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HEAVY METALS DETECTION IN WHITE BUTTON MUSHROOM (*AGARICUS BISPORUS*) CULTIVATED IN STATE OF MAHARASHTRA, INDIA

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ABSTRACT : Present studies was undertaken to investigate the heavy metals in mushroom by chemical extraction method from cultivated Button mushrooms (*Agaricus bisporus*), extensive attention has been paid during the last two decades for the management of environmental pollution caused by hazardous materials like heavy metals. Detection of heavy metal concentration in the fresh fruiting bodies of *Agaricus bisporus* is crucial in dietary intake studies. The mean concentration (mg/kg in dry weight) in the mushroom and respective substrate samples were analyzed for essential and non-essential metals, viz., Cu, Fe, Zn, Mn, Cd and Pb after acidic digestion. The results of the heavy metals concentration in each samples ranging from Cr 0.05-0.06, Fe 13.54-13.75, Ni 0.04-0.06, Zn 3.79-3.88, As 0. 05-0.06, Hg 0.04-0.06 and Pb 0.04-0.06 and found to be significant at (P<0.05). The concentration of Cr, Fe, Ni, Zn, As, Hg and Pb were observed lower than permitted limits set by WHO and FAO. All the results were under permissible limits and it was compared with the reported results and hence it may be more useful for human consumption.

Key words : White button mushroom, *Agaricus bisporus*, heavy metals, concentration, Maharashtra.

INTRODUCTION

In the past China and Japan, that have been collected, cultivated yeast and mushrooms such as lingzhi (*Ganoderma lucidum*), shiitake (*Lentinus edodes*) and yiner (*Tremella fuciformis*) and used for long years are being evaluated as edible and medicinal resources. Most classical knowledge about the mushroom as a food and medicinal agent comes from these species. Mushroom has been defined as a fleshy, aerial umbrella shaped, fruiting body comprising of macro fungi and has been consumed by Asian people for over two thousand years because of the pleasant flavor and texture (Waseer and Weis, 1999). It was widely accepted in the literature that ''mushroom'' is a macro-fungus with a distinctive fruiting body that is large enough to be seen by the naked eye and to be picked up by hand (Chang and Miles, 1992). Medicinal mushrooms are used in alternative medicine throughout the world for their presumed enhancing effect on the immune system (Jong and Birmingham, 1992; Chang and Miles, 1989).

Mushrooms are the eukaryotic, spore bearing organisms under macro-fungi, lacking chlorophyll and grow on compost matter. They derive nutrients through their mycelia. Mushroom impact on human welfare in many ways. Mushrooms are reported as rich source of proteins, minerals, and other nutrients and also been associated with boosting the immune system. Mushrooms have ability to potentiate antimutagenic and antitumorigenic activity, boost immune system weakened by radiotherapy and chemotherapy during cancer treatment.

White button mushroom consumed for various reasons such as their delicious taste and medicinal values. Due to their chemical composition and presence of bioactive compounds it has been reported as rich source against hypertension, hypercholesterolemia and cancer. Some mushrooms or their extracts have been used or studied as possible treatments for diseases, such as cardiovascular disorders also (Guillamon *et al*, 2010). Some isolates of mushrooms shows potential activity such as antioxidant, antiviral, antibacterial, antiparasitic, anti-inflammatory, and antibiotics properties in preliminary studies (Chowdhury *et al*, 2015). Other mushroom derived materials, including polysaccharides, glycoproteins and proteoglycans are the basic research for their potential to modulate immune system responses and inhibit tumor growth (Borchers *et al*, 2008). *In vitro* studies reported by (Endo *et al*, 2010) that *Agaricus bisporus* derived

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compounds induces apoptosis, inhibit angiogenesis and stimulate TNF-α production by bone marrow derived macrophages (BMM).

