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Original Article

Evaluating the Antioxidant and Heavy Metal Content of *Pleurotus ostreatus* Mushrooms Cultivated using Sugar Cane Agro-Waste

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ABSTRACT

Background: *Pleurotus ostreatus,* is one of the most cultivated mushrooms with great economic and medicinal value that can be easily grown on various bio-waste substrates. However, biosafety evaluations on these mushrooms are rarely conducted. Thus, we sought to evaluate the concentration or presence of Heavy metals in *P. ostreatus* mushrooms cultivated on agro-bio-waste products. Furthermore, the effect of adding agro wastes on wheat bran (WB) cultivated mushrooms was evaluated. **Methods:** Mushrooms grown in sugar cane tops and bagasse were supplemented with varying levels of WB. Atomic absorption spectrophotometer was applied to evaluate the concentration of heavy metals in the substrates and within mushrooms. Furthermore, DPPH free radical scavenging activity was used to determine antioxidant activity of mushroom extracts. **Results:** The transfer factor analysis (TF) showed that mushrooms have an affinity to absorb Zn, Cd, Cu and Cr from all tested substrates during cultivation (TF>1). The addition of WB supplement into substrates resulted into significant increase in mushroom yield. However, the increased addition of WB, inversely affected the DPPH scavenging activity of the *P.ostreatus* methanolic extracts. **Conclusion:** The bio-absorption of heavy metals by *P. ostreatus* is depended on the metal type. Based on these findings, mushrooms grown on these agro-waste appear to be safe and potent scavenging ability against free radicals.

Key words: Heavy metals, Mushrooms, *Pleurotus ostreatus*, DPPH, Antioxidant.

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INTRODUCTION

The oyster mushroom, namely *Pleurotus* spp., can be classified as one of the white-rot fungi under the class of basidiomycetes, which belongs to the family of Tricholomataceae. Mushrooms have been reported as potent bio-absorber of heavy metals in bioremediation studies, accumulating both essential and non-essential (toxic) heavy metals from the growth substrate.[1,2] Mushrooms have previously been shown to absorb toxic heavy metals such as mercury (Hg), lead (Pb), cadmium (Cd) and aluminum (Al), which are detrimental to human health.[3,4] These heavy metals may originate from pesticides, organic and inorganic fertilizers, livestock and poultry manure.[5] Hence, it is imperative to adequately analyze the chemical and elemental composition of growing substrates since some toxic metals could be transferred from the substrates into mushrooms during cultivation.[6] During mushroom cultivation, the growing media is usually supplemented with nitrogen sources which increase both biomass and productivity of mushrooms.[7,8] Furthermore, it has also been noted that supplementation of substrates with nitrogen cause mushrooms to become a great source of protein and minerals.[9] Hence supplements such as wheat bran have been reported to be a great source of minerals such as magnesium (Mg), zinc (Zn), manganese (Mn), iron (Fe) and phosphorus (P) for the mushrooms.[10] Recently, mushroom farmers across developing countries have resorted to using low cost agro-waste products such as sugar cane waste residues as base substrates in mushroom cultivation. However, the potential transfer of heavy metals from agro-waste products into mushrooms during cultivation is yet to be elucidated. Some of these agro-wastes may contain heavy metal elements.[11] Thus, the main goal of the present work was to investigate heavy metal absorption by mushrooms cultivated on agro-waste, namely, sugar cane tops (leaves) and bagasse in the presence of WB. Furthermore, to confirm the influence of wheat bran supplementation on the free radical scavenging properties of mushrooms.

MATERIALS AND METHODS

Mushroom spawn and bulk substrate preparation

The sugar cane tops were obtained from farms around the northern part of KwaZulu-Natal province, South Africa (UVS farm at longitudes 28°42'24.9"S 31°54'09.0"E). The bagasse substrate, WB supplement and test mushrooms (*P. ostreatus*) were all obtained at the South African Department of Agriculture and Rural Development. The mushroom strain (*P. ostreatus*) was pre-cultured on potato dextrose agar and incubated in the dark environment at 25 °C and then maintained as working spawn cultures at 4 °C.

The modified method outlined by Crisan and Sands (1978) was utilized for spawn preparation, whereby 1 kg of sorghum grains were soaked overnight in 1.5 l

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of water then excess water was drained. Gypsum ($CaSO_4 \cdot 2H_2O$), calcium carbonate ($CaCO_3$) and soaked grains were mixed, respectively, in a ratio of 4:1:300 g. The mixture was then packed into a 250 ml bottles and sterilized at 121 °C. The mixture was then aseptically inoculated with 10 mm^2 of previously grown pure cultures of test mushroom strain (*P. ostreatus*). The inoculated bottles were incubated in the dark at ± 25 °C for approximately two weeks. The sugar cane tops were milled but the bagasse substrate did not need further processing since it was already in a fine form. Tap water was added to the substrates to achieve 65 % moisture content using the following rule of thumb, 1 droplet to 2 droplets of water must be released when the substrate is squeezed. The substrates were then separately supplemented with various levels of WB, viz, 0 % WB, 2 % WB, 18 % WB and 20 % WB, respectively. After mixing supplements thoroughly with the base substrates, 1 kg of the resultant substrate was packed into polypropylene bags (22.5 cm × 30 cm) and compressed by hand to achieve compactness. The bagged substrates were pasteurized at 60 °C to 65 °C for 6 h and allowed to cool to room temperature.

