



## CHANGES IN MACRONUTRIENTS AND PHYSICAL PROPERTIES DURING THE GROWTH OF *Lentinula edodes* AND *Pleurotus ostreatus* IN A COMPOST BASED ON SUGARCANE BAGASSE AGRICULTURAL WASTE

Laura Narváez<sup>1a</sup>, Ana Cristina Bolaños<sup>1b\*</sup>, Adriana Chaurra<sup>2</sup>, and Orlando Zuñiga Escobar<sup>3</sup>

<sup>1a</sup> Facultad de Ciencias Naturales y Exactas, Departamento de Biología, Grupo de Investigación en Biología de plantas y microorganismos (GPM), Universidad del Valle. Calle 13 No. 100-00, Cali, Colombia

<https://orcid.org/0000-0002-1808-851X>

<sup>1b</sup> Facultad de Ciencias Naturales y Exactas, Departamento de Biología, Grupo de Investigación en Biología de plantas y microorganismos (GPM), Universidad del Valle. Calle 13 No. 100-00, Cali, Colombia

<https://orcid.org/0000-0003-0882-2351>

<sup>2</sup> Facultad de Ciencias básicas, Departamento de Ciencias Ambientales, Grupo de Investigación en Estudios Ambientales para el Desarrollo Sostenible (GEADES), Universidad Autónoma de Occidente. Calle 25, Vía Cali - Puerto Tejada #115-85 Km 2. Cali, Colombia <https://orcid.org/0000-0001-6277-4245>

<sup>3</sup> Facultad de Ciencias Naturales y Exactas, Departamento de Física, Grupo de Investigación en Ciencias Ambientales y de la Tierra (ILAMA), Universidad del Valle. Calle 13 No. 100-00, Cali, Colombia <https://orcid.org/0000-0003-4434-8597>

\* Corresponding author E-mail: [ana.bolanos@correounivalle.edu.co](mailto:ana.bolanos@correounivalle.edu.co)

### ABSTRACT

Spent mushroom substrate of different edible mushrooms is a nutrient-rich biomass associated with mycelial metabolic activity in the growth substrate. The objective of this study was to evaluate the changes in the nutritional content and physical properties of a sugarcane bagasse substrate after the cultivation of the edible mushrooms *Pleurotus ostreatus* and *Lentinula edodes*. Strains of both species were first propagated in potato dextrose agar culture medium, then in wheat grains, and finally inoculated in sterile sugarcane bagasse. Determinations of macronutrients, pH and C:N ratio were carried out at 0, 30 and 60 days after mushroom mycelium growth in the bagasse-based substrate. After 30 and 60 days of inoculation, nitrogen (N), phosphorous (P) and potassium (K) recorded increases of 0.26%, 0.06% and 0.14 with *Pleurotus ostreatus*, and increases of 0.33%, 0.05%, and 0.11% with *Lentinula edodes*, respectively. Regarding micronutrients, there was an increase in Calcium (Ca) and Sulphur (S) in both substrates, but amounts varied during the time evaluated. Additionally, an important increase in hydrogen ion concentration was observed when both species were inoculated, with final values of 4.26 and 3.9 for *Pleurotus ostreatus* and *Lentinula edodes* species, respectively. In conclusion, this biomass presents a high percentage of the essential micro and macro elements required in a fertilizer.

**Key words:** Biofertilizer, nitrogen, biomass, spent mushroom substrate.

## INTRODUCTION

*Lentinula edodes* and *Pleurotus ostreatus*, species of the Basidiomycota phylum, are among the five species with the highest market demand for edible fungi in the world (Royse et al., 2017). Mushroom sporocarps are characterized by their edible potential as they are a rich source of protein with high contents of essential amino acid, vitamin (B1, B2, B12, C, D, E), minerals, water and fiber as well as low levels of fat (Heleno et al., 2010; Ouzouni et al., 2009). In addition, sporocarps of many mushroom species are used for their medicinal value because of their active biological compounds with antibacterial, antifungal, antiparasitic, antidiabetic, antiallergic, anticholesterolemic, antioxidant, immunomodulating, and antitumor properties (Chang and Wasser, 2012; Sari et al., 2016). The world market for edible mushrooms was US \$ 42,419 billion dollars in 2018, but is expected to grow to US \$ 62,193 billion dollars in 2023 (<https://www.knowledge-sourcing.com/products/global-edible-mushrooms-market-industry-trends-opportunities-and-forecasts-to-2023>). This will result in an increased production of spent mushroom substrate (SMS) or spent mushroom compost (SMC), which is a by-product after the harvest of edible mushrooms (Owaid et al., 2017). This compost is made from agricultural waste such as tea leaves, banana, cotton, corn husk, coffee husks, sugarcane bagasse, cereal straw, and wood sawdust. In the cultivation of *Agaricus* spp., horse manure, chicken manure, urea, ammonium sulfate, blood meal, grape pomace, molasses, brewers' grain and feather flour are added as supplements to increase soil nitrogen content (Stamets, 1983; Kamthan and Tiwari, 2017; Pardo-Jimenez et al., 2016; Pardo-Jimenez et al., 2018).

These substrates have high content of polysaccharide, vitamin, and trace elements, such as Fe, Ca, Zn and Mg (Medina et al., 2009; Zhu et al., 2012). They also have extracellular enzymes produced by edible fungi to carry out efficient lignin degradation (Pandey et al., 2014; Singh and Singh, 2012) as well as cellulose and hemicellulose degradation (Kabet et al., 2017; Vos et al., 2017). In China, which is the largest producer of edible fungi (Liu et al., 2015), it is estimated that 4-5 kg of SMC are generated for each kg of fungi produced (Law et al., 2003; Medina et al., 2012; Phan and Sabaratnam, 2012). SMC is reused in mushroom cultivation (Wang et al., 2015) and animal feed (Ayala et al., 2011; Kim et al., 2011; Chang et al., 2016; Foluke et al., 2014), and the enzymes can be recovered (Phan and Sabaratnam, 2012; Rodriguez et al., 2012; Lim et al., 2013; Raymond et al., 2015). In

fact, the substrate is used as a substitute for peat (Abad et al., 2001) in bioremediation (Stanley et al., 2018; Marin-Benito et al., 2016), and for bioethanol production (Hiyama et al., 2011), pest management (Ahmad et al., 2016), and packaging and construction materials (Appels et al., 2018; Xing et al., 2018; Jones et al., 2017). SMC is also used as a soil conditioner and for the recuperation of degrade soils in agriculture (Gümüş and Seker, 2017; Jankowski et al., 2018; Unal, 2015). In this sense, the addition of depleted compost to the soil can result in positive effects such as the development of a granular microstructure in the A horizon and a spongy structure in the B horizon (Nakatsuka et al., 2016), and also increased biological activity (Balesdent et al., 2000), where fungi play an important role because they modulate soil structure. As the hypha grow, they stabilize, agglutinate, and decompose organic matter (binding soil grains into granular aggregates), and realign particulate materials on a micrometric scale (Tisdall and Oades, 1982; Ritz and Young, 2004). According to Gümüş and Şeker, (2018), addition of spent compost to the soil improves electrical conductivity, total nitrogen, organic carbon, aggregate stability, and soil modulus of rupture, and thus represents an environmentally friendly alternative, particularly because there is an emerging demand to increase agricultural sustainability while decreasing dependence on chemical fertilizers.

