5g of the dried and homogenized samples of mushrooms was taken and was digested in a mixture of concentrated acids (2:1) HNO_3: $HClO_4$ in the 50 ml digestion tubes over a block digestor. Small pyrex funnels were placed over the tubes and the samples were heated to 60^{0}C for 15 min, further the temperature was increased to 120^{0}C and the digestion was carried out for 75 min until the samples cleared. Afterwards the samples were cooled down and the volume was made upto 50ml with milli Q water containing 2% HNO_3 (Gupta, 1999).

Metal analysis was done through Atomic absorption spectrophotometer for Cr and Cd (Graphite furnace based model Analytic Xena Zeenit 65) according to the protocol prescribed in the manual of the apparatus. The analysis was performed in Central Instrumentation Facility (CIF), Jamia Hamdard, New Delhi. The analysis of Cu, Fe, Mn, Zn was performed in the Pomology Division, SKUAST-K, Shalimar, using atomic absorption spectrophotometer (AAS 4141). The samples were analyzed in triplicates along with sample blanks. The standard curve for each metal was analyzed utilizing analytical grade standard metal solutions (Merck Chemical Company).

Sample packing and analysis by CHNS analyzer: 10 mg of sample was packed in aluminum packs along with spatula of tungsten oxide and wolfram (VI) oxide mixture (Merck) and loaded. Analysis of carbon, hydrogen, nitrogen, sulphur and protein percentage where done through Elemental Analyzer System GmbH (Model VarioEL III) according to the protocols prescribed in the manual of the machine.

Results and Discussion

The habitat, family, edibility and medicinal activity of mushroom species are shown in Table 1. Analysis was carried in three different mushrooms species viz. *Fomes fomentarius, Ganoderma applanatum*, *Pleurotus ostreatus* from two different sites of Budgam district. They were selected based on their availability at the time of analysis. Total content of nitrogen, hydrogen, carbon, sulphur and crude protein are shown in Table 2. All the chemical concentration was determined on a dry weight basis.

Iron content ranged from 96.4 µg g^{-1}dw to 729.5µg g^{-1}dw for the site I and from 252.7µg g^{-1}dw to 632.2µg g^{-1}dw for site II. The highest concentration of Fe was found in *Pleurotus ostreatus* with 729.5µg g^{-1}dw (Site I) and *Ganoderma applanitum* with 632.2µg g^{-1}dw (Site II). Iron values for various mushrooms have been reported to be in the ranges: 31.3–1190 µg g^{-1}dw (Sesli and Tuzen, 1999), 568–3904 µg g^{-1}dw (Turkekul *et al.* 2004) and 56.1–7162 µg g^{-1}dw (Isılogglu *et al.,* 2001), respectively. Iron values for the species investigated presently are in agreement with those reported in the literature. The minimum and maximum concentration of Manganese (Mn) in collected samples ranged from 11.4µg g^{-1}dw to 17.6µg g^{-1}dw for site I and 1.7µg g^{-1}dw to 73.2µg g^{-1}dw for site II respectively. The highest concentration of Mn i.e. 73.2µg g^{-1}dw for site II and 17.1µg g^{-1}dw for site I was recorded in *Pleurotus ostreatus*. The manganese values recorded so far in the mushrooms are 7.6–56.2 µg g^{-1}dw (Demirbas, 2001), 21.7–74.3µg g^{-1}dw (Isildak *et al*, 2004) and 7.1–81.3 µg g^{-1}dw (Tuzen, 2003), respectively. Mn contents obtained in this study are in accordance with literature. Zinc (Zn) was detected in all mushroom samples, which range from 42.3µg g^{-1}dw to 76µg g^{-1}dw for site I and 70.6µg g^{-1}dw to 115.5µg g^{-1}dw for site II. The highest Zn content (115.5µg g^{-1}dw) was recorded in

Fomes fomentarius for site II. Zinc is widespread among living organisms due to its biological significance. Mushrooms are known as zinc accumulators in the fruiting body (Bano *et al.* 1981; Isılogglu *et al.* 2001). Zinc concentrations of mushroom samples in the literature have been reported to be in the ranges: 40.3–64.48 µg g^{-1}dw (Mendil *et al.* 2004) and 29.3–158 8 µg g^{-1}dw (Isılogglu *et al.* 2001). The results obtained in the present study are more or less similar to the earlier studies (Figure 1).

Table 1: Edibility and medicinal activity of three mushroom samples collected from two different sites in Budgam district of Kashmir

S. No.	Mushroom species	Family	Habitat	Edibility	Medicinal Activity
1.	*Fomes fomentarius*	Polyporaceae	Both on the stumps of cut trees and tree	In-edible	Antiinflammatory
2.	*Ganoderma applanatum*	Ganodermataceae	Grows at the base of *Salix* tree	In-edible	Anti-bacterial, anti-HIV, Immuno-modulatory
3.	*Pleurotus ostreatus*	Pleurotaceae	Populus tree and *Salix* stump	Edible	Immuno-Modulatory, Anti-tumor, anaemia

Table 2: Variation of N, H, C, S and Crude protein content in three mushroom samples collected from two different sites in Budgam district of Kashmir

Mushroom Species	Site	N content %	H content %	C content %	S content %	Crude protein content %
Fomes fomentarius	S1	4.862	6.304	39.73	ND	21.30
	S2	4.048	6.818	42.15	ND	17.73
Ganoderma applanitum	S1	4.424	4.248	26.70	ND	19.38
	S2	9.892	6.481	43.07	ND	43.33
Pleurotus ostreatus	S1	7.370	7.439	40.08	0.073	32.28
	S2	4.738	7.223	37.71	0.022	20.75

Contents are given as the arithmetical mean of three independent replications.

