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NPK Rich Leafy Tree Biomass as Dual-Purpose Substrate: Improving *Pleurotus* Spp. Nutrients Uptake and Recycling through Spent Mushroom Waste

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Abstract : The use of the NPK (nitrogen, phosphorus, potassium) rich tree based leafy biomass can be used as a sustainable food production option and can valorize the organic waste. This research was conducted to study the mineral dynamics in the cultivation of *Pleurotus* spp. on paddy straw mixed with different levels (25, 50, 75, and 100 %) of *Swietenia macrophylla*, *Gliricidia sepium*, and *Sesbania grandiflora* leaves. Mineral dynamics at three stages (original substrate, harvested fruiting bodies, and spent mushroom waste (SMW)) were measured. The foliar substrates substantially enriched the substrate medium, and the 75% supplementation fortuitously contributed to maximal nutrient content in fruiting bodies, especially for nitrogen, phosphorus, potassium, calcium, and zinc. Nutrient composition of post-harvest SMW remained high indicating its use as a rich organic soil amendment. These results emphasize the twofold advantage in recycling tree foliage for mushroom production: the enhancement of nutricious yields, with a side contribution to sustainable plant residue upcycling in the framework of a circular bioeconomy.

***IndexTerms* - *Pleurotus* spp., leafy biomass, nutrient uptake, spent mushroom waste, sustainable agriculture, circular bioeconomy.**

I. INTRODUCTION

The global interest in edible mushrooms such as *Pleurotus* spp. has been growing as new technologies and applications for sustainable agricultural systems and circular bioeconomy are demanded. *Pleurotus* spp have drawn attention for their various ecological, nutritional and economical values (Chang & Miles, 2004; Royse et al., 2004). These fungi are saprophytic species that are able to colonize various lignocellulosic materials originating from agronomic, forestry, and municipal sources. Their capacity to transform low-quality organic waste into high-protein, health-promoting foodstuffs verifies the efficiency of bioconverter in integrated biodegradation and bioconversion of agro-waste recycling systems (Philippoussis, 2009). As a result of the short time needed to grow a crop, low production cost and low land requirement, *Pleurotus* mushrooms are widely cultivated. They possess efficient enzyme systems, especially lignin-degrading oxidases (laccases, manganese peroxidases, among others) that promote the degradation of complex plant polymers (Rai et al., 2005). As a result, they are capable of colonizing various substrates, such as sawdust, corncobs, rice straw, and tree leaf litter, and converting such waste materials into nutritionally improved fruiting bodies.

Nutritionally, *Pleurotus* spp are recognized as functional food because of their significant content of proteins, dietary fiber, vitamins (especially B-complex) and essential minerals. Most importantly their bioactive components such as β-glucans, phenolics and ergothionein have been linked with antioxidant,immunomodulatory, antimicrobial, anti-inflammatory and antitumor effects (Wasser & Weis 1999; Patel et al., 2012; Borchers et al., 1999). This renders them useful, not only in combating protein-energy malnutrition, but also in some non-communicable lifestyle diseases, all over the developed and developing world. Nonetheless, mushroom productivity and nutritive quality are highly associated with the growth substrate chemical composition, which includes not only the macronutrient balance of nitrogen (N), phosphorus (P), and potassium (K) but also the balance of important micronutrients like calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) (Zadrazil & Grabbe, 1985; Ahlawat et al., 2007). Although tree-based leafy residues are plentiful and include high contents of structural carbohydrates (i.e., cellulose and lignin) the variability among them in bioavailable nutrients are often not enough to guarantee optimal fungal metabolism. Moreover, with the objective of overcoming this drawback, substrates are usually supplemented with organic supplements, such as husk of legumes, bran of wheat, poultry manure or biofertilizers. However, excess nutrient addition may affect microbial activity, contaminant production, and substrate pH and carbon to nitrogen (C:N) ratios, leading to poor yields and ineffective fruiting (Philippoussis, 2009, Sharma et al., 2013).