In Colombia, agriculture accounts for 6.3% of the gross domestic product of the country (Cárdenas et al., 2018). Sugarcane is one of the main crops in Colombia, being particularly grown in the Valle del Cauca region, where consequently large quantities of bagasse are produced. In 2015, Valle del Cauca generated an average of 7,261,526 tons of bagasse, of which 15% was used for paper production, and the remaining 85% was used in the production of bioethanol and the generation of energy in sugar mill boilers. Even though bagasse has different uses, it has not been clearly determined yet whether it might be socially and environmentally sustainable (Becerra-Quirós et al., 2016).

Sugarcane bagasse is a suitable substrate for the growth of *L. edodes* and *P. ostreatus*. Edible fungi production constitutes a process in which a low-quality residue with a negative environmental impact is converted into high-quality food and waste (SMS) with the potential to be used in the production of biofertilizers (Gerdelidani and Hosseini, 2018). These features could play a key role in the development of an integrated nutrient management system that contributes to the sustainability of regional agriculture, and to the implementation of circular

economy practices in the region. It is well known that biofertilizers increase the size and volume of plant roots, enhancing the efficiency of plants to obtain water and expanding their life span. Unlike synthetic fertilizers, biofertilizers are sources of bioavailable nutrients that can be slow released (Malusà and Canfora, 2016).

The objective of this study was to evaluate the changes in the nutritional content and physical properties of sugarcane bagasse-based compost during the mycelial growth of the edible mushrooms *P. ostreatus* and *L. edodes*. The potential of the substrate as a biofertilizer was evaluated.

## MATERIALS AND METHODS

### Obtaining degradable substrate

Sugarcane bagasse was collected at the "Palestina" sugar mill, located at Kilometer 6 on the road to the town of Candelaria in Valle del Cauca state. The collected bagasse was stored in a dry, covered area. A homogeneous sample of bagasse was taken with a fiber size of between 3 and 5 cm to facilitate the effective invasion of *P. ostreatus* and *L. edodes* mycelium.

### Strains and spawn preparation

Strains of *P. ostreatus* and *L. edodes* were obtained from the fungal culture collection of the Department of Biology of the Universidad del Valle (Colombia). At the laboratory of the Department of Biology, mycelium fragments of both fungi were inoculated in Petri dishes containing potato dextrose agar (PDA) for reactivation and to check viability. The mycelial growth of each species in this culture medium was used to prepare the spawn in wheat grain.

### Spawn production

Wheat grain was first washed, and after  $\text{CaCO}_3$  and  $\text{CaSO}_4$  were added, it was boiled for 15 minutes, drained, placed in glass jars, sealed and sterilized for one hour at 122 °C 1.5 atm. Grown mycelium pieces of 1 cm<sup>2</sup>, obtained in PDA plates, were inoculated into a glass jar containing sterile grain wheat once they reached room temperature. They were incubated at 28°C for three weeks.

### Substrate

Sugarcane bagasse was used as growth substrate. Two tests were conducted for each fungal species. Water was added to obtain 70-80% humidity. Plastic bags containing 500 g of the substrate were sealed and sterilized during one hour at 122 °C and 1.5 atm. After cooling, each sterilized substrate was inoculated with 15 g spawn (3%), and then incubated at 26 °C.

### Chemical analysis

Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) analyses were used to determine hemicellulose, cellulose, and lignin contents of the substrates. Total nitrogen (Automated Molecular Spectrophotometry with acid digestion Standard Method D8083-16), total carbon (gravimetric method with incineration at 450 °C), phosphorus (Automated Molecular Spectrophotometry with acid digestion), calcium, magnesium, sodium, and potassium (Inductively Coupled Plasma-Optical Emission Spectrometry, Standard Method 3120) were analyzed in sugarcane bagasse at 0, 30 and 60 days of the colonization process by each inoculated species. Bagasse pH was measured using a pH meter (Metrohm 914), and a water ratio of 10: 100 was used. The initial C:N ratio was obtained by dividing the nitrogen percentage by the carbon percentage obtained by the gravimetric method.

### Statistical analysis

The experiments were arranged in a completely randomized design to test three replicates of *P. ostreatus* and *L. edodes* at 0, 30 days and 60 days. The results obtained in the chemical characterization were processed using SAS 9.4 statistical software. Descriptive statistics such as means, variances, and standard deviations were calculated. Significant differences were compared by using Leneve's Test at a significance level of 5%.

## RESULTS AND DISCUSSION

### pH analysis

Values of substrate pH obtained during the colonization of *P. ostreatus* and *L. edode* tended to decrease. *L. edodes* showed the greatest reduction from an average pH of 5.64 to 3.90, while *P. ostreatus* resulted in a pH reduction to a value of 4.26 (Table 1). The one-way Anova Test carried out to compare pH values obtained for each species during the two periods evaluated showed that there were no significant differences ( $p > 0.05$ ) after 30 days. However, there was a highly significant difference ( $p > 0.01$ ) after 60 days of inoculation.

Similar pH values to those obtained in this study have been previously reported for *Pleurotus* spp. (Owaid et al., 2017), *L. edodes* (Ribas et al., 2009), *Agaricus subrufescens* (Stokness et al. 2019) and many species of fungi (Moore-Landecker, 1996; Chang and Miles, 1989; Deacon, 2006). According to these authors, the decrease in pH levels can be attributed to the production of organic acids such as gluconic, pyruvic, citric, succinic acids

**Table 1. Main physical parameters of the substrates during the growth of *Pleurotus ostreatus* and *Lentinula edodes*.**

Physical parameters	Time (days)	pH	Moisture %	Lignin (g/kg)	Cellulose (g/kg)	Hemicellulose (g/kg)	C:N Ratio
Sterile Sugarcane bagasse	0	5.64	81.67	91.40	494.00	275.00	36.12
Sugarcane bagasse + <i>Pleurotus ostreatus</i>	30	4.50	82.08	83.20	472.00	202.00	27.53
Sugarcane bagasse + <i>Pleurotus ostreatus</i>	60	4.26	82.45	48.30	284.00	208.00	66.88
Sugarcane bagasse + <i>Lentinula edodes</i>	30	4.10	82.48	87.80	491.00	186.00	31.34
Sugarcane bagasse + <i>Lentinula edodes</i>	60	3.90	82.56	68.40	374.00	134.00	64.45

and sodium oxalate crystals formed by the fungal metabolism of sugars as well as the formation of carbonic acid, which accumulates in the mycelium of the fungi during growth. Changes in pH affect the degree of dissociation of mineral salts, balance between dissolved carbon dioxide, bicarbonate ions and net charge of membrane proteins, having consequences for nutrient absorption (Deacon, 2006). On the other hand, levels of acidic pH could be related to the activity of lignin peroxidase and versatile peroxidases enzymes, produced by *P. ostreatus* and *L. edodes* to degrade the lignocellulose. These enzymes require acidic pH values ranging from 3.1 to 4.7 for catalytic functioning (Januzs, 2017). Similarly, enzymes responsible for cellulose degradation, such as endoglucanases, show optimal catalyst values between pH 4 and 5 (Baldrian, 2008).

According to the Colombian Technical Standard 5167, which regulates organic products, the pH of an organic fertilizer should be between 4 and 9. This means that the pH of the substrate degraded by *P. ostreatus* species complies with this regulation, while *L. edodes* does not meet this requirement at a 60-day period. However, when preparing a mixture for a field evaluation, this characteristic can be easily corrected by adding a carbonated material such as Calcium oxide. According to Marschner (2011), soils in arid or semi-arid zones are calcareous, have an alkaline pH and little organic matter, while plants in those places have micro and macronutrient deficiencies, especially phosphorus. Banik and Dey (1982) reported that the addition of a compost with an acid pH and depleted by fungi to calcareous soils can increase acidity and biological activity, leading to increases in CO<sub>2</sub> production, and thus to the generation of acidic conditions.