Substrate inoculation, spawn running and fruiting

After cooling, the bagged substrates were inoculated with previously prepared pure grain spawn of the test mushrooms at the rate of 2 % of wet substrate under the lamina flow hood. The inoculated bags were incubated in a dark room at 25 °C to 27 °C until they became fully colonized by mycelia. The bags were then transferred to a fruiting room which was constructed from plastic film that was covered by a single layer of 30 % grey shade cloth on the outside. The mushrooms were fruited under ambient temperatures. The mushroom fruiting bodies were harvested and dried in the same tunnel with 30 % shade cloth which had varying temperatures depending on the weather, however the temperatures did not exceed ± 45 °C and thereafter were powdered for further analysis.

Heavy metal evaluation

The heavy metals were determined both in substrates and within the actual mushroom using modified methods of Lanre-Iyanda and Adekunle (2012).[12] The powdered mushrooms and substrates (sugarcane and bagasse) of about 0.5g was firstly ashed in muffle furnace which was at 250°C for the period of 12 hours. After ashing, the samples were then digested with 9 ml of aqua regia solution (HCL and HNO3 at ratio 3:1). The digested solution was then transferred into a 25 ml volumetric flask and the flask was filled up to the mark with deionized water. Thereafter, analysis of different elements was carried out using an atomic absorption spectrophotometer.

Estimated daily intake analysis

The estimated daily intake (EDI) of metals through consumption of 100 g fresh mushrooms by individuals of 65k g in body weight were calculated using following described formula[13]:

$$EDI = C\ metal\ X\ D\ mushroom\ intake \div BW\ average$$

Whereby, C = metal concentration in mg/kg; D = daily intake of mushroom in kg person^{-1}; BW= average body weight in kg person^{-1}

Evaluation of mushroom yield

The yield of mushrooms was calculated using a modified method from Morais *et al.* (2000), whereby the following equation was used: MY = [Weight of fresh mushroom harvested (g) per fresh substrate weight]. Hence the yield of mushrooms grown from differently supplemented substrates was attained.

DPPH scavenging activity

Slightly modified method by Ayeni *et al.* (2019) was to used, to confirm the DPPH radical scavenging ability of mushroom extract[14,15]. Each methanol mushroom extract had stock solution of (25mg/ml) of which was diluted into various concentrations ranging from 10-800 µg/ml. About 500µl of DPPH solution (0.1Mm) was mixed and incubated with 1ml of extract at various concentrations and kept in dark for 30min. Then the absorbance was read at 517nm, hence the percentage of scavenging ability was calculated using the following formula:

% Scavenging Activity = (Ac - As)/ Ac *100

Where: Ac = Absorbance of the control; As = Absorbance of the sample. BHT was used as the standard.

DNA cleavage assays

The ability of *P. ostreatus* mushrooms to protect pET30 plasmid DNA from damage caused by free radicals namely Fenton's reagent (30% H_2O_2, 50Mm Ascorbic acid and 80Mm iron (III) chloride), was evaluated following previously stated methods.[14] The reaction was carried out in 96 well microtiter plate with the reaction mixture made up to a total volume of 10µl composed of 5µl pET30 plasmid DNA (concentration of 2.4 µg), 0.5 µl Fenton's reagent , 4 µl of various mushroom extract (2mg/ml) dissolved in DMSO solvent and the final volume of the reaction mixture was brought up to 10 µl using distilled water. The reaction mixture was incubated for 30 min at 25°C in the absence of light. Immediately after incubation the reaction mixtures were mixed with 5 µl loading dye, (0.25% bromophenol blue dye in 50% glycerol). Total mixture of each sample loaded into 1% agarose gel (1 g of agarose dissolved in 100ml in TAE buffer). Electrophoresis was carried out at 100 volts for 45 min, thereafter the nicked and native DNA were visualized under UV light.

Data analysis

All experiments were repeated in triplicate. Data generated were calculated using SPSS original version 6.0 and GraphPad Prism version 5.0. The results are reported as mean ± S.E.M. The statistical differences were determined using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparison test. The values were considered statistically significant where $p \leq 0.05$.

RESULTS AND DISCUSSION

The current study evaluated the concentration of nine heavy metals within both substrates supplemented with WB (Table 1) and within the *P. ostreatus* mushroom grown on these substrates (Table 2). During Mushroom cultivation supplementation with WB can be the source of minerals such as magnesium (Mg), zinc (Zn), manganese (Mn), iron (Fe) and phosphorus (P) for the mushrooms [10]. Metals such as Cd, Pb, Cr and Al are known as non-essential metals since they are toxic for both humans and the environment even at minimum concentrations.[16,17] The results displayed a varying concentration of the nine metals, as noted, however metals such as Al and Fe were observed to be in greater concentrations for all the substrates (sugar cane and bagasse) as well as within the mushrooms cultivated on these substrates. The high concentration of Al and Fe within all the substrates can be possibly be traced back to the source of the substrates i.e. sugar cane, which has been shown by previous researchers to retain and absorb heavy metals.[18,19] Furthermore, bagasse has also been found to be a potent bio-sorbent of metallic pollutants.[20] The results observed in Table 1 indicated that the sugarcane tops contained different heavy metals at varying concentrations. Metals which were in high concentration within sugar cane tops were Al at 871.39±44.71, Fe at 609.43±25.88 followed by Mn at 153.02±2.19.

Whereas metals such as cadmium (Cd), chromium (Cr) and copper (Cu) were observed to be in lower concentrations within sugar cane tops. The addition of WB supplement into substrates significantly influenced concentrations of some heavy metals within substrates,

Table 1: Heavy metal concentration of base substrate sugarcane tops and bagasse supplemented with varying levels of WB (mg/kg) prior to mushroom cultivation.

