ESSENTIAL MINERAL AND TOXIC ELEMENTS IN OYSTER MUSHROOM (*Pleurotus florida*) CULTIVATED ON WATER HYACINTH AND RICE STRAW

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ABSTRACT

The present study investigated the concentration of three mineral elements (Fe, Zn, Cu) and three toxic elements (Pb, Cd, As) in the cultivation substrate (1:1 mixture of water hyacinth and rice straw) as well as in the cultivated oyster mushrooms *Pleurotus florida* grown on this substrate. The mean concentrations (mg kg^{-1} dry weight) obtained for Fe, Zn, Cu, Pb, Cd and As in the mushrooms were 172, 52.3, 10.5, 2.3, 0.95 and 0.7 whereas in the cultivation substrate were 201.5, 56.5, 11.9, 7.2, 2.7 and 2.4 respectively. The contents of Fe, Zn and Cu minerals are in consonance with the reports in literature. Pb and Cd contents are however slightly higher than some of the reports in literature but are within the of recommended safe dietary intake limits established by FAO/WHO expert committee. The coefficient of accumulation of these elements in the mushroom were also calculated by comparing with their concentration in the cultivation substrate and was found in the order of Zn > Cu > Fe > Cd > Pb > As indicating *P.florida* as good accumulator of the minerals. In terms of nutritional aspects, taking into account the recommended dietary allowances or provisional tolerable weekly intake recommended by FAO/WHO, optimum dietary intake of average 100 g day^{-1} fresh mushroom of *P.florida* grown on water hyacinth and rice straw is beneficial for health and poses no toxicological risk.
Keywords: Mineral, *Pleurotus florida*, rice straw, substrate, toxic elements, water hyacinth.

INTRODUCTION

Mushrooms are valuable health food, low in calories, in general high in vegetable proteins, minerals, vitamins and fiber. In most countries there is a significant consumer acceptance of cultivated mushrooms for their texture, flavor, nutritional properties as well as for their therapeutic uses. Oyster mushrooms or species of *Pleurotus* are popular mushrooms with high commercial value. In India this mushroom contribute to the maximum production over other due to its ease of cultivation on a wide variety of lignocellulosic materials beside straw of wheat or rice. As such, oyster mushrooms can play an important role in managing and recycling organic waste through biodegradation. Mushrooms are known to uptake and bio-accumulate essential mineral elements as well as some polluting toxic elements in high concentrations from growth medium (Kalac and Sovodon, 2001, Demirbas, 2001). Mineral and toxic metal (as lead, cadmium, mercury) concentration in higher mushrooms are often considerably higher than those in agricultural crop plants, vegetables and fruits (Zhu et al., 2010). The principal factors influencing the accumulation of these elements in macrofungi are environmental factors such as metal concentration in substrate, pH, organic matter, contamination by atmospheric deposition and fungal factors such as species and age of mushroom, development of mycelium and fruit bodies, morphological portion (Garcia et al., 1998). Growing up on a substrate with high concentration of various toxic elements, edible mushrooms can become toxic, accumulating a large amount of those elements.

Mineral content analysis allows the evaluation of nutritional quality of food. RDA (Recommended dietary allowances) are daily intake of essential nutrients judged to be adequate to meet the known nutrient needs of practically all healthy persons (NRC, 1989). Provisional Tolerable Weekly Intakes (PTWI) are set for substances such as heavy metals, that are contaminants in food and are known to accumulate in animals and humans (Codex Alimentarius, 2011). PTWIs have been set for cadmium, lead, mercury and tin (Luis et al., 2012). The determination of toxic element (As, Pb, Cd, Hg) concentration in the food allows for the evaluation of health risk and is thus part of every food safety program and also essential

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in dietary intake studies. Food contains a wide range of elements such as potassium, sodium, iron, copper, zinc, magnesium which are essential elements since they play an important role in biological systems, whereas aluminum, lead, cadmium or arsenic are non-essential elements as they are toxic even though excessive levels of essential elements can be detrimental to organisms (Zhu et al., 2010, Akyuz and Kirbag, 2011). Therefore, it is worthwhile to evaluate the levels of essential mineral and toxic elements in mushrooms grown on any substrate (artificially / natural occurrence), to assess the contribution of the mushroom to daily intake of several toxic elements and to compare the results with the norms for these toxic elements in food stuff to report any possible contamination that would represent a health hazard.