#### Analysis of moisture content

As *P. ostreatus* and *L. edodes* grew during the evaluation period (60 days), moisture did not vary significantly ( $p > 0.05$ ). Moisture content of a substrate strongly influences the acquisition of the necessary nutrients for fungal growth and

enzymatic activity. The increase in moisture observed during the growth of both fungal species may be related to the fact that, in the degradation or decomposition process of the substrate, CO<sub>2</sub> and water are formed as by-products of the metabolism of the species and degradation of molecules with simple and complex structures (Moore-Landecker, 1996; Hurst et al., 2007).

Moisture levels between 70 and 85% are recommended to facilitate fungal growth and nutrient availability (Romero et al., 2003). For *P. ostreatus* and *L. edodes*, levels of 65-75% (Stamets, 2000) and 77% (Owaid et al., 2017) have been reported, respectively. In the present study, although moisture content was slightly higher (82%), there was no limitation or inhibition of growth (Table 1).

#### Analysis of C:N ratio

*P. ostreatus* and *L. edodes* grew on a substrate with a C:N ratio of 42:1 (Table1), which is within the range reported for species of the same genders. C:N ratio values of 30:1 and 117:1 have been reported for *Pleurotus flabellatus* (Srivastava and Bano, 1970), 18:1 and 36:1 for *P. tuber-regium* (Wu et al., 2004), and 85:1 for *P. ostreatus* (Rajarathnam and Bano, 1989). For *Lentinus edodes*, the literature has described C:N ratio values of 25:1 (Oei, 1996), 86: 1 (Ribas et al., 2009), and from 110:1 to 140: 1 (Villegas et al., 2007). Nevertheless, Chang and Miles (1989), reported that a C:N ratio of 20:1 is adequate for most fungi, while Hsu and Lo (1999) indicated that a low C:N ratio results in high degradation of organic matter and compost stability. The C:N ratio is an indicator of the nutritional level of a substrate and largely determines microbial development; therefore, a lack of balance can even impede substrate growth and colonization (Stamets, 2000). However, this parameter can vary according to the strains used, the different stages of development and type of substrate (Gaitán-Hernández et al., 2011).

#### Cellulose, hemicellulose and lignin analysis

Lignin, cellulose and hemicellulose contents



had significant decreases at 60 days for both *P. ostreatus* and *L. edodes* (Table 1). *P. ostreatus* recorded the greatest decrease in lignin (47.2%) and cellulose (42.5%), while *L. edodes* showed the greatest hemicellulose degradation (51.3%). It is noteworthy that the greatest reduction of lignin and cellulose achieved by *P. ostreatus* was observed at a pH of 4.3. Cellulose, hemicellulose and lignin are the main sources of carbon and nitrogen (Jamangapé-Ovando, 2018). During mycelium growth and development, biochemical changes result in the production of extracellular enzymes, responsible for degrading the insoluble components and a large amount of lignocellulosic material into soluble and low molecular weight compounds, which are subsequently taken up by intracellular enzymes of the fungus for their nutrition (Kurt and Buyukalaca, 2010; Kudryavtseva et al., 2008). These results support the idea that white rot fungal species vary in terms of the relative rates at which they degrade lignin and lignocellulose carbohydrates (Hatakka, 2001), enzymatic mechanisms, redox systems and radical generation (Baldrian and Valášková, 2007). Additionally, experiments carried out with the white rot fungus *Echinodontium taxodii* showed that the cellulosic delignification ability varies with the type of wood, being greater in

hardwoods than in softwoods (Yu et al., 2009).

In terms of lignin and cellulose degradation, a study on *P. ostreatus* conducted by Benavides (2013) reported values of 59.02% and 84.23%, respectively, and concluded that such decreases resulted from the increase in mycelium during cultivation, and the extracellular enzymatic activity that generated the mineralization and degradation of the lignocellulosic complex. In our study, levels of lignin degradation were higher than those of cellulose for both *P. ostreatus* and *L. edodes*, and varied slightly between the species with values of 47.2% and 42.5%, respectively. Pandey and Singh (2014) reported lignin, hemicellulose and cellulose levels of 50.21%, 27.73% and 41.20%, respectively. This is in agreement with the results obtained in the present study, confirming that there is also a decrease in hemicellulose due to mycelium growth and expansion. Montoya et al. (2014) determined that *L. edodes* is effective in the degradation of lignocellulosic materials, particularly lignin with 51.8% of degradation.

#### N, P and K analysis

Macro and micronutrients depleted by *P. ostreatus* and *L. edodes* after 60 days of growth are presented in Figs. 1, 2, 3 and 4. It is evident that the resulting substrate presents an increase in

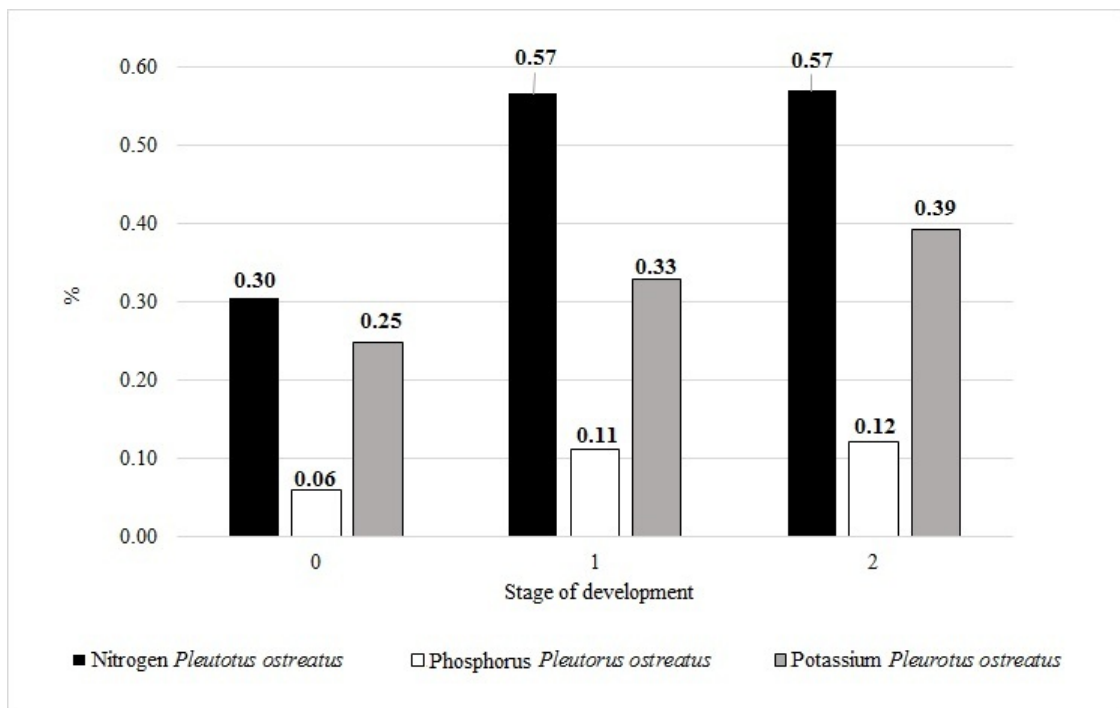


Fig. 1. Average NPK variation for *Pleurotus ostreatus*. Stage of development: 0. Sterile sugarcane bagasse, 1. Sugarcane bagasse substrate after 30 days of inoculation with *P. ostreatus*, 2. Sugarcane bagasse substrate after 60 days of inoculation with *P. ostreatus*.

