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Research article

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Assessment of heavy metal threat in *agaricus bisporus* mushrooms cultivated from water hyacinth weed of Kolleru lake, Andhra pradesh-India

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ABSTRACT

Verification of heavy metal threat owing to utilization of water hyacinth of Kolleru Lake as mushroom growth substrate instead of paddy straw has formed the objective of this investigation. Using ICP-MS, ten trace elements in *Agaricus bisporus* were determined. The mean values of trace elements in the mushrooms cultivated from the water hyacinth shoots were 16.72 ± 0.6 , 7.448 ± 0.368 , 46.86 ± 3.52 , 0.146 ± 0.6 , 0.756 ± 0.054 , 16.18 ± 2.96 , 1596 ± 81.6 , 1.434 ± 0.074 , 0.564 ± 0.056 and 0.64 ± 0.16 mg/kg for Mn, Cu, Zn, Cd, Pb, Cr, Fe, Ni, Co and arsenic respectively. The mushrooms cultivated from the paddy straw formed the controls for comparison purpose. Observed statistically no significant differences of mean values of metals between the experimental and control groups and found the heavy metals consents within the prescribed limits of safety standards. Hence, recommended the utilization of water hyacinth weed as a substitute to paddy straw for *A. bisporus* mushroom cultivation.

Keywords: Water hyacinth, Agaricus bisporus mushrooms, Heavy metals, ICP-MS, Kolleru Lake.

1. Introduction

Water hyacinth [*Eichhornia crassipes* (Mart.) Solms] is one of the top ten problematic weeds of the world (Aboul-Enein, Al-Abd, Shalaby, Abul-Ela, Nasr-Allah, Ahmoud, & El-Shemy, 2011) and is responsible for the early onset of natural succession at a relatively faster rate leading to premature death of fresh water lakes through a detrimental process called eutrophication (Khan & Ansari, 2005). The reason lies in its prolific growth, enormous multiplication potential and unusual seed dormancy. Ramsar Convention has declared Kolleru Lake of Andhra Pradesh as a Ramsar site (No.1209 on 19/08/2002). Now India is under obligation to conserve this wetland ecosystem. As the weed proliferation, eutrophication and death of the lake are linked processes, the weed control strategy attracts paramount importance. Hence, scientists are putting serious efforts to eradicate this obnoxious and invasive weed but in vain. Its eradication through physical removal is tedious, mechanical means is expensive, biological control is ineffective and spraving herbicides is environmentally objectionable (Ramaprabhu, Kumaraiah, Parameswaran, Sukumaran, & Raghavan, 1987). Under these circumstances, the concept of 'making use of aquatic weeds' (NAS, 1976; Abdel-Sabour, 2010; Aboul-Enein et al., 2011) and the philosophy of

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'eradication through utilization' (Aboul-Enein et al., 2011) seems to be workable options for ecological, environmental, social and economical point of view.

Further, food security to the ever-growing world population is going to be a major challenge of the 21st century. The scientists world over are seriously exploring ways and means to bring more food in one form or other on to the dining table. Cultivation of mushrooms using paddy straw is one such example and now the demand for Agaricus bisporus (Lange) Sing., mushrooms is on the rise. India boosted mushroom production from 5000 tons in 1970 to over 120,000 tons in 2011 (ICAR, 2011). Increased demand for mushrooms naturally results in large-scale diversion of paddy straw, a valuable fodder resource of the Indian cattle that is already in severe scarcity is going to worsen the fodder crisis further. In this backdrop, the usage of most troublesome weed of Kolleru Lake in place of the paddy straw appeared to be a wonderful solution not only to the fodder crisis but also several other crises such as food crisis, locals' livelihood insecurity and above all the lake's un-sustainability. Conversion of water hyacinth the most problematic weed biomass in to protein rich and tasty food will be a real boon to the starving world and dying lakes. Murugesan, Vijayalakshmi, Sukumaran, & Mariappan, (1995) perfected the technology to cultivate oyster mushrooms utilizing water hyacinth. They showed the weed biomass as a viable substrate considering technological and economic aspects only. However, none has tried with the white button mushrooms (Agaricus bisporus). Sometimes, the solutions prescribed in haste may invite environmental problems such as movement of toxic metals into the food chain. Independent studies on bioaccumulation of toxic metals in water hyacinth weed (Nor, 1990) and in edible mushrooms (Kalač & Svoboda, 2000) are giving scope to suspect health hazard owing to food chain contamination thereby necessitating this investigation of the health threat, if any, owing to possible movement of heavy metals from water hyacinth to white button mushrooms.

