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INTEGRATION OF WOOD ASH AND POULTRY WASTE IN *PLEUROTUS OSTREATUS* (JACQ. FR.) CULTIVATION, ASSESSMENT OF NUTRITIONAL QUALITY AND BIOACCUMULATION OF HEAVY METALS IN THE FRUIT BODIES

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ABSTRACT

The effects of wood ash and poultry waste as a replacement for wheat/rice bran and lime in mushroom cultivation and the assessment of the bioaccumulation of heavy metals in the fruiting bodies were evaluated. T1 (Sawdust, 2% wheat bran and 2% lime (control)), T2 (Sawdust+2% poultry manure + 1% wood ash), T3 (Sawdust+ 2% poultry manure+ 2% wood ash) and T4 (Sawdust +2 % poultry waste+3% wood ash) were used as treatments. The experiment was laid out in a Completely Randomised Design (CRD) in three replicates. The data generated were subjected to analysis of variance (ANOVA). Means were separated using Fisher's Least Significant Difference at p=0.05. The results obtained showed that T4 had the highest fresh weight (43.31g), dry weight (4.24 g) and length of stipe (4.72 cm) while T2 and T3 had the highest biological and bioconversion efficiencies in the value of 442 % and 72 %, respectively. Nutritional composition showed that T3 had the highest values of crude protein (3.12 %) and carbohydrate (22.71 %). The concentration of heavy metals bioaccumulated in the fruiting bodies was within the WHO/FAO acceptable limits. The results showed that wood ash and poultry waste can effectively replace the use of wheat/rice bran and lime in the cultivation of *Pleurotus ostreatus*.

Keywords: Re-integration; pood ash; poultry waste; heavy metals; bioaccumulation; mushroom

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INTRODUCTION

Edible mushrooms are naturally found in the forest on decaying organic matter such as rotten bark of trees and stump including soils rich in organic matter (Joseph and Oku, 2016). They can also be grown in commercial quantities domestically, using various agro and industrial wastes such as sawdust, corn cob and husk, etc. Many authors such as Aida *et al.* (2009) and Lu *et al.* (2020) have reported that over 200 species of edible mushrooms have been identified for food globally but only about 35 species have been artificially cultivated in commercial quantities. The growth medium for the artificial edible mushroom cultivation is one of the major factors that impact significantly on the quality and cost of production (Rai *et al.*, 2015). As saprophytes, mushrooms can obtain their nutrients by absorbing dissolved organic matter from various dead and decaying organic materials. The substrate mostly used by researchers for the cultivation of edible mushrooms is sawdust (Adedokun *et al.*, 2003; Isikhuemhen and Lebauer, 2004; Chiejina and Olufokunbi, 2010).

Most of the additives used in mushroom cultivation are not easily available as reported by Elenwo and Okere (2007), thereby increasing the cost of mushroom cultivation and making it unaffordable by many families especially in developing countries like Nigeria. There is, therefore, the need to identify certain agro-wastes such as poultry waste and wood ash as they could effectively replace lime (used to increase pH) and wheat/rice bran (as nitrogen sources).

Chicken/ poultry manure has mainly two major functions in mushroom compost. First, it is a cheap and fairly reliable source of nitrogen. In the past Ammonium sulphate, urea, cotton seed meal and malt culm or other protein-rich materials were used. However, these materials are very expensive (Okere *et al.*, 2014). Secondly, chicken/poultry manure is rich in decomposable carbohydrates. It contains various substances that increase the temperature in the compost and trigger the process of composting to supply the necessary nutrients for micro-

organisms. They also contain many beneficial microorganisms such as bacteria for the composting process (Okere *et al.*, 2015).

There is significant evidence to show that mushroom cultivation can be costly (Shroeder *et al.*, 1970; Boers, 1991). Some Countries like Japan, China and Taiwan have developed methods of growing mushrooms even with limited resources. Extension workers and individuals are adapting available techniques to local circumstance (Oci, 1986). This study was, therefore, aimed to investigate the possibility of growing *Pleurotus ostreatus var florida* on low-cost, easily available agricultural wastes like sawdust and wood ash enriched with different concentrations of poultry waste as additives.

Vegetables including mushrooms absorb heavy metals from contaminated soils and substrates including deposits of such heavy metals on the parts of the plant exposed to polluted air (Zhou, 2016). Heavy metals can easily be adsorbed by mushroom mycelia and can bioaccumulate in the edible fruit bodies at high concentrations (Jolly *et al.*, 2013). Anthropogenic activities such as agricultural crop residue, emissions from industries and vehicles are a major source of heavy metal contamination (Chen *et al.*, 2005).

