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Biodegradation And Mushroom Cultivation Studies On Peanut Shells And Corn Stalks By Pleurotus Ostreatus Under Solid State Fermentation Conditions

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Biodegradation and mushroom cultivation studies on peanut shells and corn stalks by *Pleurotus ostreatus* under solid state fermentation conditions

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Biographical Sketch

Mansuru Usif was born on March 17, 1984 in Accra, Ghana. He attended high school at Ben L. Smith in Greensboro, North Carolina and did his undergraduate studies here at A&T University as a Biology major. He was recognized as an Honors student, received the Certificate of Achievement from the Biology Department for Outstanding Academic Achievement in March 2004, and was selected as a member of Golden Key International Honor Society for outstanding Scholastic Achievement and Excellence student the following year. He earned the Bachelor of Science degree in Biology in 2007 and, a year later, enrolled into the master's program at the School of Agriculture and Environmental Science, in the Natural Resources and Environmental Design Department at Carver Hall. He maintained a GPA of 3.5 and has completed his research on bioconversion of peanut shells and corn stalks with using *Pleurotus ostreatus*, the fungus that forms the edible oyster mushrooms.

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List of Symbols

Symbol	**Meaning**
PS	Peanut shells
Pleurotus spp.	All *Pleurotus* species
WS	Wheat straw
CW	Cotton waste
CS	Corn stalk
SC	Substrate combination
BE	Biological efficiency
SW	Solid waste
50×10^9	50 billion
CO_2	Carbon dioxide
P. chrysosporium	*Phanerochaete chrysosporium*
P. columbinus	*Pleurotus columbinus*
P. ostreatus	*Pleurotus ostreatus*
P. sapidus	*Pleurotus sapidus*
P. cornucopiae	*Pleurotus cornocopiae*
P. djamor	*Pleurotus djamor*
P. florida	*Pleurotus florida*
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
8	(*Pleurotus v. florida)* strain number 8
49	(*Pleurotus ostreatus v. florida*) strain number 49

103	(*Pleurotus sajor-caju*) strain number 103
112	(*Pleurotus sp.)* strain number 112
117	(*Pleurotus sajor caju*) strain 117
400	(Pleurotus ostreatus) strain number 400
MBFBL	Mushroom biology fungal biotechnology lab
PDA	Potato dextrose agar
g	Gram
L	Liter
ml	Milliliter
°C	Degrees celsius
mins	Minutes
cm	Centimeter
s	Seconds
mm	Millimeters
C	Carbon
H	Hydrogen
N	Nitrogen
O	Oxygen
S	Sulphur
LOM	Loss of organic matter
-20°C	Below 20 degrees celsius
kg	Kilogram
C/N	Carbon to nitrogen ratio

Ca	Calcium
P	Phosphorus
Fe	Iron
Mg	Magnesium
Mn	Manganese
Pb	Lead
Zn	Zinc
Cu	Copper
K	Potassium
$HN0_3$	Nitric acid
HCl	Hydrochloric acid
MARS	Microwave accelerated reaction system
DI	Deionized water
ICP-AES	Inductively coupled plasma-atomic emission spectrophotometer
SAS	Statistical analysis system
$p < 0.05$	Calculated *p*-value is less than 0.05
Pr > F	Probability greater than F
CV	Coefficient of variance
mg/kg	Milligram per kilogram
ND	Not detected
d.w.	Dry weight
CRD	Completely randomized design
BDL	Below detectable levels

Abstract

Peanut shells (PS) and corn stalks (CS) are abundant agro-industrial wastes that are mainly disposed of by burning or soil incorporation. The biodegradation of PS and CS in various substrate combinations (SCs) was studied under solid state fermentation with *Pleurotus ostreatus.* A two factorial experiment in a completely randomized design (CRD) was used in this study. The abilities of the selected SCs to support fruit body yield was also investigated as a one factorial experiment in a CRD. Analysis of variance (ANOVA) was used to determine significant differences at 5% level of significance. The results from the biodegradation studies showed that increasing the CS proportion between 50-100% in substrate combinations increased the loss of organic matter (LOM), degradation of lignin and hemicellulose, C/N ratio, and the concentrations of minerals like Mg, Mn, Zn, and Pb. It also decreased the degradation of cellulose and mineral concentrations of Ca, Cu, Fe, K, and P. Mushroom cultivation on selected substrate combinations revealed that substrates supplemented with 5% rye (R) had higher biological efficiencies and lower C/N ratio than non supplemented substrates. The increased degradation of PS by the addition of CS suggests that the combination of the two substrates could be a useful means for biodegrading PS to obtain bio-products of interest. Also, the highest biological efficiencies of 144.09 and 128.09% obtained with substrate combinations 70PS:5R:25CS and 45PS:5R:50CS, respectively, indicates the possible commercial use of the two substrates for profitable oyster mushroom production in the mushroom industry.

CHAPTER 1

Introduction

Peanut shells (PS) are abundant agro-industrial wastes from peanut processing industries that are mainly disposed of by burning or soil incorporation (Philippoussis, Zervakis, and Diamantopoulou, 2001). Its lignin content, reported to be as high as 30-40%, limits its potential for bioconversion into nutritive value of high quality fodder with enriched protein content and improved digestibility, and other value added products (Kumar, Barrett, Delwiche, and Stroeve, 2009). In literature peanut shells were compared to other lignocellulolytic wastes as substrates for mushroom cultivation. Zervakis, Philippoussis, Ionnnidou, and Diamantopoulou (2001) studied the mycelium growth kinetics and optimal temperature conditions of *Pleurotus* spp.*, Agrocybe aegerita, Volariella volvacea, Auricularia auricular-judae, and Lentinula edodes* on wheat straw, cotton gin-trash, poplar sawdust, oak sawdust, and olive press cake, peanut shells, and corn cobs. Peanut shells were among the substrates that displayed the highest colonization rate for *Pleurotus* spp., fast growth for *V. volvacea,* and optimum growth for *A. aegerita.* Overall, PS was rated to be a satisfactory substrate for most of the strains examined. Philippoussis et al. (2001), however, determined the colonization rates of *A. aegerita, V. volvacea, and Pleurotus* spp. strains on peanut shells were significantly lower than on wheat straw (WS) and cotton waste (CW). PS was thus excluded by Phillipousis et al. (2001) from further study to evaluate the comparative growth rates on composted and noncomposted substrates. The details on substrate utilization and mushroom production with peanut shells as substrates is limited in literature and studied only by a few.

Corn stalks (CS) are other abundant wastes from corn processing industries with lignin content of at least 16% (Heltay and Zavodi, 1960). The harvested corn stalks are used in animal

feed production, organic fertilizer synthesis, or returned to the field as an agronomic resource but one-third are directly burned each year, which not only causes environmental pollution but also wastes natural resources (Guo et al, 2008).

White-rot fungal species such as *Agaricus bisporus* (button mushrooms) and *Pleurotus* spp. (oyster mushrooms) are able to bioconvert such substrates into many useful by-products through biodegradation (Chapuis and Cortieu, 1951; Hamza, Mohammady, and Majcheaczyk, 2003). The latter species, however, possess several advantages over other mushrooms that make cultivation of *Pleurotus* spp. an excellent alternative for mushroom production (Sanchez, 2009).

Pleurotus species have been known to upgrade lignocellulolytic materials by converting such wastes into substrates for mushroom production and using the spent substrate for animal feed with higher protein content (Kinfemi, Mohamed, and Ayoade, 2009). They are the third most cultivated edible mushroom in the world after *A. bisporus and Pleurotus* spp. (Valazquez-Cedeno, Farnet, Ferre, and Savoie, 2004) and have a high yield potential that could exceed 100% biological efficiency (BE) wet weight (Salmones, Mata, and Waliszewski, 2005). While wheat straw is the main substrate used for *Pleurotus* spp. production, it is possible to cultivate them on a wide variety of agricultural wastes (Kalmis and Sargin, 2004). Because of this versatility, several agro-industrial wastes can be used as potential substrates for the cultivation of *P. ostreatus*. The environmental conditions required are less demanding and they can be easily cultivated over wide geographical locations (Banik and Nandi, 2004).

The feasibility of combining two or more substrates for mushroom cultivation had been reported in literature. Isikhuemhen, Mikiashvili, and Kelkar (2008) reported that the combined utilization of poultry litter solid waste and wheat straw, supplemented by millet, resulted in higher yield production of *A. aegerita* mushrooms than using either substrate alone. Ozcelik and

Peksen (2006) obtained higher yields of shiitake mushroom cultivated on mixed substrates of hazelnut husk, wheat straw, wheat bran, and bean wood chip than non-mixed hazelnut husk substrates.

The aim of this research was to evaluate the biodegradation of peanut shells and corn stalks in mixed and non mixed combinations by *P. ostreatus* and its application in oyster mushroom fruit body production.

1.1. Objectives

1). To evaluate the bioconversion of PS and in combinations with CS substrates by *Pleurotus ostreatus* through solid state fermentation.

2). Application of PS, and in combination with CS substrates in mass cultivation of mushroom fruit bodies of *P. ostreatus.*

1.2. Hypothesis

1). Solid state fermentation with *P. ostreatus* can result in significant delignification of PS.

2). Appropriate combination of PS, CS with or without supplementation of rye will give higher fruit body yield of *P. ostreatus* than PS or CS substrate alone.

3). The nutritional content of mushrooms produced in the best substrate combination of PS, CS, and rye will result in mushrooms with the best nutritional content.

4). Spent substrate from the best substrate combination will have the least residual lignin and optimal C/N ratio.

CHAPTER 2

Literature Review

2.1. Lignocellulose in Agricultural Residues

Lignocellulose is the most abundant renewable organic resource on earth and is the major component of plant cell walls in agro-industrial wastes produced in huge tons all over the world (Villas-Boas, Esposito, and Mitchell, 2002). It represents 50% of plant biomass in cell walls and is estimated to reach annual production of 50×10^9 tons (Rajaratham and Bano, 1989). It is a compact crystalline complex of micro fibers of polysaccharides covered by lignin layers that not only protect the polysaccharides against attack by hydrolytic enzymes and other external factors but also stabilizes the complex structure (Leonowicz et al., 1999). The polysaccharide micro fibers also consist of cellulose and hemicellulose; sometimes pectin may be present. Lignin bonds or links with cellulose and hemicellulose, providing structural integrity, impermeability, and resistance to hydrolytic activity by enzymes & oxidative stress (Sanchez, 2009). The disposal of these agricultural residues after harvest is mainly accomplished through burning or incorporated into soil as organic fertilizer (Zadrazil, Brunnert, and Grabbe, 1983).

2.2. Value of Lignocellulolytic Residues

Most of the agricultural wastes have nutritive potential that can be applied to cattle diets in animal feed (Galati et al., 2004; Eun, Beauchemin, Hong, and Bauer, 2006). Because they are nutritious, a small portion can be used as feed or for industrially formulated cattle diets (Yang, Chen, Gao, and Li, 2001). Lignocellulolytic residues can be classified into 2 main groups: those in which lignocellulose is the main source of carbon and those in which besides lignocellulose, simple carbohydrates such as monosaccharides and disaccharides are present. The first group consists of agro-industrial wastes from cereals and wood processing, whereas the second consists

of wastes from the fruit processing industries (Villas-Boas, Esposito, and Mitchell, 2002). While the first group is rich in fiber they have low digestibility. The presence of lignin not only inhibits ruminal digestion of polysaccharides but also protects other highly digestible compounds (Valdez et al., 2008). Thus the digestibility is so low that they are directly unsuitable to both ruminants and non-ruminants alike (Villas-Boas et al., 2002).

The utilization of white rot fungi is a potential solution to bioconvert lignocellulolytic wastes in order to obtain products with high nutritive value, especially, for protein and vitamin contents, and increased digestibility (Anon, 1977; Durán, 1989; Durán et al., 1994; Kuhad, Singh, Tripathi, Saxena, and Eriksson, 1997). During microbial processes of bioconverting lignocellulolytic residues into feed, at least one of three objectives must be accomplished: an increase in protein level, an increase in digestibility of the lignocellulolytic material, and an improvement in dry product palatability which can also be obtained by mixing the substrate with other more palatable foods (Kamra and Zadrazil, 1988).

2.3. Utilization of White-Rot Fungi in Bioconversion of Lignocellulolytic Residues

White-rot fungi are mainly basidiomycetes, capable of degrading lignin on fallen trees and woody materials. They possess the adequate enzymes for delignification of lignocellulose (Reddy, 2005). Their main strategy, however, is to breakdown lignin so that they can gain access to cellulose and hemicellulose embedded within the ligninocellulose matrix (Hammel, 1997). White-rot fungi are not known to be capable of using lignin as the sole source of carbon (Sanchez, 2009). Some white-rot fungi attack lignin, hemicellulose, & cellulose simultaneously, and others remove lignin in advance of hemicellulose and cellulose (Blanchette, 1991; Daniel, 1994; Blanchette, Krueger, Haight, Akhtar, and Akin, 1997).

As effective degraders of lignin, fungi can be cultivated on a wide variety of lignocellulolytic wastes as substrates and have potential applications in bioconversion of lignocellulose (Ardon, Kerem, and Hadar, 1998). The ability of fungi to degrade lignocellulose efficiently is thought to be associated with a mycelia growth habit that enables the fungus to transport scarce nutrients like nitrogen and iron from a distance and into the nutrient deficient substrate that constitutes its carbon source (Hammel, 2007).

Lignin degradation takes place during microbial secondary metabolism, usually at the stationary phase, when the substrate is fully colonized and lignocellulolytic enzymes are synthesized. During the active-growth (colonization) phase, the hemicellulose fraction and water soluble carbohydrates are utilized as carbon sources. The cellulose fraction is not used for carbon source and remains generally untouched at this time to increase the digestibility of lignocellulose (Moyson and Verachtert, 1991).