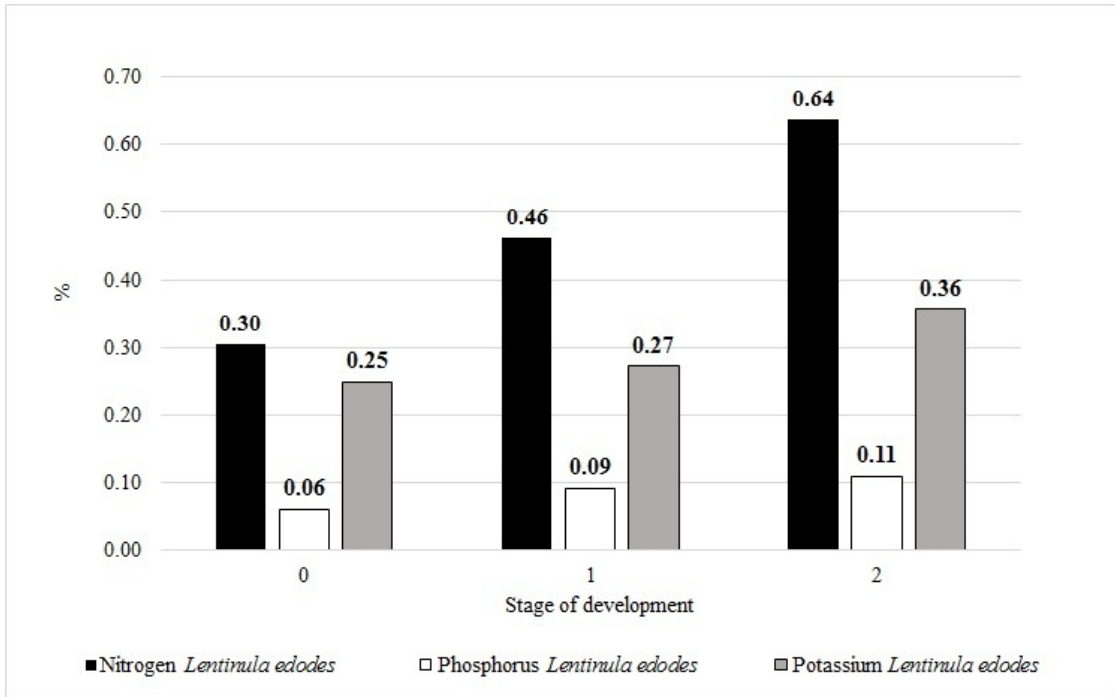


Fig. 2. Average NPK variation for *Lentinus edodes*. Stage of development: 0. Sterile sugarcane bagasse, 1. Sugarcane bagasse substrate after one 30 days of inoculation with *L. edodes*, 2. Sugarcane bagasse substrate after 60 days of inoculation with *L. edodes*.

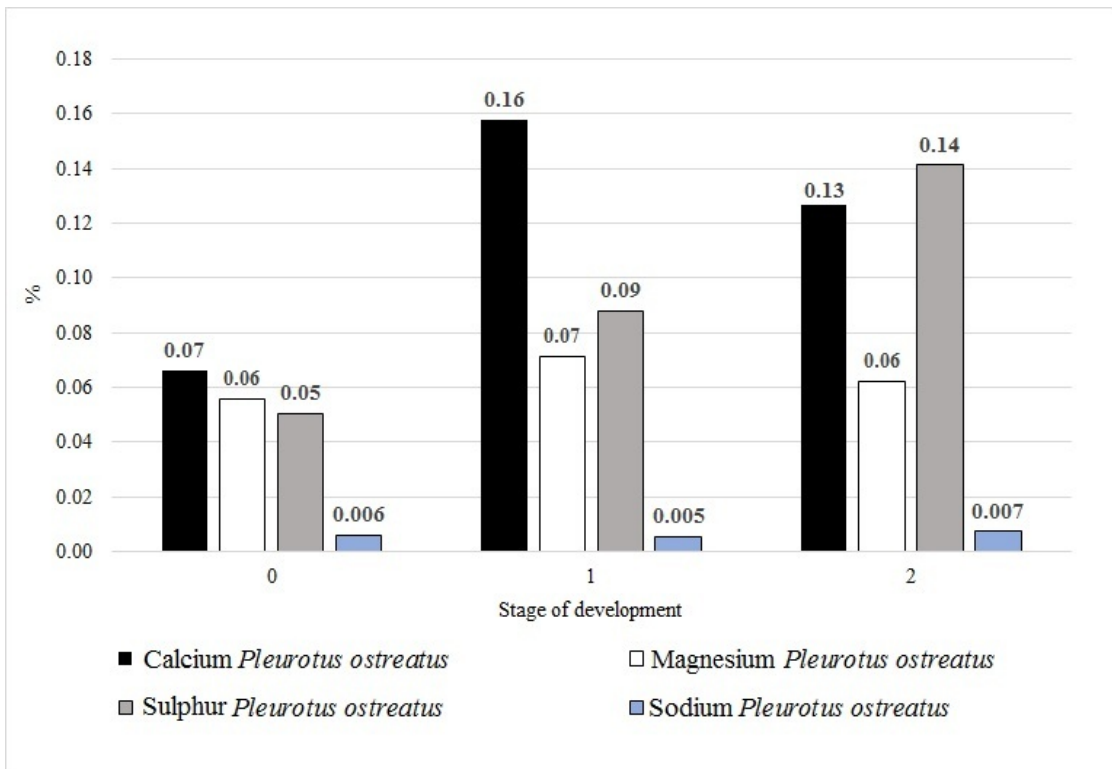
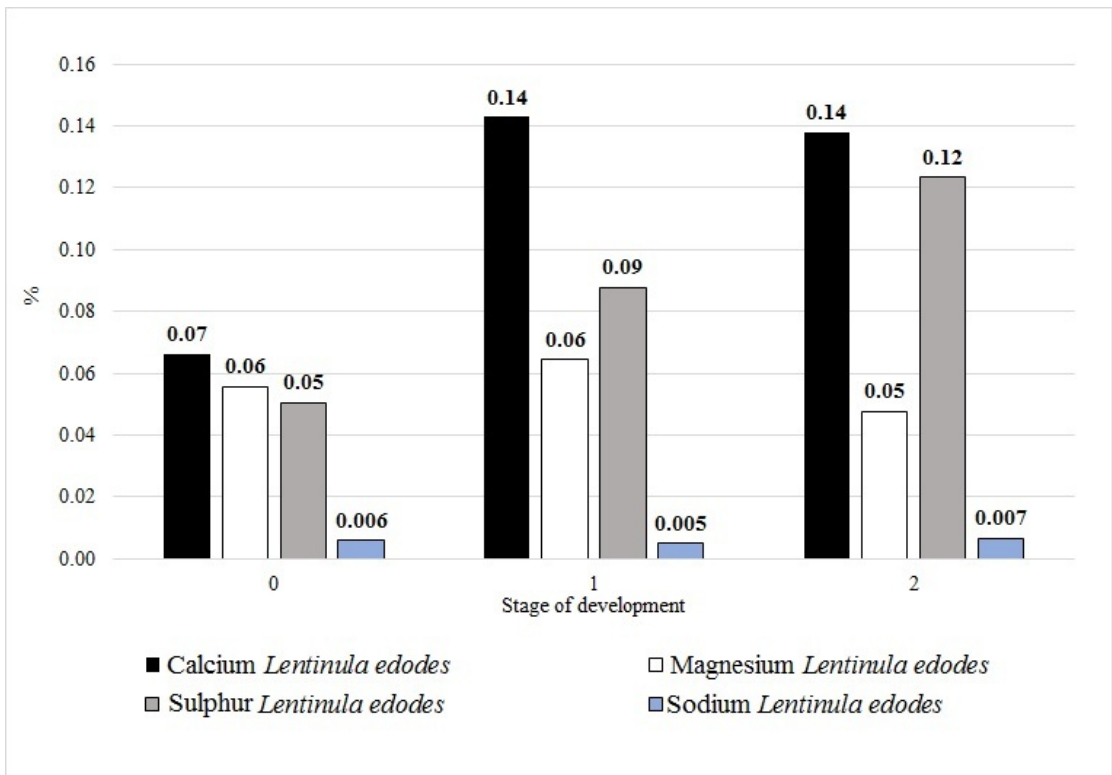