Consumption of heavy metals-contaminated food may pose a great risk to human health. They are harmful as a result of their relatively long biological half-lives, ability to accumulate in different body parts and their non –biodegradable nature (Heidarich *et al.*, 2013). Consumption of food over time with unsafe concentration of heavy metals may result in chronic bioaccumulation of heavy metals in the liver and kidney of human beings resulting in many health disorders in numerous biochemical processes leading to bone, cardiovascular, kidney and various diseases (Jarup, 2003). This study was aimed to evaluate the effect of integrating wood ash and poultry waste, as a possible replacement for wheat/rice bran and lime, on the yield and nutritional composition of *P. ostreatus*. The study also aimed at assessing the level of bioaccumulation of heavy metals in the fruit bodies.

MATERIALS AND METHODS

Study site and source of sample

This study was conducted at the Federal University of Technology Teaching and Research Farm, Owerri, Imo State Nigeria. Sawdust, wood ash and poultry waste were obtained from the University community while the mushroom spawn established on guinea corn seed was obtained from Diplomat Mushroom Farm and Services, Port Harcourt, Rivers State, Nigeria.

Preparation of Substrate

The preparation of the substrate for the cultivation of edible mushroom was carried out according to the modified method of Stamets (1993). The sawdust was moistened and mixed with 2% wheat bran and 2% lime (T1). This served as the control to enrich the substrates nutritionally and to correct the pH of the substrate, respectively. The other treatments comprising sawdust was supplemented with 2% poultry waste and different rates of wood ash. The treatments for this investigation were as follows: T1 (Sawdust, 2% wheat bran and 2% lime (control)), T2 (sawdust+2% poultry manure + 1% wood ash), T3 (sawdust+ 2% poultry manure+ 2% wood ash) and T4 (sawdust +2 % poultry waste+3% wood ash). One kilogram of the sawdust mixed with different rates of the additives was measured into high-density polypropylene bags. The bags were packed inside a drum steamer, pasteurised for three hours and allowed to cool overnight before being inoculated with spawn of *P. ostreatus* grown on guinea corn seed. The inoculated substrates were incubated at ambient temperature in a growth chamber specially constructed for monitoring relative humidity and temperature. The bags were opened and lightly watered to induce fruiting after full colonisation was achieved.

Productivity evaluation

The following parameters were measured: Mean mushroom Weight (w/w) (MMW) was obtained by measuring the fresh (wet) weight of each flush with an electronic weighing balance. Mean number of mushroom fruit bodies (MNM) was obtained by counting the number of fruit bodies from each mushroom flush. Stipe length and pileus diameter were measured using a measuring tape. Dry matter loss was measured as the ratio of the weight of the substrate at spawning to the weight of the substrate at the end of the production cycle. Dry weight of the fruit bodies was measured using an electronic weighing balance. Biological efficiency (%) (B.E) was calculated as the

weight of fresh mushroom produced per 100 g of dry substrate used and multiplied by 100. Bioconversion or bio-transformation efficiency (%) (BCE) was calculated as the weight of dry mushroom produced per 100 g of dry substrate used and multiplied by 100. The number of flushes produced was also evaluated. The production cycle for this study was 65 days.

Assessment of bioaccumulation of heavy metals in the fruit bodies

The bioaccumulation of heavy metals in the mushroom fruit bodies was assessed using standard methods according to the procedure outlined by Vincent *et al.* (2020). The heavy metals assessed in this study included Cr, Fe, Mn, Cu, Cd, Ni, Pd, Zn, As and Ag.

Experimental design and data analysis

The experiment was laid out in a completely randomised design (CRD) in three replicates. The data generated were subjected to analysis of variance (ANOVA). Means were separated using Fisher's Least Significant Difference at p=0.05 (Steel and Torrie, 1980).