2.4. White-Rot Basidiomycetes

Among the white-rot fungi that effectively degrade lignin are Shiitake (*Lentinula edodes*), Black Poplar Mushrooms (*Agrocybe aegerita*), Turkey Tail (*Trametes versicolor*), *Lentinus squarosolus*, Judas Ear Fungus (*Auricularia auricular-judae*), Oyster Mushrooms (*Pleurotus* spp.), etc. The natural ability of some fungi, particularly *Pleurotus* species, to upgrade lignocellulolose materials into animal feeds through mushroom production has been documented in literature (Kinfemi et al., 2009).

2.5. Oyster Mushrooms (*Pleurotus* spp.)

Cultivation of oyster mushrooms (*Pleurotus* spp.) has increased throughout the world and its rise in popularity is due to its ease of cultivation, high yield potential, and high nutritional as well as medicinal value (Banik and Nandi, 2004). Though commonly grown on pasteurized

wheat or rice straw, *Pleurotus* spp. are cultivatable on several lignocellulolytic residues. This enables them to play an important role in management of organic wastes, whose disposal has become problematic (Gregori, Svagelj, and Pohleven, 2007).

The biological efficiencies (BE) of *Pleurotus* strains can exceed 100% wet weight (Salmones, Mata, and Waliszewski, 2005). Mandeel, Al-Laith and Mohammed (2005) obtained 134% and 100.8% BE with *Pleurotus pulmonarius* cultivated on carboard and office papers, respectively. They also obtained BEs of 117.5% and 112.4% with *Pleurotus ostreatus* cultivated on cardboard and office papers, respectively.

2.6. Variety and Contents of Lignocellulolytic Residues used for *Pleurotus* spp. Cultivation

The amount of lignin depends on the type of lignocellulolytic residue (substrate); its content ranges could also be influenced by variety as well as regional condition (Rymsza, 2010). Thus, an increase in lignin content directly affects the degradation of neutral detergent fiber (NDF) (Caballero et al., 2001). With the increase in lignin content as plant matrices, degradation of dry organic matter correlates more closely with NDF digestibility than with NDF content (Dechamps, 1999). The amount of lignin content in a lignocellulolytic residue can be high, such as peanut shells (Matsunobu, Horishita, and Konaka 1994; Bota and Wang, 2005; Sanchez, 2009) and can lower the rate of bioconversion of such residues.

Mushrooms can be cultivated on single or mix of lignocellulolytic substrates. In the latter case, growing mushrooms on mixed substrates could outperform fruit body yield production on single substrates in comparison. Mane, Patil, Syed, and Baig (2007) compared individual and mixed substrates of cotton stalks, groundnut haulms, soybean straw, pigeon pea stalks and leaves and wheat straw for cultivation of *Pleurotus sajor-caju* (*Pleurotus*

pulmonarium var. *sajor-caju*) and determined that supplemented or not, some mixed substrates produced more fruit body yields than the non mixed substrates.

Yildiz, Gezer, Yildiz, Temiz, and Dizman, (2001) also investigated the effects of different substrate combinations of needle of spruce, rice stalk, wheat straw, sawdust, waste paper, grass, and bran on the mycelial growth period of *P. ostreatus*. They determined that mixed ratios such as 75% tilia leaves:25% bran and 50% populus leaves:50% wheat straw displayed the shortest growth period (19 days) and individual substrates such as100% wheat straw displayed the longest growth period (40 days).

CHAPTER 3

Materials and Methods

3.1. Biodegradation

3.1.1. Strain and inoculum preparation. *Pleurotus ostreatus* (MBFBL 400) was selected from the mushroom biology fungal biotechnology laboratory (MBFBL) cultures collection at North Carolina A&T University at Greensboro, N.C., and maintained on potato dextrose agar (PDA) media. The media was prepared according to the manufacturer's instruction (DIFCO, USA) by mixing 39 g of potato dextrose agar with 1 L (1000 ml) deionized water (DI) and autoclaved at 121°C for 15 mins. After cooling the media to 55 °C under a laminar flow hood, it was poured into 10×15 mm polystyrene petri dishes and left to solidify. The inoculated plates were incubated in a 25 °C incubator. For liquid inoculum, a petri dish (9 cm diameter) containing actively growing mycelia culture (14 days) was partitioned into 4 quadrants (6 g) and homogenized with 200 ml sterilized water in a waring laboratory blender for 2 to 3 times with a resting period of 30 s per interval.

3.1.2. Substrates and experimental design. Milled peanut shells were obtained from the Good Peanut Company in Aulander, NC and Severn Peanut Company in Severn, NC. Corn stalks were obtained at the fields from the swine unit at North Carolina A&T School farm, oven-dried at 80 °C for 24 hours. Both substrates were milled to 2 mm sieve size in a Thomas Willey Mill. The substrate mixtures in this experiment were seven combinations of peanut shells and corn stalks. Each of the substrate combinations (15 g dry weight and 70% moisture content) was loaded into 500 ml Erlenmeyer flasks and then sterilized at 121 °C for 1hr. Substrates were inoculated by pipetting 8 ml liquid inoculums through an indented hole at the center of the substrates.

The biodegradation performance of the seven substrate combinations (SC) was observed during incubation periods of 0, 30, 60, 90, & 120 days. The experimental design was a 7 × 5 factorial experiment in a completely randomized design with 4 replications. The treatments were 2 factorial arrangements of seven substrate combinations and five incubation periods into 35 treatments (Table 1). Each of the treatments was replicated in four Erlenmeyer flasks for a total of 140 experimental units.

Table 1

7×5 factorial treatment arrangements of seven substrate combinations and five incubation periods (days)

Substrate Combinations	Incubation periods (Days) (0)	(30)	(60)	(90)	(120)
(SC1)PS100:CS0	100:0	100:0	100:0	100:0	100:0
(SC2)PS 90:CS10	90:10	90:10	90:10	90:10	90:10
(SC3)PS75: CS25	75:25	75:25	75:25	75:25	75:25
(SC4)PS50:CS50	50:50	50:50	50:50	50:50	50:50
(SC5)PS25:CS75	25:75	25:75	25:75	25:75	25:75
(SC6) PS10:CS90	10:90	10:90	10:90	10:90	10:90
(SC7) PS0:CS100	0:100	0:100	0:100	0:100	0:100

Immediately after inoculation, the “0 day” (control) flasks in each substrate combination were oven-dried at 90 °C until constant weight was attained and recorded. The experimental flasks (for 30, 60, 90, and 120 days) were covered with foam stoppers, labeled, sealed with aluminum foil, and incubated at 25 °C in the dark. At each 30 day intervals, selected flasks from each substrate combination were sampled. The resulting biomass (fungus + substrate) were

weighed and recorded as per control above. Loss of organic matter (LOM) was calculated as the percentage difference in weight between the experimental and the control flasks in each SC. The samples were then milled to fine texture with a coffee grinder and stored at 4 °C for later analysis.

3.2. Mushroom Cultivation

3.2.1. Strain and inoculum preparation. *P. ostreatus* (MBFBL400), from PDA cultures, was used for grain spawn preparation. Sorghum grains (1 kg, wet weight) were loaded into polyethylene bags (47 × 17 cm, type 4 Unicorn bags, TX, USA) to which 700 ml water was added. The grains were autoclaved for 5 mins, spread out on a laboratory bench top and mixed with gypsum (1%). The resulting mixture was loaded into micro-pore patch fitted poly-propylene bags (Unicorn bag type 422-1 A) and sterilized at 121°C for 1 hr. After the bags were cooled to room temperature, each bag was inoculated with agar blocks of actively growing mycelia of MBFBL400, sealed and incubated at 25 °C. Following apparent colonization (2 weeks), grain spawn was used to inoculate the SCs.

3.2.2. Substrates and experimental design. Substrates of 2 kg (dry weight) and 65% moisture content were prepared in 13 combinations and stratified into three groups: substrates with no supplement from 100PS:0CS to 0CS:100PS, substrates supplemented with 5% rye (R) 85PS:5Rye:10CS to 50PS:5Rye:45CS and substrates supplemented with 10% rye from 80PS:10Rye:10CS to 50PS:10Rye:40CS (Table 2). Each substrate combination was replicated 4 times for a total of 52 experimental units. The substrates were loaded into Type 422-1 A cultivation bags (93 × 36 cm unicorn bags) and sterilized at 121°C for 1 hr. After cooling the bags under the hood they were inoculated with 5% (100 g) of grain spawn. The contents were thoroughly mixed and sealed and incubated at 25 °C in the dark until colonization. Upon full

colonization of the bags in 27±5 days they were transferred to the fruiting house for primordial induction and fruiting. Fruit bodies were collected up to five flushes. When the primordia appeared from various sides of the blocks the top of the plastic bags were removed to increase gas exchange. During harvesting the bags were completely removed to expose the blocks for further fruit yield. The total fresh weight of the fresh mushrooms was used to calculate the biological efficiency (BE). After harvest the collected mushrooms and blocks were oven dried at 60°C and milled to 1-2 mm for both mineral and C/N ratio analysis.

Table 2

Thirteen treatment substrate combinations of peanut shells and corn stalks

Substrate combinations		
Group 1	Group 2	Group 3
SC1 100PS:0CS (4)	SC6 85PS:5R:10CS (4)	SC10 80PS:10R:10CS (4)
SC2 90PS:10CS (4)	SC7 70PS:5R:25CS (4)	SC11 65PS: 10R:10CS (4)
SC3 75PS:25CS (4)	SC8 40PS:5R:50CS (4)	SC12 40PS:10R:50CS (4)
SC4 50PS:50CS (4)	SC9 50PS:5R:40CS (4)	SC13 50PS: 10R:40CS (4)
SC5 0PS:100CS (4)		

3.3. Sample Analysis

Milled samples were analyzed for lignin, hemicellulose, cellulose, at the Rumen Fermentation Profiling Lab, West Virginia University (WV) USA, using the procedures for NDF and acid detergent fiber (ADF) analysis (AOAC, 1990; Goering et al., 1970; and Van Soest et al., 1991). The carbon to nitrogen ratio (C/N) were analyzed with the Perkin Elmer CHNOS Series 2400 Elemental. Samples (5 to 10 mg) were weighed into 5 × 8 mm tin capsules and were loaded into the analyzer. The basis for determining C and N is the “dynamic flash combustion”

that converts organic or inorganic samples into combustible products. The released gases from the combusted products pass through a reduction furnace and are separated into individual gases and analyzed in a chromatographic column separated and detected by a thermal conductivity detector that gives an output signal proportional to the concentration of the individual components of the mixture.

The mineral concentrations of Calcium (Ca), Phosphorus (P), Iron (Fe), Magnesium (Mg), Manganese (Mn), Zinc (Zn), Copper (Cu), and Potassium (K) and Lead (Pb) were determined through an Inductively coupled plasma-atomic emission spectrophotometer (ICP-AES) at the Murdok Research Institute at Kannapolis, NC. Solid samples of 0.1 g were predigested with 7.5 ml concentrated nitric acid (HNO_3) and 2.5 ml concentrated hydrochloric acid (HCl) in unsealed omni vessels without the application of heat for 15 minutes. This step was necessary to expel highly volatile compounds in organic samples. The vessels were loaded into a high performance microwave accelerated reaction system (MARS 5) 5 and heated for digestion at 200 °C for 15 minutes. The resulting liquid samples were diluted with deionized water (DI) to bring the volume up to 50 ml for ICP analysis.

3.4. Statistical Analysis

The experimental data in the biodegradation and mushroom cultivation were subjected to statistical analysis system (SAS) and analysis of variance (ANOVA) at 5% level of significance. Duncan's multiple range test was used to make multiple comparisons. Significant differences between means and interaction between the factors (where applicable) were indicated by the p-value ($p < 0.05$). Means with different superscript letters were significantly different from each other, means with same letters were not significantly different, and means with two letters such as "ab" were not significantly different from means with "a" and means with "b".

CHAPTER 4

Results and Discussion

4.1. Biodegradation

4.1.1. Loss of organic matter. The results from loss of organic matter in different substrate combinations (SCs) are shown in Table 3. LOM in the substrate combinations varied between 0.02% to 24.09%. The highest LOM was observed in 50PS:50CS (24.09%), followed by 10PS:90CS (22.74%), 0PS:100CS (22.10%) and 25PS:75CS (21.42%) after 120 days.

Table 3

Loss of organic matter means from substrate combinations of peanut shells and corn stalks

	LOM† (%) Days				
Combination	0	30	60	90	120
100PS:00CS	0.05^{j}	1.69^{j}	2.83^{j}	8.39^{j}	17.09^{de}
90PS:10CS	0.02^{k}	3.31^{j}	3.70^{j}	9.66^{h}	18.63^{dc}
75PS:25CS	0.03^{k}	2.37^{jk}	4.23^{ij}	11.63^{gh}	19.90^{bc}
50PS:50CS	0.38^{k}	4.02^{j}	6.94^{i}	15.28^{ef}	24.09^{a}
25PS:75CS	0.32^{k}	5.10^{ij}	11.49^{h}	14.97^{ef}	21.42^{ab}
10PS:90CS	0.28^{k}	3.91^{j}	11.20^{gh}	14.06^{fg}	22.74^{ab}
0PS:100CS	0.04^{k}	3.77^{j}	9.87^{h}	12.59^{gh}	22.10^{ab}

†-Means having a letter in common are not significantly different at the 5% level of significance according to Duncan's multiple range test.

These results showed that the least degradation occurred in peanut shells alone (100PS:0CS) resulting in low LOM. However, increasing the CS content in substrates resulted in increasing loss of organic matter. The maximum LOM was achieved at the SC ratio 50PS:CS50 but further addition of cornstalks beyond 50% reduced the LOM. This makes it evident that combining peanut shells with cornstalks was essential for obtaining higher LOM. The analysis of

variance for LOM is presented in Table 4. The LOM between the different SCs were significantly different from each other ($p < 0.0001$). The interaction between the combination and day factors (combination × day) also indicated that the appropriate substrate combination and length of days were important to obtain significant loss of organic matter through degradation.

