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EFFECT OF SELECTED HEAVY METALS ON THE GROWTH PERFORMANCE AND YIELD OF COMMERCIALLY CULTIVATED AMERICAN OYSTER MUSHROOM (*Pleurotus ostreatus)*

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ABSTRACT

Mushrooms can accumulate heavy metals from the growth substrates. This study compared the growth and yield performances of commercially grown American Oyster mushrooms (*Pleurotus ostreatus*) in the presence of different heavy metals in the substrate. The substrate of the mushroom media contained different heavy metals (Cadmium (Cd), Lead (Pb), Arsenic (As), and Mercury (Hg)) in three concentration levels (25, 50, and 75 mg/kg of sawdust). An experiment was carried out in a Complete Randomized Design (CRD) with five replicates. Both growth parameters (total biological efficiency, total colonization time, time for primordial formation, number of fruiting bodies in the first flush, and weekly mycelium growth rate) and yield parameters (weight and the total number of fruiting bodies) of the mushroom were recorded. Three flushes were made during the study period from October to December 2016. All the data were analyzed statistically using ANOVA ($p<0.05$) and Duncan's multiple range test by SAS statistical software (version 9.1.3). The results revealed that biological efficiency of Cd, Hg, and Pb metal concentrated mycelium showed significantly higher values at all three different concentration levels compared to As that lowest at 75 mg/kg concerted level. The highest time of primordial formation, the highest total colonization time, and numbers of fruiting body's development in the first flush was observed at 75 mg/kg level As treated substrate. Further studies are required to find the bioaccumulation of the heavy metal in the mushrooms.

Keywords: Bioaccumulation, Heavy metals, Human health, *Pleurotus ostreatus*, Substrate

INTRODUCTION

Macro fungi or mushrooms belong to fungal groups that form characteristic fruiting bodies in which spores reside (Lee *et al.* 2009) and are important in the ecosystem (Ouzouni *et al.* 2009) due to their ability to biodegrade substrates such as wastes of agricultural production. Besides the primary role in the recycling of plant materials in an ecosystem, also they are appreciated as good sources of food and medicine (Lee *et al.* 2009). Fruiting bodies of mushrooms are valued as food due to their texture, flavor, chemical properties, and nutritional properties (Beluhan and Ranogajec 2011). The therapeutic effects, usefulness in preventing diseases such as hypertension, hypercholesterolemia, and cancer have been reported by Netravathi *et al.* (2006). *Agaricus bisporus* and *Pleurotus ostreatus* are popular edible mushrooms with high commercial values and are thus cultivated worldwide (Lee *et al.* 2009).

Mushrooms have a characteristic fruiting body consist of a stem (Stipe) bearing a cap (Pileus) that has the potentiality to bioaccumulate most heavy metals and can uptake heavy metals from the substrate through substrate mycelia (Sharma *et al.* 2010; Bellettini *et al.* 2016). Therefore, growing on a substrate with a high concentration of various heavy metals, the

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mushroom can become toxic to the consumers by accumulating a larger amount of heavy metals (Stihi *et al.* 2011).

Heavy metal concentrations in mushrooms (macro fungi) are reported to be considerably higher than those in agricultural crop plants, vegetables, and fruits (Kalac *et al.* 1991; Svoboda *et al.* 2000). Many wild edible mushroom species have been known to accumulate great concentrations of heavy metals such as lead, cadmium, iron, copper, manganese, zinc, chromium, nickel, aluminum, and mercury (Kalac *et al.* 1991; Svoboda *et al.* 2000; Kalac and Svoboda 2000; Bellettini *et al*. 2016; Dulay *et al*. 2015a).

The accumulation of heavy metals in macro fungi has been affected by environmental (*e.g.* organic matter content, pH, substrate type, and metal concentrations) and fungal factors (*e.g.* species of the mushroom, morphological part of the fruiting body, developmental stage, age of the mycelium, the interval between fructification and biochemical composition, *etc.*) (Garcia *et al.* 1998; Kalac and Svoboda 2000; Chen 2004; Ita *et al.* 2006; Yilmaz *et al.* 2003; Dulay *et al.* 2015a).