RESULTS

Mushroom Yield

Table 1 shows that T1 (control) produced 9.3, 21.2 and 21.7 g less mushroom fresh weight when it was compared with T2, T3 and T4, respectively. T2 produced 11.9 and 12.4 g less mushroom fruit bodies than T3 and T4. T4, however, produced the highest fresh weight of mushroom fruit bodies than T1, T2 and T3. The result showed that T4 produced 2.12, 1.21 and 0.046 g more dry weight of mushroom fruit bodies than T1, T2 and T3, respectively. T3 produced higher dry weight of mushroom fruit bodies than T1 and T2 by 2.08 and 1.17 g while T2 produced 0.91g higher than T1 which produced the least dry weight of mushroom fruit bodies. The result of the number of mushroom fruit bodies produced by the treatments revealed that T2 produced 11.0, 12.7 and 18.0 higher fruit bodies than T1, T3 and T4. T1 produced 1.7 and 5.0 fruit bodies higher than T3 and T4 while T3 produced 3.3 higher fruit bodies than T4. T4 produced the least number of mushroom fruit bodies when compared with all the other treatments.

The treatment T4 produced fruit bodies with 2.4, 2.71 and 1.40 cm stipes longer than those produced by T1, T2 and T3, respectively. T3, on the other hand, produced fruit bodies with 1.0 and 1.31 cm longer stipes than those produced from T1 and T2. T1 produced fruit bodies with a stipe length of 0.28 cm longer than those produced by T2, while the shortest fruit bodies were produced from T2. The result of the pileus diameter showed that T3 produced the largest pileus of 3.54, 3.03 and 0.10 cm, which were larger than those produced from T1, T2 and T4, respectively, while T4 produced fruit bodies with pileus diameters 3.3 and 3.9 cm, larger than those produced from T1and T2. T2 produced fruit bodies with pileus diameter 0.51 cm larger than those produced from T1 while the least was produced from T1.

The result of the evaluation of time of harvest after inoculation showed that T3 produced fruit bodies 51.3 days after inoculation while T4, T2 and T1 produced fruit bodies 57.0, 60.0 and 62.0 days after inoculation. T3 and T4 produced fruit bodies faster than T1 and T2. Result of the number of days to the formation of pin heads at inoculation showed that T3 and T1 produced pin heads 52.7 and 55.6 days while T2 produced pin heads after 62 days after inoculation. T1 had the highest dry matter loss of 50% followed by T3 (44%) while T2 and T4 had 37 and 40 %, respectively. Also, T3 had the highest biological efficiency of 4.42% followed by T4 (4.33%) while T1 and T2 had 2.16 and 3.09 %, respectively. T2 had the highest bioconversion efficiency of 0.71% followed by T1(0.59%), T3 and T4 which had 0.54 and 0.32%, respectively. T1, T2 and T4 had the highest mean number of mushroom flushes of 1.33 while T3 had the lowest value of 1.0. T2 and T4 produced the second mushroom flush 53 days after harvesting the first flush, followed by T1 while T3 did not produce fruit bodies after the first flush.

Table 1 Effects of different substrates on the yield and yield attributes of mushroom

Treatment	Fresh weight (g)	Dry weight (g)	NMFB	Length of stipe (cm)	PD (cm)	NDTHAI	DTPHFAI	Dry matter loss (%)	B. E (%)	B.C.E (%)	MNOF	NODTSF
T1	21.64[b]	2.12[b]	11.00[b]	2.33[b]	3.72[b]	62.00[a]	55.67[a]	50.0[a]	216[b]	59[a]	1.33[a]	4.33[a]
T2	30.90[a,b]	3.03[a,b]	22.00[a]	2.06[b]	4.23[b]	60.00[a]	62.00[a]	37.0[a]	309[a,b]	72[a]	1.33[a]	5.33[a]
T3	42.82[a]	4.20[a]	9.33[b]	3.37[a,b]	7.27[a]	51.33[a]	52.67[a]	44.0[a]	442[a]	54[a,b]	1.00[a]	0.00[b]
T4	43.31[a]	4.24[a]	6.00[b]	4.77[a]	7.17[a]	57.00[a]	51.00[a]	40.0[a,b]	433[a]	32[b]	1.33[a]	5.33[a]
LSD (0.05)	17.15	1.68	8.86	1.47	1.76	6.91	9.18	16.0	168	10	0.41	6.15

Key:
T1-100% sawdust + 2% wheat bran+ 2% lime
T2-100% sawdust+2% poultry waste +1% wood ash
T3-100% sawdust+2% poultry waste +2% wood ash
T4-100% sawdust+2% poultry waste +3% wood ash
NMFB- Number of mushroom fruit bodies
BE- Biological efficiency
B.C.E- Bioconversion efficiency
NDTHAI- Number of days to harvest after inoculation
DTPHFAI- Days to Pin head formation after inoculation
MNOF- Mean number of flushes
NODTSF-Number of days to the second flush
PD- Pileus diameter
Values within the same column with the same superscript are not statistically significant at 5% level of probability