Table 4

Analysis of variance for loss of organic matter in substrates degraded by Pleurotus ostreatus

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	313.195303	52.199217	48.93	< 0.0001
Day	4	5542.506730	1385.626682	1298.74	< 0.0001
Combination × Day	24	188.978630	7.874110	7.38	< 0.0001
Error	70	74.682800	1.066897		
Total	104	6119.463363			

In literature, Locci et al. (2007) reported an increase of 17% organic matter loss in a 13C-NMR spectrum degradation of wheat bran during 62 days of cultivation with *P. ostreatus*, Mukherjee, Ghosh, and Nandi (2004) reported 23.6% LOM in water hyacinth after 48 days using *P. ostreatus*, and Isikhuemhen and Mikiashvili (2009) reported 53.2-56.2% LOM in substrate combinations of solid waste, wheat straw and millet degraded by *P. ostreatus* after 33 days.

4.1.2. Lignocellulose macromolecules.

4.1.2.1. Lignin.

Lignin content ranged between 5.99% and 30.82% (Table 5). In each of the SCs tested an increase in CS content increased the lignin loss. Lignin degradation varied from 0.29-41.50% throughout the incubation period. The highest lignin loss was determined in 0PS:100CS, (41.50%) after 120 days.

Table 5

Lignin content in PS and CS substrates degraded by Pleurotus ostreatus

Combination	Lignin† (%) Days				
	0	30	60	90	120
100PS:00CS	30.82^{a}	30.73^{a}	30.71^{a}	27.87^{bc}	26.74^{c}
90PS:10CS	28.31^{b}	27.96^{bc}	27.81^{bc}	26.75^{c}	23.45^{ef}
75PS:25CS	26.92^{bc}	25.30^{d}	24.09^{d}	22.23^{gf}	21.00^{gh}
50PS:50CS	22.26^{f}	19.87^{hi}	19.03^{ij}	17.89^{ljk}	17.66^{lk}
25PS:75CS	18.96^{ijk}	16.60^{l}	15.06^{m}	14.04^{m}	11.27^{no}
10PS:90CS	11.97^{n}	11.63^{n}	8.86^{qr}	8.72^{qr}	8.05^{r}
0PS:100CS	10.24^{op}	9.46^{pq}	8.15^{qr}	6.62^{s}	5.99^{s}

†.Means having a letter in common are not significantly different at the 5% level of significance according to Duncan's multiple range test.

The first hypothesis stated that SSF with *Pleurotus ostreatus* can result in significant delignification of PS. In 100PS:0CS the change of lignin content in 90 days was significantly different from the control (0 days). Although these results supported the hypothesis this SC had the lowest lignin degradation range compared to the other SCs. Gradual addition of cornstalks in different percentages resulted in the subsequent loss of lignin. This improvement also showed that the combination of PS and CS was necessary for higher delignification of PS.

Sherief, Tanash, and Temraz (2010) reported lignin loss of 30% and 16% from sawdust and rice straw, respectively, after 50 days of cultivation with *P. ostreatus*. Tanuguchi et al. (2005) obtained 41% lignin loss in rice straw after treatment with *P. ostreatus.* The analysis of variance for lignin showed that there was interaction between the substrate combination and day factors (combination × day) for lignin degradation. The interaction *p*-value ($p < 0.0001$) also indicated

that the lignin content in the substrate combinations following degradation were significantly different from each other throughout the incubation period (Table 6).

Table 6

Analysis of variance for the presence of lignin in substrate degradation by Pleurotus ostreatus

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	6170.842853	1028.473809	1718.91	< 0.0001
Day	4	338.757213	84.689303	141.51	< 0.0001
Combination × Day	24	46.814413	1.950601	3.26	< 0.0001
Error	70	41.883133	0.598330		
Total	140	6598.297613			

4.1.2.2. Cellulose. The cellulose content in the tested SCs varied from 32.92% to 44.41% (Table 7). Cellulose content in SCs with 75-100% PS was lower than SCs with 50% PS or less. At the 50PS:50CS ratio the cellulose content in some of substrate combinations was found to be over 40%. Further addition of CS (75-100%) resulted in higher cellulose content in the substrates.

Table 7

Cellulose content in PS and CS substrates degraded by Pleurotus ostreatus

	Cellulose† (%) Days				
Combination	0	30	60	90	120
100PS:00CS	37.32^{ij}	37.32^{ij}	36.00^{jkl}	33.37^{mn}	32.92^{n}
90PS:10CS	38.86^{hi}	35.97^{jkl}	34.88^{klm}	33.76^{mn}	33.51^{mn}
75PS:25CS	37.22^{ij}	36.75^{j}	36.57^{jk}	36.15^{jkl}	34.57^{lmn}
50PS:50CS	42.22^{b-f}	41.88^{c-f}	41.16^{c-g}	41.13^{d-g}	34.87^{klm}
25PS:75CS	43.05^{abc}	43.01^{a-d}	42.92^{a-d}	40.92^{efg}	39.44^{gh}
10PS:90CS	42.28^{b-e}	41.70^{c-f}	41.55^{c-f}	41.01^{efg}	40.36^{fgh}
0PS:100CS	44.41^{a}	43.93^{ab}	43.93^{ab}	41.37^{c-f}	38.81^{hi}

†-Means having a letter in common are not significantly different at the 5% level of significance

according to Duncan's multiple range test.

Degradation of cellulose among SCs ranged from 0-17.43%. In 0PS:100CS 0.88-12.61% cellulose was lost via degradation. These results showed that in contrast to high lignin loss the cellulose degradation in SCs was low. The *p*-value of the interaction of factors (combination × day) was $p < 0.0001$. This also indicated that significant differences in cellulose content were found among the SCs (Table 8).

Table 8

Analysis of variance for the presence of cellulose in substrates degraded by Pleurotus ostreatus

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	918.6643295	153.1107216	156.10	< 0.0001
Day	4	225.6772571	63.9193143	65.17	< 0.0001
Combination × Day	24	84.3886895	3.5161954	3.58	< 0.0001
Error	70	68.6578000	0.980826		
Total	140	1327.388076			

These results are in agreement with Tsang, Reid, and Coxworth (1987) who obtained 20% (average) loss of cellulose from degradation of wheat straw by *Pleurotus sajor-caju* (*Pleurotus pulmonarius* var. *sajor-caju*), *P. sapidus, P. cornucopiae, and P. ostreatus* mushrooms and Jafari, Nikkah, Sadeghi, and Chamani (2007) also reported 0.02%, 0.55%, 0.26% loss of cellulose by *P. ostreatus, P. florida, and P. djamor* during cultivation on wheat straw.

4.1.2.3. Hemicellulose. Hemicellulose content in the SCs varied between 7.90% and 27.02% (Table 9). Following degradation the loss of hemicellulose varied from 1.21% (100PS:0CS, 30 days) to 52.70% (50PS:50CS, 120 days). This shows that adding cornstalks to the SCs resulted in increasing loss of hemicellulose. In 10PS:90CS hemicellulose degradation

ranged from 12.71-38.25% and 10.81-35.60% in 0PS:100CS. It is evident that the degradation rates of these two SCs are not significantly different at 5% level of significance.

Table 9

Hemicellulose content in PS and CS substrates degraded by Pleurotus ostreatus

	Hemicellulose † (%) Days				
Combination	0	30	60	90	120
100PS:00CS	9.12^{opq}	9.01^{pqr}	8.88^{pqr}	8.11^{qr}	7.90^{r}
90PS:10CS	14.32^{hi}	12.43^{ljk}	11.16^{lmn}	10.11^{nop}	9.36^{opq}
75PS:25CS	16.44^{efg}	15.91^{fg}	14.40^{hi}	13.75^{ij}	13.14^{ijk}
50PS:50CS	20.09^{c}	14.19^{hi}	11.83^{klm}	10.14^{nop}	9.63^{op}
25PS:75CS	18.61^{d}	16.03^{efg}	15.48^{gh}	12.70^{jk}	10.52^{mno}
10PS:90CS	26.90^{a}	23.47^{b}	20.02^{c}	17.19^{ef}	16.61^{efg}
0PS:100CS	27.02^{a}	24.10^{b}	20.04^{c}	17.44^{de}	17.39^{de}

†-Means having a letter in common are not significantly different at the 5% level of significance according to Duncan's multiple range test.

Compared to 17% loss of cellulose approximately 52.70% hemicellulose was observed to be lost from the SCs. This implied that hemicellulose is utilized more than cellulose by *P. ostreatus.* Thompson et al. (2003) reported that hemicellulose was degraded preferentially to cellulose by *P. ostreatus* cultivated on wheat straw. Wang, Sakoda, and Suzuki (2001) also reported low degradation of cellulose by *P. ostreatus* cultivated on spent beer grains. The interaction between the combination and day factors (combination × day) for the degradation of hemicellulose also indicated significant differences ($p < 0.0001$) between the SCs during the incubation period (Table 10).

Table 10

Analysis of variance for the presence of hemicellulose in substrates degraded by Pleurotus ostreatus

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	1940.292640	323.382107	526.31	< 0.0001
Day	4	655.325318	163.831330	266.64	< 0.0001
Combination × Day	24	196.345655	8.181069	13.31	< 0.0001
Error	70	43.010467	0.61		
Total	104	1327.388076			

4.1.3. Minerals

4.1.3.1. Calcium. Calcium in the SCs varied between 1029.45 and 2637.27 mg/kg (Table 11). High values of calcium (> 2000 mg/kg) were mostly found in substrate combinations with 25-100% PS. In 10PS:90CS and 0PS:100CS calcium values were below 2000 mg/kg ranging between 1418.87-1769.54 mg/kg and 1222.80-1619.91 mg/kg, respectively.

Between 0 to 120 days calcium levels gradually increased in each SC but in 100PS:0CS calcium contents were not significantly different from its control (0 days). As the proportion of cornstalks increased calcium levels in SCs subsequently decreased. This shows that the addition of cornstalks decreases calcium content in SCs. The interaction between combination and day factors (combination × day) also indicated significant differences at $p < 0.0001$ in calcium contents between the seven SCs (Table 12). These results were higher than 654 mg/kg Ca, reported by Moda, Horii, and Spoto, (2005) in sugarcane bagasse cultivated by *Pleurotus sajor-caju (Pleurotus pulmonarius* var. *sajor-caju*) but lower than 8470 and 4170 mg/kg Ca in cowpea shells degraded by *P. ostreatus and P. pulmonarius*, repectively (Kinfemi et al., 2009).

Table 11

Calcium content in substrate combinations degraded by Pleurotus ostreatus

Combination	Days				
	0	30	60	90	120
	mg/kg[†]				
100PS:00CS	2191.79^{cde}	2637.27^{a}	2223.59^{b-e}	2424.39^{abc}	2324.79^{bcd}
90PS:10CS	2279.16^{b-e}	2431.00^{abc}	2404.14^{abc}	2401.78^{abc}	2613.61^{a}
75PS:25CS	2079.00^{efg}	1878.47^{gh}	1878.43^{gh}	2082.22^{efg}	2446.24^{ab}
50PS:50CS	1784.97^{hij}	1769.91^{hij}	1831.09^{hi}	1919.21^{fgh}	2418.65^{abc}
25PS:75CS	1541.92^{jk}	1558.56^{jk}	1709.67^{hij}	1831.30^{hi}	2142.85^{def}
10PS:90CS	1418.87^{kl}	1299.88^{l}	1408.22^{kl}	1667.44^{hij}	1769.54^{hij}
0PS:100CS	1222.80^{lm}	1029.45^{m}	1278.15^{l}	1401.53^{kl}	1619.91^{ijk}

†-Means having a letter in common are not significantly different at the 5% level of significance according to Duncan's multiple range test.

Table 12

Analysis of variance for the presence of calcium in PS & CS substrates

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	15562755.14	2593792.52	149.74	< 0.0001
Day	4	2445873.67	611468.42	35.30	< 0.0001
Combination × Day	24	1410180.71	58757.53	3.39	< 0.0001
Error	70	1212499.77	17321.43		
Total	104	206311309.29			

4.1.3.2. Copper. The highest and lowest copper contents were found in 75PS:25CS (46.31 mg/kg) and 0PS:100CS (4.96 mg/kg). Although substrates with 75 to 100% PS had higher copper values they did not significantly differ from the SCs with lower values (Figure 1).

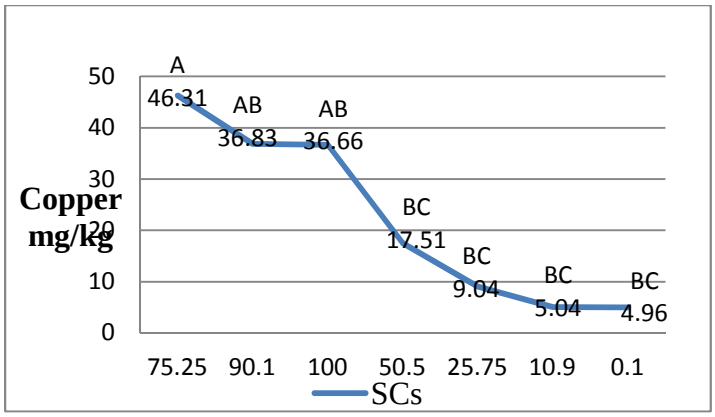


Figure 1 Copper content in substrate combinations degraded by *Pleurotus ostreatus*

Analysis of variance for copper is shown in Table 13. There was no interaction between the combination and day factors at $p = 0.42$. While significant differences were not found within the day factors at $p = 0.12$, p-value at 0.0018 indicates that significant differences in Cu varied by combination.