2. Materials and method

The collected water samples and the water hyacinth shoots from Kolleru Lake (16°37'N 081°12'E) and analyzed for ten heavy metals Mn, Cu, Zn, Cd, Pb, Cr, Fe, Ni, Co and arsenic by using Agilent 7700 series Inductively Coupled Plasma Mass Spectrometer (ICP-MS), which is highly sensitive and capable of determination of a range of metals. Water hyacinth (Eichhornia crassipes), a problematic weed of Kolleru Lake, aging >50 days was used as a substrate in cultivating A. bisporus mushrooms. Abdel-Sabour, (2010) observed excessive accumulation of toxic metals such as Cd, Cr, Cu, Ni and arsenic in roots and rhizomes than in the shoots of water hyacinth. Hence, the exclusion of the roots and rhizomes in the growth substrate was intentional to minimize the food chain contamination with heavy metals. Maintained control samples using conventionally used paddy straw as substrate. To cultivate white buttoned mushrooms followed the method of Krishnamurthy, Nakkeeran, Prakasam, & Marimuthyu, (2000) with slight modification. These first crop mushrooms of eighteen days old were harvested independently and analyzed to study bioaccumulation of heavy metals (Mn, Cu, Zn, Cd, Pb, Cr, Fe, Ni, Co and As) using ICP-MS after microwave digestion with proper validation. The trace elements were determined on dry weight basis. Also analyzed the lake water and the weed biomass (excluding roots and rhizomes) and showed the heavy metal contents in Table 1. This helps in tracking the flow of trace metals from lake water to mushrooms via water hyacinth (Table 1). We converted the ppb level data in to mg/L in case of lake water and mg/kg in case of water hyacinth and mushrooms on dry weight basis. Divided mean values by 50 with the assumption that one adult will eat 200 grams mushrooms per serving on fresh weight basis and 10 kgs fresh weight mushrooms will become 1 kg on

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d.w.basis and shown in parenthesis of Table 2. We compared the heavy metal contents of the mushrooms usually grown on paddy straw as controls and the mushrooms experimentally cultivated from the weed and verified with FAO/WHO standards of food products (FAO/WHO, 1989; WHO, 1996). As a part of statistical analysis, we compared the mean values of individual trace elements present in the experimental and control samples of five each using student's paired t test with a two-tailed distribution. As the accumulative capability of heavy metals is species specific (Kalač & Svoboda, 2000), we have restricted comparisons strictly related to *A. bisporus* mushrooms from the literature (Table 3).

Name of the	Kolleru Lake Biomass of water		Ratio
heavy metal	water KLW) (mg/L)	hyacinth (BWH)	Between
neavy nietai		(mg/kg d.w basis)	BWH&KLW
Manganese (Mn)	1.09 ± 0.08	121.00 ± 0.7	111.00
Copper (Cu)	3.06 ± 1.06	84.60 ± 0.9	27.67
Zinc (Zn)	5.04 ± 1.07	93.70 ± 1.8	18.60
Cadmium (Cd)	2.07 ± 0.08	2.83 ± 0.8	1.37
Lead (Pb)	1.08 ± 0.06	131.40 ± 0.6	121.67
Chromium (Cr)	4.06 ± 1.08	18.58 ± 0.1	4.58
Iron (Fe)	14.05 ± 1.06	1891.00 ± 45	134.59
Nickel (Ni)	5.09 ± 0.03	2.48 ± 1.7	0.49
Cobalt (Co)	3.09 ± 0.03	4.68 ± 1.3	1.51
Arsenic (As)	1.07 ± 0.08	1.91 ± 2.5	1.79

