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Abstract. The environmental imbalance exerted by the continuous release of phenolic substances necessitates a return of polluted sites to natural and safe status. In this study, fungal isolates obtained from sawdust-contaminated soils were screened for laccase production capacities, using tannic acid, as an index to the bio-stimulatory potentials of the sawdust. Soil and sawdust samples collected from wood-processing plants in Morogbo-Agbara (M), Iju (I), and Oja (O) of Ado-Odo/Ota, Ogun State, Nigeria were subjected to physicochemical analysis. The phenolic content estimated using gallic acid calibration curve, showed 0.90%, 0.79% and 0.33% for the soil samples labeled MSL, ISL, OSL, respectively. Phenol content was observed to be 0.63%, 0.91%, and 0.53% for sawdust samples labeled MSD, ISD, OSD, respectively. In the same labeling order, the percentage nitrogen content was 0.77%, 0.38%, and 0.21% for soil; and 0.0025%, 0.0035% and 0.0028% for sawdust; while the percentage carbon was 0.25%, 0.62% and 0.49% for soil samples; and 88.11%, 85.56%, and 88.69% for the sawdust samples. Fungal species of *Aspergillus, Penicillium, Candida* and *Saccharomyces* among the ten isolates presented a positive reaction for laccase production by showing a brownish-black coloration. The ability of the fungal isolates to produce laccase makes them useful laccase sources for industrial and environmental applications.

Keywords: Sawdust, laccase, fungi, soil, phenolic content.

1. Introduction

Over the last decade, environmental bioremediation remains a major concern in the world. The continuous release of harmful by-products into the environment exerts devastating effects on the quality of life of inhabitants of such environments [1]. This has made it imperative to keep up research for sustainable solutions to the menace of environmental pollution. The United Nations Environmental Assembly (UNEA-3) has passed a resolution urging faster action and collaboration to combat and control soil contamination. This agreement, signed by over 170 nations, demonstrates the global importance of soil contamination and the countries' determination to find tangible solutions to address the causes and consequences of this serious concern [2].

Lignocelluloses make up the majority of agricultural wastes, and are the most abundant natural substance on the planet, yet they are underutilized [3, 4]. This material was discovered to be a rich source of organic components, including cellulose, hemicellulose, and lignin, which account for 45-55 percent, 24-40 percent, and 18-25 percent of the total composition of these residues, respectively [5]. These agro-industrial wastes are dumped into the environment, causing floods, pollution, and the ruin of the ecosystem's aesthetic quality [6]. A viable method of addressing these issues is to utilize them as bioconversions substrates into value-added products like enzymes [7]. Sawdust is a lignocellulosic waste made up of tiny wood particles produced during the cutting and shredding of wood using saws and other wood processing machinery [8]. Being the major by-product of sawmill wood processing, it may be re-processed into particle-board, used as fuel in a sawdust burner, or as a heat source for other milling processes. However, it is commonly viewed as waste and is disposed of in the environment. Improper disposal of sawdust results in pollution of the surrounding soils and water bodies. The use of sawdusts by microorganisms to produce an essential industrial enzyme such as laccase is a positive finding that will aid in pollution reduction and waste valorization [9].

Laccases are important industrial enzymes with great biotechnological potentials, belonging to a family of enzymes referred to as polyphenol oxidases, also called multicopper oxidases (MCOs) [10].

Laccases oxidize their substrates by transferring electrons to a trinuclear copper center from a mononuclear copper core [11]. Laccases have a broad industrial and environmental application range - they react with a diverse range of substrates and xenobiotics. First discovered in *Rhus vernicifera*, the Japanese lacquer tree sap, with its distinguishing metal-containing oxidase feature [12], laccases have found varied usage in food, textile, pharmaceutical, and paper and pulp industries. Laccases have since been detected in various basidiomycetes and ascomycetes fungi as well as in different bacteria and plant species. Fungal laccases account for the most significant class of multicopper oxidases based on number and characterization; they are by far the most intensively studied [13, 14]. A wide range of fungal taxa is involved in the development of laccases especially white-rot fungi – is the most widely known as the most efficient laccase producers and lignin degraders [14]. These laccases produced by fungi are responsible for physiological processes (intracellular as well as extracellular), including pigmentation, delignification, morphogenesis, stress defense, and pathogenesis [15]. Some known laccase-producing fungi include: *Trametes versicolor*, *Cerrena unicolor*, *Trichoderma harzianum*, *Pycnoporus coccineus*, etc.