Table 13

Analysis of variance for the presence of copper in PS and CS substrates

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	28685.111096	4780.85183	3.96	0.0018
Day	4	8977.92921	2244.48230	1.86	0.1271
Combination × Day	24	30345.81353	1264.40890	1.05	0.4228
Error	70	84449.6518	1206.4236		
Total	140	152458.5055			

Copper plays a role in delignification of lignin as a cofactor for laccase. Laccase activity is stimulated mainly by copper, however certain minerals such as manganese, iron, zinc, and cadmium had been reported to be involved as well (Baldrian and Gabriel, 2003). In this study the low levels of copper noted in the two SCs (10PS:90CS and 0PS:100CS) with the best

lignocellulose degradation suggest that copper at high concentrations can decrease substrate colonization of *P. ostreatus*. This was supported by Baldrian et al. (2005) who found high concentrations of Cu increased laccasse activity resulted but decreased the substrate colonization rate. In literature, the copper values in this study is higher than those of Moda et al. (2005) who reported 2.4 mg/kg Cu in bagasse substrates degraded by *P. ostreatus* and Kinfemi et al. (2009) who obtained 0.043 and 0.0462 mg/kg Cu in cowpea shells that were degraded by *P. ostreatus* and *P. pulmonarius*, respectively.

4.1.3.3. Iron. Iron values varied from 34.38-1202.47 mg/kg (Table 14). High levels in iron were found in substrates with 50-100% PS. Low iron values ranged 37.07-43.96 mg/kg (25PS:75CS), 36.93-39.52 mg/kg (10PS:90CS), and 34.38-36.81 mg/kg (0PS:100CS) and were not significantly different from each other at the 5% level of significance.

Table 14

Iron content in substrate combinations degraded by Pleurotus ostreatus

Combination	Days				
	0	30	60	90	120
	mg/kg†				
100PS:0CS	777.11^{cde}	971.88^{b}	879.37^{bc}	1202.47^{a}	837.22^{bcd}
90PS:10CS	792.67^{cde}	864.77^{bc}	910.39^{bc}	882.39^{bc}	1133.20^{a}
75PS:25CS	670.65^{ef}	694.58^{def}	542.74^{fg}	642.55^{efg}	841.11^{bcd}
50PS:50CS	505.27^{g}	543.57^{fg}	500.36^{g}	550.66^{fg}	826.76^{bcd}
25PS:75CS	42.46^{h}	41.76^{h}	43.96^{h}	38.71^{h}	37.07^{h}
10PS:90CS	39.52^{h}	37.03^{h}	36.64^{h}	37.76^{h}	36.93^{h}
0PS:100CS	35.07^{h}	34.68^{h}	34.38^{h}	35.65^{h}	36.81^{h}

†-Means having a letter in common are not significantly different at the 5% level of significance as indicated by Duncan's multiple range test.

The iron content in 100PS:0CS throughout the incubation period was not significantly different from the controls (0 days). Increasing CS in substrate combinations decreased the iron content in SCs. Iron is speculated to participate in the cleavage of the lignocellulolytic

biopolymers by the mechanism of Fenton's reaction involving Fe (II) but regarding metal content and availability in lignocellulose, the information has never been directly addressed (Perez and Jefferies, 1992; Perie and Gold, 1991; Singhal and Rathore, 2001; Wood, 1994). The interaction between the combination and day factors also indicated significant differences between the SCs varying across the incubation period (days). The *p*-value is $p < 0.0001$. (Table 15).

Table 15

Analysis of variance for the presence of iron in PS and CS substrates

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	15461494.23	2576915.71	361.86	< 0.0001
Day	4	219129.52	54782.38	7.69	< 0.0001
Combination × Day	24	674950.0	28122.92	3.95	< 0.0001
Error	70	498485.24	7121.22		
Total	104	16854059.05			

In literature Moda et al. (2005) obtained 96 mg/kg iron in bagasse degraded by *Pleurotus sajor-caju* (*Pleurotus pulmonarius* var. *sajor-caju*) and Kinfemi et al. (2009) obtained 6.84 mg/kg and 0.02 mg/kg in cowpea shells degraded by *P. ostreatus* and *P. pulmonarius*, respectively.

4.1.3.4. Potassium. Potassium content in SCs varied from 74.31-10410.98 mg/kg (Table 16). Potassium values were high in 100PS:0CS-50PS:50CS. The highest K values were found in 50PS:50CS (10410.98 mg/kg) and 75PSPS:25CS (10189.20 mg/kg) in 120 days. The lower values of potassium in 25PS:75CS-0PS:100CS were below 500 mg/kg and were not significantly different from each other. Adding corn stalks to the SCs was observed to increase potassium values until the PS:CS ratio reached 50:50. Beyond that, potassium levels decreased with further

addition of cornstalks. The combination and day factors (combination × day) were found to interact at *p*-value 0.0023. The *p*-value also indicated that the potassium concentrations in each SC were significantly different from each other and varied throughout the incubation period (Table 17).

Table 16

Potassium content in substrate combinations degraded by Pleurotus ostreatus

Combination	Potassium				
	0	30	60	90	120
	mg/kg†				
100PS:00CS	5848.97^{hi}	6231.80^{ghi}	5720.07^{i}	6220.78^{ghi}	6015.76^{hi}
90PS:10CS	6611.54^{f-i}	6849.68^{f-i}	6823.02^{f-i}	6626.19^{f-i}	7726.49^{ef}
75PS:25CS	7196.32^{efg}	6916.54^{fgh}	6928.83^{fgh}	7582.34^{ef}	10189.20^{ab}
50PS:50CS	8157.42^{ed}	9002.13^{cd}	9162.50^{bcd}	9261.23^{bc}	10410.98^{a}
25PS:75CS	374.21^{j}	387.99^{j}	376.96^{j}	375.51^{j}	484.99^{j}
10PS:90CS	240.86^{j}	207.71^{j}	185.17^{j}	219.17^{j}	293.46^{j}
0PS:100CS	90.55^{j}	87.71^{j}	74.31^{j}	100.17^{j}	101.73^{j}

†-Means having a letter in common are not significantly different at the 5% level of significance as indicated by Duncan's multiple range test.

Table 17

Analysis of variance for the presence of potassium in PS and CS substrates

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	1429006542	238167757	632.14	< 0.0001
Days	4	12148530	3037132	8.06	< 0.0001
Combination × Days	24	21800331	908347	2.41	< 0.0023
Error	70	26373679	376767		
Total	104	14893329083			

Moda reported 1200 mg/kg K in bagasse substrates degraded by *Pleurotus sajor-caju* (*Pleurotus pulmonarius* var. *sajor-caju*). Kinfemi et al. (2009) also reported 3210 mg/kg and

11200 mg/kg K cowpea shells degraded by *Pleurotus pulmonarius* and *Pleurotus ostreatus.* The results in this study are in agreement with those in literature.

4.1.3.5. Magnesium. Magnesium values in substrates varied from 1267.46-12482.35 mg/kg (Table 18). In contrast to calcium, copper, iron, and potassium, increasing magnesium values were observed in SCs with 75-100% CS. The highest magnesium values were 12482.35 mg/kg (0PS:100CS) and 12080.26 mg/kg (10PS:90CS) in 120 days. The lowest magnesium content was 1267.65 mg/kg (100PS:0CS) at 0 days .

Table 18

Magnesium content in substrate combinations degraded by Pleutotus ostreatus

Combination	Days				
	0	30	60	90	120
	mg/kg†				
100PS:00CS	1267.56^{l}	1447.27^{jkl}	1299.70^{kl}	1439.27^{jkl}	1335.94^{kl}
90PS:10CS	1423.40^{kl}	1535.36^{jkl}	1535.41^{jkl}	1485.10^{jkl}	1782.00^{jkl}
75PS:25CS	1547.34^{jkl}	1448.63^{jkl}	1434.80^{jkl}	1569.51^{jkl}	1826.57^{jk}
50PS:50CS	1773.64^{jkl}	1833.44^{ij}	1899.87^{ij}	1919.24^{ij}	2354.35^{i}
25PS:75CS	8367.04^{h}	9070.46^{g}	9321.52^{fg}	10426.86^{cd}	11549.33^{b}
10PS:90CS	9402.91^{fg}	9710.08^{ef}	10186.11^{de}	11330.06^{b}	12080.26^{a}
0PS:100CS	9804.56^{ef}	10207.45^{de}	10745.53^{c}	11388.24^{b}	12482.35^{a}

†-Means having a letter in common are not significantly different at the 5% level of significance as indicated by Duncan's Multiple Range Test.

The subsequent increase of magnesium content with increasing proportion of cornstalks showed the inclusion of corn stalks in SCs increases magnesium in substrates. The interaction factors (combination × day) also indicated significant differences ($p < 0.0001$) in magnesium values between the different SCs (Table 19). Kinfemi et al. (2009) reported 4260 and 3950 mg/kg Mg in cowpea shells degraded by *Pleurotus ostreatus* and *Pleurotus pulmonarius,* respectively.

Table 19

Analysis of variance for the presence of magnesium in PS and CS substrates

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	2003594245	333932374	4204.35	< 0 .0001
Day	4	25968752	6492188	81.74	< 0.0001
Combination × Day	24	22665594	944400	11.89	< 0.0001
Error	70	5559783	79425		
Total	104	2057788374			

4.1.3.6. Manganese. Manganese in the substrate combinations were 45.72 mg/kg to 2965.77 mg/kg (Table 20). Similar to magnesium, increasing manganese values were observed in SCs with 75-100% CS. The low manganese values were found in 100PS:0CS to 50PS:50CS and were not significantly different at the 5% level of significance.

Table 20

Manganese content in substrate combinations degraded by Pleurotus ostreatus

Combination	Days				
	0	30	60	90	120
	mg/kg†				
100PS:00CS	74.23^{h}	82.42^{h}	76.62^{h}	84.86^{h}	78.85^{h}
90PS:10CS	73.67^{h}	73.90^{h}	76.78^{h}	74.59^{h}	89.68^{h}
75PS:25CS	61.34^{h}	57.06^{h}	58.81^{h}	63.15^{h}	71.77^{h}
50PS:50CS	45.72^{h}	47.29^{h}	48.59^{h}	49.91^{h}	61.37^{h}
25PS:75CS	2007.81^{g}	2194.86^{f}	2286.40^{ef}	2405.50^{de}	2806.75^{b}
10PS:90CS	2279.54^{ef}	2291.81^{ef}	2357.79^{de}	2641.26^{c}	2833.15^{b}
0PS:100CS	2402.03^{de}	2260.86^{ef}	2442.49^{d}	2650.39^{c}	2965.77^{a}

†-Means having a letter in common are not significantly different at the 5% level of significance as indicated by Duncan's multiple range test.

Manganese in substrates is very crucial because it plays an important role in biological oxidation and lignin degradation (Cui and Dolphin, 1990). For several white rot- fungi

manganese was responsible for manganese peroxidase (MnP) induction (Steffen, Hattaka, and Hofrichter, 2002; Steffen et al., 2002). Interestingly, while high degradation favored high manganese content in this study, Zohar and Yithak (1993) had reported that significant lignin degradation and mineralization by *P. ostreatus* also occurs under manganese deficiency. The low manganese levels found in 100PS:0CS to 0PS:100CS suggests Mn could be one of the elements that is critical in lignin degradation. This seems to be supported by the fact that increasing the cornstalks to 75% and beyond increased Mn content resulting in higher degradations observed. The interaction between combination and day factors for manganese content is shown in Table 21. Significant differences for the interaction effect (combination × day) regarding manganese were noted by the *p*-value $p < 0.0001$. Kinfemi et al. (2009) reported 180 and 770 mg/kg in cowpea shells degraded by *Pleurotus ostreatus* and *Pleurotus pulmonarius*, respectively. Moda et al. (2005) reported 20 mg/kg Mn in *Pleurotus sajor-caju* (*Pleurotus pulmonarius* var. *sajor-caju*) degraded bagasse.

Table 21

Analysis of variance for the presence of manganese in PS & CS substrates

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	146918666.0	24486444.3	3877.32	< 0.0001
Days	4	1150033.0	287508.2	45.53	< 0.0001
Combination × Days	24	1544184.6	64341.0	10.19	< 0.0001
Error	70	442070.9	6315.3		
Total	104				

4.1.3.7. Phosphorus. Phosphorous content ranged between 8.55mg/kg to 1096.22 mg/kg (Table 22). Phosphorus levels were high in 100PS:0CS to 50PS:50CS. Low phosphorous values

were observed in 25PS:75CS to 0PS:100CS. Although these values in the latter SCs decreased further with addition of cornstalks there were not significantly different from each other.

These results showed that increasing cornstalks in substrates decreases phosphorus content. Interaction between combination and day (combination × day) showed significant differences in phosphorous content between the seven substrate combinations at *p*-value $p < 0.0001$ (Table 23). In bagasse substrates degraded by *Pleurotus sajor-caju* (*Pleurotus pulmonarius* var. *sajor-caju*) Moda et al. (2005) obtained 225 mg/kg phosphorous.

Table 22

Phosphorus content in substrate combinations degraded by P .ostreatus

Combination	Days				
	0	30	60	90	120
	mg/kg†				
100PS:00CS	797.84^{fg}	904^{bcd}	816.69^{fg}	910.02^{bcd}	831.14^{d-g}
90PS:10CS	873.33^{c-f}	899.41^{b-e}	908.60^{bcd}	858.74^{c-f}	1096.22^{a}
75PS:25CS	838.79^{def}	753.67^{gh}	717.26^{h}	813.71^{fg}	966.66^{b}
50PS:50CS	795.01^{fg}	817.87^{fg}	817.09^{fg}	823.92^{efg}	925.43^{bc}
25PS:75CS	25.34^{i}	28.27^{i}	35.57^{i}	31.35^{i}	37.78^{i}
10PS:90CS	17.47^{i}	18.34^{i}	16.97^{i}	22.10^{i}	24.95^{i}
0PS:100CS	9.02^{i}	8.55^{i}	9.58^{i}	11.55^{i}	15.13^{i}

†-Means having a letter in common are not significantly different at the 5% level of significance as indicated by Duncan's multiple range test.