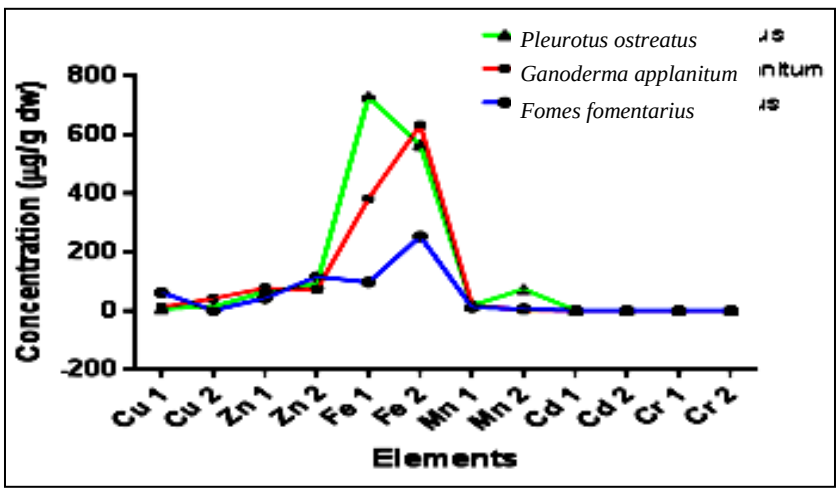

Figure 1: Variation of metal content in three mushroom samples from two different sites in Budgam district of Kashmir

Copper concentration was between 6.3 µg g^{-1}dw to 61.4 µg g^{-1}dw for site I and 0.4µg g^{-1}dw to 41.5µg g^{-1}dw at site II; the highest concentration 41.5µg g^{-1}dw was recorded in *Ganoderma applanatum* at site II. Very least amount of copper (0.4µg g^{-1}dw) was detected in *Fomes fomentarius* at site II. Copper contents of mushroom samples in the literature have been reported to be in the ranges: 4.71–51.0µg g^{-1}dw (Tuzen, *et al.* 1998), 12–181µg g^{-1}dw (Tuzen *et al.* 2003) and 10.3–145µg g^{-1}dw (Sesli and Tuzen, 1999), respectively. Other studies also report copper from different mushrooms in the range of 34.5–83.0µg g^{-1}dw (Demirbas, 2002), 10.0–14.0 µg g^{-1}dw (Isılogglu *et al.* 2001) and 21.1–42.6µg g^{-1}dw (Sivrikaya *et al.* 2002), respectively. Copper is one of the essential minerals that help iron in making red blood cells and delivering oxygen to every part of the body. Chromium observed ranged from 0.049µg g^{-1}dw to 0.079µg/g dw for site I and 0.049 µg/g dw to 0.136µg/g dw for site II. Chromium values in mushroom samples have been earlier reported to be in the ranges: 0.16–4.86 µg g^{-1}dw (Malinowska *et al.* 2004), 0.87–2.66µg g^{-1}dw (Tuzen, 2003) and 7.0–11.0 µg g^{-1}dw (Siverikaya *et al.* 2002), respectively. The chromium levels in mushrooms analysed for this study were found to be lower than those reported in the literature. Cadmium (Cd) concentrations in mushroom species ranged from 0.127µg g^{-1}dw to 0.332µg g^{-1}dw at site I and 0.041µg g^{-1}dw to 0.652 µg g^{-1}dw at site II. The highest concentration of cadmium was found in *Ganoderma applanatum* (0.652 µg g^{-1}dw at site II). Cadmium contents of mushroom samples in the literature have been reported to be in the ranges: 0.81–7.50µg g^{-1}dw (Svoboda *et al.* 2000), 0.10–0.71 µg g^{-1}dw (Mendil *et al.* 2004), 0.28–1.6µg g^{-1}dw (Mendil *et al.* 2004) and 0.12–2.60µg g^{-1}dw (Malinowska *et al.* 2004). Our cadmium levels were found in accord with the results reported in the literature; however, value recorded for some species in the present study is much lower than those reported earlier in the literature. The concentration of nitrogen, hydrogen and carbon ranged from 4.048% to 9.892%, 4.248% to 7.439%, 26.70% to 43.07%, across all the mushrooms analyzed from site I and site II respectively. Only *Pleurotus ostreatus* contained Sulphur that too very meager amount i.e. 0.022% and 0.073% from site I and site II respectively. The low concentration of sulphur in mushrooms is in accordance with the observations made for analysis of sulphur containing amino acids by different scientist. Most of the species of mushrooms are deficient in sulphur containing amino acids. The crude protein content ranged from 17.73% to 43.33% from mushrooms in site I and site II respectively. Now- a-days mushroom is considered as the corner stones of health care system due to presence of many helpful phytochemicals in alleviating some serious diseases. In modern system of disease control, mushrooms containing strong antioxidants properties or phytochemicals neutralize the injurious effects of free radicals as scavengers and thus help in specific body functions in reducing the risk of incidence of many diseases like cardiovascular problems, various types of arthritis, cancer, AIDS and various other degenerative diseases. The predominant mushrooms showing promise for their antiviral and other medicinal activities are polypores- the so-called bracket fungi or woody conks like species belonging to Genus *Ganoderma, Fomes* and *Trametes* (Collins and Ng, 1997; Hattori *et al.* 2011). The current study focuses to mushrooms especially those belonging to *Polyporaceae*, as a rich frontier of new medicines. Many of these are long term residents of nutrient recycling by decomposing aged trees. In a time when new antiviral medicines are critically needed, mushrooms stand out as an untapped resource and deserve intensive studies.

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