WB-Sugarcane tops substrate heavy metal analysis					
Substrate composition	**Al**	**Cu**	**Cd**	**Fe**	**Mn**
0% WB-Sugarcane	588.16 ± 63.85^{a}	3.90 ± 0.15^{a}	3.04 ± 0.50^{a}	370.15 ± 29.15^{a}	184.99 ± 7.65^{b}
2% WB-Sugarcane	790.41 ± 66.18^{ab}	4.04 ± 0.22^{a}	2.51 ± 0.06^{a}	495.27 ± 23.38^{b}	151.69 ± 3.41^{a}
18% WB-Sugarcane	819.0 5± 40.93^{b}	5.11 ± 0.28^{ab}	2.52 ± 0.15^{a}	549.68 ± 17.19^{bc}	140.10 ± 2.30^{a}
20% WB-Sugarcane	871.39 ± 44.7^{1}b	5.67 ± 0.31^{b}	2.39 ± 0.02^{a}	609.43 ± 25.8^{8}c	153.02 ± 2.1^{9}a
	Ni	**Pb**	**Cr**	**Zn**	
0% WB-Sugarcane	13.94 ± 0.64^{a}	7.30 ± 1.70^{a}	4.1 2± 2.90^{a}	16.14 ± 0.61^{a}	
2% WB-Sugarcane	13.48 ± 1.90^{a}	9.80 ± 1.83^{a}	2.27 ± 1.06^{a}	18.56 ± 1.40^{a}	
18% WB-Sugarcane	12.99 ± 2.09^{a}	11.7 6± 2.20^{a}	5.24 ± 1.49^{a}	29.73 ± 0.31^{b}	
20% WB-Sugarcane	14.21 ± 0.98^{a}	10.94 ± 1.31^{a}	3.78 ± 0.11^{a}	32.8 4± 1.40^{b}	
Bagasse-WB substrate heavy metal analysis					
Substrates composition	**AL**	**CU**	**Cd**	**Fe**	**Mn**
0% WB-Bagasse	3345.66 ± 253.13^{b}	0.44 ± 2.15^{a}	3.51 ± 0.10^{a}	3476.32 ± 436.66^{a}	103.89 ± 6.74^{a}
2% WB-Bagasse	2433.11 ± 75.26^{a}	7.11 ± 0.77^{a}	4.05 ± 0.21^{a}	2837.58 ± 332.^{a}	131.79 ± 29.14^{a}
18% WB-Bagasse	2298.90 ± 24.08^{a}	8.54 ± 0.21^{a}	2.16 ± 1.47^{a}	2296.20 ± 58.83^{a}	107.20 ± 1.32^{a}
20% WB-Bagasse	2306.79 ± 125.79^{a}	9.02 ± 0.46^{a}	1.56 ± 0.88^{a}	2015.87 ± 179.42^{a}	122.23 ± 4.73^{a}
	Ni	**Pb**	**Zn**	**Cr**	
0% WB-Bagasse	27.29 ± 3.24^{b}	22.17 ± 7.96^{a}	103.48 ± 81.64^{a}	36.41 ± 2.35^{a}	
2% WB-Bagasse	16.71 ± 2.71^{ab}	14.45 ± 2.77^{a}	21.40 ± 1.76^{a}	26.04 ± 2.28^{a}	
18% WB-Bagasse	12.51 ± 1.20^{a}	10.97 ± 0.51^{a}	34.90 ± 1.13^{a}	14.96 ± 2.11^{a}	
20% WB-Bagasse	12.67 ± 0.07^{a}	14.11 ± 2.93^{a}	37.11 ± 1.78^{a}	20.04 ± 2.32^{a}	

Superscript with different letter(s) are significantly different ($P \leq 0.05$) within same column. Superscript with different letter(s) are significantly different ($P \leq 0.05$) within same column.

Table 2: Heavy metal concentration within *P. ostreatus* grown on sugarcane tops supplemented with varying levels of WB (mg/kg).

Heavy metal analysis on *P. ostreatus* grown with the sugarcane tops					
Substrates composition	**AL**	**CU**	**Cd**	**Fe**	**Mn**
0% WB-Sugarcane	46.21 ± 4.35^{b}	10.37 ± 0.52^{ab}	7.64 ± 4.77^{a}	330.39 ± 220.94^{a}	12.80 ± 5.37^{a}
2% WB-Sugarcane	48.58 ± 2.98^{b}	10.28 ± 0.19^{ab}	4.87 ± 0.52^{a}	117.54 ± 5.99^{a}	26.04 ± 1.75^{a}
18% WB-Sugarcane	10.15 ± 3.17^{a}	8.60 ± 0.39^{a}	4.46 ± 0.19^{a}	155.30 ± 59.66^{a}	13.56 ± 6.23^{a}
20% WB-Sugarcane	18.46 ± 3.30^{a}	12.64 ± 1.13^{b}	5.52 ± 1.12^{a}	698.99 ± 469.53^{a}	9.83 ± 2.42^{a}
	Ni	**Pb**	**Cr**	**Zn**	
0% WB-Sugarcane	6.69 ± 0.19^{a}	4.82 ± 0.17^{a}	18.94 ± 10.68^{a}	90.98 ± 2.70^{a}	
2% WB-Sugarcane	4.54 ± 0.31^{a}	3.99 ± 0.76^{a}	6.17 ± 0.15^{a}	110.56 ± 2.39^{b}	
18% WB-Sugarcane	3.61 ± 0.40^{a}	3.3 ± 0.73^{a}	11.76 ±7.82^{a}	89.314 ± 4.74^{a}	
20% WB-Sugarcane	5.98 ± 3.28^{a}	3.16 ± 0.17^{a}	90.31 ± 70.79^{a}	95.78 ± 1.66^{a}	
Heavy metal analysis on *P. ostreatus* grown with the bagasse supplement					
Substrates composition	**AL**	**CU**	**Cd**	**Fe**	**Mn**
0% WB-Bagasse	12.63 ± 1.16^{a}	5.02 ± 0.33^{a}	3.79 ± 0.16^{a}	115.11 ± 3.18^{a}	26.98 ± 2.28^{a}
2% WB-Bagasse	21.7 ± 4.77^{a}	5.49 ± 0.86^{a}	4.43 ± 0.07^{ab}	117.94 ± 8.55^{a}	30.85 ± 1.20^{a}
18% WB-Bagasse	34.46 ± 1.86^{a}	5.82 ±0.36^{a}	3.96 ± 0.04^{a}	145.46 ± 23.75^{a}	8.82 ± 0.56^{a}
20% WB-Bagasse	23.25 ± 7.92^{a}	17.67 ±10.33^{a}	5.20 ± 0.35^{b}	995.85 ± 870.04^{a}	30.17 ± 21.21^{a}
	Ni	**Pb**	**Zn**	**Cr**	
0% WB-Bagasse	1.93 ± 0.34^{a}	0.67 ± 0.17^{b}	98.79 ± 4.53^{a}	3.29 ± 0.05^{a}	
2% WB-Bagasse	2.42 ± 0.14^{a}	*<DL	94.33 ± 9.18^{a}	5.86 ± 1.97^{a}	
18% WB-Bagasse	4.0 5± 0.22^{a}	*<DL	86.98 ± 2.30^{a}	9.97 ± 1.69^{a}	
20% WB-Bagasse	29.53 ± 26.15^{a}	*<DL	100.49 ± 6.60^{a}	944.83 ± 871.21^{a}	