Table 23

Analysis of variance for the presence of phosphorous in PS and CS substrates

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	18142043.58	3023673.93	1655.07	< 0.0001
Day	4	92460.29	23115.07	12.65	< 0.0001
Combination × Day	24	194005.91	8083.58	4.42	< 0.0001

Table 23 *(cont).*

Error	70	127883.83	1826.91
Total	140	18556393.60	

4.1.3.8. *Zinc*. Zinc contents in SCs are shown in Figure 2. The highest and lowest levels of zinc were 27.69 mg/kg (100PS:0CS) and 19.78 mg/kg (50PS:50CS). Significant differences were not found between those of 75PS:25CS and 50PS:CS and zinc levels in 25PS:75CS, 10PS:90CS and 0PS:100CS were not detected (ND).

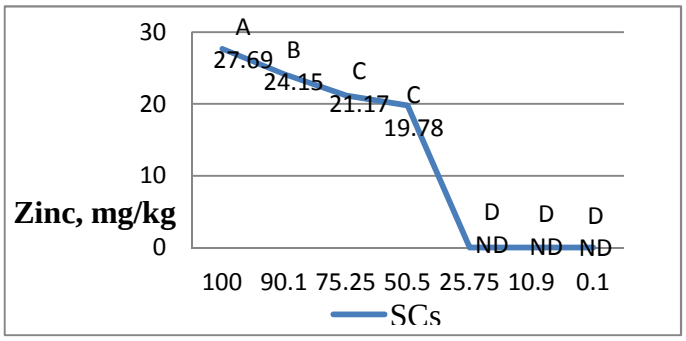


Figure 2 Presence of zinc in PS and CS substrate combinations

In degradation, the presence of zinc was reported to stimulate production of cellulases endo-1,4-β-glucanase and 1,4-β-glucosidase along with Cu but also like Cu, high concentrations of zinc were noted to decrease the substrate colonization rate of *P. ostreatus* (Baldrian et al., 2005). Decreasing Zn contents with addition of cornstalks in the SCs with known values is in agreement with Baldrian's results. There was no interaction between the factors ($p = 0.16$) Similar to copper, significant differences were only found within the combination factor ($p < 0.0001$) (Table 24). These results are in agreement with Moda et al. (2005) who obtained 23 mg/kg Zn in bagasse.

Table 24

Analysis of variance for the presence of zinc in PS and CS substrates

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	14421.24854	2403.54142	231.36	< 0.0001
Days	4	31.59407	7.89852	0.73	0.5762
Combination × Day	24	353.35487	14.72312	1.30	0.1633
Error	70	14806.19749	435.47640		
Total	104	15566.24882			

4.1.3.9. *Lead.* Lead concentrations were found in the substrates (Table 25). Low traces were found in 100PS:0CS varying between 1.49 to 1.61 mg/kg. Higher traces were observed in substrates 25PS:75CS to 0PS:100CS. Lead has been known to inhibit fungal biodegradation at certain levels accumulated by white-rot fungi.

Table 25

Lead content in substrate combinations degraded by P. ostreatus

Combination	Days				
	0	30	60	90	120
	mg/kg†				
100PS:0CS	1.49^{f}	1.61^{f}	0^{f}	1.61^{f}	0^{f}
90PS:10CS	0^{f}	0^{f}	0^{f}	0^{f}	0^{f}
75PS:25CS	0^{f}	0^{f}	0^{f}	0^{f}	1.62^{f}
50PS:50CS	0^{f}	1.61^{f}	0^{f}	0^{f}	0^{f}
25PS:75CS	683.99^{e}	751.76^{cde}	770.43^{bcd}	813.43^{bc}	980.08^{a}
10PS:90CS	730.90^{de}	743.96^{cde}	725.78^{de}	819.38^{bc}	936.77^{a}
0PS:100CS	813.63^{de}	730.57^{de}	776.35^{bcd}	842.45^{b}	948.11^{a}

†-Means having a letter in common are not significantly different at the 5% level of significance as indicated by Duncan's multiple range test.

The presence of heavy metals like lead can be toxic for white rot fungi and affect their growth, extracellular enzyme activity, and penetration and nutrition in the soil environment (Baldrian, 2003). Baldrian et al. (2005) and Tuomela et al. (2005) reported the low production of MnP in the presence of lead in SSF and liquid culture, respectively. In this study the two best SCs (10PS:90CS and 0PS:100CS) with the best degradation of lignin, cellulose, and hemicellulose were among the SCs with high lead concentrations. The degradation losses, however, showed that the degradation of lignocellulose by *P. ostreatus* was not significantly affected by lead. The heavy metal may be in an inactive form or state that would otherwise affect the degradation of substrate. Significant differences in lead levels were found between the SCs at $p < 0.0001$ (Table 26). There was interaction between the combination and day factors (combination × day). Other lead reports in literature were 0.40-2.80 mg/kg (Svoboda, Zimmermannova, and Kalac 2000), 0.75-7.77 mg/kg (Tuzen, Turkenkul, Hadsdemir, Mendil, and Sari, 2003) 1.43-4.17 mg/kg (Tuzen, 2003) and 0.75-1.99 mg/kg (Soylak, Saracoglu, Tuzen, and Mendil, 2004).

Table 26

Analysis of variance for the presence of lead in PS and CS substrates

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	16634393.18	2772398.86	1519.09	< 0 .0001
Days	4	127142.71	31785.68	17.42	< 0.0001
Combination × Days	24	197582.47	8232.60	4.51	< 0.0001
Error	70	127752.68	1825.04		
Total	104	17086871.04			

4.1.4. C/N ratio. The carbon to nitrogen ratio in the substrates was analyzed following biodegradation. C/N varied in each substrate combination and increased with further addition of

cornstalks in the mixtures (Table 27). The SCs with higher content of PS had low C/N ratios ranging from 29.23-34.34. The SCs with the best LOM and lignocellulose degradation (50PS:50CS-0PS:100CS) displayed C/N values of 35.71-51.43. The latter C/N ratio is between the C/N ratio of peanut shells and corn stalks 55-77.3 (Phillippousis, 2009).

Table 27

Carbon to Nitrogen ratio in substrates (combination) degraded by Pleurotus ostreatus

Combination	C/N, d.w.
100PS:0CS	29.23[f]
90PS:10CS	32.02[e]
75PS:25CS	34.34[de]
50PS:50CS	35.71[d]
25PS:75CS	40.49[c]
10PS:90CS	47.21[b]
0PS:100CS	51.43[a]

†-Means having a letter in common are not significantly different at the 5% level of significance as indicated by Duncan's multiple range test.

For C/N there was no interaction between the factors (concentration × day) at *p*-value $p = 0.2890$. However, significant differences were found in each individual factor at $p < 0.0001$. As shown in Table 28, C/N ratio in substrates can vary either by combination or the days required for the degradation of lignocellulose as these factors are independent of each other shown. Regarding the fourth hypothesis that spent substrate from the best substrate combination will have the least residual lignin and optimal C/N ratio, these results supported that 0PS:100CS displayed the least residual lignin 5.99% and optimal C/N ratio of 51.43, this was then followed by 10PS:90CS with the second least residual lignin 8.05% and C/N ratio of 47.21.

Table 28

Analysis of variance of C/N ratio in biodegraded substrates

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Combination	6	5998.998006	999.833001	82.02	< 0.0001
Day	4	1478.767606	369.691901	30.33	< 0.0001
Combination ×Day	24	345.621794	14.400908	1.18	0.2890
Error	70	853.353533	12.190765		
Total	104	8676.740939			

4.2. Mushroom Cultivation

4.2.1. Mushroom yield and biological efficiency. Faster colonization was observed in bags with more PS content in all the 3 groups. Full colonization was achieved during 27±5 days. Primordia that appeared during the fruiting stage matured into fruit bodies and were harvested 5-7 days later in the first flush, 14-16 days in the second flush, 28-30 days in the third flush, additional fruit bodies were collected after 33-35 days in the fourth flush, and 45-47 days in the fifth flush (Table 29). Fruit body yield began to significantly cease in some of the blocks during the fourth flush. Approximately most of the total yield was obtained from the first flush and mostly varied between 50% to 74%. Elisashvili, Chichua, Kachlishvili, Tsullauri, and Khadziani (2003) reported that most of the yield obtained from three flushes via cultivation of *P. ostreatus* on cotton wastes (CW) was from the first flush and corresponded to 50%. Philippoussis (2001) also reported 50% of total yield obtained from the first flush harvested from cultivation of *P. ostreatus* on cotton wastes.

Table 29

Fruit body yield and biological efficiency of Pleurotus ostreatus on PS and CS substrates

Substrate Combinations	Yield of mushroom (g)					Total*	Biological Efficiency* (%)
	Flush 1	Flush 2	Flush 3	Flush 4	Flush 5		
SC1100PS:0CS	780.91	320.24	120.05	72.76	23.45	1371.41^{cd}	65.85^{cd}
SC2 90PS:10CS	780.99	198.86	89.32	12.67	0	1081.84^{d}	54.09^{d}
SC3 75PS:25CS	704.72	721.29	266.97	80.51	37.79	1811.28^{bc}	90.56^{bc}
SC4 50PS:50CS	683.30	618.75	164.91	7.67	36.58	1511.21^{bcd}	75.56^{bcd}
SC5 0PS:100CS	959.76	569.66	203.56	57.18	0	1790.16^{bc}	89.50^{bc}
SC6 85PS:5R:10CS	1040.91	282.24	238.95	0	0	1562.10^{bcd}	78.10^{bcd}
SC7 70PS:5R:25CS	1828.62	726.83	147.98	178.47	29.72	2881.90^{a}	144.09^{a}
SC8 45PS:5R:50CS	1491.42	702.22	271.58	96.61	42.19	2561.83^{a}	128.09^{a}
SC9 50PS:5R:40CS	1029.76	505.44	123.56	0	0	1658.76^{bc}	82.93^{bc}
SC10 80PS:10R:10CS	1297.93	286.63	127.27	31.85	0	1743.68^{bc}	87.18^{bc}
SC11 65PS:10R:25CS	1029.83	335.27	152.62	31.53	0	1549.25^{bcd}	77.46^{bcd}
SC1240PS:10R:50CS	1238.81	336.84	109.52	64.49	0	1779.66^{bc}	88.93^{bc}
SC1350PS:10R:40CS	998.53	490.66	375.78	121.71	25.38	1986.74^{b}	99.33^{b}

PS Peanut Shells; CS Corn Stalk; R Rye. Controls: 100PS:0CS and 100CS. Ratio in column 1 represents percentage of substrate content of SCs.*Means having a letter in common are not significantly different at the 5% level of significance as indicated by Duncan's multiple range test.

BE of control substrates (100PS:0CS and 0PS:100CS) did not differ from each other although a higher BE was observed on 100% CS. With the inclusion of 5% rye, the highest mushroom yield and BE were obtained in 70PS:5R:25CS with a total yield of 2881.90 g and BE of 144.09%, followed by 45PS:5R:50CS with a total yield of 2561.8 3 g and BE of 128%. As previously mentioned by Salmones, bioconversion of substrates by *Pleurotus* spp. can exceed 100% (wet weight). The best SCs in this study had indeed surpassed the 100% BE by 44% and 28% increase, respectively. Other authors had reported BE >100% via cultivating *Pleurotus* spp. on certain substrates. Banik and Nandi (2003) obtained several BEs ranging from 48.5% - 186.3% in *P. sajor-caju* (*Pleurotus pulmonarius* var. *sajor-caju*) cultivated in various types of

slurry manure and rice straw supplement. Isikhuemhen and Mikiashvilli (2009) also reported a BE of 181.5% from cultivation of *Pleurotus ostreatus* on 70% wheat straw: 20% solid waste substrates supplemented with 10% millet. Mushroom yield and biological efficiency in SCs with 10% rye did not significantly improve.

The second hypothesis stated that the appropriate combination of PS, CS with or without supplementation with rye will give higher fruit body yield of *P. ostreatus* than PS or CS substrate alone. From our results it is evident the combination of both PS and CS without supplement produced higher yields (75PS:25CS) than either substrates alone. However with the inclusion of 5% rye the mushroom yield and BE of two best SCs increased by almost 2 times that of the control SCs mentioned above. Although the yield of 45PS:5R:50CS is lower than that of 70PS:5R:25CS they were not significantly different from each other at 5% level of significance.

4.2.2. C/N ratio in substrates. Amount of carbon, hydrogen, nitrogen present in substrates are listed in Table 31. Each of the three compositions was determined on a percentage dry weight basis. Contents of carbon ranged from 39.62-43.87% C, 5.41-6.75% H, and 1.70-2.99 % N. C/N ratio in substrates ranged 18.33 to 27.67 (Table 30). Soto Cruz, Casteneda, Hach, Rojas, and Torres, (1999) observed 22.4- 23.2 C/N from mixtures of oat straw, oat bran, and copra cake. C/N was higher in non supplemented substrates, when rye was added C/N values decreased except for 40PS:10R:50CS. The SCs mentioned above with the best yields (70PS:5R:25CS & 45PS:5R:50CS) had C/N ratios of 18.50 and 16.54 respectively. The results obtained in this study were higher than those of Soto Cruz et al. (1999). Adequate C/N is said to be critical for the rate of lignocellulose degradation and mushroom production (Madelin, 1956).

Table 30

Amount of carbon, nitrogen, hydrogen, and C/N ratio in the spent substrate blocks

Substrate Combinations	Carbon %	Hydrogen %	Nitrogen %	C/N *
SC1 100 PS:0 CS	43.87	5.53	2.16	23.80^{ab}
SC2 90 PS:10 CS	42.95	5.41	1.95	25.91^{ab}
SC3 75 PS:25 CS	41.51	6.68	2.00	24.17^{abc}
SC4 50 PS:50 CS	40.19	6.72	1.97	23.98^{abc}
SC5 0PS:100CS	40.38	6.75	1.70	25.76^{ab}
SC6 85 PS:5Rye:10CS	43.25	5.65	1.98	19.10^{ed}
SC7 70 PS:5Rye:25CS	40.11	6.32	2.49	18.50^{ed}
SC8 45PS:5Rye:50CS	39.62	6.54	2.13	16.54^{e}
SC9 50PS:5 Rye:45CS	41.26	6.37	2.61	21.73^{bcd}
SC10 80PS:10 Rye:10CS	42.13	5.82	2.99	19.90^{cde}
SC11 65PS:10 Rye:25CS	40.76	6.62	2.41	18.33^{ed}
SC 12 40PS:10 Rye:50CS	41.00	6.55	2.10	27.67^{a}
SC13 45PS:10 Rye:50CS	41.75	5.42	2.66	22.76^{ab}

*Means having a letter in common are not significantly different at the 5% level of significance as indicated by Duncan's multiple range test.