Nevertheless, Sharma *et al.* (2010) reported that the age and size of the fruiting body of any mushroom have an eligible role in the uptake of heavy metals because the life span of the fruiting body is only 10-14 days hence time consumed for the uptake of these metals from the substrate is limited. Moreover, *Pleurotus* species strains showed higher resistance to heavy metals like Cu, Cd, Zn, Ni, Co, and Hg than the other species (Sanglimsuwan *et al.* 1993).

Mushrooms are also excellent in heavy metal biotransformation and are considered good recyclers (Demirbas 2001). In recent years, considerable attention has been focused on the bioaccumulation of heavy metals in fruiting bodies of mushrooms (Zhu *et al.* 2011; Bellettini *et al.* 2016; Llorente-Mirandes *et al.* 2016). Compared to green plants, mushrooms can build up large concentrations of the same heavy metals such as Pb, Cd, and Hg (Demirbas 2001), while considerably higher heavy metal concentrations can be present in mushrooms than those in agricultural crop plants, vegetables, and fruits (Dursun *et al.* 2006). Heavy metal contamination of food is one of the most important aspects of food quality assurance. Intake of heavy metal-contaminated mushrooms may pose a risk to human health. On the other hand, heavy metal concentrations in the wastes used as a substrate may also influence the growth and the yield of commercially cultivated Oyster mushrooms.

Agro-industrial waste produces in huge amounts and it becomes an interesting substrate, due to its commercial exploitations as well as associated environmental problems. They also can be an added value to low-cost production. Many studies have been conducted to test the ability of *Pleurotus* spp. to grow on different agro wastes such as rice bran, sawdust, and other agricultural residues and accumulation of toxic metals (Bellettini *et al.* 2016; Dulay *et al.* 2015a). Therefore, awareness of the heavy metal composition and accumulation of heavy metals in the cultivated mushroom on agro-waste substrates is important before making its use in mushroom cultivation (Patil *et al.* 2010; Bellettini *et al.* 2016).

This study was conducted to investigate the influence of different heavy metal levels on the growth and the yield of commercially cultivated American oyster mushrooms under farmers' field conditions in Sri Lanka. It is hypothesized that (1) the levels of the heavy metal in the substrate do not affect the growth and yield of the mushroom and, (2) there is no impact of heavy metals on the biological efficiency of the selected substrate.

METHODOLOGY

Study area

The study was conducted at the laboratory of the Department of Agriculture Biology, and mushroom cropping houses at the Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka, from October to December 2016.

Substrate processing and spawning

All substances and ingredients (100 kg of sawdust, 10 kg of rice bran, 2 kg of $CaCO_3$, and 1 kg of green gram flour) were mixed well with water. Then 200 g of $MgSO_4$ was dissolved in water and it was sprinkled onto the mixture. The substrates were allowed to dry in the sun and regularly weighed (daily) for five consecutive days to determine the change in the air-dried weight of the substrates. Substrates were divided into five and heavy metals were mixed (Table 1). After that, they were transferred to 10 individual (32.5 cm × 17.5 cm size and 200 gauges) polypropylene bags. The optimum moisture was determined by pressing a handful of the mixture making sure that water does not leak through the fingers and a handful of the substrate remains without breaking down. The moisture content was brought to 65–70 % (Iqbal *et al*. 2005). The bags were filled with substrates (three-quarter of the bag) and the mouth was plugged with PVC ring and cotton wool. It was covered by a piece of paper and a rubber band. The substrates were sterilized (three and half-hours time) in steam at 120 °C temperature under 15 psi pressure and allowed to cool to room temperature for several hours.