The occurrence of heavy metals in soil, water and living objects is a major public health concern and due to their detrimental effects on human health and their removal from the environment is deemed important for the protection of environmental health. Mushrooms are capable of bio accumulating more heavy metals in their fruit bodies since some of these heavy metals are natural components of the earth's crust. The key to eating mushroom and avoiding the possibility of heavy metal poisoning is to eat them in moderation and avoid species like the chanterelle that are more prone to collecting heavy metals from the soil. Eating mushroom may still provide health benefits, but this new study suggests that eating them in moderation may be important to reduce the risk of heavy metals poisoning. Heavy metals such as iron, zinc and manganese are essential metals since they play an important role in biological systems (Yılmaz *et al*, 2010). Detection of heavy metal concentration such as Cu, Zn, Fe, Mn, Cd, Cr, Ni and Pb studied in 14 different wild growing edible mushrooms reported by (Singh *et al*, 2011). Studied found that Fe content was higher than other metals in all mushroom species. Studies on *Agaricus bisporus*, which contains high amounts of antioxidants, has been reported to additionally harbor a pro-oxidative phenolic compound, that is the 4-(hydroxymethyl)-phenyl radical (Zhu *et al*, 2011). These microspheres exhibit excellent potential for application in biomedical fields and life science, especially for the delivery and controlled release of bioactive compounds (Shnyreva *et al*, 2010).

Studied reported by (Huang *et al*, 2012) that *Agaricus bisporus* is an excellent source of several essential amino acids, vitamins such as (B2, niacin and folate) and mineral supplements (potassium, phosphorus, zinc and copper). However, the commercial value of *Agaricus bisporus* can be decreased or fully lost within few days if it is stored at ambient temperature. To the best of our knowledge, there were no reports on the heavy metal detection in cultivated mushroom in Maharashtra, India.

MATERIALS AND METHODS

Materials

The fruiting bodies of Button mushroom (*Agaricus bisporus*) were taken from Pune and Nashik district of Maharashtra, India. Nitric acid (HNO_3) and perchloric acid ($HClO_4$) were purchased from Sigma-Aldrich. All reagents/chemicals were analytical grade. Triple distilled water (TDW) used for the preparation of various solvents during analysis. These mushrooms species were cultivated under controlled condition in organic manures.

Methods

Sample area and sample locations

The study area includes Pune and Nasik region of State of Maharashtra, India. Samples of *Agaricus bisporus* were collected from the various mushroom producers.

Preparation of samples

The collected samples were washed with distilled water to remove the debris particles. Thereafter, samples were grounded into smaller particles using commercial blender and stored in polyethylene bags at which temperature, until used for acid digestion.

Acid digestion and heavy metals detection

2.0 g of the grounded samples was introduced into the digestion vessels. The ration of 2:1 of 10 ml of nitric and perchloric acid; was added to the sample and digested at 80^0C for 1 hour till a transparent solution was achieved. After cooling, the digested samples were filtered into a 100 ml volumetric flask using Whatman (No.1) filter paper and the filtrate was diluted to 100 ml mark with distilled water. The samples were analyzed for heavy metals (Fe, Zn, Pb, Cr, As, Hg and Ni) by using AOAC 20th Edition (2016).

Statistical analysis

All the statistical analysis was carried out using SPSS statistical software package. The data on heavy metals concentration in *Agaricus bisporus* were analyzed using one way analysis of variance (ANOVA). The accumulation factors were assessed as the ratio of the concentration in the White Button Mushroom. Correlations between heavy metals in the white button mushroom were analysis in triplicate (n = 3) and found to be significant at ($P<0.05$).

RESULTS AND DISCUSSION

The results from this study showed the concentrations of five heavy metals which include Pb, Cr, As, Hg and Ni in *Agaricus bisporus* collected from different mushroom cultivators. Concentration of Pb, Cr, As, Hg and Ni in the samples are given in Table 1. The main of this study to determine the heavy metal content of *Agaricus bisporus* collected from various mushroom cultivators in Maharashtra and to ascertain whether their composition complies with standards set by the WHO/FAO and other international tolerance limits on heavy metals in food as given below in Table 1.

Table 1 : WHO/FAO and other international tolerance limits on heavy metals in food.