Nutritional Composition of Fruit Bodies

The result of the nutritional composition of the mushroom fruit bodies is shown in Table 2. T1 had the highest value of ash (3.52%), followed by T2 (2.41%) and T3 (2.25%), while the lowest value of 1.43% was obtained from T4. T4 had the highest value of fat (1.44%) followed by T2 (1.32%) while T1 and T3 had 1.22 and 1.21%. The result of the moisture content showed that T1 had 30% moisture content, which was higher than T3 (28%) while T2 and T4 had 20 % each. T3 had the highest value of crude protein of 3.12%, followed by T1 (2.99%) while T2 and T4 had 1.86 and 1.69 %. The carbohydrate content of the fruit bodies was highest in T3 (22.71%), followed by T4 (21.74%) while T1 and T2 had 21.71 and 18.49 %. Fruit bodies produced from T1 contained the highest values of percentage ash and moisture content, which were significantly different when compared with the values obtained from the other treatments. T3 contained the highest values of crude protein and carbohydrate.

Table 2: Effects of different substrates on nutritional composition of the fruit bodies

Treatment	Ash (%)	Fat (%)	MC (%)	Crude protein (%)	Carbohydrate (%)
T1	3.52[a]	1.22[a]	30.0[a]	2.99[b]	21.71[a]
T2	2.41[a,b]	1.32[a]	20.0[b]	1.86[c]	18.49[b]
T3	2.25[a,b]	1.21[a]	28.0[a]	3.12[a]	22.71[a]
T4	1.43[b]	1.44[a]	20.0[b]	1.69[d]	21.74[a]
LSD (0.05)	0.819	0.823	1.08	0.010	0.815

Key:
T1-100% sawdust + 2% wheat bran+ 2% lime
T2-100% sawdust+2% poultry waste +1% wood ash
T3-100% sawdust+2% poultry waste +2% wood ash
T4-100% sawdust+2% poultry waste +3% wood ash
Values within the same column with the same superscripts are not statistically significant at 5% level of probability

Bioaccumulation of heavy metals in the fruit bodies

The result of the bioaccumulation of heavy metals in the mushroom fruit bodies is presented in Table 3. The result shows that T1 contained the highest value of chromium (Cr) (2.95 mg/kg), followed by T3 (2.24 mg/kg), T2 (1.60 mg/kg) and T4 (1.85 mg/kg). T2 contained the highest value of Iron (Fe) (56.0 mg/kg) followed by T4 (32.1 mg/kg) and T3 (22.6 mg/kg) while the lowest value of 14.6 mg/kg was obtained from T1. T3 and T2 contained the highest level of Manganese (Mn) (7.42 and 7.30 mg/kg) followed by T1 (6.20 mg/kg) and T4 (5.55 mg/kg). The highest value of Copper was obtained from T1 (2.65 mg/kg) followed by T3 (2.60 mg/kg), T2 (2.23 mg/kg) and T4 (1.31 mg/kg). T1 had 0.47 mg/kg Cadmium (Cd), higher than all the other treatments namely T2, T3 and T4 with values 0.34, 0.28 and 0.26 mg/kg, respectively. T1 contained a higher value of Nickel (Ni) (1.22 mg/kg) than T2 (1.17 mg/kg), T3 (1.12 mg/kg) and T4 (0.72 mg/kg). The lowest value was obtained from T4. T4 had the highest value of 0.51 mg/kg for Lead (Pb) followed by T2 (0.42 mg/kg) while T1 and T3 had 0.24 and 0.12 mg/kg. The result of Zn content of the fruit bodies showed that T4 had the highest value of 32.57mg/kg followed by T3 which had 27.13 mg/kg while T1 and T2 had 26.20 and 22.01 mg/kg, respectively. Analysis of the fruit bodies for Arsenic (As) showed that T4 had 0.028 mg/kg, which was higher than T1 (0.022 mg/kg), T3 (0.017 mg/kg) and T2 (0.015mg/kg). T2 and T3 had a similar value of 0.003mg/kg while T1 had 0.002 mg/kg.