Substrate optimization is an increasingly recog nized discipline and a large number of reports have been published on this topic, with an emphasis on increasing yield as well as some morphological characteristics (cap size, biological efficiency) of the fruiting body (Pathmashini et al., 2008; Sharma et al., 2013). Only few authors have systematically investigated the nutrient dynamics

through the growth cycle of the fungi, that is, from early substrate preparation to mushroom development, exclusion of the mushrooms, and analysis of the spent mushroom waste (SMW). This information void is especially serious as a broad nutrient profile could shed light not only on mineral uptake by mushrooms, but also on the potential agricultural value of SMW as a secondary bioresource. The recycling of nutritionally valuable SMW for organic soil amendments can reduce the dependency on chemical fertilizers, increase soil fertility and structure and also stimulate the activity of specialized microorganisms in degraded soils (Mikiashvili et al., 2024). In addition, it is consistent with the concept of a zero farm waste and circular economy as in the second approach the agro-waste is re-integrated within production-use system cycles (Hasan & Abdulhadi, 2023). So, the evaluation of mineral status of SMW post cultivation is important to understand whether this water is suitable for crop production or soil cleaning activities.

In line with above, the present investigation was under taken to study the mineral dynamics linked with the growth of *Pleurotus* spp. on leaves of the trees *Swietenia macrophylla*, *Gliricidia sepium*, and *Sesbania grandiflora*. These were chosen because they were locally available, grew rapidly not to mention previous reports of NPK rich mineral contents (Bajpai et al., 2017). Importantly, our approach is the first to study nutrient profiles of substrates in a 3-fold manner: (1) before the cultivation process (substrates composition), (2) as an output (harvested mushrooms), and (3) after the process (nutrients left in SMS). We believe that the present study is the first to investigate these particular substrates in this integrated way. Through characterizing nutrients flow and transformation dynamics in these stages the study intends to refine substrate formulations for improved yields and quality of mushrooms and for sustainable reuse of post-cultivation residues. Results are anticipated to inform best practice for sustainable mushroom farming, in particular for tropical and subtropical regions possessing amounts of tree biomass as waste resource.

II. MATERIALS AND METHODS

2.1 Cultivation of *Pleurotus* spp.

The strain of *Pleurotus* spp. used in this study was acquired from the Regional Agricultural Research Station's Division of Microbiology, Mushroom Research Laboratory in Tirupati, India. The culture was maintained on potato dextrose agar (PDA) slants and subcultured regularly. The sorghum grains were treated with the fungus to be used as spawn. The grains were boiled in a water bath for 15 minutes, and then they were mixed with calcium sulphate (2% w/w) and calcium carbonate (4% w/w). Three hundred grams of grain were placed in polythene bags that were 40 microns thick and had a 1000g capacity. After autoclaving for 30 minutes at 15 psi (pounds per square inch), the bags were let to cool to room temperature. After the sterile grains were combined with the mother spawn grains, they were cultivated for 20 days at 28±2°C. The grains were completely covered by white mycelium to create mushroom beds. The technique described by Bano et al., (1963) was used to cultivate *Pleurotus* spp. with modifications. The *Swietenia macrophylla* (SM), *Gliricidia sepium* (GS) and *Sesbania grandiflora* (SG) plant leaves were mixed with the well-dried paddy straw (on a dry weight basis) in different proportions (25%, 50%, 75%, and 100% v/v plant leaves residues). The mixture was then chopped into pieces that were 2-3 cm long and left to soak in water for overnight. The slightly moist casing substrate was sterilized for 30 minutes at 121°C after the excess water was drained, and then it was left to cool to room temperature. The casing substrate was inoculated with 30 grams of spawn per kg. Following the making of tiny holes in the spawning beds, they remained 15 days at 28±2°C and 70±5% relative humidity in a dark chamber. The cropping room, which kept the temperature at 24±2°C and the relative humidity at or above 90%, was where the fully enclosed beds were relocated. Watering was done twice daily in the cropping room; the day before the first harvest, watering was skipped over. The first crop of mushroom fruit bodies was harvested after a period of 25 days. The fruit bodies were harvested after a cycle of 60 days, the mineral dynamic in fruiting bodies and spent mushroom waste, were determined. With aforementioned four proportions and three replicates, a factorial experiment was set up in a fully randomized design.