Table 1: Treatment allocation showing substrate mixing with different concentrations of heavy metals in the compost medium

	Treatment	Concentration (mg/ kg of sow dust)		
Control	T1	-		
$CdSO_4$	T2	25	50	75
$Pb(NO_3)_2$	T3	25	50	75
$AsSO_4$	T4	25	50	75
$HgCl_2$	T5	25	50	75

Following this, substrates bags were transferred to a sterilized mushroom growing house and each bag was inoculated with a spoonful of spawn prepared in paddy seed substrate. The bags were sealed and plugged with PVC rings and cotton wool covered by a piece of paper and placing a rubber band, and kept under a dark condition.

Cultivation conditions and cropping system

The cultivation and cropping method described by Chang and Miles (2004) was adopted. After mycelial growth in the bags under the dark condition became abundant and/or pinheads emerged, the portions of the bags were cut-off to create perforations to facilitate the development of fruiting bodies and were placed on racks at a spacing of 15–20 cm. Inoculated bags were watered 2–3 times a day to keep the mycelia moist. Relative humidity (RH) and room temperature were monitored and maintained with thermo hydrometer and RH was maintained between 80 and 85 % by spraying a fine mist of water (Oei and Mass 2003) as required.

Data Collection

Harvesting was done three times during the study period. The first harvest was done after four weeks from the inoculation. The second and third harvesting time varied with the treatment combinations. Both Mycelial growth parameters (Biological efficiency, average colonization time, time for primordial formation, number of fruiting bodies of the first flush, and weekly mycelia growth rate) and Yield parameters (weight, and the total number of fruiting bodies) were measured and recorded. Biological efficiency (BE) was calculated as follows (Chang and Miles 2004),

$$B.E. = \frac{Fresh\ weight\ of\ mushroom}{Dry\ weight\ of\ mushroom} x100$$

Table 2: Fresh weight of Pleurotus ostreatus mushroom on different heavy metal level treated substrates

Heavy metal Treated Level	Treat-ment	1st harvest Fresh weight (g)	2nd harvest Fresh weight (g)	3rd harvest Fresh weight (g)	Total harvest Fresh weight (g)
	Control	62.0±8.00ab	31.0±09.27bcd	27.0±09.17a	120.0±08.51a
25 mg/kg	Cd	66.0±11.66ab	29.0±09.54bcd	12.0±07.35bc	107.0±10.44ab
	Hg	70.0±19.75ab	30.0±10.95bcd	06.0±06.00bc	106.0±29.93ab
	Pb	76.0±11.22ab	21.0±05.57bcde	08.0±05.83bc	101.0±14.87ab
	As	64.0±07.48ab	00.00±00.00e	00.00±00.00c	64.0±07.48bc
50 mg/kg	Cd	70.0±22.80ab	27.0±08.00bcd	06.0±04.00bc	103.0±17.72ab
	Hg	84.0±04.00a	34.0±08.72bc	06.0±04.00bc	102.0±24.98ab
	Pb	80.0±11.83ab	16.0±02.92cde	16.0±2.92ba	112.0±12.81ab
	As	36.0±11.67b	08.0±08.00ed	00.00±00.00c	44.0±11.66c
75 mg/kg	Cd	70.0±25.30ab	34.0±08.72bc	02.0±02.00c	106.0±23.58ab
	Hg	100.0±04.47a	44.0±04.00b	04.0±04.00bc	148.0±06.63a
	Pb	72.0±08.60ab	68.0±04.90a	00.00±00.00c	140.0±05.48a
	As	36.0±16.00b	00.00±00.00e	00.00±00.00c	36.0±16.00c

Data were analyzed by Duncan's Multiple Range Test. Each value is expressed as the mean ± S.E. (n=5). Means within a column followed by the same letters are not statistically significantly different (P<0.05).

Experimental Design and Statistical analysis

The experiment was carried out in a 5×3 factorial Completely Randomize Design (CRD) with five replicates of each treatment. Four heavy metal types with three different concentrations were used along with the control (Table 1). Statistical analysis was performed with Duncan's multiple range test (DMRT) using SAS statistical software (version 9.1.3).

