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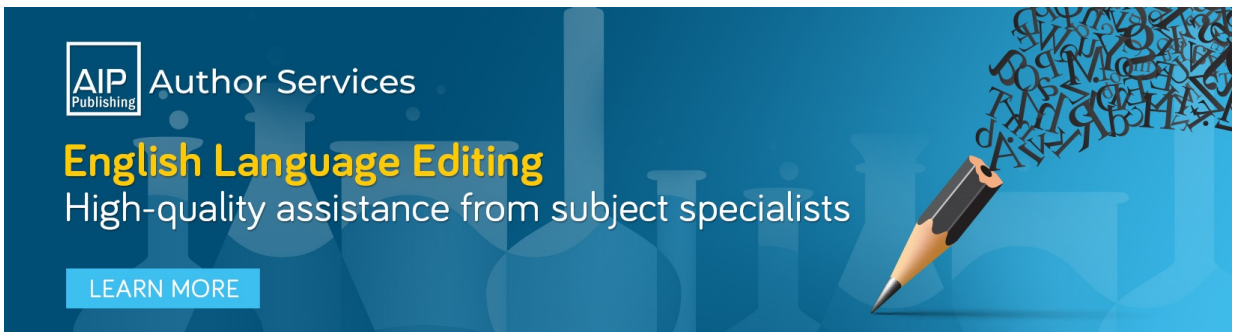

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Evaluating Carbon, Nitrogen and Heavy Metal Content in Different Agriculture Biomass for Mushroom Substrate

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Abstract. A substrate is an essential requirement for mushroom cultivation as it provides the nutrients for the growth of the mushroom mycelia to produce fruiting bodies. Abundant agricultural biomass such as oil palm waste, paddy straw, timber and sugarcane bagasse, lead to the uses for the mushroom substrates. These abundant biomasses are very less used for human activities whereas the remaining part refers to agriculture waste. The agriculture waste can be used as alternative substrates for mushroom cultivation due to the commercial substrates of rubber sawdust prices have been increasing that can affect the cost of the mushroom production. However, choosing different alternative substrates will have a different effect on the yields and quality of the mushrooms. The mushroom substrates mainly composed of the sources of carbon and nitrogen that act as the essential element for the growth of the mushroom. Apart from the variation of carbon and nitrogen content in the mushroom substrates, the substrates might contain toxic metal that can contaminate the fruiting bodies through the nutrient uptakes. Mushrooms have been observed to absorb and store variety of chemical elements where they can lead to contamination towards the edible fruiting bodies. Hence, this study choose different agricultural biomass of paddy straw and oil palm frond with the commercial substrate of sawdust in order to analyse the content of the substrates for mushroom cultivation suitability. Results were demonstrated that paddy straw have potential as alternative substrates for mushroom cultivation based on the C/N ratio value (0.5907) due to the high nitrogen content (84.92±9.71%) whereas mushroom need low C/N for better growth. The heavy metal content for paddy straw is follow WHO/FAO safe limit quantity Cu, Fe, Zn and Pb were 0.1433mg/L, 1.5993mg/L, 0.8090mg/L and 0.0233mg/L, respectively. However, arsenic content for paddy straw recorded the lowest amount between the treatments which is 2.0423mg/L and nearly reaching the permissible limit.

INTRODUCTION

The agricultural sector becomes one of the contributors to the economy of the country. Due to the increasing of the agriculture sector, it generates crop yields that lead to an increase of agricultural biomass, such as paddy straw, coconut husk, corn silage, sugarcane bagasse, etc. According to Tambichik et al. [1], agricultural and construction industries were growing year by year and considered agricultural waste because significantly less of this biomass is used as a by-product. Malaysia disposed of about 1.2 million tonnes of agricultural waste into landfills annually [2] and there are large amounts of unused lignocellulosic by-products available in the tropical and subtropical areas [3]. Poor management of agricultural waste can increase various problems, especially environmental pollution. For example, the burning of paddy straw is one of the most severe problems which can cause environmental pollution. Agricultural wastes are generated in large amounts worldwide and often cause environmental and health issues. In order to control the environmental pollution caused by the unpractically agro-waste management, the waste is reused as a substrate of mushroom cultivation. The usage of agricultural waste as the substrates for mushroom cultivation can lead to agriculture and agro-industrial waste management [4].