S. No.	Heavy Metals	mg/Kg	References
1	Cromium (Cr)	2.3	Codex Alimentarius Commission, Joint FAO / WHO(2001) Food additives and contaminants, Joint FAO/ WHO food Standards program;ALINORM 01/12A:1-289.
2	Ferrous (Fe)	15. 0	Turkish Food Codex Anonymous Regulation (2002). Regulation of setting maximum level for certain contaminants in food stuff, Official Gazette, Iss: 24908.
3	Nickel(Ni)	1.5	Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme andWorld Health Organization (2007). Codex *alimentarius Commission: procedural manual*. Food & Agriculture Org.
4	Zinc (Zn)	50	Joint FAO/WHO Expert Committee on Food Additives, & World Health Organization. (1995). Evaluation of certain food additives and contaminants: forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives.
5	Arsenic (As)	0.2	Codex Alimentarius Commission, Joint FAO/WHO. (2018). Joint FAO/WHO Food Standards Programme Codex Committee On Contaminants In Foods. 12th Session Utrecht, The Netherlands, 12 - 16 March 2018, CF/12 INF/1
6	Mercury (Hg)	10	Codex Alimentarious Commission (1996) Joint FAO / WHO food standards programme, Codex General Standard for Contaminants and Toxins in Foods, Doc. No. Cx/FAC 96/17.
7	Lead (Pb)	0.3	European Commission. (2001). Commission Regulation (EC) No. 466 / 2001. Directive 2001/22/EC, EU.

Table 2 : Results obtained from analyzed samples.

Samples	Chromium (Cr)	Nickel (Ni)	Arsenic(As)	Mercury (Hg)	Lead (Pb)	Zinc (Zn)	Iron (Fe)
1	0.05	0.05	0.05	0.06	0.04	3.87	13.75
2	0.05	0.04	0.06	0.05	0.06	3.88	13.54
3	0.06	0.06	0.05	0.04	0.05	3.79	13.55
Mean	**0.05**	**0.050**	**0.05**	**0.05**	**0.05**	**3.85**	**13.61**
Standard deviation(± SD)	**0.004**	**0.007**	**0.004**	**0.007**	**0.007**	**0.035**	**0.084**
Standard error(±SE)	2.25	1.45	0.15	9.95	0.25	46.15	1.39
Covariance	2.22222E-05	6.6667E-05	2.22222E-05	6.66667E-05	6.667E-05	0.001622	0.009356
Correlation coefficient		0.8660254	-0.5	-0.8660254	1.86E-16	-0.9948	-0.463

The heavy metal concentration order of elements in white button mushroom was found to be in order of Cr<Ni<As<Hg<Pb<Zn<Fe (Table 1, Fig. 1). The variation in the heavy metal concentrations in these samples may be as a result of differences in substrate composition which is determined by the ecosystem and great differences in uptake of individual metals by the mushroom species (Manzi *et al*, 2001). However, the size of fruiting body is of less importance in the accumulation of heavy metals by mushrooms (Michelot *et al,* 1998).

Chromium (Cr)

The Cr concentrations of White Button Mushroom in this study were <0.05 mg/kg, much less than those in a previously-published database (Tables 2 and 3) on cultivated mushrooms except a study in Bangladesh (Mohiuddin *et al*, 2016). The highest concentration of chromium (63.0 mg/kg, dw) was observed in Mexico (Kalac *et al*, 2000). While in *P. ostreatus* and lowest (4.06 mg/kg, dw) was in *Agaricus bisporus* in the same country.

Ferrous (Fe)

The concentration of Fe in this study was (13.61 mg/kg) lower than pervious reported (Tables 2 and 3). The highest level of Fe was found in Poland (432.24 mg/kg) (Munoz *et al*, 2005). Fe concentration was higher in previous study (Fig. 2).

3Nickel (Ni)

The Ni concentration of white button mushroom in this study were less (<0.05 mg/kg) than those reported in previous literature (Tables 2 and 3). High levels of Ni (56.1 mg/kg) were reported in Turkey.

Zinc (Zn)

In this study, the highest mean level (3.85 mg/kg) were observed as compared to previous reported (Table 2, Fig. 2). *Agaricus bisporus* in Hungary where very high concentration of Zn were obtained for the same species in different locations (Bosiacki *et al*, 2018) (Tables 2 and 3).