RESULTS AND DISCUSSION

Fresh weight of *Pleurotus ostreatus* on the substrate having different heavy metal concentration levels

According to the results (Table 2), the yield has reduced significantly with the increase of metal concentration in the substrate. The lowest total fresh weight was found at 75 mg/kg concentration level in As treated substrate and showed the reduction of the mushroom yield in the subsequent harvests. Arsenic (As) showed a significant difference between other heavy metals and there were no significant differences within the concentrations of As.

More than 50% of the yield was obtained in the first two flushes in all of the substrates. Considering the total fresh harvest, the highest yield (148 g; 140 g; 120 g) parameter of total fresh weight was shown in 75 mg/kg concentration level of Hg, 75 mg/kg concentration level of Pb, and control treatment respectively. However, the total harvest obtained with the substrate treated with 75 mg/kg of both Pb and Hg separately, was not significantly different from the total harvest of the control. This study was found that when increasing the level of heavy metal, the yields of subsequent harvesting stages (first, second, and third flushes) have been decreased.

According to Radulescu *et al*. (2010), the accumulation of metals in the mushrooms was found to be species metabolism-dependent and likewise strongly affected by the chemical composition of the substrate. Cd, Hg, and Pb increased the yield with the increasing level of heavy metal in the substrates. The reasons could not be explained clearly by the evidence here, however, there was some negative effect on yield or fresh

Table 3: Comparisons of mycelial growth parameters of *Pleurotus ostreatus*

Heavy metal Level	Treatment	Biological Efficiency (%)	Average colonization time (days)	Time to primordial formation (days)	No of fruiting bodies in the 1st flush
	Control	123.46±2.19c	25.8±0.5e	28.8±0.5e	04.80±01.66bc
25 mg/ kg	Cd	118.10±1.73d	26.6±0.7de	29.4±0.6e	04.40±00.60bc
	Hg	098.15±6.12i	26.6±0.5de	29.2±0.6e	05.60±00.40bc
	Pb	101.10±3.21h	25.0±0.0e	27.2±0.4e	06.60±01.50abc
	As	064.00±1.70k	40.2±1.7c	42.2±1.7c	06.40±01.78abc
50 mg/ kg	Cd	109.57±4.52e	28.0±1.6de	30.2±1.6e	04.20±01.78bc
	Hg	097.89±4.91j	25.4±0.2e	27.4±0.5e	08.40±01.86ab
	Pb	107.28±2.85g	24.6±0.4e	27.0±0.5e	05.60±00.93bc
	As	038.60±2.06l	43.8±1.3b	45.6±1.3b	01.60±00.67c
75 mg/ kg	Cd	108.16±6.06f	30.2±2.5d	33.8±2.1d	07.20±03.14ab
	Hg	147.12±1.71a	25.0±0.0e	27.2±0.4e	07.80±02.85ab
	Pb	145.53±1.33b	25.4±0.4e	27.8±0.5e	06.40±01.21abc
	As	030.87±2.87m	65.6±2.1a	72.2±0.6a	11.20±00.49a

Data were analyzed by Duncan's Multiple Range Test. Each value is expressed as the mean ± S.E. (n=5). Means within a column followed by the same letters are not statistically significantly different (P<0.05).

weight of the mushroom with As treated substrates compared to Pb, Hg, and Cd, which needed to be investigated.

Mycelial growth parameters of *Pleurotus ostreatus* on substrates treated with different heavy metal concentration

The total biological efficiency (%), total colonization time (in days), time to the primordial formation (days), and the number of fruiting body development at 1st flush were measured and data were reported in Table 3.

The highest time of primordial formation, the highest total colonization time, and numbers of fruiting body development in the first flush was found in heavy metal concentration level 75 mg/kg of As, and the lowest total biological efficiency was also reported at 75 mg/kg As concentration level.