Table 1: Heavy metal concentrations in water and water hyacinth weed of Kolleru Lake

Table 3: Heavy metal concentrations (mg/kg d.w basis) in the Agaricus bisporus reported from the literature and by the authors in the present study

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Author	Mn	Cu	Zn	Cd	Pb
Zhu et al., 2011	28.8 ± 2.1	14.8 ± 1.1	81.4 ± 6.4	0.35 ± 0.02	2.21 ± 0.13
Ita et al., 2008	30.63 ± 3.77	18.13 ± 2.90	43.21 ± 5.91	0.62 ± 0.14	1.06 ± 0.23
Demirbaş 2001	22.3 ± 4.7	5.22 ± 0.72	17.8 ± 5.8	3.48 ± 0.58	2.41 ± 0.83
Mendil et al., 2004	20.9 ± 1.3	11.9 ± 1.0	51.8 ± 4.6	0.10 ± 0.01	6.9 ± 0.3
Authors' study-					
Water hyacinth					
substrate	16.72 ± 0.6	7.448 ± 0.368	46.46.86 ± 3.52	0.146 ± 0.6	0.756 ± 0.054
Authors' study-					
Paddy straw					
substrate	14.3 ± 0.56	6.926 ± 0.342	47.32 ± 3.8	0.152 ± 0.56	0.738 ± 0.05

Table 3 (continued) : Heavy metal concentrations (mg/kg d.w basis) in the Agaricus bisporus
reported from the literature and by the authors in the present study

Author	Cr	Fe	Ni	Со	As
Zhu et al., 2011	22.6 ± 1.8	190 ±13	0.92 ± 0.06	а	а
Ita et al., 2008	а	а	1.94 ± 0.46	а	а
Demirbaş 2001	а	126 ±14	56.1 ± 12.6	0.32 ± 0.09	0.76 ± 0.19
Mendil et al., 2004	3.1 ± 0.2	332 ± 22.6	8.2 ± 0.6	а	а
Authors' study- Water hyacinth substrate	16.18 ± 2.96	1596 ± 81.6	1.434 ± 0.074	0.564 ± 0.056	0.64 ± 0.16
Authors'study- Paddy straw substrate	16.32 ± 0.58	1322.4 ±70.8	1.472 ± 0.062	0.582 ± 0.072	0.68 ± 0.062

^a not detectable

3. Results and Discussion

The results of the Table 1 reveal the contents of trace metals in the Kolleru Lake water and the shoots of the water hyacinth samples, and the ratios between them. The accumulation of trace elements such as Mn, Cu, Zn, Pb, Cr and Fe in the shoots of the weed was several times greater than that of the lake waters. In case of Cd, Co, and As relatively lesser accumulation took place whereas nickel recorded the least (Table 1). Dixit & Tiwari, (2007) also reported multifold accumulation with Pb, Cr, Zn and Mn in their study on Shahpura Lake of Bhopal. According to Zhu, Zayed, Qian, de Souza, & Terry, (1999) accumulation of Cd, Cr, Ni and arsenic was higher in water hyacinth roots than in its shoots. Largely, the water hyacinth weed showed greater affinity for the metals. Thus, it has become necessary to analyze the heavy metal concentration in the experimental and control samples of *A. bisporus* mushrooms.

The results of the Table 2 reveal the contents of trace elements of the mushrooms grown experimentally on water hyacinth and controls grown on paddy straw. The metal uptake by the mushrooms from water hyacinth substrate in decreasing order was found to be as Fe>Zn>Mn>Cr>Cu>Ni>Pb>As>Co>Cd. The boiling of the shoots of the weed while preparing the substrate for mushroom cultivation; and shorter crop duration might be the other reason (Kalač & Svoboda, 2000) for lesser accumulation of trace elements in the mushrooms cultivated from the water hyacinth weed. Table 2 also reveals the contents of trace elements in the control samples of mushrooms cultivated from the paddy straw substrate. The metal uptake by the mushrooms from paddy straw substrate in the decreasing order was found to be as Fe>Zn>Cr>Mn>Cu>Ni>Pb>As>Co>Cd. As for as the contents of trace elements are concerned not much variation was noticed between the experimental as well as the control samples.