Fig. 3. Average of Ca, Mg, S and Na variation for *Pleurotus ostreatus*. Stage of development: 0. Sterile sugarcane bagasse, 1. Sugarcane bagasse substrate after 30 days of inoculation with *P. ostreatus*, 2. Sugarcane bagasse substrate after 60 days of inoculation with *P. ostreatus*.



**Fig. 4. Average of Ca, Mg, S and Na variation for *Lentinula edodes*. Stage of development: 0. Sterile sugarcane bagasse, 1. Sugarcane bagasse substrate after 30 days of inoculation with *L. edodes*, 2. Sugarcane bagasse substrate after 60 days of inoculation with *L. edodes*.**

primary macronutrients (N, P, K) and secondary macronutrients (Ca, Mg, S and Na) after the growth of the two species studied.

N content in the depleted substrate was similar for both species during the evaluation period. Average values of 5.69 g / kg (0.569%) and 6.38 g / kg (0.638%) were obtained for *P. ostreatus* and *L. edodes*, respectively. Phosphorus content behaved in a similar way, reaching values of 1.21 g / kg (0.121%) for *P. ostreatus* and 1.09 g / kg (0.109%) for *L. edodes*. The final potassium content in the substrate was 3.92 g / kg (0.392%) and 3.56 g / kg (0.356%) for *P. ostreatus* and *L. Edodes*, respectively. The one-way Anova performed to compare the values obtained for each species showed no significant differences ( $p > 0.05$ ). These results agree with those obtained by Owaïd et al. (2017), who reported nitrogen levels of 6.65g / kg in a substrate depleted by *P. salmoneostramineus*, made of 50% wheat straw + 30% sawdust, and 20% palm fiber. Additionally, N content reached 9.98 g / kg in another substrate depleted by *P. ostreatus* based on 50% wheat straw, 30% sawdust and 20% palm fiber, due to the fact that they started from twice the initial content of the substrate used in the present study (3.05 g / kg). In the case of *L. edodes*, Ribas et al. (2009) found an equal value for

N in a substrate made up of eucalyptus sawdust + 10% rice bran + gypsum and  $\text{CaCO}_3$ . Experiments carried out by Medina et al. (2009) showed that the N content in different mixtures of compost spent by *P. ostreatus* + commercial *Sphagnum* varied from 11-13g / kg.

Rao et al. (2007) highlighted that as a spent substrate presents high variability and chemical imbalance, it is necessary to replace the missing chemicals in order to be used as a fertilizer. In the present study, the nitrogen level in the compost degraded by *P. ostreatus* and *L. edodes* was below the optimal range required for organic fertilizers, and therefore a mixture with other plant residues with higher N content can be used to start with a substrate richer in N from its initial formulation when the substrate was not supplemented during the initial phase. On the other hand, it would be interesting to make mixtures of compost depleted by these species and compost depleted by *Agaricus subrufescens*, which has an N content of 1.8%, in order to achieve acceptable levels of N for an organic fertilizer, which range from 1 to 2% (10-20g / kg) (Alexander, 1994).

Regarding levels of phosphorus (P) and potassium (K), Alexander (1994), defined that the optimal range for organic compost varies from

0.6% to 0.9% for P and from 0.2% to 0.5% for K. In the present study, the SMS of *P. ostreatus* and *L. edodes* showed low levels of phosphorus, with values of 0.12% and 0.11%, respectively. However, they may represent a good source of K, with values of 0.39% and 0.36%, respectively. Ribas et al. (2009) also reported levels of 0.58% and 0.17% for P and K, respectively, in a substrate of *L. edodes* based on eucalyptus sawdust and 10% rice bran. The disparity in the results suggests that more studies are needed to balance the appropriate levels of these elements in a substrate. It is important to note that P bioavailability depends on the contents of organic matter, Ca, Fe and Al as well as pH of the medium since P is more bioavailable to plants at pH between 6 and 7 (Chang et al., 2014).

### Ca, Mg, S and Na Analysis

Values of secondary macro elements such as calcium, magnesium, sulfur and sodium showed different trends from the formulation stage until completion of the 60-day evaluation period for both *P. ostreatus* and *L. edodes*. Fig. 2 shows the percentage of each element during the three evaluation periods (0, 30 and 60 days).

Calcium increased in a very similar way in both species, reaching an average content of 0.7%, while there was no considerable increase for sodium and magnesium. Sulfur behaved very similarly in the two species, recording an increase of 0.09% and 0.07% for *P. ostreatus* and *L. edodes*, respectively; the content of this element in the agro-industrial substrate was 0.051% (0.51 g / kg). *P. ostreatus* recorded an average value of 0.141% (1.41 g / kg), while the final average value reached by *L. edodes* was 0.123% (1.23 g / kg).

Maszkiewicz (2010) extensively documented the high Ca, Mg, Na and S contents in the residual compost of *Agaricus bisporus*. Postemsky and López-Castro (2016) reported that a compost degraded by *P. ostreatus* based on sunflower shell and supplemented with 2% and 0.5% of calcium sulfate ( $\text{CaSO}_4$ ) and calcium carbonate ( $\text{CaCO}_3$ ), respectively, contained 1305 mg / kg of Ca, 30 mg / kg of Na, 173 mg / kg of Mg and 170 mg / kg of S.

### CONCLUSIONS

The increase in nutrients observed in the material resulting after the growth of *Pleurotus ostreatus* and *Lentinula edodes* in sugarcane bagasse can be used to develop a suitable organic amendment by adding plant residues rich in nitrogen, phosphorus, potassium, calcium, total minerals, fat and fiber beginning from the initial stage of compost formulation to obtain an appropriate C:N ratio. An optimum C:N

relation allows for better mycelial growth, and consequently an improved synthesis of proteins and other minerals in the substrate. The addition of this substrate can contribute to the recovery of soils that have undergone processes of degradation, salinization, extensive use of chemical fertilizers, or that are deficient in nutrients. It is well known that this material is rich in organic matter so that its use could improve parameters related to soil structure such as porosity, aeration, and water movement. Similarly, given the decrease in pH, which occurs naturally as fungal mycelium grows due to the production of various metabolites, the resulting substrate could be used to treat and modify calcareous soils once appropriate doses and mixtures are determined. Finally, the decomposition and delignification carried out by these two species of fungi are crucial steps in the process of organic matter humidification.

### ACKNOWLEDGMENTS

The author would like to thank the Universidad del Valle, Cali, Colombia for the financial support from Internal Call 2017 for research projects.