The utilization of natural catalysts such as laccase for different biotechnological processes has been on expansion lately; a significant need that has come about into this is that of biodegradation of complex substances [16, 17]. In this study fungal isolates obtained from sawdust contaminated soils at wood-processing plants in Morogbo-Agbara (M), Iju (I), and Oja (O) of Ado-Odo/Ota, Ogun State, Nigeria, were screened for laccase production capacities, as an index to the bio-stimulatory potentials of the sawdust.

2. Methodology

2.1. Sampling

Sawdust and Sawdust-polluted soils were obtained from sawmills in Morogbo-Agbara, Iju and Oja in Ota, Ogun State, Nigeria. It is located along 6.50437ºN, 3.09267ºE, 6.6802°N, 3.1407°E, and 6.6691°N, 3.2741°E. Samples were labeled according to the locations from which they were obtained: M, I and O for Morogbo-Agabra, Iju and Oja, respectively. This was followed by the labeling as soil or sawdust sample. Soil samples were abbreviated as 'SL' and Sawdust samples abbreviated as 'SD'.

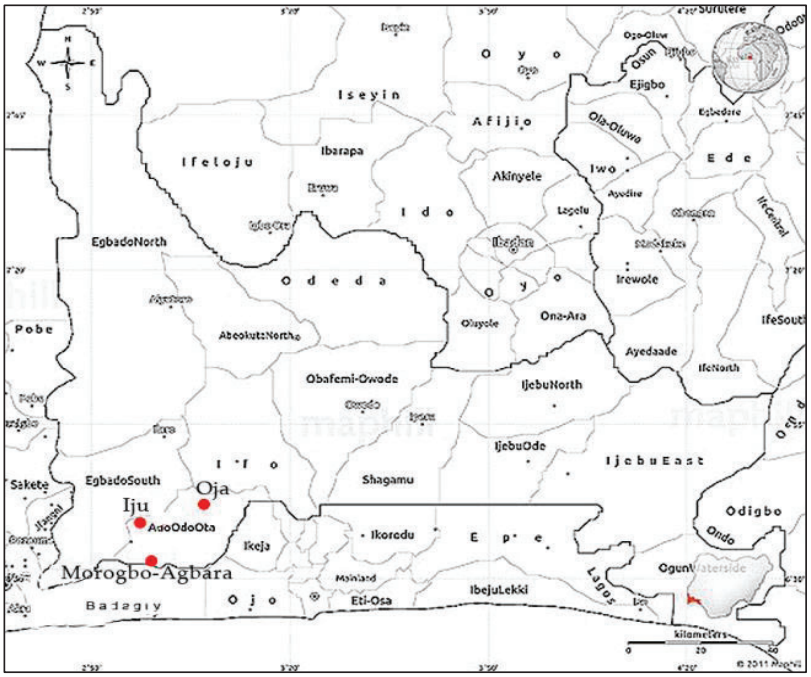

Figure 1: Map showing sampling sites (source: www.maphill.com)

Figure 2: Sawdust and sawdust-contaminated soil samples

The common types of wood processed in these sawmills include *Hevea brasiliensis* (rubber wood), *Terminalia superba* (Frake or African limba wood), *Terminalia ivorensis* (Idigbo), *Tectona grandis* (Teak), *Triplochiton scleroxylon* (Obeche), *Milicia excels* (Iroko), *Afzelia africana* (Apa or African Mahogany).

2.2. Carbon, Nitrogen and Phenolic Content Analyses

The soil and sawdust were subjected to physicochemical analysis to determine the Carbon, Nitrogen, and Phenolic content using standard methods.

2.2.1. Carbon content. The loss on ignition procedure was applied to analyze carbon content, with 5 g of the sample cooked to between 350ºC and 440ºC overnight in a ceramic crucible placed inside a muffle furnace [18, 19]; and weighed after cooling in a desiccator. The weighted difference between the initial and end samples divided by the initial sample weight, and multiplied by 100 percent, was used to deduce the organic matter content. Before calculating the organic matter content, all weights were adjusted to account for moisture content.

2.2.2. Nitrogen Content. Percentage nitrogen content in soil samples was estimated using the Kjeldahl method [20, 21]. Samples were digested with concentrated sulfuric acid to convert Nitrogen in the samples to $(NH_4)_2SO_4$. Nitrogen content was determined by distillation and titration using sodium hydroxide and boric acid with mixed indicators.