Superscript with different letter(s) are significantly different ($P \leq 0.05$) within same column.

hence it was evident from Table 1 that the addition of WB ($p < 0.05$) caused a significant increase in concentration of Fe, Zn and Cu within sugar cane tops (Table 1).

However, the addition of WB showed no significant influence on heavy metals such as Cd, nickel (Ni), Pb and Cr. The results in Table 2 suggest that the uptake of heavy metals by the *P. ostreatus* was not significantly influenced by the varying levels of WB or Baggase supplementation, with the exceptions of Al and Cu.. For Cu only 20% WB had contrary outcomes. Metals such as Al, Fe and Zn were in higher concentration within the *P. ostreatus* mushroom compared to other metals (Table 2). In Table 2, the metals which appeared to be at a high concentration within the *P. ostreatus* was Fe (995.85 ± 870.04) and Zn (100.49 ± 6.60), while Pb (2%, 18% and 20% WB) was found to be below the detection limit of 0.01 mg/kg. Table 2 further shows that the addition of varying levels of supplements had no statistically significant effect on the concentration of heavy metals such as Al, Cu, Fe, Mn, Ni, Zn and Cr within mushrooms were not influenced by WB. The observed data was in line with previously obtained data for the bagasse substrate, this might be due to the natural abundance of these metals within the environment (Opaluwa *et al.*, 2012). The

results in Table 3 show that there was no major significant effect on supplementing the bagasse substrates with WB in terms of heavy metal concentration. Regarding Al it was observed that the control (0%) had significantly ($p < 0.05$) higher concentration (3345.66 ± 253.13) compared to other levels of supplementation. For Ni, the control also had significantly higher concentration (27.2 9± 3.24) compared to other levels of supplementation. Some heavy metals such as Pb and Cd within *P. ostreatus* mushroom were significantly influenced by addition of varying levels of WB since the control culture (0%) had a minimum concentration of Cd. This study found that the accumulation of heavy metals within *P. ostreatus* varied for different metals and for mushrooms grown on different substrates. It was observed that metals such as Fe and Zn were in high concentrations within *P. ostreatus* mushrooms grown on both sugarcane tops and bagasse substrates (Table 2). This was in line with the findings of Zhu *et al.* (2011) showing that Fe and Zn are found in higher concentrations within mushrooms.[21] The Zn metal concentration ranged from 89.314 mg/kg to 110.56 mg/kg for *P. ostreatus* grown on sugar cane tops, and for *P. ostreatus* grown on bagasse substrate it ranged from 86.98 mg/l to 100.49 mg/l which was above the WHO permissible limit of 60 mg/kg.[19,22] Concentrations of Fe and Zn indicate that the *P. ostreatus* mushrooms may have high affinity towards Zn and Fe.

In general, when comparing metal concentration for mushrooms grown on both substrates it was observed that the metals which were higher for sugar cane tops grown mushrooms (AL, Fe and Zn) and were slightly lower for the bagasse grown mushrooms. This corroborates with the findings of Ogbo and Okhuoya (2011) who stated that the biosorption of metals by species vary depending on the type of metal, metal concentration and most importantly the composition of the substrate.[22] To trace the source of heavy metals found within mushrooms, the transfer factor (TF) is used since it is one of the important factors which indicate the ability of metals to be transferred from substrate into mushroom. The TF > 1 indicates that the mushroom gains metals from the soil/substrate, while a TF < 1 means that the mushroom excludes metals from the substrates.[23] The results of this study indicate that the mobility of metals varies with type of metal and type of substrate. Hence, it was observed in Figure 1 that some elements had high mobility, and some had lower mobility. Heavy metals such as Zn, Cd, Cu and Cr, had TF > 1; although Cu and Cr on sugar cane tops substrate had TF >1, however on bagasse they had TF < 1. Other metals such as Fe, Al, Ni, Pb and Mn had TF < 1. Such differences in the transfer or biosorption of heavy metals may be due to factors such a variation in the composition of substrates,[4] type of metal and concentration.[5,22,24] According to researchers in the field, transfer factor does not necessarily represent the risk of a heavy metal, but it shows the possible source of contamination.[25] This study can then conclude that the nonessential metal, Cd, found in mushrooms is likely to have been transferred or absorbed by the mushroom from both substrates that were used for cultivation.