2.2 Nutrient (elemental) Analysis

The representative samples were extracted from three different origins viz., (i) pre-cropping casing substrate before inoculation, (ii) fruiting bodies, and (iii) spent mushroom substrate at the end of cropping. All samples were oven-dried at 60 °C to constant weight, and then ground into fine powder with a stainless-steel mill and kept in sealed cans before analysis. The dried samples were digested in a tri-acid solution composed of HNO_3, H_2SO_2 and $HClO_2$ in a ratio of 9:2:1 for mineral analysis. The digested products were filtered and then diluted as appropriate. The amounts of phosphorus (P) were determined by the Vanadomolybdate yellow color method and carbon (C) and nitrogen (N) were estimated with the CHN analyzer. Potassium (K) was estimated using a flame photometer (GFU2202), Further macro and microelements calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn) were determined by ICP-OES (8000 Perkin Elmer). Nutrient concentrations were expressed on a dry weight (mg/kg or %) basis. All samples were examined in triplicate for method accuracy and precision.

2.3 Statistical Analysis

All data was maintained on analysis of variance (ANOVA) using SPSS statistics software. Means were compared at 5% level of significance.

III. RESULTS AND DISCUSSION

This research has demonstrated the elemental composition of three components of mushroom cultivation system: (i) tree-based leafy substrates (*Swietenia macrophylla*, *Gliricidia sepium* and *Sesbania grandiflora*) (ii) nutrient supplemented casing substrates for *Pleurotus* spp. cultivation, and (iii) after harvesting spent mushroom waste (SMW). The current study provided the information about the dynamics of the nutrients throughout the growth period, and suggested that the NPK rich tree-based leafy substrates can be integrated into sustainable production.

3.1 Elemental composition of leafy substrates

The concentration of elemental in *Swietenia macrophylla* (SM), *Gliricidia sepium* (GS) and *Sesbania grandiflora* (SG) has been summarized in Table 1. In comparison to the three species, the highest carbon concentration was observed in SM (48.63±1.31%), followed by GS (45.60±1.58%) and SG (42.71±1.18%). Higher carbon concentration in SM is likely related to higher relative proportion of structural polymers (lignin and cellulose and hemicellulose) that are more resistant to microbial degradation (Palm et al., 2001; Tian et al., 1992). As a consequence, high C-content substrates such as SM tend to have slow mineralization with long carbon retention in the soil, and have potential to be used in soil amendment toward long-term efforts of organic matter accretion (Zhang et al., 2014). Nitrogen composition also differed markedly among species, GS (4.13±0.12%) and SG (3.65±0.09%)

containing more nitrogen than SM (2.28±0.05%). These levels are either similar or better than the popularly used agricultural residues: soybean straw, maize stalks, and groundnut haulms which contain less than 2% nitrogen (Obodai et al., 2003; Fanadzo et al., 2010).

In mushroom cultivation, higher content of nitrogen is of great value because it plays a major roles to support the metabolism of the fungi and play essential roles in mycelial growth, enzyme synthesis and sporophore formation (Giller, 2001; Chang & Miles, 2004; Ahlawat et al., 2007). In terms of mineral contents, SG contained the highest amounts of calcium (1.40±0.11%), magnesium (0.52±0.03%), iron (249.4±9.87mg/kg), and zinc (70.31±1.99 mg/kg), indicating that it was a mineral-enriched substratum, which would be suitable for high-humidity and temperature regulated environments like mushroom casing layers. These micronutrients are indispensable cofactors in fungal where enzymatic systems, oxidative metabolism, structural integrity, and sporulation processes (Kalac, 2010; Gutiérrez et al., 2009; Kumhomkul & Panich-Pat, 2013). However, the contents of phosphorus and potassium in GS and SM were higher, where SM had the highest potassium, 1.65±0.16%. It should be mentioned that potassium promotes sporophore (fruiting body) formation in *Pleurotus* spp. by controlling water movement and carbohydrate relocation (Zhu et al., 2013). In general, macronutrients are involved in nucleic acids metabolism, ATP synthesis, and osmotic regulation and thus also central to plant and fungal physiology (Sharma et al., 2013; Uzun, 2004). Analyzing the overall mineral nutritional profiles, the SM, GS and SG offer good potential to be used for production of bioresource substrates in integrated nutrient management. High content of nitrogen and elements useful for plants make them especially interesting in sustainable mushroom production and in other bio-based agricultural use. These results corroborate earlier studies recommending the use of nitrogen fixing or NPK rich trees such as *Swietenia*, *Gliricidia* and *Sesbania* for the organics waste valorization and the composition of a substrate (Reddy et al., 2013; Mkhize et al., 2020).