Water hyacinth [*Eichhornia crassipes* (Mart.) Solms.] is one of the top ten problematic weeds of the world (Aboul-Enin et al., 2011) which adversely affect both human and animal due to its enormous rate of propagation. So, many workers (Nageswaran et al., 2003, Bandopadhyay, 2009) attempted to eradicate the vast quantity of this weed through its cost-effective and eco-friendly utilization as a substrate for cultivation of mushroom or as an ingredient in vermicompost. It is also used in waste water treatment for its unique property to absorb heavy metal on its roots (Dos Santos, 2001). In the present study water hyacinth along with rice straw (1:1) has been used as the substrate for cultivation of oyster mushroom *Pleurotus florida.*

In the light of above, the objective of this study is (i) to determine the levels of three essential mineral elements and three toxic elements in samples of cultivation substrate of water hyacinth (WH) mixed (1 : 1) with rice straw (RS) as well as in the oyster mushroom (*P.florida*) cultivated on this substrate and (ii) to estimate the contribution of this cost-effective oyster mushroom consumption to the RDA or PTWI set for mineral and toxic elements respectively by expert council or committee (NRC, 1989, EC No 1881/2006, Codex Alimentarius, 2011) of food safety program. Iron, copper and zinc (Fe, Cu, Zn) have been chosen as representative essential mineral and lead, cadmium, arsenic (Pb, Cd, As) as representative toxic elements whose levels in environment represent a reliable index of environment pollution.

MATERIALS AND METHODS

The samples used in this study were obtained from previous cultivation work (Bandopadhyay 2009). Pure culture of *Pleurotus florida nomen nudum* (Eger, strain P-1) was procured from NCMRT, Solan, H.P., India. Rice straw and sundried water hyacinth (without root) was mixed in 1:1 proportion to use as substrate for cultivation of *P. florida*. Preparation of substrate and cultivation of *Pleurotus* sp. were carried out following standard polythene bag method (Upadhyay, 1997). Spawns of *P. florida* were prepared on wheat grains in polypropylene packets following Suman and Sharma (2005) and spawned @5% wet substrate. Temperature and relative humidity regimes were maintained between 14 – 22°C and 75 - 80% respectively. Mushrooms were harvested upto 3rd flush to prepare mushroom samples for determination of essential mineral and toxic element contents.

Mushrooms were cut into slices, air dried at 60°C for 24 h. and homogenized to fine powder. Sample of substrates (WH+RS 1 : 1) was dried at 70°C for 72 h , then homogenized to fine powder and weighed for acid digestion.

Samples from each of dried mushroom powder (500 mg) and substrate powder (1 gm) were digested at 100°C for 30 min. in acid solution containing mixture of HNO_3 : H_2SO_4 : H_2O_2 (10 : 1 : 1, 12 ml for 1g sample). After cooling to room temperature, the volume of clear digested solution was made upto 50 ml using deionized water. A blank was also prepared using similar experimental procedure (Anonymous, 1990). The concentrations of Cu, Zn, Fe, Pb and Cd were determined using Atomic Absorption Spectrometer (AAS) [Perkin Elmer 5100 PC] in Bose Institute, Kolkata, while Arsenic (As) was determined by AVANTA GBC AAS with flamed hollow cathode lamp (HCL).

The samples after acid digestion were passed through the AAS system using different lamps, and calibrated with different minerals in different concentrations for different micronutrients (Anonymous, 1990). The concentrations of all the essential and toxic metals are the mean of three replicates and are expressed in mg related with kg of dry mushroom or substrate. The minimum detection limits of the device ranged according to analyzed elements and were found to be (mg kg^{-1} dry weight) 0.077 for Cu, 0.018 for Zn, 0.11 for Fe, 0.028 for Cd and 0.45 for Pb.

Table 1: Content (mg kg^{-1} dw*) of mineral and toxic elements in cultivated *P.florida* in the present study and the range of contents of these elements in selective references on other cultivated spp. of *Pleurotus*