Furthermore, the study indicated that supplementing the substrates with WB does not significant correlate with the concentration of most metals found both in substrates and within mushrooms. For heavy metals found in substrates it was observed that the addition of WB into substrates significantly correlated with the concentration of heavy metals found in substrates such as sugar cane tops (Cu, r=0.982; Zn, r=0.996) and bagasse (Zn, r=0.993; Fe, r=-0.977) (Table 4). Addition of WB in substrates caused some negative correlation with concentration of heavy metals found in mushrooms. It was observed that there was negative correlation of WB and Al (r=-0.980) in mushrooms grown in sugar cane tops supplemented with WB. Also, some negative correlation was observed on Fe (r=-0.978) and Pb (r=-0.982) for mushrooms grown on bagasse supplemented with WB (Table 2). Such negative correlation may be linked to the fact that the fungi usually produce oxalic acid as means of immobilizing metals ions and complexes into insoluble oxalates which cause reduced bioavailability of these metals hence tolerance to the metals is increased.

Further analysis confirmed all the heavy metals tested within the mushrooms are that were below or within the recommended daily intake (RDI) set by regulatory body such as the FAO/WHO (Table 2). Nonetheless, caution needs to be exercised when consuming in following daily intake limits to avert any health risk associated with the consumption of these mushrooms might be reduced.

Some authors have indicated on previous studies that the practice of supplementation of substrate is beneficial in order to obtain satisfying yields together with good development of mushrooms [26]. Similarly, in this study that the addition of wheat bran as supplement into mushroom growing substrate contributed greatly into an increase in mushroom yield for both substrates (Figure 2). It was observed that as supplements were increasing also the yield increased up to certain point then had some slightly decrease. These findings corroborate with study conducted by Moonmoon *et al.*, 2011 who also found similar trend in terms of addition of supplements such as wheat barn, maize flower and rice bran as supplements to improve mushroom yield.[27] Thus, the practice of adding wheat bran as supplement seems to be beneficial for improving mushroom yield, however its effect on improving the therapeutic property of the mushroom (*P. ostreatus*) is not yet well recognized.

The results on Tables 5 and 6 indicated that *P. ostreatus* extracts were effective in scavenging DPPH radicals in a dose dependent manner, meaning the increase in concentration of the extract resulted in higher percentage in radical scavenging activity. Such activity might probably be due to the fact that *P.ostreatus* mushroom is rich in phenolic compounds which significantly contributes to numerous biological pathways, which could possibly scavenge free radicals [28]. By definition IC_{50} is the concentration of antioxidant required to scavenge 50% of DPPH radical, therefore smaller IC_{50} is ideal since it correspond

Table 3: Pearson's coefficient of correlation among supplement (WB), mushrooms and concentration of various heavy metals.

Concentration of Heavy metals									
Supplemented Substrate only	**Cu**	**Zn**	**Al**	**Cd**	**Fe**	**Mn**	**Ni**	**Pb**	**Cr**
WB-Sugarcane	**0.982***	**0.996****	ns	ns	ns	ns	ns	ns	ns
WB-Bagasse	ns	**0.993****	ns	ns	**0.977***	ns	ns	ns	ns
Mushrooms in WB Supplemented substrates	**Cu**	**Zn**	**Al**	**Cd**	**Fe**	**Mn**	**Ni**	**Pb**	**Cr**
P. ostreatus grown in WB-Sugarcane	Ns	ns	**-0.980***	ns	ns	ns	ns	ns	ns
P. ostreatus grown in WB-Bagasse	Ns	ns	ns	ns	**-0.978***	ns	ns	**-0.982***	ns

Ns, Correlation is not significant; *Correlation is significant at the 0.05 level (two-tailed); **Correlation is significant at 0.001 level (two-tailed); WB, Wheat bran.