Table 1: Elemental analysis including carbon and nitrogen in NPK rich tree leafy substrates

Elements	***Swietenia macrophylla* (SM)**	***Gliricidia sepium* (GS)**	***Sesbania grandiflora* (SG)**	**F ($p<0.05$)**
Carbon (%)	48.63±1.31	45.60±1.58	42.71±1.18	28.14
Nitrogen (%)	2.28±0.05	4.13±0.12	3.65±0.09	663.57
Phosphorus (%)	0.18±0.06	0.27±0.05	0.25±0.08	3.68
Potassium (%)	1.65±0.16	1.58±0.13	0.76±0.04	99.9
Calcium (%)	1.10±0.09	1.30±0.14	1.40±0.11	10.55
Magnesium (%)	0.30±0.02	0.45±0.05	0.52±0.03	59.84
Iron (mg/kg)	119.6±9.55	212.4±8.08	249.4±9.87	316.99
Zinc (mg/kg)	45.37±1.92	69.07±2.03	70.31±1.99	302.16

3.2 Elemental dynamics in mushroom fruiting bodies

Mineral composition of *Pleurotus* spp. mushrooms grown with the use of different combinations (25–100%) of NPK-enriched leafy biomass as casing substrate has been presented in Table 2. The content of carbon (%) in mushroom fruiting bodies increased from 39.3±1.57% (at 25% casing substrate) to a maximum of 42.3±1.14% (at 75% casing substrate), and then decreased slightly to 41.0±1.06% (at 100% casing substrate). It follows that incorporation of fungal biomass of organic matter was enhanced by increasing availability of nutrient in the substrate, up to an optimum level. Nevertheless, at high casing concentrations, the excess organic material may hinder the aeration of substrates or favor competition, which may in turn hinder carbon assimilation efficiency (Royse et al., 2004; Zhang et al., 2014; Upadhyay et al., 2002). Nitrogen content decreased incrementally with increased casing substrate (3.5±0.11% in 25% casing substrate to 3.0±0.13% in 100% casing substrate). This tendency may be due to 'N' dilution due to higher fungal biomass or immobilisation of competing microorganisms in the denser substrates (Mane et al., 2007; Philippoussis, 2009). Notwithstanding the decrease in these components, all the values for all the formulations were within the acceptable range for mushroom yield (high quality mushroom), as nitrogen is important for fungal enzymatic and protein synthesis (Chang & Miles 2004; Akinyele & Akinyosoye, 2005).

The contents of phosphorus and potassium were also enhanced with increasing proportions of casing substrate, with maxima of 0.35±0.02% and 1.60±0.06% at 75% casing substrate. These macronutrients are obligatory for metabolic processes such as production of ATP, synthesis of nucleic acids, osmotic balance and cell division during the fruiting body formation (Crisan & Sands, 1978; Sharma et al., 2013; Jiskani, 2001). Highest peaks at 75% casing indicated that it has been the optimal ratio with better nutrient availability for rapid growth of mushroom. Calcium and magnesium contents were also higher at the increased levels of casing substrate (0.90±0.10% and 0.40±0.04%, respectively). Both elements are important secondary macronutrients for structural stability of fungal cell walls, mycelial development, and are involved as cofactor in enzymatic reaction (Gutiérrez et al., 2009; Kalac, 2010). Their enrichment also emphasizes the potential of nutrient-rich leafy substrates to efficiently develop productive mushrooms.