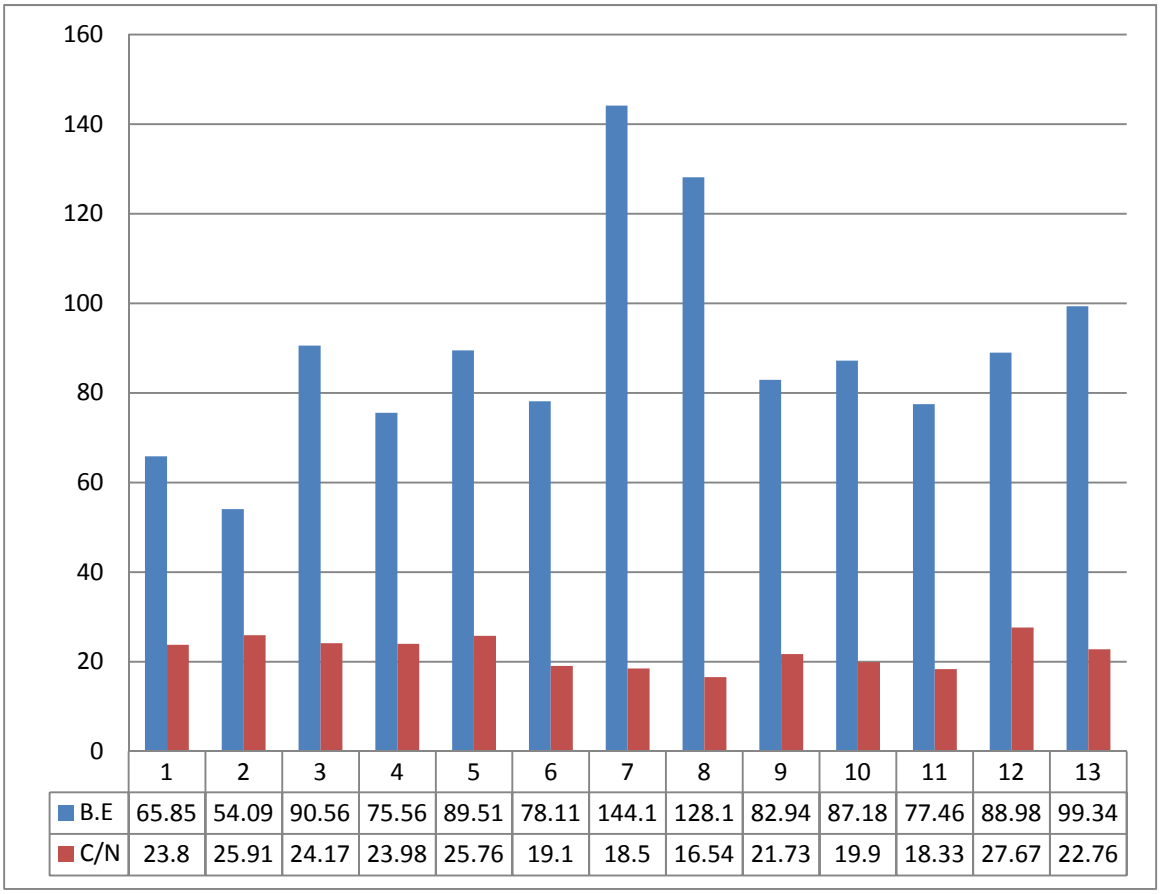
Analysis of variance in C/N showed significant differences between the treatments (SCs) in Table 31. The calculated *p*-value for significant differences was $p < 0.0001$. As seen in Table 29 and 30, the PS:CS ratio 45:50 to 70:25 with 5% rye and C/N ratio of 16.54-18.50 favors the optimum yield of *P. ostreatus* cultivated on PS and CS substrates.

Table 31

Analysis of Variance of C/N ratio in the spent substrates blocks

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	585.0721769	48.7560147	6.28	< 0.0001
Error	39	302.7910000	7.7638718		
Total	51	887.8631769			

The comparison of the C/N ratio and BE of the thirteen substrates are shown in Figure 3.



	1	2	3	4	5	6	7	8	9	10	11	12	13
B.E	65.85	54.09	90.56	75.56	89.51	78.11	144.1	128.1	82.94	87.18	77.46	88.98	99.34
C/N	23.8	25.91	24.17	23.98	25.76	19.1	18.5	16.54	21.73	19.9	18.33	27.67	22.76

Figure 3 Pleurotus ostreatus strain 400, biological efficiency, and C/N ratio of the different PS and CS substrate combinations

4.2.3. Minerals in substrates. The grinded substrate blocks were analyzed for the nine minerals and were shown in Table 32. Mineral content levels were found to be less than those in the biodegradation experiment. Concentrations were 29.74-45.14, 0.80-2.43, 16.50-56.44, 5.83-9.48, 0.05-0.20, 1.39-7.64, 0.01-0.14, mg/kg for calcium, iron, potassium, magnesium, manganese, phosphorous, and zinc, respectively. Copper and lead levels were below detectable levels (BDL). While significant differences may or not be found among the treatments (SCs) in each mineral analysis, no statistical analysis was needed for minerals that were not detected.

Table 32

Trace mineral concentrations in the analyzed substrate blocks (mg/kg)

Substrate combinations	Minerals*								
	Ca	Cu	Fe	K	Mg	Mn	P	Zn	Pb
1 100PS:0CS	33.71^{ab}	BDL	2.43^{a}	16.50^{e}	6.29^{cd}	0.20^{a}	2.01^{ef}	0.01^{f}	BDL
2 90PS:10CS	33.20^{ab}	-	2.43^{a}	22.71^{ed}	6.89^{bcd}	0.19^{a}	2.20	0.02^{ef}	-
3 75PS:25CS	38.21^{ab}	-	1.72^{abc}	38.85^{bc}	8.20^{ab}	0.15^{a}	4.96^{bc}	0.03^{def}	-
4 50PS:50CS	34.91^{ab}	-	1.50^{bcd}	47.03^{ab}	8.41^{ab}	0.11^{cd}	6.74^{a}	0.06^{cd}	-
5 0PS:100CS	30.03^{b}	-	2.21^{ab}	17.40^{e}	5.89^{d}	0.19^{a}	1.39^{f}	0.01^{f}	-
6 85PS:5ye:10CS	45.14^{a}	-	1.53^{bcd}	40.29^{bc}	7.34^{bcd}	0.14^{abc}	3.60^{cde}	0.04^{def}	-
7 70PS:5Rye:25CS	36.00^{ab}	-	1.22^{cd}	41.52^{bc}	6.93^{bcd}	0.10^{bcd}	3.51^{cde}	0.06^{cde}	-
8 45PS:5Rye:50CS	35.64^{ab}	-	1.20^{cd}	52.46^{a}	7.92^{abc}	0.10^{bcd}	7.01^{a}	0.12^{ab}	-
9 50PS:5Rye:45CS	29.74^{b}	-	1.18^{cd}	25.23^{de}	5.83^{d}	0.05^{d}	2.94^{def}	0.06^{cde}	-
10 80PS:10Rye:10CS	38.57^{ab}	-	1.19^{cd}	37.64^{bc}	7.29^{bcd}	0.08^{d}	4.68^{cd}	0.09^{cd}	-
11 65PS:10Rye:25CS	37.15^{ab}	-	1.04^{cd}	33.14^{cd}	7.43^{bcd}	0.08^{cd}	4.11^{cd}	0.11^{ab}	-
12 40PS:10Rye:50CS	43.50^{ab}	-	0.90^{d}	56.44^{a}	9.48^{a}	0.11^{bcd}	7.64^{a}	0.14^{a}	-
13 45PS:10Rye:45CS	35.01^{ab}	-	0.80^{d}	55.50^{a}	7.17^{bcd}	0.07^{d}	6.52^{ab}	0.09^{bc}	-

BDL: Below detectable levels.*Means having a letter in common are not significantly different at the 5% level of significance as indicated by Duncan's multiple range test.

4.2.3.1. *Calcium.* The mineral contents of calcium ranged from 29.74-45.14 mg/kg (Figure 4). The SCs with the highest BE (70PS:5Rye:25CS and 45PS:5Rye:50CS) had 36.00 and 35.64 mg/kg of calcium, respectively. The highest calcium content was found in 85PS:5Rye:10CS substrates. The addition of rye (5-10%) in substrates showed increase of calcium levels than non supplemented substrates except SC9 50PS:5Rye:45CS that had the lowest calcium content.

The *p*-value 0.35 for the treatments indicates that the calcium concentrations found in the SC treatments did not significantly vary from each other during fruiting (Table 33). The reported results were higher than those reported by Kinfemi (2010) who obtained 0.89 and 0.69 mg/kg Ca in peanut husks when treated with *P. ostreatus* and *P. pulmonarius*, respectively.

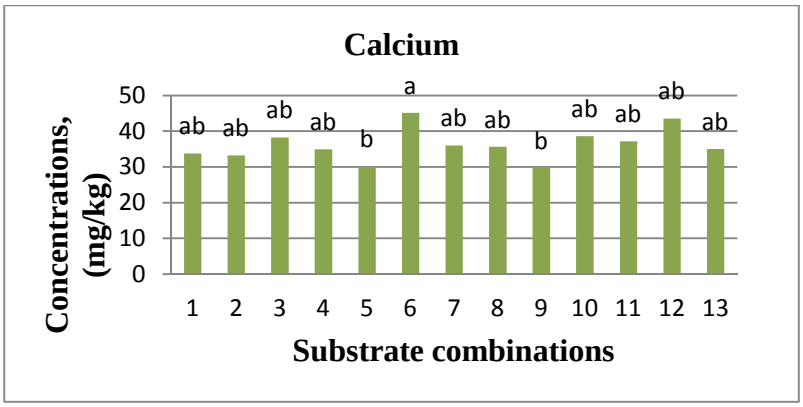


Figure 4 Calcium concentrations in the thirteen substrates blocks

Table 33

Analysis of variance for calcium in substrates blocks

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	968.185481	80.682123	1.14	0.3599
Error	39	2766.288125	70.930465		
Total	51	3734.473606			

4.2.3.2. *Copper.* Traces of copper levels could not be detected in SC and thus could not be measured. Kinfemi (2010) reported levels of copper traces of 0.02 mg/kg in PS substrates untreated with fungi (control). After treatment with *Pleurotus ostreatus* and *Pleurotus pulmonarius* copper levels increased to 0.03 and 0.04 respectively.

4.2.3.3. *Iron.* The iron levels in the substrates ranged between 0.80-2.43 mg/kg (Figure 5). Iron levels were higher in non supplemented substrates than substrates supplemented with rye. The highest Fe content with the highest values (2.43 mg/kg) was found in both 100PS:OCS and 90PS:10CS. Iron content in 70PS:5Rye:25CS and 45PS:5Rye:50CS were 1.22 and 1.20 mg/kg, respectively. Significant differences in iron levels were found between the SCs at $p <$ 0.0001. These iron values were higher than those of Kinfemi (2010) who obtained 1.13 mg/kg

from *Pleurotus ostreatus* & 0.06 mg/kg from *Pulmonarius pulmonarius* (Table 34). This might be possible due to different PS varieties used by Kinfemi and this experiment.

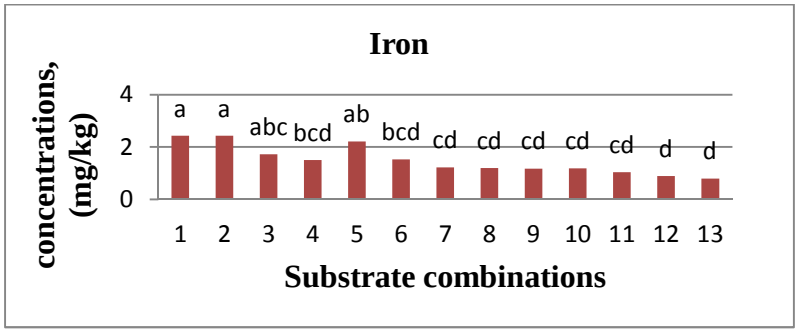


Figure 5 Iron concentrations in the thirteen substrates blocks

Table 34

Analysis of variance for iron in substrates blocks

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	14.90251923	1.24187660	5.70	< 0.0001
Error	39	8.50442500	0.21806218		
Total	51	23.40694423			

4.2.3.4. *Potassium.* Potassium concentrations ranged between 16.50-56.44 mg/kg (Figure 6). The highest potassium concentration was observed in 40PS:10Rye:50CS (56.44 mg/kg) followed by 50PS:10Rye:40CS (55.50 mg/kg) whose values were not significantly different from each other at 5% level of significance. Potassium levels in the two best SCs were 41.52 (SC7) and 52.46 (SC8) mg/kg.

The potassium contents in the SCs were significantly different from each other at $p <$ 0.0001 (Table 35). Potassium values obtained by Kinfemi (2010) from PS substrates were 0.22 (*P. ostreatus*) and 0.16 mg/kg (*P. pulmonarius*). Compared to the non supplemented SCs those supplemented with generally had higher K contents.