Table 3: Composition of heavy metals in the mushroom

Treatment	Cr (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Cd (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	As (mg/kg)	Ag (mg/kg)
T1	2.95[a]	14.6[d]	6.20[a,b]	2.65[a]	0.47[a]	1.22[a]	0.24[c]	26.20[b]	0.022[a]	0.002[a]
T2	1.60[d]	56.0[a]	7.30[a]	2.23[b]	0.34[b]	1.17[b]	0.42[b]	22.01[c]	0.015[a]	0.003[a]
T3	2.24[b]	22.6[c]	7.42[a]	2.60[a]	0.28[c]	1.12[c]	0.12[d]	27.13[b]	0.017[a]	0.003[a]
T4	1.85[c]	32.1[b]	5.55[b]	1.31[d]	0.26[d]	0.72[d]	0.51[a]	32.57[a]	0.028[a]	0.0[b]
LSD(0.05)	0.053	0.797	0.687	0.041	0.007	0.008	0.0081	0.733	0.009	0.0004

Key:
T1-100% sawdust + 2% wheat bran+ 2%lime
T2-100% sawdust+2% poultry waste +1% wood ash
T3-100% sawdust+2% poultry waste +2% wood ash
T4-100% sawdust+2% poultry waste +3% wood ash
Values within the same column with the same superscripts are not statistically significant at 5% level of probability

DISCUSSION

The results showed that the treatments with poultry waste and wood ash performed better than the T1 or control (with wheat bran and lime) which have been reported by many authors to be the standard medium for the cultivation of *P. ostreatus* on different substrates (Madan *et al.*,1987; Victoria and Agina, 2002; Elenwo and Okere, 2007). The results suggest that increasing the quantities of wood ash in the substrate leads to increase in the yield of *P. ostreatus.* This could be attributed to the increase in the pH of the mushroom substrate. This observation is in line with our earlier findings (Elenwo and Okere, 2007). Zaghi *et al.* (2010) reported that the productivity of different substrates was dependent on the water-holding capacity as well as porosity of the substrates, which allows for efficient respiration of mycelia and easy access of enzymes to the nutrients present in them. Naupane *et al*. (12018) observed that substrate type and additives influence the length of the stipes and the pileus diameter. The practical implication of this finding is that poultry wastes and wood ash at 2 and 3%, respectively, could effectively replace the use of wheat bran and lime commonly used in mushroom cultivation. These findings will drastically reduce the cost of mushroom cultivation to the barest minimum.

The result also revealed that T3 had a higher crude protein and carbohydrate than all the other treatments, which is in agreement with the findings of Inyod *et al.* (2016). This could be attributed to the optimum C/N ratio and pH of the substrate which facilitated rapid colonisation and degradation of the nutrients in the substrate. This is in agreement with the findings of Nelson and Cox (2004), who reported that improved growth and high crude protein in the fruit bodies could be due to higher Nitrogen content and lowered C/N ratio which are readily available for the biosynthesis of purines and pyrimidines needed for amino acid synthesis, the building blocks of cellular protein. The result suggests that increasing the lime and wood ash above 2% leads to decrease in the crude protein content of the mushroom fruit bodies. The addition of wheat bran and lime did not significantly increase the ash content of the fruit bodies when it was compared with poultry waste and wood ash at 1 and 2%. However, it appears that increasing the level of wood ash in the substrate decreased the percentage ash in the fruit bodies.

The result showed that the fruit bodies obtained from T1 contained a significantly higher Cr level than all the other treatments, which could be attributed to the lime, wheat bran and the nature of the soil where the wheat was grown or the tree from which the sawdust was obtained. However, the four treatments contained Cr below the WHO/FAO stipulated limit of 5.0 mg/kg (WHO/FAO, 2007). Also, the values obtained for Fe, Mn and Cu were significantly below the WHO/FAO (2007) stipulated limit of 450, 500 and 40 mg/kg, respectively. The results further revealed that T1 contained the highest concentration of Cd when compared with the other treatments while its concentration from the four treatments were below the WHO/FAO (2015) stipulated limit of 0.05-2.0 mg/kg. T4 had the highest value of Pb followed by T2 which were also below the WHO/FAO (2015) stipulated limit of 0.01-3.0 mg/kg. T4 contained the highest value of Zn than all the other treatments though the values from all the treatments were below the WHO/FAO (2007) stipulated limit of 60.0 mg

CONCLUSION

The results obtained from this study showed that wood ash and poultry waste can effectively replace the use of wheat/rice bran and lime in the cultivation of *P. ostreatus*. The result obtained from the assessment of the heavy metals in the fruit bodies revealed that the heavy metals were within the WHO/FAO acceptable limits, making them safe for human consumption. However, further study is recommended especially in identifying the level of heavy metals from different sawdust sources used in the cultivation of edible mushrooms.

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