There are many types of agricultural residue available in the world. The main component of the agriculture residue is the lignocellulose, where the lignocellulose itself consists of cellulose, hemicellulose, and lignin [5]. These three major structural components are available in the entire vascular plants where it has the function of structural support systems. The primary lignocellulose agriculture residues are paddy straw, wheat straw, barley straw, corn stover, sorghum stalks, coconut husk, sugarcane bagasse, oil palm wastes, pineapple skin and banana leaves [6]. However, the agricultural residue can be easily breakdown with the help of the decomposition process by decomposers such as fungi [7]. Lignocellulose materials occur in paddy straw and oil palm frond for carbon

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sources that can help the mushroom growth and increase the potential for alternative substrates for the mushroom cultivation. In this study, detection and comparison between different agricultural biomass of paddy straw and oil palm frond with the commercial substrate were made based on the composition of carbon, nitrogen, and heavy metal in the agriculture waste for alternative as mushroom substrates.

MATERIALS AND METHODS

Collection and Preparation of Samples

The agriculture biomass used were sawdust of rubber tree, paddy straw and oil palm frond. The sawdust of the rubber tree was purchased from a saw mill in Perak, Malaysia while for paddy straw was collected from a paddy grower in Bachok, Kelantan. Oil palm frond (OPF) was collected from the oil palm estate in Felda Kemahang, Tanah Merah, Kelantan. For the sawdust, the ground sawdust was sun dried for 24 hours to remove moisture that can caused fungus infestation towards the sawdust while for paddy straw, it was cut for 5cm long using a cutter. Oil palm frond was cut into blocks using machine cutter and the OPF size were reduce into 5cm long using machete. The paddy straw and OPF were cleaned using deionised water in order to remove any debris and the excess water. The raw samples were weighed using analytical balance model Sartorius BT224S for 100g and were dried in oven at 70ºC to a constant weight. For paddy straw and OPF, the raw samples were ground using miller until small size then were proceed to electric grinder for fine particles. The samples (i.e., sawdust, paddy straw and OPF) were ground using electric grinder and were sieved using siever (1.0mm). The fine samples were stored in polypropylene plastic air tight container at room temperature (25ºC) before analysed.

Analysis of Total Organic Carbon in Agriculture Biomass

The total organic carbon analysis was followed by the loss on ignition method by Mathiessen et al. [8]. First, the empty crucibles without lid were weighed (W_c). The powdered samples were weighed about 1g (W_i) and placed into the crucible. The crucibles were ashed in a 360 ºC furnace for 4 hours with the lid on. After 4 hours of ashing, the temperature of the furnace was reduced until it reached 100ºC before taking the samples out and placing them into the desiccator before reweighed. The crucibles without lid were reweighed (W_f) after fully cooled. The total organic carbon was calculated using the following formula:

$$\text{Total organic matter (\%)} = \frac{(Wi+Wc)-Wf}{Wi}\ x\ 100 \quad (1)$$

*where W_i is the initial weight of the samples without crucible, W_f the is final weight of samples with the crucible, W_c is the weight of the crucible