The highest biological efficiency was shown at 75 mg/kg Hg concentration treated bags. However, total colonization time, primordial formation time, and the number of fruiting bodies in first flush values showed low values. Nevertheless, these results showed a negative relationship to heavy metal concentration compared with 75 mg/kg As concentration level. Jain *et al*. 1988 observed that the production of fruiting bodies (sporocarp) in Pleurotus spp. was reduced by the high concentration of heavy metals. Purkayastha and Mitra (1992) reported that Co and Pb caused a maximum reduction in the fruiting body's production of *V. volvacea* and *Pleurotus* spp.

The mycelium growth rate of *Pleurotus ostreatus* on different levels of heavy metal concentrations

Mycelium length was measured by the end of each week and a comparison of the mycelium growth rate of *Pleurotus ostreatus* on the different heavy metal levels is given in table 4. When the beginning of the third week, colonization and initiated primordial formation were completed in most of the substrates. The mycelia growth rate in the different heavy metal treated substrates significantly ($p < 0.05$) lower than the control treatment in the first week. According to the results, As metals affected a significant

Table 4: Comparison of the mycelial growth rate of *Pleurotus ostreatus* on different heavy metal concentration levels

Concentration Level	Heavy metal	1st week Growth rate mm/day	2nd week growth rate mm/day	3rd week growth rate mm/day
	Control	1.87±1.40a	0.99±0.00a	1.00±0.01a
25 mg/kg concentration Level	Cd	0.54±0.01b	0.98±0.01a	1.00±0.01a
	Hg	0.54±0.02b	0.97±0.01a	1.00±0.00a
	Pb	0.39±0.03b	0.97±0.01a	1.00±0.01a
	As	0.26±0.06b	0.78±0.06b	1.00±0.00a
50 mg/kg concentration Level	Cd	0.42±0.05b	0.98±0.01a	1.00±0.02a
	Hg	0.48±0.03b	0.95±0.02a	1.00±0.02a
	Pb	0.44±0.20b	0.98±0.01a	1.00±0.00a
	As	0.22±0.33b	0.64±0.05c	0.97±0.03a
75 mg/kg concentration Level	Cd	0.43±2.24b	0.87±0.11ab	0.93±0.07a
	Hg	0.50±0.02b	0.99±0.01a	1.00±0.00a
	Pb	0.43±0.00b	0.98±0.01a	1.00±0.00a
	As	0.05±0.00b	0.50±0.05d	0.82±0.06b

Data were analyzed by Duncan's Multiple Range Test. Each value is expressed as the mean ± S.E. (n=5). Means within a column followed by the same letters are not statistically significantly different (P<0.05).

reduction of the mycelial growth rate compared with the other metals.

Generally, heavy metal contents at low and medium levels have been shown to inhibit the mycelial growth rate. The higher concentration of As may inhibit the growth or even death of mushrooms. Some the metals like Zinc (Zn) and Iron (Fe) ion may act as growth stimulators (Das 2005). The mycelium growth is increased significantly at Pb, Cd and Hg treated substrates. Dulay *et al*. (2015a) reported that the lowest mycelial growth of P. *ostreatus*, and other *Pleurotus* spps. have been resulted by Pb in the concentration of 100 ppm. Cd was reported to have the ability to inhibit the growth of mushrooms by restricting the bio-contact of the heavy metals in the soil (Mitra 1994).

Mandal *et al*. (1998) quoted that the growth of the mushroom was significantly inhibited by Hg including P. *ostreatus*, while Purkayastha and Mitra (1992) observed more than 85% reduction of growth in *Pleurotus* spps. at 15 and 6 µg/ml of Pb and Hg, respectively, where Pb had reduced mycelial protein significantly (36%), while Hg caused a maximum reduction (30%) of proteins in sporocarp.

When concerning the mushroom growth, Baldrian (2003) indicated that the Hg is the most potent growth inhibitor, where a 3-day lag phase was observed in Hg causing a decrease of growth rate down to 8 %. Baldrian (2003) reported white-rot fungi to require trace amounts of essential heavy metals such as Cd, Mn, or Zn for their growth, but these metals are toxic when present in excess. Toxic heavy metals can inhibit the mycelial growth and the decrease of the growth rate of fungi caused by heavy metals is often accompanied by morphological changes of the growing mycelium (Baldrian 2003; Dulay *et al*. 2015b). Yilmaz *et al*. (2003) reported the uptake rate of heavy metals in mushrooms could be correlated with the contact time and distributed unevenly within a fruiting body. Moreover, Mitra (1994) mentioned that the uptake of heavy metals also depends on the concentrations of other metals in the substratum.