### LITERATURE CITE

- Abad, M., P. Noguera, and S. Bures. 2001. National inventory of organic wastes for use as growing media for ornamental potted plant production: case study in Spain. *Bioresource Technology* 77:197-200. [https://doi.org/10.1016/S0960-8524\(00\)00152-8](https://doi.org/10.1016/S0960-8524(00)00152-8)
- Ahmad, Z.M., I. Khan, B. Shah, A. Naeem, N. Khan, W. Ullah, et al. 2016. Study on the management of *Ralstonia solanacearum* (Smith) with spent mushroom compost. *Journal of Entomology and Zoology Studies* 4(3): 114-121. <https://www.researchgate.net/publication/302411979>
- Alexander, R.A. 1994. Standards and guidelines for compost use. *Biocycle* 35:37-41.
- Appels, F.V.W., J. Dijksterhuis, C.E. Lukasiewicz, K.M.B. Jansen, H.A.B. Wösten, and P. Krijgsheld. 2018. Hydrophobin gene deletion and environmental growth conditions impact mechanical properties of mycelium by affecting the density of the material. *Scientific Reports* 8:470. <https://doi.org/10.1038/s41598-018-23171-2>
- Ayala, M., S.S. Gonzalez-Munoz, J. M. Pinos-Rodríguez, C. Vázquez, M. Meneses, O. Loera, and G.D. Mendoza. 2011. Fibrolytic potential of spent compost of *Agaricus bisporus* to degrade forages for ruminants. *African Journal of Microbiology Research* 5:643-650. <https://doi.org/10.5897/AJMR10.528>



- Baldrian, P., and V. Valášková. 2008. Degradation of cellulose by Basidiomycetous fungi. *FEMS Microbiology Reviews* 32:501-521. <https://doi.org/10.1111/j.1574-6976.2008.00106.x>
- Balesdent, J., C. Chenu, and M. Balabane. 2000. Relationship of soil organic matter dynamics to physical protection and tillage. *Soil & Tillage Research* 53:215-230. [https://doi.org/10.1016/S0167-1987\(99\)00107-5](https://doi.org/10.1016/S0167-1987(99)00107-5)
- Banik, S., and B.K. Dey. 1982. Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate-solubilizing microorganisms. *Plant and Soil* 69:353-364. <https://doi.org/10.1007/BF02372456>
- Becerra-Quiroz, A. P., A. L. Buitrago-Coca, y P. Pinto-Baquero. 2016. Sostenibilidad del aprovechamiento del bagazo de caña de azúcar en el Valle del Cauca, Colombia. *Ingeniería solidaria*, 12: 133-149. <https://doi.org/10.16925/in.v12i20.1548>
- Benavides O., L. 2013. Aprovechamiento de residuos lignocelulósicos para el cultivo de orellnas (*Pleurotus ostreatus*). Tesis Magister en Ciencias Agrarias, Universidad de Nariño, Colombia. <http://sired.udenar.edu.co/2527/1/89544.pdf>
- Cárdenas, M., J. Echavarrí, G. Hernández, A. Maiguashca, A. Meisel, J. Ocampo, and J. Zárate. 2018. Recuadro 2 del informe al congreso marzo de 2018: Coyuntura del sector agropecuario colombiano. Banco de la Republica de Colombia. <https://www.banrep.gov.co/sites/default/files/publicaciones/archivos/informe-congreso-marzo-2018-recuadro-2>. Consulted July 7th 2020.
- Chang, S.Ch., M.J. Lin, Y.P. Chao, CH.J. Chiang, Yu-Sh. Jea, and T.T. Lee. 2016. Effects of spent mushroom compost meal on growth performance and meat characteristics of grower geese. *Revista Brasileira de Zootecnia* 45:281-287. <https://www.scielo.br/pdf/rbz/v45n6/1516-3598-rbz-45-06-00281>
- Chang, H.Y., O.H. Ahmed, and N.M.A. Majid. 2014. Improving phosphorus availability in an acid soil using organic amendments produced from agroindustrial wastes. *The Scientific World Journal*, 2014. <https://doi.org/http://dx.doi.org/10.1155/2014/506356>.
- Chang, S.T., and S.P. Wasser. 2012. The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. *International Journal of Medicinal Mushrooms*, vol. 14:95-134. <https://doi.org/10.1615/IntJMedMushr.v14.i2.10>
- Chang, S.T., and P.G. Miles. 1989. *Edible Mushrooms and their Cultivation*. CRC Press, Florida, USA.
- Deacon, J. 2006. *Fungal Biology*. 4th ed. Blackwell Publishing Ltd. Oxford, UK.
- Foluke, A., A. Olutayo, and A. Olufemi. 2014. Assessing spent mushroom substrate as a replacement to wheat bran in the diet of broilers. *American International Journal of Contemporary Research* 4: 178-183. <https://www.researchgate.net/publication/269334643>
- Gaitán-Hernández, R., M. Esqueda, A.H. Gutiérrez-Saldaña, and M. Beltrán-García. 2011. Quantitative changes in the biochemical composition of lignocellulosic residues during the vegetative growth of *Lentinula edodes*. *Brazilian Journal of Microbiology* 42:30-40. <https://doi.org/10.1590/S1517-83822011000100004>
- Gerdelidani, A.F., and A.H.M. Hosseini. 2018. Effects of sugar cane bagasse biochar and spent mushroom compost on phosphorus fractionation in calcareous soils. *Soil Research*. 56:136-144. <https://doi.org/10.1071/SR17091>
- Gümüş, I., and C. Şeker. 2017. Effects of spent mushroom compost application on the physicochemical properties of a degraded soil. *Solid Earth*, 8:1153-1160. <https://doi.org/10.5194/se-8-1153-2017>
- Hatakka, A. 2001. Biodegradation of lignin. p. 129-167. In: M. Hofrichter, A. Steinbüchel (Ed.), *Lignin, Humic Substances and Coal*: Wiley-VCH, Helsinki, FI.
- Heleno, S.A., L. Barros, M.J. Sousa, A. Martins, and I. Ferreira. 2010. Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry* 119:443-1450. <https://doi.org/10.1016/j.foodchem.2009.09.025>
- Hiyama, R., S. Gisusi, and A. Harada. 2011. Evaluation of waste mushroom medium from cultivation of shiitake mushroom (*Lentinula edodes*) as feedstock of enzymatic saccharification. *Journal of Wood Science* 57:429-435. <https://doi.org/10.1007/s10086-011-1185-y>
- Hsu, J-H., and S-L. Lo. 1999. Chemical and spectroscopic analysis of organic matter transformations during composting of pig manure. *Environmental Pollution* 104:189-196. <https://scholars.lib.ntu.edu.tw/bitstream/123456789/77320/1/22.pdf>
- Hurst, C., R.Crawford, J. Garland, D. Lipson, A. Mills. and L. Stetzenbach. 2007. *Manual of environmental microbiology*. ASM Press, Washington, USA.
- Jamangapé, O. R. G. 2018. Cultivo y elaboración de productos a base de setas. Universidad de Ciencias y Artes de Chiapas, Mexico.