$$\text{Total organic carbon (\%)} = \text{organic matter x } 0.58 \quad (2)$$

Total Nitrogen Analysis in Agriculture Biomass

The total nitrogen analysis standard methods outlined by the Association of Official Analytical Chemists (AOAC) [9] using the Kjeldahl method. The digestion process was done using Foss Digestor 2508. Approximately 0.5g of the powdered samples were weighed and placed in a digestion tube with 2g of Kjedahl tablet in 12mL of concentrated sulphuric acid. Blank sample were done by placing 2g Kjedahl tablets and 12ml of concentrated sulphuric acid without sample into the digestion tube. Then, the tubes with samples and blank were digested at 400ºC for 1hour 30 minutes. During the digestion process, the Kjeldahl tablets act as a catalyst in order to increase the reaction rates. The digestion process was done when the sample solution turn from black to clear blue or green solution. Then, the samples with the digestion tubes were cooled for 15 minutes on the plates. About 80mL of distilled water was poured into each digestion tubes followed by 50mL of 40% sodium hydroxide.

The samples proceeded to the distillation process that was done using Gerhardt Vapodest 30s. The digested samples were distilled in 330mL of 4% boric acid, 3.3mL bromocresol green and 2.31mL methyl red. The distillation process was done for 3 minutes where the distillation process turned the red coloured boric acid into the green. Dark green indicates the nitrogen been transferred from the samples to the boric acid mixture. The mixture was titrated against 50mL of 0.1M hydrochloric acid where the solution changed from green to pink for the final colour as the end point for the titration process. The titrates reading were recorded and the percentage of the total nitrogen was obtained by using the formula:

$$\text{Percentage of total nitrogen} = \frac{(\text{ml of HCl sample} - \text{ml of HCl blank sample}) \text{x Molarity of HCl x } 1.4007}{\text{weight of sample (g)}} \; x\; 100 \quad (3)$$

Analysis of Heavy Metal in Agriculture Biomass

The analysis of heavy metal for the samples was followed the dry ashing method by Gebrelibanos et al. [10]. The powdered samples were weighed approximately 0.5g intro crucible and ashed in the furnace at 500ºC for 4 hours until a white or grey ash residue was obtained. The residue was dissolved in 2mL 69% nitric acid and filtered into a 50mL volumetric flask using Whattman paper no four sized 90mm. The deionised water was used to wash the residue on the filter paper to make sure all of the element enter the volumetric flask. Then, the volumetric flask was made up until mark with deionised water. For blank sample, the method was the same but the sample was not included. The samples were stored in chiller at 4ºC. Then, dilution process was done by transferring the samples from 50mL volumetric flask into 50mL polypropylene tube. From the 50mL stock samples, 15mL of the samples were transferred into 15mL polypropylene tube using 15mL measuring cylinder. Then, four times serial dilution process were done by transferring 1.5mL of samples into 15mL polypropylene tube using 1000µL micropipette into 15mL polypropylene tube containing 13.5mL of 5% nitric acid. Then, the samples were analysed for zinc (Zn), copper (Cu), iron (Fe) and lead (Pb) analysis using Atomic Absorption Spectroscopy (AAS). While for arsenic (As) element analysis in the samples, about 1mL from the stock samples were transferred into test tubes. Then, 1mL of concentrated hydrochloric acid was added into the same tubes followed by 1mL of 5% KI + ascorbic acid solution. Next, the samples were placed at ambient temperature for 45 minutes and were diluted using 7mL of deionised water. After that, the samples were undergone four times serial dilution. The samples were analysed using Atomic Absorption Spectroscopy (AAS) for arsenic detection. The results were obtained and recorded.

Statistical Analysis

All of the data were subjected by multiple comparisons of the ANOVA test and were ranked by Duncan's homogeneity subsets if the F test will be significant at the 95% probability level by using Statistical Package for Social Science Software (SPSS software) (IBM SPSS Statistics Version 21).