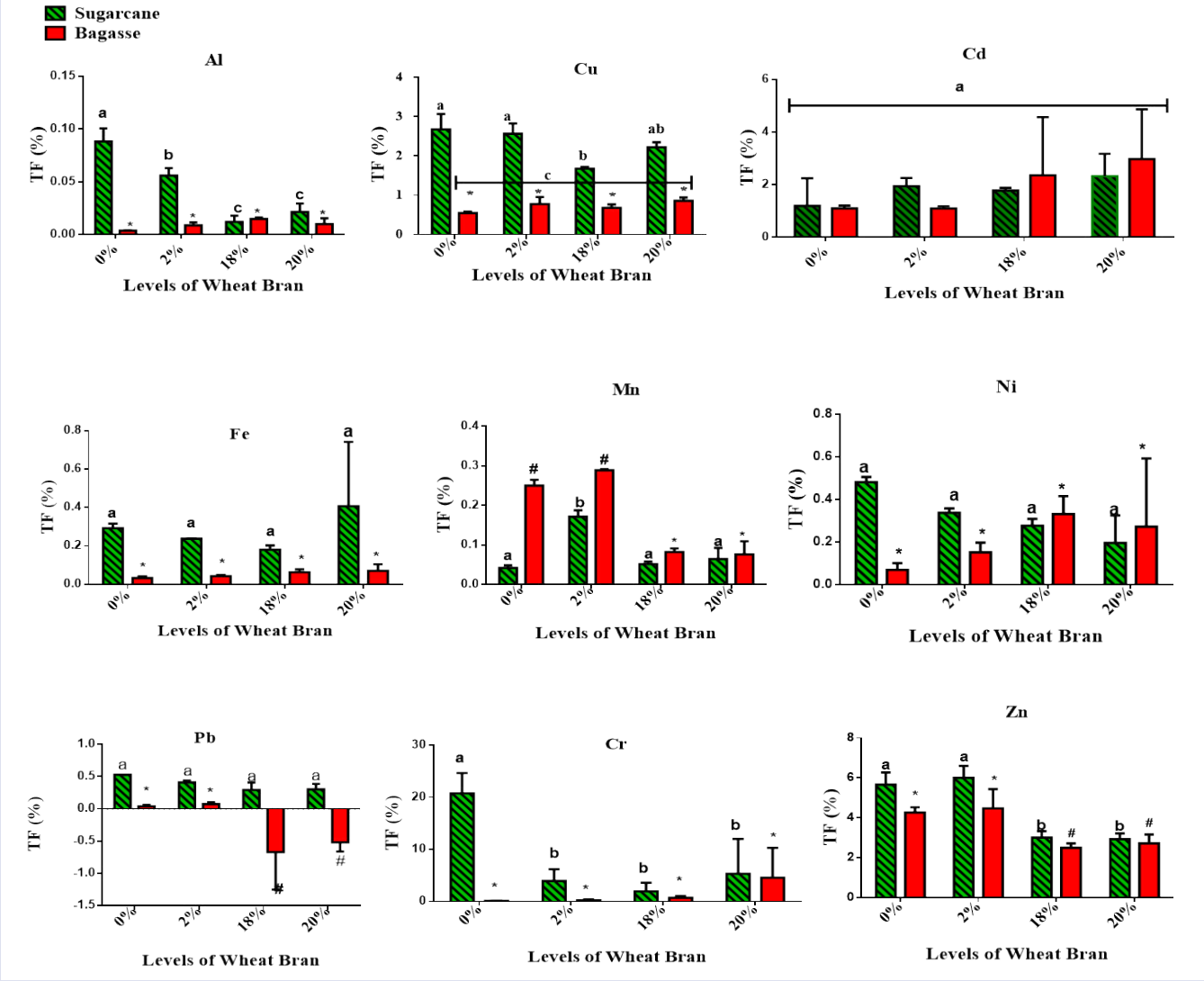

Figure 1: Transfer factor (TF) of heavy metals from sugar cane tops and bagasse into *P. ostreatus* mushroom.

higher antioxidant activity of plant extract [29]. In-terms of the IC_{50} it was noted that mushrooms grown on un-supplemented substrates had a more significant IC_{50} value when compared to the mushrooms grown on supplemented substrate. Furthermore, it was observed that an increase in supplement resulted in higher IC_{50} (not ideal), which could probably be influenced by different factors such as the content of phenolic compounds since they are the major compounds in mushrooms antioxidants which can scavenge free radicals.[30,31] Hence, Gąsecka *et al.*, 2016 have previously stated that substrates could have some influence on phenolic content of the mushrooms.[32] Therefore, our clearly demonstrate that the type and content of substrates used in growing mushooms, may directly influence the antioxidant properties and phenolic content of the resultant mushrooms.

Radicals such as hydroxyl radicals have the potential to damage DNA, lipids and proteins.[14,33–35] Results on Figure 3 proved that exposing pET30 plasmid DNA to Fenton's reagent caused complete damage of plasmid DNA. This is due to hydroxyl radical since it reacts with nitrogen bases of DNA causing production of both base radicals and sugar radicals which then react and results in breakage of sugar phosphate leading to DNA damage.[36] The results on Figure 3 showed that addition of WB on different mushroom extract had no significant protective role on DNA protection. As expected, sugarcane mushroom extract grown in the absence of WB successfully prevented DNA damage caused by Hydroxyl radical (Figure 3). The DNA protective efficiency of this mushroom extract may probably be due to compounds such as phenolics and flavonoids which usually prevent production of ROS by forming complex with cations such as CU^{+2} and Fe^{+3} which participate in the formation of hydroxyl radical [36]. Other extract did not show DNA protective activity probably due to lower content of these compounds (phenolics and flavonoids), hence previous studies by Fatima *et al.* (2018) indicated that extract with less phenols and flavonoids were not found to be effective in DNA protection. Nonetheless, results of this study indicate that mushroom extract have the potential antioxidant activity.

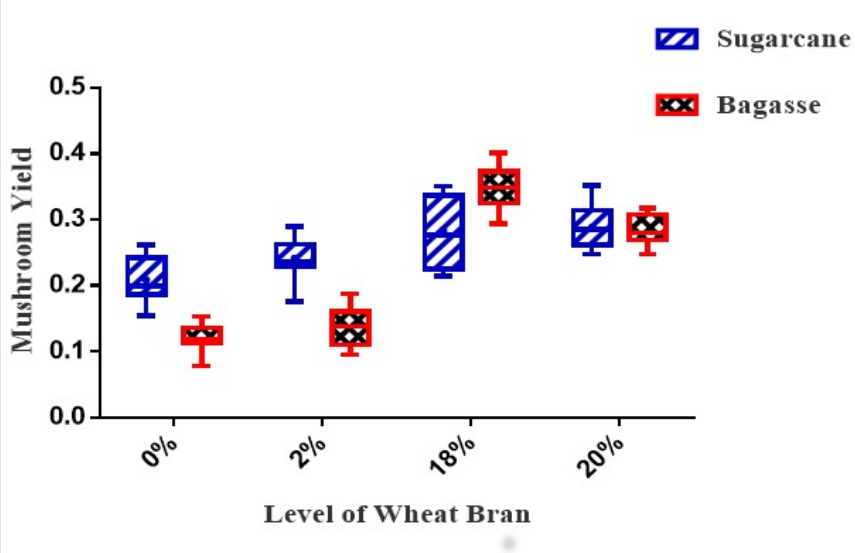

Figure 2: Increasing levels of wheat bran supplementation directly influence production yield of *P. ostreatus*.

Table 4: Estimated Daily Intake (EDI) of metals from consuming 0.1 kg of fresh mushroom by the 65 kg body weight individual (mg/kg day-1 bw). The *P. ostreatus* mushroom grown on Sugar cane tops supplemented with varying levels of WB.