In fact, the presence of trace metals such as iron, copper, zinc, manganese cobalt, chromium (trivalent) and nickel is desirable as they play important role in biochemical processes of various biological systems (Unak, Lambrecht, Biber, & Darcan, 2007). Manganese is one of the vitally important elements of several enzymes. Copper is essential for a variety of biochemical processes. Zinc is essential for all forms of biochemical processes including the process of genetic expression. Plant based mechanism of reduction of Cr(VI) into Cr(III) in water hyacinth roots was observed by Lytle, Lytle, Yang, Qian, Hansen, & Zayed, (1998). Consequently, the non-toxic Cr(III) finds in leaf tissues. Trivalent chromium is a trace metal having nutritional significance and necessary for the normal metabolism of cholesterol, fat, and glucose (WHO, 1996).

Iron was the dominant element in comparison to heavy metals in the mushrooms followed by zinc and manganese (Table 2). Iron is vital in a variety of metabolic processes, including hemoglobin synthesis. The iron levels in the mushrooms of both the experimental and the control samples were higher than the levels of the other metals and the reported values for iron from the literature (Table 3) by Demirbaş (2001); Mendil, Uluözlü, Hasdemir, & Çaglar, (2004); Zhu, Qu, Fan, Qiao, Hao, & Wang, (2011) also reported the highest concentration of iron in almost all mushroom species studied by them. In general, iron deficiency is prevalent owing to low iron foods and poor absorption rates. As a result, most of the people are becoming anemic. High intake is desirable to compensate excretion and un-utilization (Lynch & Baynes, 1996). Furthermore, high iron intake helps in reducing absorption, utilization and retention of the most toxic metals particularly cadmium and lead with its antagonistic nature (WHO, 1996). Thus, excess iron in the mushrooms is a blessing in disguise. Cobalt is an essential component of vitamin B_{12} and together it fights pernicious anemia. Nickel is

beneficial as an activator of some enzyme systems. It could improve insulin production with the help of vitamin C. Ni and Co are essential in vaso-dilation of left and right coronary arteries respectively.

Cadmium is a highly toxic metal. It seems to be the most deleterious among heavy metals in mushrooms. Luckily, it is the least in quantity out of the ten metals worked out (Table 2). Lead is a toxic metal that has no beneficial role in human metabolism. Arsenic causes skin disorders. However, arsenic will not occur in undesirable levels in mushrooms (Kalač & Svoboda, 2000).

Using Student's *t* test compared the contents of the trace elements of the mushrooms cultivated from the water hyacinth shoots with those of the mushrooms cultivated from paddy straw, which served as a control and found no statistically significant differences of mean values (Table 2). As the calculated *t* values (Table 2) are less than the table values ($t_{4; 0.025} = \pm 2.776$) the null hypothesis is accepted i.e. no statistically significant differences are found between the means of the individual trace elements from the experimental and control samples. The contents of all the elements were in agreement with those reported in the literature (Table 3). Further, all these are well below the FAO/WHO, (1989) and WHO, (1996) prescribed limits for food products (Table 2).