RESULTS AND DISCUSSION

Total Carbon Contents

Carbon is one of the main macronutrient for mushroom. The results for carbon content in agriculture biomass (i.e., sawdust, paddy straw and oil palm frond) are shown in Table 1. The result shows that saw dust recorded the highest amount of carbon content followed by oil palm frond, and paddy straw for 56.89±0.12%, 55.19±0.01% and 50.17±0.17%, respectively. There is in agreement with the result reported by Dayegamiye et al. [11], where sawdust carbon content was the highest (55.2%) among the other agriculture materials. Sawdust mainly composted by carbon which is 60.8% [12]. This could be due to the lignin-cellulosic character in the agriculture biomass. Lignocellulosic materials are made up of carbon, hydrogen and oxygen [13]. The lignin-degrading fungi or the mushroom utilised the carbon by using the lignocellulolytic enzyme to breakdown the lignin of the agriculture biomass [14]. High in carbon content makes the lignin degradation in sawdust easier, where the saw dust decomposition activities in sawdust become more efficient [15].

TABLE 1. Total carbon and nitrogen content in each of the agriculture biomass for mushroom substrates

Agriculture Biomass	Carbon Content (%)	Nitrogen Content (%)
Sawdust	56.89±0.12	28.52±1.54
Paddy straw	50.17±0.17	84.92±9.71
Oil palm frond	55.19±0.01	34.33±6.48

Total Nitrogen Contents

Besides carbon, nitrogen is also an essential macronutrients required by fungi especially for the mushroom for structural and energy requirements [16]. Substrates with high organic or inorganic nitrogen contents can help in increasing the mushroom yield [17]. The results of total nitrogen content in (sawdust, paddy straw and oil palm frond) are shown in Table 1. This study shows that paddy straw had the highest amount of total nitrogen followed by oil palm frond and sawdust for 84.92±9.71, 34.33±6.48 and 28.52±1.54 respectively. The fungi can easily use nitrogen because the absorption of these molecules is more energetically efficient than synthesising the molecules which allow the fungi to obtain more energy for mycelial growth and mushroom formation.

Although a high amount of nitrogen can help on growth of the mushroom, high doses of nitrogen also can cause thermogenesis which is increasing of temperature. A high temperature is not suitable for the mycelium growth where it is sufficient enough to kill the mycelium. The optimum temperature for mycelium growth is at 15-35^{o}C depending on the nutrient medium used [18]. Therefore, paddy straw can be utilised without the addition of supplement to cultivate the mushroom. Low in total nitrogen content shows that lignocellulosic materials such as saw dust and oil palm frond are low in protein content, which also affect towards the mushroom cultivation [19]. From the result, saw dust recorded the lowest total organic nitrogen content which is 28.52±1.54%. In order to get sufficient amounts of nitrogen, phosphate, potassium and vitamins for the mushroom growth, different supplements or additives are required. Rice bran usually been used in commercial sawdust substrate as supplements towards the mushroom substrates in order to increase the biological efficiency of the mushroom. In Malaysia, commercial cultivation of *Pleurotus* sp. usually by using sawdust from rubber tree as substrates supplemented with rice bran [20]. It is because high lignin substrates such as saw dust can lead to low yield production due to the resistance to degradation of the substances by the enzymes [21]. According to Fasidi et al. [22] mushroom substrates supplemented with 30% rice bran can increase the productivity of shiitake mushroom.

From the result in Table 2, oil palm frond is stated to have a higher C/N ratio (1.6074) compared to sawdust and paddy straw which are 0.9721 and 0.5907 respectively. The C/N ratio for oil palm frond exceeds the agreement from the research made by Ali et al. [23] where oil palm frond has higher C/N ratio compared to rubber saw dust. C/N ratio can influence the development of mycelium of the mushroom and the formation of the pinhead. However, low C/N ratio can lead to better production performance of fruiting bodies and the yield of the mushroom [24]. It is due to the amount of total nitrogen content in the oil palm frond is low, it lead to reduction of mycelium growth where nitrogen is the important element for the growth of the mycelium [23].