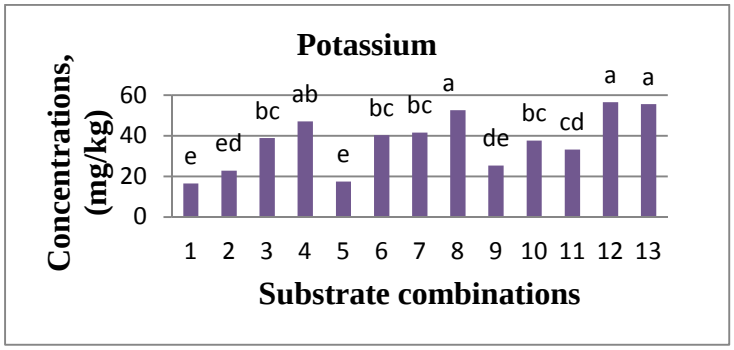


Figure 6 Potassium concentrations in the thirteen substrates blocks

Table 35

Analysis of variance for potassium in substrate blocks

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	9012.67777	751.05648	15.41	< 0.0001
Error	39	1900.79632	48.73837		
Total	52	10913.47410			

4.2.3.5. *Magnesium.* Magnesium content ranged from 6.29 mg/kg in 100PS:0CS to 9.48 mg/kg in 40PS:10Rye:50CS (Figure 7). In non supplemented substrates magnesium levels increased upon increasing the proportion of CS in substrates to 50%. Beyond 50% further addition of cornstalks reduced magnesium levels. The pattern was also similar in supplemented groups. This may be that Mg levels in PS and CS SCs were not largely affected by rye supplementation.

Significant differences were observed between the SCs for the presence of magnesium in the substrates at *p*-value 0.0013 (Table 36). From Figure 6 and Table 36 differences in K content are evident in rye. Kinfemi (2010) reported 0.22 mg/kg and 0.16

mg/kg K in substrates treated with both *P. ostreatus* and *P. pulmonarius.* These values in this study were higher than the literature values.

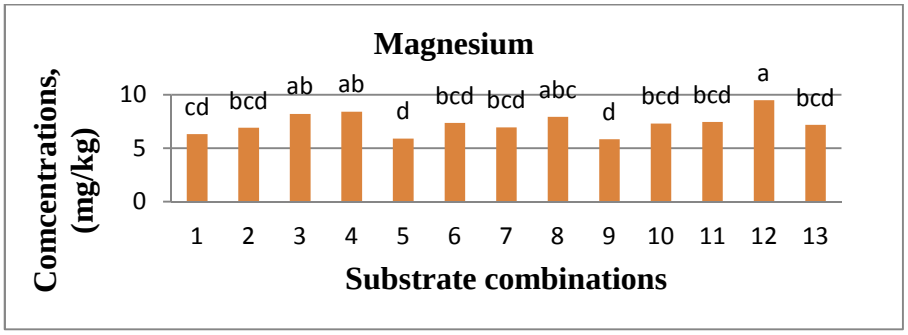


Figure 7 Magnesium concentrations in the thirteen substrate blocks

Table 36

Analysis of variance for magnesium in substrate blocks

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	50.73916923	4.22826410	3.57	0.0013
Error	39	46.20397500	1.18471731		
Total	51	96.94314423			

4.2.3.6 ***Manganese.*** The minimum manganese concentration was 0.05 mg/kg in 50PS:5Rye:45CS substrates and the maximum concentration was 0.20 mg/kg in 100PS:0CS substrates (Figure 8). The two best substrates had 0.10 mg/kg Mn and were not significantly different from each other. Manganese levels were observed to be lower in supplemented than non supplemented groups.

The manganese content in substrates significantly varied between SCs at *p*-value $p < 0.0001$ (Table 37). Manganese contents reported by Kinfemi (2010) in PS husks were 0.04 (*P. ostreatus*) and 0.09 (*P. pulmonarius*) mg/kg. While manganese for MnP production were low in the best SCs after degradation, it was demonstrated by Eichlerova

et al. (2000) that high production cannot be clearly correlated with substantial degradation of target molecules.

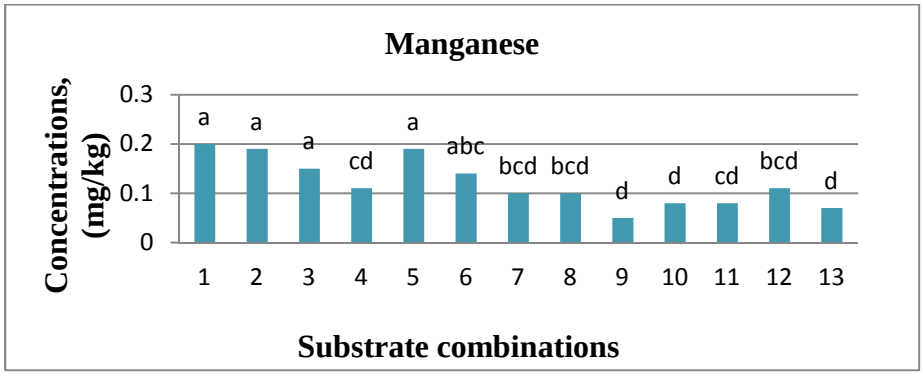


Figure 8 Manganese concentrations in the thirteen substrate blocks

Table 37

Analysis of variance for manganese in substrate blocks

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	0.10785000	0.00898750	6.25	< 0.0001
Error	39	0.05605000	0.00143718		
Total	51	0.16390000204			

4.2.3.7. Phosphorous. The minimum phosphorous content in substrates was 1.39 mg/kg in 0PS:100CS and the maximum was 7.01 mg/kg in the second best SC 45PS:5Rye:50CS (Figure 9). The phosphorous content in the best SC, however, was only half (3.51 mg/kg) of the content in the second best SC.

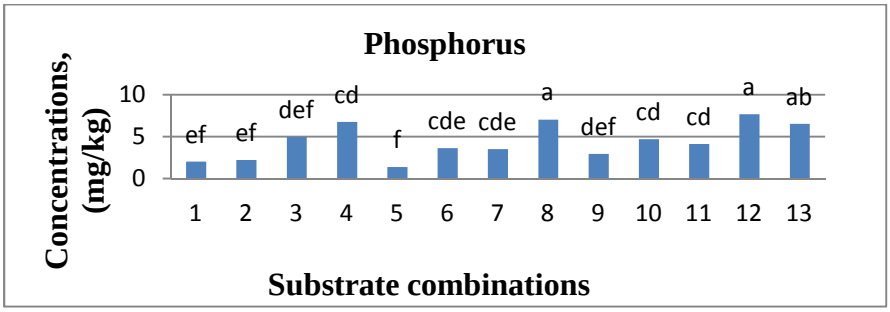


Figure 9 Phosphorous concentrations in the thirteen substrate blocks

Figure 9 showed that the inclusion of rye in PS and CS combinations increased phosphorous content. Kinfemi (2010) reported 0.08 and 0.09 mg/kg in PS husks treated with *P. ostreatus* and *Pleurotus pulmonarius,* respectively. Significant differences were found between the SCs for phosphorous at *p*-value $p < 0.0001$ (Table 38).

Table 38

Analysis of variance for phosphorous in substrate blocks

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	203.8380077	16.9865006	13.89	< 0.0001
Error	39	47.6889000	1.2227923		
Total	51	251.5269077			

4.2.3.8. *Zinc.* Zinc content in substrates ranged from 0.01 mg/kg found in both 100PS:0CS and 0PS:100CS to 0.67 mg/kg in 50PS:50CS (Figure 10). The substrates with the best mushroom yields and BEs had 0.06 mg/kg (75PS:5Rye:25CS) and 0.12 mg/kg (45PS:5Rye:50CS) Zn. The addition of rye in substrates combinations increased zinc levels; most notably in the group supplemented by 10% rye (SC10-SC13).

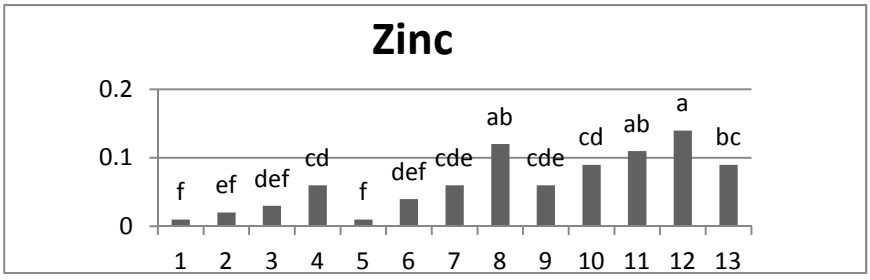


Figure 10 Zinc concentrations in the thirteen substrate blocks

Significant differences for zinc were found to vary among the SCs during the incubation period. These differences were indicated by the *p* value in the ANOVA ($p < 0.0001$) (Table 39).

Kinfemi (2010) observed 0.03 mg/kg Zn in the two *Pleurotus* species cultivated on PS substrates.

Table 39

Analysis of variance for zinc in substrate blocks

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	0.08810769	0.00734231	10.05	< 0.0001
Error	39	0.02850000	0.00073077		
Total	51	0.11660769			

4.2.3.9. *Lead.* Lead concentrations in substrates were below detectable levels like copper, as a result there was no data reported on lead. The third hypothesis stated that nutritional content of mushrooms produced in the best substrate combination of PS, CS, and rye will result in mushrooms with the best nutritional content. The two best SCs with the highest BEs did not show the best nutritional content because for each mineral analyzed in the SCs mineral content varied.

4.2.4. C/N ratio in fruit bodies. Significant differences in C/N of fruit bodies were not found to vary among the SCs (treatments) according to the *p*-value 0.0876 (Table 40). In fruit body yields (Table 41), carbon content in fruit bodies ranged between 29.23 and 39.68%, nitrogen level ranges were 4.48-5.73% and hydrogen levels were in the ranges 5.41-6.75%. The fruit bodies collected from the two best substrates (SC 7 & 8) displayed C/N ratios of 8.09 and 8.05%, respectively. Nitrogen levels in fruit bodies appeared to be higher than in substrates, whereas C in fruit bodies was lower than in the substrates. The C and N results were in agreement

those of Yildiz, Yesil, Yavuz, Temiz, and Karaplan (2005) who analyzed 31.75% to 41.73% C, 2.88% to 6.42% N, and 4.56% to 6.13% H in fruit bodies.

Table 40

Analysis of variance of C/N ratio in fruit bodies

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	38.7182423	3.22652019	1.78	0.0876
Error	39	70.87778250	1.8173801		
Total	51	109.5960673			

Table 41

Amount of carbon, hydrogen, nitrogen, and C/N in fruit bodies of Pleurotus ostreatus collected from PS and CS substrates

Substrate Combinations	Carbon %	Hydrogen %	Nitrogen %	C/N %*
1 100PS:0CS	38.24	7.23	5.33	8.37^{abc}
2 90PS:10CS	39.48	7.87	5.67	8.10^{bc}
3 75PS:25CS	39.68	7.82	5.08	9.10^{abc}
4 50PS:50 CS	38.29	7.12	4.24	10.47^{a}
5 0PS:100CS	38.65	7.07	4.48	8.34^{abc}
6 85PS:5Rye:10CS	39.22	6.36	5.49	8.70^{abc}
7 70PS:5Rye:25CS	38.68	7.17	5.19	8.09^{bc}
8 45PS:5Rye:50CS	29.23	5.53	4.79	8.05^{bc}
9 50PS:5Rye:45CS	39.51	6.76	5.67	7.21^{c}
10 80PS:10Rye:10CS	39.35	8.11	5.73	9.08^{abc}
11 65PS:10Rye:10CS	39.34	7.03	5.17	8.36^{abc}
12 40PS:10 Rye:50CS	36.55	7.63	5.09	9.53^{ab}
13 50PS:10 Rye: 40CS	38.77	7.13	4.76	10.06^{ab}

*Means having a letter in common are not significantly different at the 5% level of significance as indicated by Duncan's multiple range test.

4.2.5. Minerals in fruit bodies. *Pleurotus ostreatus* mushrooms cultivated on the substrates were also analyzed for minerals that were assimilated into the fruit bodies. Concentration ranges were 0.07-0.33, 57.03-75.60, 3.61-5.49, 22.04-30.90, and 0.06-0.19 mg/kg for iron, potassium, magnesium, phosphorous, and zinc, respectively (Table 42). Copper, manganese, and lead levels were below detectable levels (BDL) and were not found in fruit bodies.

Table 42

Trace mineral levels in fruit bodies of Pleurotus osteratus (mg/kg)

Substrate combinations	Minerals*								
	Ca	Cu	Fe	K	Mg	Mn	P	Zn	Pb
1 100PS:0CS	BDL	BDL	0.24^{bcd}	57.39^{c}	4.41^{cde}	BDL	23.98^{ed}	0.19^{a}	BDL
2 90PS:10CS	BDL	-	0.25^{abc}	58.66^{bc}	4.95^{abcd}	-	24.65^{dec}	0.17^{ab}	-
3 75PS:25CS	BDL	-	0.17^{bcde}	65.43^{abc}	5.00^{abcd}	-	27.95^{abcd}	0.12^{bc}	-
4 50PS:50CS	BDL	-	0.21^{bcde}	72.79^{a}	5.49^{a}	-	29.43^{ab}	0.13^{bc}	-
5 0PS:100CS	BDL	-	0.33^{a}	68.40^{abc}	5.48^{a}	-	27.99^{abcd}	0.15^{abc}	-
6 85PS:5ye:10CS	BDL	-	0.26^{ab}	75.60^{a}	5.15^{abc}	-	29.13^{ab}	0.12^{cd}	-
7 70PS:5Rye:25CS	BDL	-	0.26^{ab}	70.90^{a}	5.28^{ab}	-	30.90^{a}	0.15^{abc}	-
8 45PS:5Rye:50CS	BDL	-	0.21^{bcde}	59.35^{bc}	3.99^{ef}	-	24.10^{de}	0.11^{cd}	-
9 50PS:5Rye:45CS	BDL	-	0.16^{cdef}	64.35^{abc}	4.27^{def}	-	27.93^{abcd}	0.12^{bc}	-
10 80PS:10Rye:10CS	BDL	-	0.15^{def}	66.20^{abc}	4.65^{bcde}	-	28.41^{abc}	0.13^{bc}	-
11 65PS:10Rye:25CS	BDL	-	0.14^{ef}	69.55^{ab}	4.45^{cde}	-	29.43^{ab}	0.13^{bc}	-
12 40PS:10Rye:50CS	BDL	-	0.14^{ef}	65.14^{abc}	4.21^{def}	-	25.79^{bcde}	0.10^{cd}	-
13 45PS:10Rye:45CS	BDL	-	0.07^{f}	57.03^{c}	3.61^{f}	-	22.04^{e}	0.06^{d}	-

BDL: Below detectable levels. *Means having a letter in common are not significantly different at the 5% level of significance as indicated by Duncan's Multiple Range Test.