Micronutrient content, including iron and zinc, increased with increasing substrate composition. Fe(II) contents had increased from 160.4±7.23 mg/kg (25% casing substrate) to 185.1±11.8 mg/kg (75% casing substrate), zinc had reached a maximum concentration of 62.71±3.02 mg/kg. These elements are part of electron transport chains, antioxidant enzymes and other ash-required synthesis of vital fungal proteins (Mattila et al., 2001; Kalac, 2010; Kumhomkul & Panich-Pat, 2013). Their occurrence in fruiting bodies does not only improve fungal metabolism, but also increases the nutritional and functional food quality of *Pleurotus* mushrooms. Collectively, these results corroborate previous assertion that optimized casing substrates can improve the biochemical and nutritional composition of edible mushrooms (Royse et al., 2004; Sharma et al., 2013; Pathmashini et al., 2008). It appeared that the 75% casing substrate proportion balances well on nutrient supply and aeration of substrate and is suitable for high-yield, and high-nutrition mushroom cultivation at a controlled environment.

Table 2: Nutrient (elemental) uptake by *Pleurotus* spp. cultivated on NPK rich tree leafy substrate

Elements	25% Casing Substrate	50% Casing Substrate	75% Casing Substrate	100% Casing Substrate	F (p<0.05)
Carbon (%)	39.3±1.57	40.0±0.98	42.3±1.14	41.0±1.06	6.96
Nitrogen (%)	3.5±0.11	3.4±0.21	3.2±0.19	3.0±0.13	10.80
Phosphorus (%)	0.30±0.04	0.32±0.03	0.35±0.02	0.34±0.03	3.10
Potassium (%)	1.20±0.09	1.35±0.11	1.60±0.06	1.50±0.12	19.2
Calcium (%)	0.75±0.07	0.82±0.06	0.90±0.10	0.88±0.13	3.09
Magnesium (%)	0.30±0.06	0.35±0.09	0.40±0.04	0.38±0.03	3.19
Iron (mg/kg)	160.4±7.23	170.2±9.98	185.1±11.8	180.6±10.5	7.29
Zinc (mg/kg)	55.32±2.44	58.95±3.81	62.71±3.02	60.83±1.57	7.44

3.3 Elemental composition of spent mushroom waste (SMW)

The chemical composition of spent mushroom waste (SMW) of *Pleurotus* spp. growth was substantial and slightly less than the beginning casing composts and harvested mushrooms (Table 3). The carbon content was significantly reduced (between 29.8±2.28% in 50% casing substrate derived SMW and 24.6±1.35% in 100% casing substrate derived SMW), indicating that organic substrates were actively decomposed and much carbon was assimilated into the fungal mycelium during the colonization and fruiting stages (Royse et al., 2004; Zhang et al., 2014). A decrease of the residue of carbon is typical for efficient biodegradation by fungal enzymes, and it is part of the metabolic transformation and microbial respiration (Philippoussis, 2009; Akinyele & Akinyosoye, 2005). The nitrogen level was 0.67±0.07%-0.84±0.03%, much lower than that in the original casing materials. It is also possible that the reduction in 'N' is due to uptake of' 'N' into the fungal mycelium for protein synthesis and sporophore formation, and the loss of 'N' by volatilization or by microbial immobilization in the substrate (Giller, 2001; Chang & Miles, 2004; Jang et al., 2003). Nevertheless, they are still of agronomic interest, especially in organic and low input systems where residual fertilisers such as these can be returned to the field as part of a waste-recycling strategy in order to improve soil fertility and structure (Ahlawat et al., 2007; Zied et al., 2011). Notably, P and K concentrations did not decrease or increased slightly among the treatments. The highest values for 0.22± 0.03% P and 1.10±0.03% K were present in the 75% casing substrate derived SMW. This observed relative persistence might result from a lower mobility and mineralization rate of these nutrients, compared with nitrogen, which makes them still available in the soil after the harvest (Ahlawat et al., 2007; Sharma et al., 2013). Those nutrients are essential for energy metabolism, root development and osmotic regulation in plants, so 75% casing substrate derived SMW can be successfully used as a part of organic nutrient management programs (Muley et al., 2020).