- Jankowski, K., E. Malinowska, J. Sosnowski, B. Wiśniewska-Kadżajan, and A. Kaczorek. 2018. Effects of spent mushroom substrate and slurry on nutritional value of grass and Medicago x varia T. Martyn mixtures. *International Journal Agricultural and Biological Engineering* 11:61-66. <https://doi.org/10.25165/j.ijabe.20181103.3726>
- Janusz, G., A. Pawlik, J. Sulej, U. Świdorska-Burek, A. Jarosz-Wilkolazka, and A. Paszczyński. 2017. Lignin degradation: microorganisms, enzymes involved genomes analysis and evolution. *FEMS Microbiology Reviews* 41: 941-962. <https://doi.org/10.1093/femsre/fux049>
- Jones, M.P., T. Huynh, C. Dekiwadia, f. Daver, and J. Sabu. 2017. Mycelium composites: a review of engineering characteristics and growth kinetics. *Journal of Bionanoscience* 11:241-257. <https://doi.org/10.1166/jbns.2017.1440>
- Kabel, M.A., E. Jurak, M.R. Mäkelä, R. P. de Vries. 2017. Occurrence and function of enzymes for lignocellulose degradation in commercial *Agaricus bisporus* cultivation. *Applied Microbiology and Biotechnology* 101:4363-4369. <https://doi.org/10.1007/s00253-017-8294-5>
- Kamthan, R., and I. Tiwari. 2017. Agricultural Wastes- potential Substrates for Mushroom cultivation. *European Journal of Experimental Biology*, 7:1-4. <https://doi.org/10.21767/2248-9215.100031>
- Kim, Y.I., W.M. Cho, S.K. Hong, Y.K. Oh, and W.S. Kwak. 2011. Yield, nutrient characteristics, ruminal solubility, and degradability of spent mushroom (*Agaricus bisporus*) substrates for ruminants. *Asian-Australia Journal of Animal Science* 24:1560-1568.
- Kudryavtseva, O., Y. Dunaevsky, O. Kamzolkina, and M. Belozersky. 2008. Fungal Proteolytic Enzymes: Features of the Extracellular Proteases of Xylotrophic Basidiomycetes. *Microbiology* 77:643-653. <https://doi.org/10.1134/S0026261708060015>
- Kurt, S., and S. Buyukalaca. 2010. Yield performances and changes in enzyme activities of *Pleurotus* spp. (*P. ostreatus* and *P. sajor-caju*) cultivated on different agricultural wastes. *Bioresource Technology*, 101: 3164. <https://doi.org/10.1016/j.biortech.2009.12.011>
- Law, W., W. Lau, K. Lo, L. Wai, and S. Chiu. 2003. Removal of biocide pentachlorophenol in water system by the spent mushroom compost of *Pleurotus pulmonarius*. *Chemosphere* 52: 1531-1537. [https://doi.org/10.1016/s0045-6535\(03\)00492-2](https://doi.org/10.1016/s0045-6535(03)00492-2)
- Lim, S.H., Y-H. Lee, and H.W Kang. 2013. Efficient Recovery of Lignocellulolytic Enzymes of Spent Mushroom Compost from Oyster Mushrooms, *Pleurotus* spp., and Potential Use in Dye Decolorization. *Mycobiology* 41: 214-220. <https://doi.org/10.5941/MYCO.2013.41.4.214>
- Liu, Y., CH. Zhao, D. Lin, H. Lin, and Zh. Lin. 2015. Effect of water extract from spent mushroom substrate after *Ganoderma balabacense* cultivation by using JUNCAO technique on production performance and hematology parameters of dairy cows. *Animal Science Journal* 86: 855-862. <https://doi.org/10.1111/asj.12371>.
- Malusà, E., F. Pinzari, and I. Canfora. 2016. Efficacy of biofertilizers: challenges to improve crop production. p. 17-40. In D.P. Singh et al. (ed.). *Microbial inoculants in sustainable agricultural productivity*. Springer, New Delhi, IN.
- Marín-Benito, J.M., M.J. Sánchez-Martín, and M.S. Rodríguez-Cruz. 2016. Impact of Spent Mushroom Substrates on the Fate of Pesticides in Soil, and Their Use for Preventing and/or Controlling Soil and Water Contamination: A Review. *Toxics* 4:2-24. <https://doi.org/10.3390/toxics4030017>
- Marschner, H. 2011. *Mineral nutrition of higher plants*. 3rd ed. Academic press, London UK.
- Maszkiewicz, J. 2010. Used mushroom substrate as fertilizer and fuel. In *Mushrooms. Mushroom Producer Bulletin*. Wyd. Hortpress 1:59-60. (in Polish)
- Medina, E., C. Paredes, M.A. Bustamante, R. Moral, and J. Moreno-Caselles. 2012. Relationships between soil physico-chemical, chemical and biological properties in a soil amended with spent mushroom substrate. *Geoderma* 173-174:152-161. <https://doi.org/10.1016/j.geoderma.2011.12.011>
- Medina, E., C. Paredes, M.D. Pérez-Murcia, M.A. Bustamante, and R. Moral. 2009. Spent mushroom substrates as component of growing media for germination and growth of horticultural plants. *Bioresource Technology* 100:4227-4232. <https://doi.org/10.1016/j.biortech.2009.03.055>
- Montoya, B., S. Sánchez and L. Levin. 2014. Evaluation of endoglucanase, exoglucanase, laccase, and lignin peroxidase activities on ten white-rot fungi. *Bioteconología en el Sector Agropecuario y Agroindustrial* 12:115-124. Retrieved from <http://www.scielo.org.co/pdf/bsaa/v12n2/v12n2a13.pdf>
- Moore-Landecker, E. 1996. *Fundamentals of the fungi*. 4th ed. Prentice-Hall, Inc. New Jersey, USA.