Mineral/ Toxic element	**Contents of elements in the substrate of water hyacinth and rice straw (1:1)in the present study (mg kg^{-1} dw of substrate)**	**Contents of elements in *P.florida* in the present study (mg kg^{-1} dw of mushroom)**	**Range of contents in cultivated spp. of *Pleurotus* in published literature (mg kg^{-1} dw of mushroom)**	**References**
Iron (Fe)	201.5 ± 104.3	172 ± 61.5	67-1524	Chang et al., 1981; Bisaria et al., 1992; Caglarirmak, 2007; Akyuz and Kirbag, 2010; Zahid et al., 2010.
Zinc (Zn)	56.5 ± 6.2	52.3 ± 3.7	54-180	Wang, 2001; Rasad and Abdon, 2002; Gong et al., 2003; Guo et al., 2007; Caglarirmak, 2007; USDA, 2010; Akyuz and Kirbag, 2010.
Copper (Cu)	11.9 ± 2.6	10.5 ± 1.1	11-36	Rasad and Abdon, 2002; Dogan et al., 2006; Akyuz and Kirbag, 2010; Zahid et al., 2010; USDA, 2010.
Lead (Pb)	7.2 ± 1.5	2.3 ± 1.6	n.d. – 4.4	Dogan et al., 2006, Regula and Siwulski 2007, Garcia et al. 2009.
Cadmium (Cd)	2.7 ± 1.8	0.95 ± 1.3	0.3 – 2.9	Dogan et al. 2006; Regula and Siwulski, 2007; Garcia et al., 2009.
Arsenic (As)	2.4 ± 1.2	0.7 ± 0.3		No reference in literature for As content in cultivated mushroom

*dw =Dry weight

Table 2: Coefficient of accumulation (K_a) of the mineral and toxic elements in *P.florida* cultivated on water hyacinth and rice straw (1:1).

Mineral/ Toxic element	Average yield* of *P.florida* (g fresh weight per kg dw of substrate (WH+RS)	Mineral/ Toxic element content (g) in mushroom (dw) produced per kg dw of substrate (considering 82% moisture content of mushroom)	Coefficient of accumulation (K_a)
Iron (Fe)	1522	47.1	0.23
Zinc (Zn)	1522	14.3	0.25
Copper (Cu)	1522	2.9	0.24
Lead (Pb)	1522	0.63	0.09
Cadmium (Cd)	1522	0.26	0.1
Arsenic (As)	1522	0.2	0.08

* Referred to Bandopadhyay (2013).

Table 3: Recommended daily dietary intake values (mg day^{-1} $person^{-1}$) *

Mineral/ Toxic element	Recommended values of daily dietary intake (mg day^{-1} $person^{-1}$ derived from RDA[a]/PTWI[b]/)	Mineral/ Toxic element content(mg) per kg fresh weight of experimental mushroom of *P. florida*	Mineral/ Toxic element content (mg) per 100g fresh weight of experimental mushroom of *P. florida*
Iron (Fe)	18[a]	31	3.1
Zinc (Zn)	15[a]	9.4	0.9
Copper (Cu)	2[a]	1.9	0.2
Lead (Pb)	0.2[b]	0.4	0.04
Cadmium (Cd)	0.06[b]	0.17	0.02
Arsenic (As)	0.12[b]	0.13	0.01

* Referred to [a]NRC, 1989, [b]Codex Alimentarius, 2011.
[a]RDA: Recommended dietary allowances.
[b]PTWI: Provisional Tolerable Weekly Intake.

RESULTS AND DISCUSSION

The concentrations (mg kg^{-1}) of representative essential and toxic elements in substrate and in mushrooms are detailed in Table 1, which reveal gradual decrease of the element contents in the descending order of Fe > Zn > Cu > Pb > Cd > As. Among all the analyzed elements in the mushrooms, iron was the most important trace element with highest concentration

(172mg kg^{-1} dry weight of mushroom) followed by zinc (52.3 mg kg^{-1}) and copper (10.5 mg kg^{-1}). The table also summarizes the references of a selection of studies on the range of content of these elements in other cultivated oyster mushrooms published in literature.

The contents of all the essential minerals considered in this study fall within the range in literature and consistent with previous studies on cultivated mushrooms of *Pleurotus* spp. As the micronutrients (both mineral and toxic elements) flows from substrate to mushroom and accumulate, the co-efficient of accumulation of essential minerals and toxic elements are also calculated using relation:

$$K_a = \frac{C_m}{C_s}$$

Where C_m is the concentration of elements in mushrooms and C_s is their concentration in initial substrate of cultivation. Table 2 represents the coefficient of accumulation in mushroom indicating highest accumulation for Zn followed by Cu and Fe indicating *P. florida* as good accumulator of these elements.

According to Demirbas (2000) the level of mineral and heavy metal accumulation depends on the ability of the species to extract the elements from the substrate and on their selective uptake and deposition of such elements in the tissues. In the present study higher concentrations of toxic elements have been detected in initial substrates of water hyacinth and common paddy straw though they are present in much lower concentration in the mushrooms. Therefore, they are not getting accumulated in toxic level in the mushrooms which supports cultivation of mushroom on water hyacinth supplemented rice straw.