TABLE 2. C/N ratio in each of the agriculture biomass for mushroom substrates

Agriculture Biomass	C/N Ratio
Sawdust	0.9721
Paddy straw	0.5907
Oil palm frond	1.6074

Heavy Metal Analysis

The uses of fertilisers and pesticides are widely in Malaysia especially in the agriculture sector to increase the productivity [25]. This agricultural practice caused substantial amounts of waste from phosphate fertilizers or any pesticides mostly diffused into the environment that lead to the soil and water contamination. The plants can uptakes the contaminated water and soil. The contaminants can accumulate in the biomass and remained in the organism by transferring from one trophic level to another in the food chain [26]. So, in order to find an alternative mushroom substrates by using agriculture biomass, heavy metal analysis was done in this study. As shown in Figure 1, the mean concentration of copper (Cu) in oil palm frond was the highest (0.2180 mg/L) and the least was in paddy straw (0.1433 mg/L). The result from this study was found to be comparable based on the result reported by Oviasogie et al. [27] where the mean concentration of Cu on oil palm frond and leaves of 60 years palm recorded the highest mean which is 3.91 mg/L. The bioaccumulation of cu in oil palm is due to the sufficient bioavailable and reserved amount of Cu in the soils. However, low of Cu content is due to the deficiency of Cu elements in the plants. The availability of Cu in the soil also low and caused the uptake of Cu by the plant is low and leads to a deficiency of Cu [28]. The concentration of Cu for this study achieved the permissible copper value of plants by FAO/WHO 2015 which is 30mg/L [29].

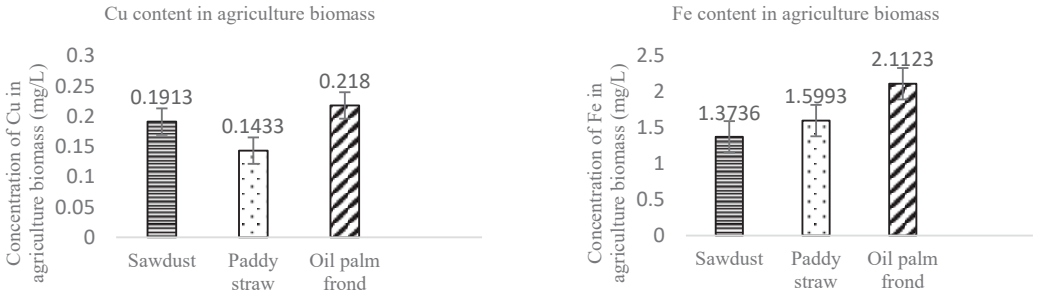

FIGURE 1. Concentration of heavy metals in samples (a) Concentration of copper (Cu) in the samples, (b) concentration of iron (Fe) in the samples

TABLE 3: FAO/WHO (2015) guidelines for metals in foods and vegetables

Metals	WHO/FAO (2015) (mg/L)	Normal Range In Plant (mg/L)
Cu	30.0	2.5
Pb	2.0	0.50-30.0
Zn	60.0	20-100
Fe	48.0	400-500
As	0.2	0.2-1.5

The result for iron content is stated in Fig. 1 where the mean concentration of iron (Fe) in oil palm frond is the highest (2.1123 mg/L) and the sawdust recorded the lowest concentration which is 1.3736 mg/L. The amount of iron content in the study is good in agreement with Olafisoye et al. [30] where the amount of iron content in oil palm sampled in Agbarho is 1.29 mg/L. The utilisation of fertiliser is one of the factors that contributes towards the iron content in the plants and become essential in chlorophyll synthesis [31]. However, the iron content recorded in this study is below the permissible level of 48 mg/L [29].