Arsenic (As)

Mean concentration of As (mg/kg, dw) in *Agaricus*

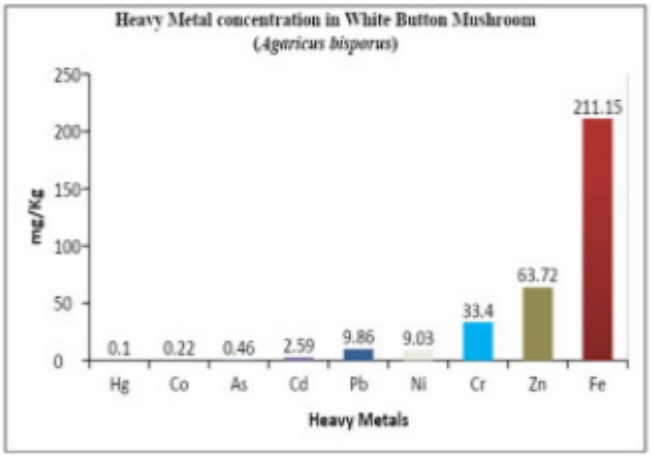

Fig. 1 : Comparison of heavy metals contents in white Button Mushroom in previous studied.

Correlations between heavy metals in white button mushroom

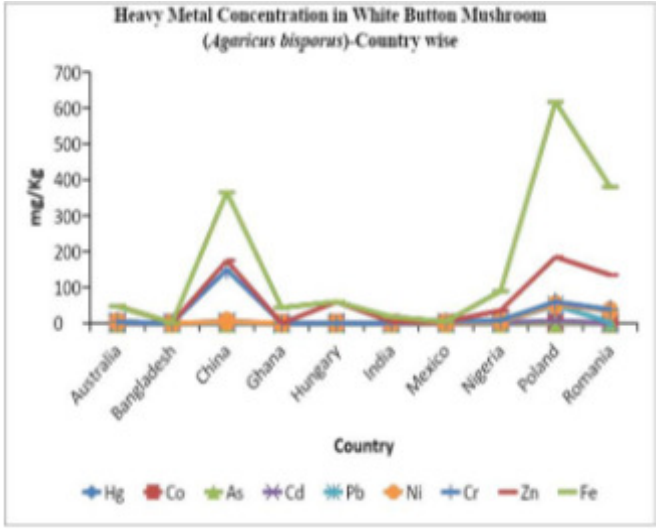

Fig. 2 : Heavy metal concentration in White Button Mushroom (*Agaricus bisporus*) reported in country.

Table 3 : Heavy metal concentration (mg/kg) in the *Agaricus bisporus* reported from the literature and in present study.

Sr. No.	Year	Country	Reference	Hg	Co	As	Cd	Pb	Ni	Cr	Zn	Fe
1	2001	Turkey	a	NA	0.320	0.760	NA	NA	56.100	ND	NA	126.000
2	2003	Hungary	b	NA	NA	NA	NA	NA	NA	NA	60.000	NA
3	2004	Turkey	c	NA	ND	ND	NA	NA	8.200	3.100	NA	332.000
4	2005	Mexico	d	NA	NA	NA	0.540	NA	NA	4.060	NA	NA
5	2008	Nigeria	e	NA	ND	NA	NA	NA	1.940	ND	NA	ND
6	2010	China	f	NA	NA	NA	0.057	NA	4.600	264.000	26.300	NA
7	2010	Romania	g	NA	NA	NA	0.190	1.560	NA	NA	96.600	261.700
8	2011	China	h	NA	ND	NA	NA	NA	0.920	22.600	NA	190.000
9	2013	Ghana	i	NA	NA	NA	0.350	0.040	NA	NA	NA	43.770
10	2013	Australia	j	NA	0.126	NA	NA	3.900	NA	NA	43.800	NA
11	2014	Nigeria	k	NA	NA	NA	4.880	1.250	2.500	NA	27.330	58.250
12	2015	Nigeria	l	NA	NA	NA	0.410	0.620	0.180	NA	26.550	50.030
13	2016	Bangladesh	m	0.080	NA	NA	NA	0.220	NA	0.300	NA	NA
14	2017	Poland	n	NA	NA	0.710	NA	NA	NA	NA	NA	NA
15	2018	Poland	o	NA	NA	NA	6.140	42.830	3.090	6.930	124.840	432.240
Mean				**0.080**	**0.223**	**0.710**	**1.795**	**7.203**	**9.691**	**9.943**	**57.917**	**186.749**
Standard Deviation(SD)				0.00	0.10	0.03	2.38	14.59	17.69	95.90	36.05	135.24
Standard Error SE±				0.00	0.07	0.02	0.90	5.52	6.26	39.15	13.63	47.82
16	2019	India	Study	**0.05**	**0**	**0.05**	ND	**0.05**	**0.05**	**0.05**	**3. 85**	**13. 61**