- Nakatsuka, H., M. Oda, Y. Hayashi, and K. Tamura. 2016. Effects of fresh spent mushroom substrate of *Pleurotus ostreatus* on soil micromorphology in Brazil. *Geoderma* 269: 54-60. <http://dx.doi.org/10.1016/j.geoderma.2016.01.023>
- Oei, P. 1996. *Mushroom cultivation*. Tool Publications, Leiden, The Netherlands.
- Outzouni, P.K., D. Petridis, W.D. Koller, and K.A. Riganakos. 2009. Nutritional Value and metal content of wild edible mushrooms collected from west Macedonia and Epirus Greece. *Food Chemistry* 115: 1575-1580. <https://doi.org/10.1016/j.foodchem.2009.02.014>
- Owaid, M.M., I.A. Abed, and S.S.S. Al-Saeedi. 2017. Applicable properties of the bio-fertilizer spent mushroom substrate in organic systems as a byproduct from the cultivation of *Pleurotus* spp. *Information processing in Agriculture* 4:78-82. <https://doi.org/10.1016/j.inpa.2017.01.001>
- Pandey, V.K., and M.P. Singh. 2014. Biodegradation of wheat straw by *Pleurotus ostreatus*. *Cellular and Molecular Biology* 60:29-35. <https://doi.org/10.14715/cmb/2014.60.5.6>
- Pardo-Giménez, A., L. Catalán, J. Carrasco, M. Álvarez-Ortí, D. Zied, and J. Pardo. 2016. Effect of supplementing crop substrate with defatted pistachio meal on *Agaricus bisporus* and *Pleurotus ostreatus* production. *J. Sci. Food Agric.* 96(11): 3838-3845. <https://doi.org/10.1002/jsfa.7579>
- Pardo-Giménez, A., J. Carrasco, J.M. Roncero, M. Álvarez-Ortí, D.C. Zied and J.E. Pardo-González. 2018. Recycling of the biomass waste defatted almond meal as a novel nutritional supplementation for cultivated edible mushrooms. *Acta Scientiarum. Agronomy* 40: e39341. DOI: 10.4025/actasciagron.v40i1.39341
- Phan, C.W., and V. Sabaratnam. 2012. Potential uses of spent mushroom substrate and its associated Lignocellulosic enzymes. *Applied Microbiology & Biotechnology* 96:863-873. <https://doi.org/10.1007/s00253-012-4446-9>
- Postemsky, P. D., and R. I. López-Castro. 2016. Aplicaciones de sustrato residual del cultivo de hongos en la producción hortícola. *Horticultura Argentina* 35: 44-63. <https://www.researchgate.net/publication/310648661>
- Rajaratnam, S., and Z. Bano. 1989. *Pleurotus* mushrooms: part III. Biotransformation of natural lignocellulosic wastes: commercial applications and implications. *Critical Reviews in Food Science and Nutrition* 28:31-113.
- Rao, J.R., M. Watabe, T.A. Stewart, B.C. Millar, and J.E. Moore. 2007. Pelleted organomineral fertilizers from composted pig slurry solids, animal wastes and spent mushroom compost for amenity grasslands. *Waste Management* 27:1117-1128.
- Raymond, P., A.M. Mshandete, and A.K. Kivaisi. 2015. Enzyme Profiles of *Pleurotus* HK-37 During Mycelia Vegetative Growth and Fruiting on Solid Sisal Waste Fractions Supplemented with Cow Manure. *Advances in Biochemistry* 3:57-65.
- Ribas, L.C.C., M.M. Mendonça, C.M. Camellini, and C.H.L. Soares. 2009. Use of spent mushroom substrates from *Agaricus subrufescens* (syn. *A. blazei*, *A. brasiliensis*) and *Lentinula edodes* productions in the enrichment of a soil-based potting media for lettuce (*Lactuca sativa*) cultivation: growth promotion and soil bioremediation. *Bioresource Technology* 100:4750-4757. <https://doi.org/10.1016/j.biortech.2008.10.059>
- Ritz, K., and I.M. Young. 2004. Interactions between soil structure and fungi. *Mycologist* 18: 52-59. [https://doi.org/10.1017/S0269-915X\(04\)00201-0](https://doi.org/10.1017/S0269-915X(04)00201-0)
- Rodríguez-da luz, J.M., M. Nunes-Días, S. Albino-Paes, D. Pereira-Torres, M. Soares da Silva, and M.C. Megumi-Kasuya. 2012. Lignocellulosic enzyme production of *Pleurotus ostreatus* growth in agroindustrial wastes. *Brazilian Journal of Microbiology* 43: 1508-1515. <http://dx.doi.org/10.1590/S1517-83822012000400035>
- Romero, A., A. Rodríguez, and M. Pérez. 2003. *Pleurotus ostreatus*. Importancia y tecnología de cultivo. Universidad de Cienfuegos "Carlos Rafael Rodríguez", Cuatro Caminos, Cdad. Cienfuegos, Cuba.
- Royse, D.J., J. Baars, and Q. Tan. 2017. Current overview of mushroom production in the world. P. 5-13. In D.C. Zied, Pardo-Giménez A. (ed.). *Edible and medicinal mushrooms: technology and applications*. John Wiley & Sons Ltd. Hoboken, New Jersey, USA. <https://doi.org/10.1002/9781119149446.ch2>
- Sari, M., R. Hambitzer, J. Lelley, K. Toepfer, N. Teusch, and A. Nickisch-Hartfiel. 2016. Characterization of cross flow ultrafiltration fractions from *Grifola frondosa* revealed distinct cytotoxicity in tumor cells. *International Journal for Medicinal Mushrooms* 18:671-680.
- Singh, M.P., and V.K. Singh. 2012. Biodegradation of vegetable and agrowastes by *Pleurotus sapidus*: A noble strategy to produce mushroom with enhanced yield and nutrition. *Cellular and Molecular Biology* 58:1-7.

- Srivastava, H. C., and Z. Bano. 1970. Nutrition requirements of *Pleurotus flabellatus*. Applied and Environmental Microbiology 19:166-169.
- Stamets, P. 2000. Growing gourmet and medicinal mushroom. Third ed. Ten Speed Press, Toronto, CA.
- Stanley, H.O., S.N. Maeba, D.K. Gbenekanu, and C.J. Ugboma. 2018. Crude Oil Degradation Using Spent Mushroom Compost (SMC) of *Pleurotus florida*. Asian Journal of Advanced Research and Reports 2:1-7. <https://doi.org/10.9734/AJARR/2018/44326>
- Stoknes, K., E. Wojciechowska, A. Jasinska, and R. Noble. 2019. Amelioration of Composts for Greenhouse Vegetable Plants Using Pasteurized *Agaricus* Mushroom Substrate. Sustainability 11: 6779. <https://doi.org/10.3390/su11236779>
- Tisdall, J.M., and J.M. Oades. 1982. Organic-matter and water-stable aggregates in soils. Journal of Soil Science 33:141-163. <https://doi.org/10.1111/j.1365-2389.1982.tb01755.x>
- Unal, M. 2015. The utilization of spent mushroom compost applied at different rates in tomato (*Lycopersicon esculentum* Mill.) seedling production. Emirates Journal of Food and Agriculture 27:692-7. <https://doi.org/10.9755/ejfa.2015-05-206>
- Villegas, V., A.M. Pérez, and C. Arredondo. 2007. Evaluación de la producción del hongo *Lentinula edodes* Pegler en bloques sintéticos a partir de residuos agroindustriales. Ingeniería y Ciencia 6:23-39. <https://www.researchgate.net/publication/28220574>
- Vos, A.M., E. Jurak, J.F. Pelkmans, K. Herman, G. Pels, J.J. Baars, et al. 2017. H<sub>2</sub>O<sub>2</sub> as a candidate bottleneck for MnP activity during cultivation of *Agaricus bisporus* in compost. AMB Express 7:124. <https://doi.org/10.1186/s13568-017-0424-z>
- Wang, S., F. Xu, Z. Li, S. Zhao, S. Song, Ch. Rong, et al. 2015. The spent mushroom substrates of *Hypsizigus marmoreus* can be an effective component for growing the oyster mushroom *Pleurotus ostreatus*. Scientia Horticulturae 186: 217-222. <https://doi.org/10.1016/j.scienta.2015.02.028>
- Wu, J.Z., P.C.K. Cheung, K.H. Wong, and N.I. Huang. 2004. Studies on submerged fermentation of *Pleurotus tuber-regium* (Fr.) Singer. Part 2: effect of carbon-nitrogen ratio of culture medium on the content and composition of the mycelial dietary fibre. Food Chemistry. 85:101-105. <https://doi.org/10.1016/j.foodchem.2003.06.009>
- Xing, Y., M. Brewer, H. El-Gharabawy, G. Griffith, and P. Jones. 2018. Growing and testing mycelium bricks as building insulation materials. IOP Conf Ser: Earth Environmental Science 121:022032. <https://doi.org/10.1088/1755-1315/121/2/022032>
- Yu, H., G. Guo, X. Zhang, K. Yan, and C. Xu. 2009. The effect of biological pretreatment with the selective white-rot fungus *Echinodontium taxodii* on enzymatic hydrolysis of softwoods and hardwoods. Bioresource Technology 100:5170-5.
- Zhu, H., k. Sheng, E. Yan, J. Qiao, and F. Lv. 2012. Extraction, purification and antibacterial activities of a polysaccharide from spent mushroom substrate. International Journal of Biological Macromolecules 50:840- 843. <https://doi.org/10.1016/j.ijbiomac.2011.11.016>