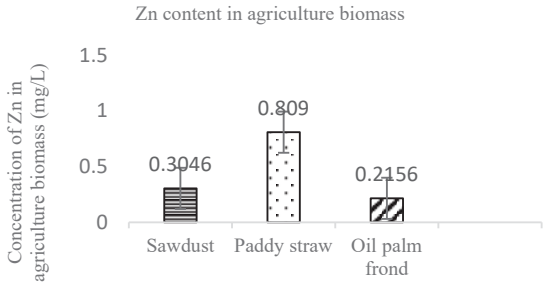

FIGURE 2. Concentration of zinc (Zn) in the samples

Zinc has important role in both primary and secondary metabolites. Mushroom can uptake the zinc ions for their growth. Fig. 2 shows the highest concentration of zinc content recorded by paddy straw which is 0.809 mg/L while the lowest zinc content recorded by oil palm frond which is 0.2156 mg/L. The paddy straw has the highest zinc content due to the application of fertiliser of zinc sulphate [32]. However, the zinc content recorded in this study is below the permissible level of 60 mg/L [29].

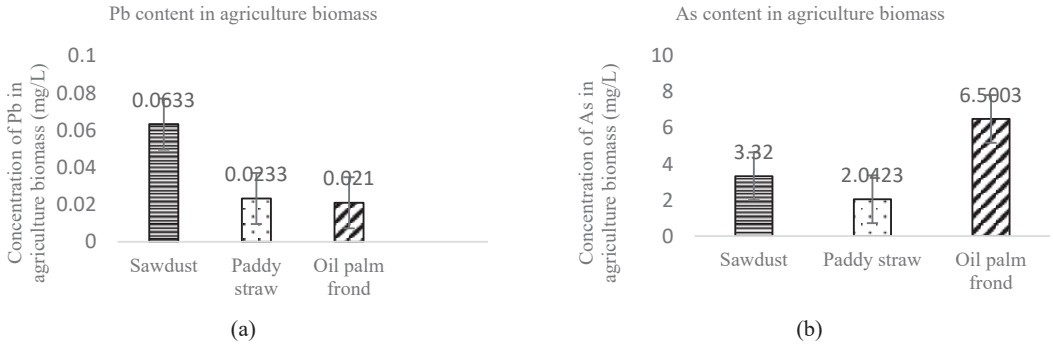

FIGURE 3. Heavy metals contents in each samples, (a) Concentration of lead (Pb) in the samples, (b) Concentration of arsenic (As) in the samples

The mushroom fruiting bodies also have a tendency to absorb the heavy metal from the substrates because they can act as an effective biosorbent of toxic metals [33]. The heavy metal concentration in the mushroom fruiting bodies varies between species and substrate composition [34]. This study shows the result for lead (Pb) content (Fig. 3) in sawdust is the highest (0.0633 mg/L) compared to paddy straw and oil palm frond (0.0233 mg/L and 0.0210 mg/L) respectively. However, lead content recorded in this study is below the permissible level of 2.0 mg/L [29]. From the result in Fig. 3, the oil palm frond is high in arsenic (As) content (6.5003mg/L) compared to sawdust and paddy straw (3.32 mg/L and 2.0432 mg/L) respectively. This study has contradicted the result with Abedin *et al.* [35] where the arsenic in paddy straw recorded the highest content which is 91.8 mg/L. The organic arsenic compound are usually used as pesticides [26]. The oil palm frond recorded the high arsenic content might due to the application of the pesticides on the palm oil plantation. The arsenic come from the pesticides and leach into the soil and been uptaken by the tree. However, arsenic content recorded for paddy straw in this study is the nearly permissible level of 0.2 mg/L [29].

CONCLUSION

It is found that the cultivation of edible mushrooms depends on the mushroom growing on the suitable cultivation substrates that are based on carbon content, nitrogen content, C/N ratio of the substrates, and the heavy metal accumulation in the substrates. Among all tested substrates in this study, it showed that paddy straw had a standard level in terms of C/N ratio (0.5907) due to the high nitrogen content (84.92±9.71%) and low carbon content (50.17±0.17%). In contrast, mushroom need low C/N for better growth. Also, the heavy metal content for paddy straw nearly at the safe limit as for the substrate for mushroom cultivation.

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