P. ostreatus mushroom grown on Sugar cane tops					
Heavy metals	0% WB	2% WB	18% WB	20%WB	RDI (mg/day)
Al	0.07	0.07	0.02	0.03	-
Cu	0.02	0.02	0.01	0.02	0.9 (Ros et al., 2011)
Cd	0.01	0.007	0.007	0.008	0.007 (FAO/WHO)
Fe	0.51	0.18	0.24	1.08	8.0-18.0 (Ros et al., 2011)
Mn	0.02	0.04	0.02	0.02	1.8-2.3 (Ros et al., 2011)
Ni	0.01	0.006	0.006	0.009	0.13-0.4 (US RDA)
Pb	0.007	0.006	0.005	0.005	0.025 (FAO/WHO)
Cr	0.03	0.009	0.018	0.14	0.02-0.2 (US RDA)
Zn	0.14	0.17	0.14	0.15	8.0-11.0 (Ros et al., 2011)
***P. ostreatus* mushroom grown on Baggase cane tops**					
Heavy metals	**0% WB**	**2% WB**	**18% WB**	**20%WB**	**RDI (mg/day)**
Al	0.02	0.03	0.05	0.04	-
Cu	0.008	0.008	0.009	0.03	0.9 (Ros et al., 2011)
Cd	0.006	0.007	0.006	0.008	0.007 (FAO/WHO)
Fe	0.18	0.18	0.22	1.53	8.0-18.0 (Ros et al., 2011
Mn	0.04	0.05	0.01	0.05	1.8-2.3 (Ros et al., 2011)
Ni	0.003	0.004	0.006	0.05	0.13-0.4 (US RDA)
Pb	0.001	*<DL	*<DL	*<DL	0.025 (FAO/WHO)
Cr	0.005	0.009	0.02	1.45	0.02-0.2 (US RDA)
Zn	0.15	0.15	0.13	0.15	8.0-11.0 (Ros et al., 2011)

*<DL (Below detection limits); Recommended Daily intake (RDI);

Table 5: Percentage scavenging activity of DPPH by *P. ostreatus* cultivated on bagasse with varying levels of wheat bran supplementation.

Conc (ug/ml)	0% WB	2% WB	18% WB	20% WB	BHT
10	61.24 ± 0.83	44.38 ± 0.33	49.62 ± 0.33	48.55 ± 1.06	89.67 ± 0.28
25	67.02 ± 1.95	45.96 ± 0.42	52.67 ± 0.10	48.59 ± 0.11	89.71 ± 0.11
50	65.13 ± 0.53	48.69 ± 0.64	55.88 ± 0.33	50.50 ± .17	89.81 ± 0.05
100	67.96 ± 11.62	57.56 ± 0.61	64.62 ± 0.23	55.34 ± 0.23	90.97 ± 1.14
250	74.58 ± 0.29	80.09 ± 0.37	74.00 ± 0.96	64.54 ± 0.17	89.71 ± 0.29
500	84.72 ± 3.07	84.61 ± 3.18	79.50 ± 0.54	77.02 ± 0.36	85.92 ± 3.68
800	84.72 ± 0.71	84.50 ± 0.14	71.85 ± 0.08	85.95 ± 0.54	88.60 ± 1.00
IC_{50}	**1.89**	**29.76**	**13.09**	**27.40**	**6.98**

(n = 3, X ± SEM), IC50 – Inhibitory concentration

Table 6: Percentage scavenging activity of DPPH by *P. ostreatus* cultivated on sugar cane tops with varying levels of wheat bran supplementation.

Conc (ug/ml)	0% WB	2% WB	18% WB	20% WB	BHT
10	50.36 ± 0.100	50.02 ± 0.16	47.27 ± 0.84	46.06 ± 0.84	89.67 ± 0.28
25	52.81 ± 0.100	52.66 ±1.08	50.17 ± 0.29	51.90 ± 3.15	89.71 ± 0.11
50	57.06 ± 0.24	56.31 ± 0.30	56.19 ± 0.25	55.29 ± 0.33	89.81 ± 0.05
100	63.80 ± 0.38	65.57 ± 0.65	67.98 ± 0.57	65.84 ± 0.83	90.97 ± 1.14
250	75.52 ± 0.36	84.03 ± 0.79	87.80 ± 1.58	84.33 ± 0.48	89.71 ± 0.29
500	70.47 ± 1.70	87.38 ± 1.21	90.28 ± 0.75	87.68 ± 0.36	85.92 ± 3.68
800	67.10 ± 0.53	84.44 ± 0.41	89.64 ± 0.59	81.05 ± 0.83	88.60 ± 1.00
IC50	**9.191139**	**15.2869**	**18.55854**	**18.23718**	**6.98**