a. Demirbas (2001)
b. Vetter (2003)
c. Mendil *et al* (2004)
d. Munoz *et al* (2005)
e. Ita *et al* (2008)
f. Fangkun *et al* (2010)
h. Zhu *et al* (2011)
g. Radulescu *et al* (2010)
i. Quarcoo and Adotey (2013)
j. Koyyalamudi *et al* (2013)
k. Undochukwu *et al* (2014)
l. Umeri *et al* (2015)
m. Mohiuddin *et al* (2016)
n. Rzymski *et al* (2017)
o. Bosiacki *et al* (2018)

bisporus were found <0.05mg/kg (Table 1). Amongst different cultivated white button mushroom in different countries, total As concentration of this study were lower (Tables 2 and 3). However, an investigation in Bangladesh found As in *Agaricus bisporus* below detection level (Mohiuddin *et al*, 2016). Furthermore the concentration of As present in mushroom of this study was within limit of Australia and New Zealand Food Standard Code.

Mercury (Hg)

Mercury is highly toxic element of the environment poses health risk depending on its concentration, duration and method of expose. The concentration of Hg in the

mushroom production as a high concentration in the substrate accelerates its accumulation into the fruiting body. The Hg concentration in *Agaricus bisporus* in this study were <0.05 mg/kg (Table 1). which were much lower than that of Bangladesh (Kalac *et al*, 2000) (Tables 2 and 3).

Lead (Pb)

Lead concentration of mushrooms found in this study were <0.05 mg/kg in *Agaricus bisporus* which were slightly higher than another study conducted in Bangladesh (Mohiuddin *et al*, 2016). Cultivated *Agaricus bisporus* showed about 50 time higher concentration of Pb whereas *Agaricus bisporus* of Australia (Vetter *et al*, 2003) demonstrated about 10 to 20 fold higher than that of *P.high* king and *P. ostreatus* in our study, respectively.

There were significant statistical correlations between concentration in *Agaricus bisporus* which shown in Table 1. Significantly positive correlations were established between Cr and Ni (r = 0.86), Fe and Hg (r = 0.88), Ni and As (r = 0.86), Zn and Hg (r = 0.81), As and Pb (r = 0.86). Similarly negative correlation were established between Cr and Zinc (r = -0.99), Cr and Hg (r = -0.86), Fe and Pb (r = -0.88), Ni and Zn (r = -0.91). The other correlations between heavy metals were not significantally correlated. Correlationship significant level were set at (P<0.05).

CONCLUSION

The order of the levels of heavy metals in the white button mushroom samples were found to be Cr<Ni<As<Hg<Pb<Zn<Fe. The concentration of heavy metals varied between elements. Moreover the estimated daily intakes were based on the best available production and consumption data. Due to the low consumption rate of mushrooms, it is, therefore important to examine other foods sources. Different country has different level of heavy metals in *Agaricus bisporus* as per the tolerance/ permissible value of Cr, Fe, Ni, Zn, As, Hg and Pb from FAO/WHO guidelines. Future work should focus on formulating the values for other metals, nutritive values and cytotoxicity assessment, which will provide more clear information for risk assessment.

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Conflicts of interest : The authors declare that they have no conflicts of interest.

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