4.2.5.1 Calcium. The calcium levels in the fruit bodies were below detectable levels.

4.2.5.2 Copper. No assimilated traces of copper had been found in the fruit bodies from the substrate blocks.

4.2.5.3 Iron. The maximum iron concentration was 0.33 mg/kg in the 0PS:100CS substrates. The minimum concentration was 0.07 mg/kg in the 45PS:10Rye:50CS substrates

combination. The substrates with the best BEs had 0.26 and 0.21 mg/kg of iron, respectively (Figure 11).

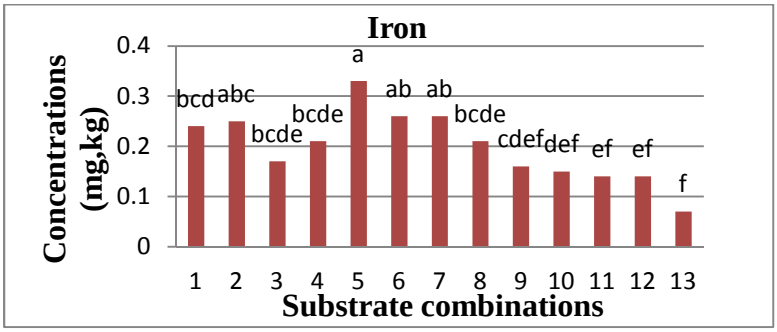


Figure 11 Iron concentrations in the fruit bodies

The iron concentrations in fruit bodies of each SC were significantly different. The *p*-value was found to be $p < 0.0001$. (Table 43). Other iron values in literature were 71 (Wang, Sakoda, and Suzuki, 2001) and 75 (STFC 1982) mg/kg. In living tissues iron is needed for transport of oxygen as well several enzymatic reactions in living tissues. When compared to chicken, beef, pork, oyster mushrooms are reported to contain twice the iron. (HMR, 2010).

Table 43

Analysis of variance for iron in fruit bodies

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	0.23292308	0.01941026	5.81	< 0.0001
Error	39	0.13040000	0.00334359		
Total	51	0.36332308			

4.2.5.4 *Potassium.* The potassium levels in fruit bodies varied from 57.03 (45PS:10Rye:50CS) to 75.90 mg/kg (85PS:5Rye:10CS) in substrates (Figure 12). Compared to non supplemented substrates SCs supplemented with rye, on average, improved K content. In the

two best SCs K levels were 70.90 mg/kg in the 70PS:5Rye:25CS and 59.35 mg/kg in 45PS:5Rye:50CS.

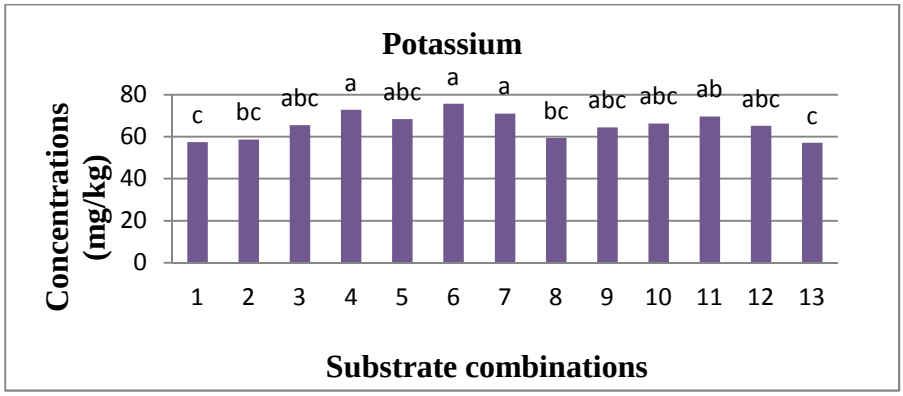


Figure 12 Potassium concentrations in the fruit bodies

Significant differences were found in potassium levels in fruit bodies between the SCs (Table 44). The *p*-value was 0.0043. Reported potassium values in literature by Wang et al. (2001) and the STFC (1982) by *P. ostreatus* were 2171 mg/kg on spent beer grain and 2720 mg/kg, respectively.

Table 44

Analysis of variance for potassium in fruit bodies

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	1733.435677	144.452973	3.04	0.0043
Error	39	1855.974775	47.589097		
Total	51	3589.410452			

4.2.5.5 ***Magnesium.*** The maximum and minimum magnesium levels observed in fruit bodies were 5.49 mg/kg (50PS:50CS) and 3.61 mg/kg (45PS:5Rye:50CS) (Figure 13). Magnesium in the best SC 70PS:5Rye:25CS was 5.28 mg/kg. The second best SC

45PS:5Rye:50CS had 3.99 mg/kg Mg. Wang et al. (2002) and STFU (1982) obtained 1819 and 1560 mg/kg Mg, respectively.

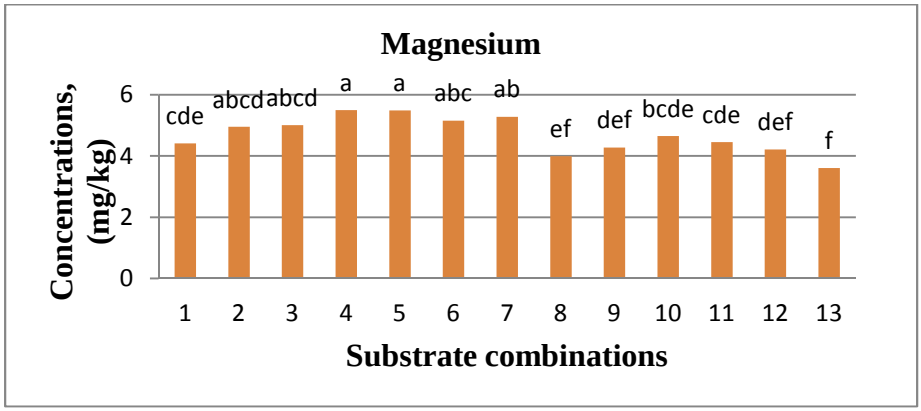


Figure 13 Magnesium concentrations in the fruit bodies

Magnesium concentrations in fruit bodies between the thirteen substrate combinations were significantly different according to the *p*-value <0.0001 by the ANOVA (Table 45). When rye was added at the PS:CS ratio 45:50 magnesium levels decreased compared to those without supplement.

Table 45

Analysis of variance for magnesium in fruit bodies

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	16.69060769	1.39088397	5.92	< 0.0001
Error	39	9.16770000	0.23506923		
Total	51	25.85830769			

4.2.5.6 ***Manganese.*** Manganese concentrations assimilated into the fruit bodies from the substrates were below detectable levels.

4.2.5.7. Phosphorus. Phosphorus in fruit bodies ranged from 22.04 to 30.90 mg/kg. The lowest value was found in 40PS:10Rye:50CS and the highest value was found in

70PS:5Rye:25CS substrates with the highest BE value. The second best SC had45PS:5Rye:50CS 24.10 mg/kg P (Figure 14). Wang et al. (2001) obtained 1647.6 mg/kg whereas STFC obtained 1061 mg/kg of P in fruit bodies.

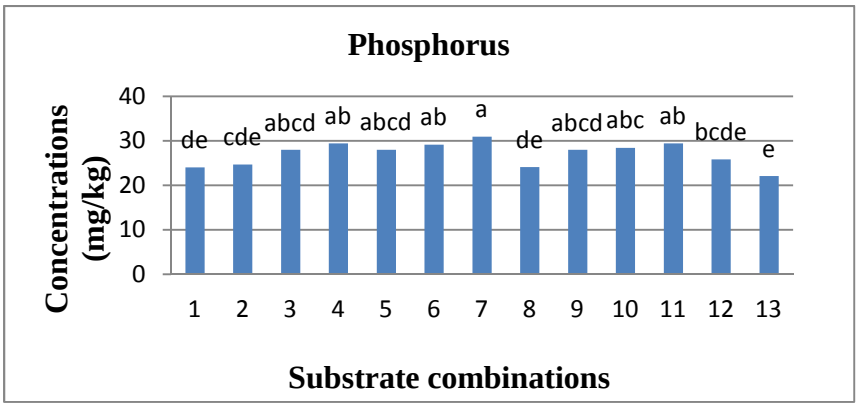


Figure 14 Phosphorous concentrations in the fruit bodies

The phosphorus content in fruit bodies was significantly different between the substrate combinations. The *p*-value indicated by the ANOVA was 0.0003 (Table 46). This pattern was similar to that of potassium. Wang et al. (2001) reported that potassium and phosphorous were the main constituents of ash in *P. ostreatus*, however, Jwanny, Rashad, and Abdg (1995) stated that magnesium and phosphorous constitute ash, followed by iron and sodium.

Table 46

Analysis of variance for phosphorous in fruit bodies

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	341.1590577	28.4299215	4.22	0.0003
Error	39	262.6652500	6.7350064		
Total	51	603.8243077			

4.2.5.8 ***Zinc.*** The zinc concentrations in fruit bodies varied and ranged between 0.06 mg/kg (45PS:5Rye:50CS) to 0.19 (100PS:0CS) mg/kg (Figure 15). Zinc levels in the 75PS:5Rye:25CS and 45PS:5Rye:50CS had 0.15 mg/kg and 0.11 mg/kg, respectively. Traces of zinc found in fruit bodies collected from the thirteen SCs were reported to have significant differences between them. The *p*-value indicated was 0.0009 (Table 47). Wang et al. (2011) who obtained values of 137 mg/kg and STFC (1982) that obtained 108 mg/kg Zn.

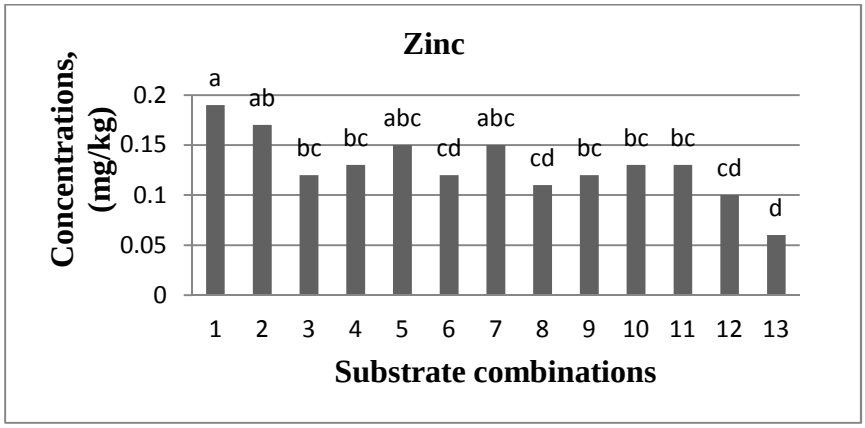


Figure 15 Zinc concentrations in the fruit bodies

Table 47

Analysis of variance for zinc in fruit bodies

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr > F
Tmt	12	0.05168077	0.00430673	3.72	0.0009
Error	39	0.04512500	0.00115705		
Total	51	0.09680577			

Lead. Lead concentrations were below detectable levels and were not detected in fruit bodies. Compared to fruit bodies mineral content was higher in spent substrates in which mycelium of the fungus stores minerals for fruit body formation (Zadrazil, 1978). During fruit

body production the minerals are then translocated to the fruit bodies by the mycelium (Chang and Miles, 1989).

Vetter, Hadju, Gyorfi, and Maszlaver (2005) reported that calcium, potassium, phosphorus, and magnesium constitute 97-98% whereas elements such manganese and copper make up 2-3% of the total element concentration of three species: *Agaricus bisporus, Pleurotus ostreatus, and Lentinula edodes.* Variation of mineral contents in this study and the reported literatures might be explained by certain factors such as the type or variety of the substrate (Tshinyangu, 1996; Wang et al., 2001), substrate composition or the nature of the fungal species used for study which can also result in wide variability of mineral concentrations even within the same species (Svoboda et al., 2000).

CHAPTER 5

Conclusion

Solid state fermentation with *P. ostreatus* was very effective in the selective removal of lignin in PS. Substrate combination of peanut shells with cornstalks significantly increased the loss of organic matter followed by higher significant degradation of lignin than using either substrates alone at SC ratios 10PS:90CS and 0PS:100CS. The variations in degradation of lignocelluloses macromolecules in *P. ostreatus* are largely influenced by the combination of substrates as well as the length of incubation (solid state fermentation) period.

The supplementation of substrates with rye was effective in enhancing mushroom yield and the biological efficiency. The highest fruit body yields of *P. ostreatus* were achieved with the appropriate PS and CS substrate 45PS:50CS-75PS:20CS combination and the inclusion of rye at 5% level of supplementation.

C/N ratio is affected by substrate combinations. Biodegradation of lignocellulose in PS and CS substrates was favored by C/N ratio 47.219-51.437 and 16.54-18.50 supported fruit body production of oyster mushrooms in these substrates.

Nutritional content in substrates was variable depending on several factors such as substrate ratios, days of incubation, or the fungus used. Nonetheless, substrate mixture were successful as substrates for the cultivation of *P. ostreatus* and its advantages in this study could promote further research with PS and CS profitable for the commercial use of the two substrates in the production of oyster mushrooms in the mushroom industry.

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