Table 3: Nutrient (elemental) retention in spent mushroom waste (SMW) of *Pleurotus* spp. grown NPK rich tree leafy substrate

Element	25% Casing Substrate Derived SMW	50% Casing Substrate Derived SMW	75% Casing Substrate Derived SMW	100% Casing Substrate Derived SMW	F (p<0.05)
Carbon (%)	28.4±1.54	29.8±2.28	27.0±1.19	24.6±1.35	10.91
Nitrogen (%)	0.71±0.04	0.84±0.03	0.70±0.05	0.67±0.07	13.73
Phosphorus (%)	0.15±0.02	0.18±0.04	0.22± 0.03	0.20±0.02	6.48
Potassium (%)	0.80±0.05	0.90±0.07	1.10±0.03	1.00±0.08	27.21
Calcium (%)	0.60±0.02	0.65±0.03	0.75±0.02	0.70±0.04	30.30
Magnesium (%)	0.22±0.04	0.25±0.02	0.30±0.02	0.28±0.03	8.90
Iron (mg/kg)	130.4±6.54	140.6±5.72	150.2±4.75	145.8±7.83	10.94
Zinc (mg/kg)	40.26±1.15	43.68±1.29	47.83±1.01	45.17±1.44	30.34

Meanwhile, the concentration of calcium (0.75±0.02%) and magnesium (0.30±0.02%) also remained moderate across all treatments. It indicates that they were partially absorbed by the mushrooms and held by the substrate matrix, which might be because of their low solubility or absorption into unhydrolyzed lignocellulosic structures (Gutiérrez et al., 2009; Gupta et al., 2018). Both of them are known to be important for enzymatic activation, cell wall structure and soil pH regulation, and are therefore desirable components of composted organic matter. Some of the mineral contents were also quite high; e.g., iron was as high as 150.2±4.75 mg/kg and zinc was up to 47.83±1.01 mg/kg in 75% casing substrate derived SMW. These minor elements are essential for microbial metabolism, enzyme cofactor action as well as physiological development in plants such as chlorophyll formation and reduction of oxidative stress (Kalac, 2010; Mattila et al., 2001). Their persistence in SMW also confirms its potential as a bioavailable N-rich organic input. The nutrient balance of SMW reinforces its value as a secondary organic amendment. Its use in the post-harvest stage is also in line with circular bioeconomy viewpoint as it keeps nutrients cycle, waste minimize and sustainable agriculture by improving soil fertility and structure (Liu et al., 2018; Owaid & Abed, 2019). The provision of SMW in the composting system or to the field directly may potentially be beneficial in the frame of integrated nutrient management for improved crop productivity and sustainable environmental resilience.

IV. CONCLUSION

The present study investigated element contents and nutrient translocation in a sustainable *Pleurotus* spp. cultivation system on leafy substrate enriched with NPK using *Swietenia macrophylla*, *Gliricidia sepium* and *Sesbania grandiflora* as the casing materials. Of the substrates, *S.macrophylla* had the highest carbon content, but that of *G.sepium* and *S. grandiflora* was richer in nitrogen and minerals, supporting fungal metabolism and growth. The increase in the nutrient uptake of the mushroom fruiting bodies with

increasing level of substrate enrichment reached its maximum at 75% casing substrate. This proportion provided optimum quantities of carbon, phosphorus, potassium, calcium, magnesium and essential micronutrients necessary for increased yield and quality. The SMW after harvesting, contain significant amounts of phosphorus, potassium, calcium, and other elements indicating its importance as a natural organic amendment for soil. These results advocate the involvement of NPK-rich leafy biomass and SMW recycling in circular bioeconomy approaches, which contribute to sustainable cultivation of mushroom and nutrient management in the agroecosystems.

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