In order to contribute to the safe consumption of the experimental mushroom (grown on WH+RS) to the dietary intake of essential minerals and to evaluate the risk of dietary exposure to the toxic elements, the concentration of the mineral and toxic elements obtained in this study were compared with RDA (for Fe, Cu, Zn, As) or PTWI (for Pb, Cd) for an average adult of 60 kg body weight (b.w) as established by the Joint National Research Council (NRC, 1989), FAO / WHO (2010) , Codex Alimentarius (2011) Expert Committee on Food Additives and European Commission (toxicological fact sheet series, 2009 EC No 1881/2006) (Table 3). The recommended dietary values are on the fresh weight of foodstuff as such the obtained values for the mineral and toxic element contents on dry weight basis were recalculated on fresh weight basis considering 82% moisture content of experimental mushroom of *P. florida* (Bandopadhyay, 2013). Mean concentrations of Fe, Zn, Cu, Pb and Cd obtained in the experimental mushrooms were approximately equivalent to 31 mg kg^{-1}, 9.4 mg kg^{-1}, 1.9 mg kg^{-1}, 0.4 mg kg^{-1} and 0.17 mg kg^{-1} fresh weight of mushroom. Arsenic was obtained in trace amount of 0.7 mg kg^{-1} dry weight or 0.13 mg kg^{-1} fresh weight of mushroom. Recommended allowances for iron, zinc and copper for average adult person are 15, 12-15 and 1.5-3 mg day^{-1} respectively (NRC, 1989) while their provisional maximum tolerable daily intake (PMTDI) are 45, 40 and 10 mg day^{-1} respectively (Codex Alimentarius, 2011). The PTWI recommended by the Joint FAO/WHO Expert Committee on Food Additives for cadmium was previously 7 μg Cd kg^{-1} body weight (JECFA, 2003). In 2010 the JECFA (Joint FAO / WHO Expert Committee on Food Additives) reviewed the evaluation on cadmium and established a new provisional tolerable monthly intake (PTMI) of 25 μg kg^{-1} body weight which corresponds to a weekly intake of 5.8 μg kg^{-1} body weight or 0.3 mg for an adult. The PTWI for Pb, set by JECFA (25 μg Pb kg^{-1} body weight) was withdrawn in 2010. Regulation (EC) of European commission has set maximum levels for certain contaminants in vegetables establishing a maximum level of 0.2 mg kg^{-1} and 0.3 mg kg^{-1} for Cd and Pb respectively in oyster mushrooms like *Pleurotus ostreatus* (Toxicological factsheet series, 2009). For arsenic, inorganic As is more toxic than organic As. The safe range in toxicological guidance value is 2-7 μg kg^{-1} body weight per day (Codex Alimentarius, 2011) which is equivalent to 0.12 mg day^{-1} for a 60kg adult (Toxicological factsheet series, 2009). Considering the above recommended values, an adult person (of average 60kg b.w.) can therefore safely consume average 100g fresh mushroom of *P.florida* per day. The estimated intake of referred elements from dietary intake of this fresh mushroom will be 3.1mg day^{-1} Fe, 0.9mg day^{-1} Zn and 0.02mg day^{-1} Cu while 0.04 mg day^{-1} Pb , 0.02 mg day^{-1} Cd and 0.01 mg day^{-1} As, which are well below the permissible levels set by standard expert committee or commission for human consumption.

In the present study, Fe and Zn were found as the dominant essential mineral elements in comparison to other minerals. Iron is vital in a variety of metabolic processes. High intake of iron is desirable to compensate excretion and unutilization (Lynch and Baynes, 1996) and to reduce absorption, utilization and retention of most toxic metals particularly Pb and Cd with its antagonistic nature. Sufficient Zn in food is also essential to neutralize the toxic effects of Cd.

Comparison of the levels of essential minerals and toxic elements in the studied mushroom of *P. florida* indicated that permissible levels must be followed for safe food. Regular monitoring of toxic elements from mushrooms cultivated on substrates collected from roadside fields or even from rural areas is required to do vegetables safety. Monitoring accompanied by regular reevaluation of acceptable levels must continue but with the realization that some toxic heavy metals will probably always be found in very low quantities and they are considered to be unavoidable contaminants. It can therefore be concluded that cultivation of *P.florida* on mixture of water hyacinth and rice straw is a cost-effective, eco-friendly project and controlled consumption of this mushrooms daily does not pose any toxicological risk.

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