CONCLUSION

According to data, increased Arsenic (As) level in the substrate, reduced the fresh weight of the mushroom, while increased Pb, Cd, and Hg levels increased the fresh weight of P. *ostreatus*. The highest time of primordial

formation, highest total colonization time, and highest numbers of primordial formation time (days) taken in the first flush were found at 75 mg/kg As concentration level. The lowest total biological efficiency was reported at 75 mg/kg As concentration level than Cd, Pb, and Hg. Accumulated heavy metal in mushrooms will have a toxic effect on the human body and needs to further investigate heavy metal accumulation for the safety of human consumption. Further studies are required to find the absorption level and the rate of the heavy metal levels which admit in the mushrooms.

REFERENCES

Baldrian P and Gabriel J 2003 Lignocellulose degradation by *Pleurotus ostreatus* in the presence of cadmium. FEMS Microbiol. Lett. 220: 235-240.

Bellettini MB, Fiorda FA, Maieves HA, Teixeira GL, Ávila S, Hornung PS, Júnior AM and Ribani RH 2016 Factors affecting mushroom *Pleurotus* spp. Saudi J. Biol. Sci. 26(4): 633-646.

Beluhan S and Ranogajec A 2011 Chemical composition and non-volatile components of Croatian wild edible mushrooms. Food Chem. 124(3): 1076-1082.

Chang ST and Miles PG (2 eds.) 2004 Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact. Boca Raton: CRC Press, USA.

Chen WA 2004 Growing specialty mushroom Shiitake. Mushworld Publishers, Heineart Inc. Republic of Korea: 248-260.

Das N 2005 Heavy metals biosorption by mushrooms. Indian J. Nat. Prod. Resour. 4(6): 454-459.

Demirbas A 2001 Concentrations of 21 metals in 18 species of mushrooms growing in the East Black Sea region. Food Chem. 75: 453-457.

Dulay RMR, De Castro MAEG, Coloma NB, Bernardo AP, Cruz AGD, Tiniola RC, Kalaw SP and Reyes RG 2015a Effects and mycoremediation of lead (Pb) in five *Pleurotus* mushrooms. Int. J. Biol. Pharm. Allied Sci. 4(3): 1664-1677.

Dulay RMR, Pascual AHL, Constante RD, Tiniola RC, Areglo JL, Arenas MC, Kalaw SP and Reyes RG 2015b Growth response and mycoremediation activity of *Coprinus comatus* on heavy metal contaminated media. Mycosphere 6(1): 1-7.

Dursun N, Ozcan MM, Kasık G and Ozturk C 2006 Mineral contents of 34 species of edible mushrooms growing wild in Turkey. J. Sci. Food Agric. 86: 1087-1094.

Iqbal MSH, Rauf A and Sheikh IM 2005 Yield performance of oyster mushroom on different substrates. Int. J. Agric. Biol. 7: 900-903.

Ita BN, Essien JP and Ebong GA 2006 Heavy metal levels in fruiting bodies of edible and non-edible mushrooms from the Niger Delta Region of Nigeria. J. Agric. Soc. Sci. 2: 84-87.

Jain SK, Gujral GS, Jha NK and Vasudevan P 1988 Heavy metal uptake by *Pleurotus* sajor-caju from metal-enriched duckweed substrate. Biol. Wastes. 24: 275-282.

Kalac P, Burda J and Staskova I 1991 Concentration of lead, cadmium, mercury and copper in mushrooms in the vicinity of a lead smelter. Sci. Total Environ. 105: 109-119.

Kalac P and Svoboda L 2000 A review of trace element concentrations in edible mushrooms. Food Chem. 69: 273-281.