There was no additional threat of heavy metal bioaccumulation by replacing the conventional paddy straw with the water hyacinth shoots in the cultivation of *A. bisporus* mushrooms. Hence, recommended the water hyacinth of Kolleru Lake as a growth substrate in cultivating *A. bisporus* mushrooms. Even, if any threat persists, according to Zrodlowski, (1995) these metals can be reduced up to 30-40% by simply washing and hand peeling of caps and stalks of *A. bisporus*. Short term boiling also can reduce the threat to some extent (Svoboda, Kalač, Spicka, & Janouskova, 2002). Losses of 45, 6, 23 and 4% were observed by Coşkuner, & Özdemir, (1997) for Mn, Fe, Zn and Cu respectively on boiling of *A. bisporus* mushrooms for 15min, at 95-100°C. High calcium and iron diet will be acting antagonistic in absorption, utilization and retention of Zn, Mn, Cd, Pb, and Zn, Cu, Cr respectively (WHO, 1996). Mushrooms are therapeutically valued because of their chemical composition (Manzi, Aguzzi, & Pizzoferrato, 2001), rich proteins (Agrahar-Murugkar & Subbulakshmi, 2005) and minerals (Çayir, Coşkun, & Coşkun (2010). Their consumption is on rise since benefits are many fold.

Table 2: Bioaccumulation of heavy metals in the mushrooms cultivated from water hyacinth(experimental sample) and paddy straw (control sample); calculated t-values to compare the
mean values; and prescribed safe limits for adult/day

Name of the heavy metal	Mean of five values of each heavy metal in mushrooms cultivated from paddy straw (MPS) mg/kg d.w basis	Mean of five values of each heavy metal in mushrooms cultivated from water hyacinth (MWH) mg/kg d.w basis	MPS and MWH Means compared with <i>t</i> test Calculated <i>t</i> values	WHO (1996) Safe limits / 60kg body weight / day
Mn	$14.300 \pm 0.56 \\ (0.286)$	$16.720 \pm 0.6 \ (0.3344)$	0.0647	35.00 ppm
Cu	6.926 ± 0.342	7.448 ± 0.368	0.2211	75.00 ppm

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	(0.13852)	(0.14896)		
Zn	$47.320 \pm 3.8 \\ (0.9464)$	46.860 ±3.52 (0.9372)	0.7830	100.00 ppm
Cd	$\begin{array}{c} 0.152 \pm 0.56 \\ (0.00304) \end{array}$	$\begin{array}{c} 0.146 \pm 0.020 \\ (0.00292) \end{array}$	0.5733	0.50 ppm
Pb	$\begin{array}{c} 0.738 \pm 0.05 \\ (0.01476) \end{array}$	$\begin{array}{c} 0.756 \pm 0.054 \\ (0.01512) \end{array}$	0.2205	0.43 ppm
Cr	$ \begin{array}{r} 16.320 \pm 0.58 \\ (0.3264) \end{array} $	$ \begin{array}{r} 16.180 \pm 2.96 \\ (0.3236) \end{array} $	0.8079	0.24 ppm
Fe	1322.400 ± 70.80 (26.448)	1596 .000 ± 81.6 (31.92)	0.0003	200.00 ppm
Ni	$\begin{array}{c} 1.472 \pm 0.062 \\ (0.02944) \end{array}$	$ \begin{array}{r} 1.434 \pm 0.074 \\ (0.02868) \end{array} $	0.7450	2.00 ppm
Со	$\begin{array}{c} 0.582 \pm 0.072 \\ (0.01164) \end{array}$	$\begin{array}{c} 0.564 \pm 0.056 \\ (0.01128) \end{array}$	0.3530	0.60 ^a ppm
As	$\begin{array}{c} 0.680 \pm 0.062 \\ (0.0136) \end{array}$	0.640 ± 0.16 (0.0128)	0.0337	0.12 ^b ppm

Data given in parenthesis are virtual intake of heavy metals calculated by dividing mean values with 50 on the assumption that one adult will eat 200gms of mushrooms per serving on fresh weight basis and 10kgs fresh weight mushrooms will become 1kg mushroom on drying; ^aMubeen, et al., (2009); ^bFAO/WHO, (1989).

4. Conclusion

There is no additional threat by replacing the paddy straw with the shoots of the water hyacinth weed of Kolleru Lake for the mushrooms production. Further, the contents of the heavy metals are well below the prescribed limits of World Health Organization. Hence, we recommend water hyacinth weed as a safe substitute to paddy straw for the cultivation of white buttoned (*A. bisporus*) mushrooms.

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