(n = 3, X ± SEM), IC50 – Inhibitory concentration

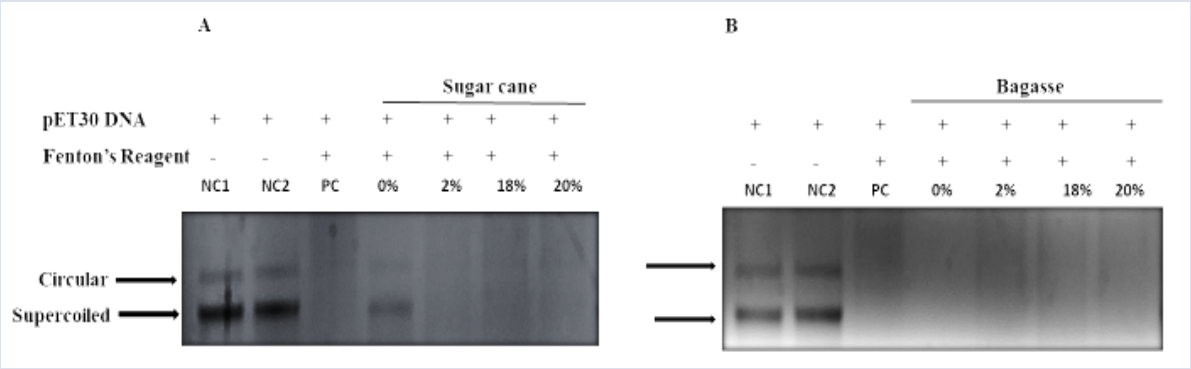

Figure 3: Plasmid DNA protective ability of *P. ostreatus* mushrooms grown on substrates namely sugar cane (A) and bagasse (B) supplemented with varying levels of wheat bran. The pET30 plasmid was incubated with extract in the absence (-) and in the presence (+) of DNA oxidizing reagent (Fenton's reagent). Lane NC1 (Negative control with DNA only); Lane NC2 (Negative control with DNA and DMSO); Lane PC (Positive control with DNA and Fenton's Reagent); Lane 0% - 20% [Extract with DNA and Fenton's reagent; (Extracts are from mushrooms grown on substrates supplemented with 0%, 2%, 18% and 20% wheat bran, respectively)].

CONCLUSIONS

This study found that sugar cane tops and bagasse substrates were enriched with heavy metals which were probably as a result of emissions by industries, fertilizers and pesticides which farmers use during sugar cane farming. This study showed that the *P. ostreatus* mushroom absorbs the heavy metals from the substrates but at varying rates, meaning that *P. ostreatus* mushrooms have different affinities for different metals. Supplementation of the substrate with WB influenced some of the metals within both substrates and the mushrooms. This data further supports the literature stating that the absorption of metals by species is influenced by substrate composition. The addition of the WB supplements to mushroom resulted in significantly higher yield, however, inversely reduced antioxidant reducing activity of the grown mushroom. This suggests that bagasse and sugar cane tops supplementation does not influence heavy metal accumulation in mushroom cultivation. Further studies are still required to investigate the presence of phenolic and flavonoid compounds which are capable of scavenging free radicals.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest in this work.

AUTHOR'S CONTRIBUTIONS

The manuscript was written and read by all the authors mentioned. OJP, MBCS and SSM structured and designed the experiments, wrote and proof read the paper for possible corrections. SSM and NLG conducted the laboratory experiments, collected and analyzed data.

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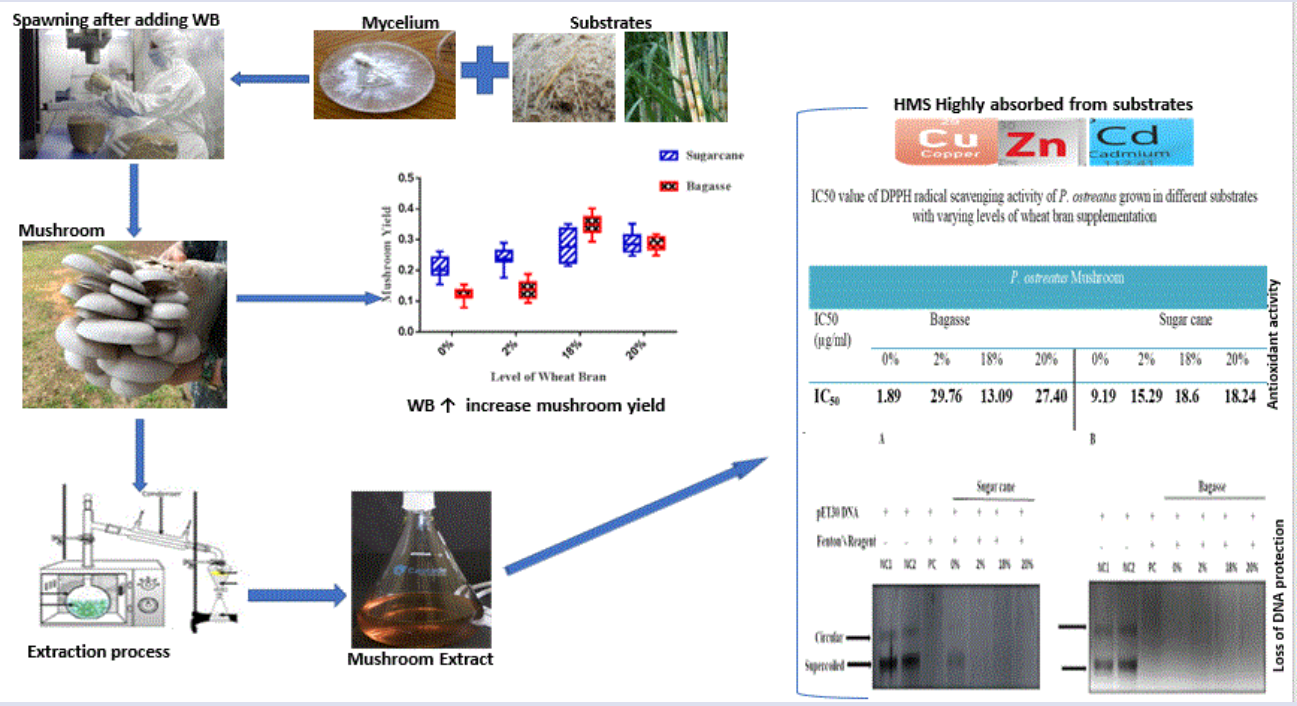
GRAPHICAL ABSTRACT



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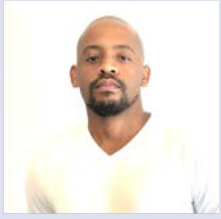

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