Lee CY, Park JE, Kim BB, Kim SM and Ro HS 2009 Determination of mineral components in the cultivation substrates of edible mushrooms and their uptake into fruiting bodies. Mycobiology 37(2): 109-113.

Llorente-Mirandes T, Llorens-Muñoz M, Funes-Collado V, Sahuquillo À and

López-Sánchez JF 2016 Assessment of arsenic bioaccessibility in raw and cooked edible mushrooms by a PBET method. Food Chem. 194: 849-856.

Mandal TK, Baldrian P, Gabriel J, Nerud F and Zadraz ˇil F 1998 Effect of mercury on the growth of wood-rotting basidiomycetes *Pleurotus ostreatus*, Pycnoporus cinnabarinus and Serpulal acrymans. Chemosphere 36(3): 435-440.

Mitra AK 1994 Studies on the uptake of heavy metal pollutants by edible mushrooms and its effect on their growth, productivity and mammalian systems. PhD thesis, University of Calcutta.

Netravathi GP, Sathisha UV, Shylaja MD, Shashirekha MN and Rajarathnam S 2006 Antioxidant activity of indigenous edible mushrooms. J. Agric. Food Chem. 54(26): 9764-9772.

Oei P and Maas M 2003 Mushroom cultivation, appropriate technology for mushroom growers. Back-hughs Publishers, Leiden, The Netherlands: 110-150.

Ouzouni PK, Petridis D, Koller WD and Riganakos KA 2009 Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece. Food Chem. 115(4): 1575-1580.

Patil SS, Ahmed SA, Telang SM and Baig MMV (2010) The nutritional value of *Pleurotus ostreatus* (Jacq.:fr.) kumm cultivated on different lignocellulosic agro wastes. Innov. Rom. Food Bio-technol. 7: 66-76.

Purkayastha RP and Mitra AK 1992 Metal uptake by mycelia during submerged growth and by sporocarps of an edible fungus Volvariell avolvacea. Indian J. Exp. Biol. 30(12): 1184-1187.

Radulescu C, Stihi C, Busuioc G, Popescu IV, Gheboianu AI and Cimpoca VG 2010 Evaluation of essential elements and heavy metal levels in fruiting bodies of wild mushrooms and their substrate by EDXRF spectrometry and FAA spectrometry. Rom Biotech. Lett. 15 (4): 5444-5456.

Sanglimsuwan S, Naoto Y, Tsutomu M and Yoshikatsu M 1993 Resistance to and Uptake of Heavy Metals in Mushrooms. J. Ferment Bioeng. 75 (2): 112-114.

Sharma R, Rakesh K and Akhilesh KP 2010 Dynamics of Acid Phosphatase Production of the Ectomycorrhizal Mushroom Cantharellus tropicalis. J. Biotech.15: 1563-1564.

Stihi C, Radulescu C, Busuioc G, Popescu IV, Gheboianu A and Ene A 2011 Studies on accumulation of heavy metals from substrate to edible wild mushrooms. Rom. Journ. Phys. 56: 257-264.

Svoboda L, Zimmermannova K and Kalac P 2000 Concentrations of mercury, cadmium, lead and copper in fruiting bodies of edible mushrooms in an emission area of a copper smelter and a mercury smelter. Sci. Total Environ. 246: 61-67.

Yilmaz F, Isiloglu M and Merdivan M 2003 Heavy metal levels in some macrofungi. Tus. J. of Botany 27: 45-56.

Zhu F, Qu L, Fan W, Qiao M, Hao H and Wang X 2011 Assessment of heavy metals in some wild edible mushrooms collected from Yunnan Province, China. Environ. Monit. Assess. 179: 191-199.

Author Contribution

LDMZ, PCDP and KLWK conceptualized and designed the study. LDMZ, SSMP and PCDP performed the experiments. LDMZ, SSMP and PCDP analyzed the data. LDMZ, PCDP, SSMP and KLWK wrote the paper with input from all authors. All authors discussed the results and commented on the manuscript.