2.2.3. Phenolic Content. The overall phenolic content of the soil and sawdust samples was evaluated using the Folin-Ciocalteu method (F-C reaction) [22] and represented as mg GAE/g DW

2.3. Isolation of Fungi

Malt extract agar (MEA) and potato dextrose agar (PDA) were employed in fungal isolation. To limit bacterial growth, 2% (w/v) glycerol was added to the medium. Isolation of fungi involved culturing serial dilutions of samples in triplicates on PDA and MEA plates supplemented with 2% glycerol, before incubation at 25 ± 2 ^{0}C for 5-7 days [23, 24]. The fungi were sub-cultured repeatedly on PDA till pure cultures were obtained.

2.4. Screening for Laccase Production

The indicator compound used in screening for laccase producers was tannic acid at a molarity of 4 mM; a modification of the method according to Kiiskinen et al. [25]. Before autoclaving, tannic acid was introduced to PDA medium. Fungal laccase producers were screened from soil samples on agar plates containing media and indicator compound, incubated for 7 days at 25 ± 2 ^{0}C. Plates containing media without indicator compound were also used to culture fungi as a control to differentiate positive and negative reactions.

3. Results

3.1. Carbon, Nitrogen and Phenolic Contents

The percentage nitrogen, phenolic an carbon contents of both soil and sawdust samples can be seen in Table 1. The percentage soil carbon ranged from 44.24 – 49.28 % and that of sawdust ranged from 85.56 – 88.69 %. OSL and OSD had the highest percentage carbon for soil and sawdust samples respectively. The least percentage carbon was seen in site I. However, soil ISL had the highest nitrogen content having 0.34 % nitrogen. All analyses were carried out in triplicates.

Table 1: Carbon, nitrogen and phenolic contents of the soil and sawdust samples

Samples		Carbon content %	Nitrogen content %	Phenolic content %
Soil	MSL	48.81 ± 0.16	0.245 ± 0.009	0.900 ± 0.021
	ISL	44.24 ± 0.11	0.340 ± 0.04	0.788 ± 0.092
	OSL	49.28 ± 0.11	0.280 ± 0.06	0.327 ± 0.023
Sawdust	MSD	88.11 ± 0.16	0.77 ± 0.024	0.63 ± 0.004
	ISD	85.56 ± 0.11	0.38 ± 0.029	0.91 ± 0.001
	OSD	88.69 ± 0.11	0.21 ± 0.077	0.53 ± 0.002

* MSL – Morogbo-Agbara soil, ISL – Iju soil, OSL – Oja Soil, MSD – Morogbo-Agbara sawdust, ISD – Iju sawdust, OSD – Oja sawdust

3.2. Isolation of fungi

Overall, ten fungal isolates were obtained from the soil samples. The Morogbo-Agbara soil samples had the higher number of fungal isolates, with 5 of the isolates obtained from it. The soil samples from Iju and Oja locations had 3 and 2 fungal isolates, respectively. Identification of the most probable microorganisms by culture and microscopic characteristics are described in Table 2.

Table 2: Characteristics of fungal isolates for identification

Isolate Code	Cultural features	Microscopic features	Probable Organisms
ML1	Filamentous, medium-sized colonies with elevated mycelia that appear light green. Underneath the plate showed a cream color.	Brush-like conidia head and conidiosphores with a blue-green microscopic appearance.	*Penicillum spp*
ML2	Colonies that were fluffy and had raised mycelia appearing black in color, with a pale yellowish colouration at the reverse side of plates.	Chain-like conidia carried at the vesicle end; and septate hyphae with long conidiophores.	*Aspergillus niger*

ML3	Dusty greenish colonies with a pale yellow reverse color.	Columnar conidial head on Non-septate conidiosphore with spherical spore, septate hyphae.	*Aspergillus flavus*
ML4	White, filamentous, medium flat colonies with black spores.	Thick and branching septate hyphae	*Rhizopus sp*
ML5	Round, small, raised, smooth pale blue colonies	Large sporangium with non-septate hyphae	*Penicillium expansum*
OL1	Circular, pink, small mucoid colonies	Oval large cells	*Cryptococcus sp*
OL2	Irregular, small, raised, undulate, smooth, opaque, custard yellow colonies	Small-sized oval cells	*Candida sp*
IL1	Irregular, small, raised, undulate, mucoid, opaque, lemon-yellow colonies	Oval cells	*Saccharomyces sp*
IL2	Small creamy white mucoid colonies.	Small yeast-like cells	*Saccharomyces cerevisiae*
IL3	Orange and dusty colonies.		*Fusarium sp*

*ML – Morogbo-Agbara isolates, OL – Oja isolates, IL – Iju isolates

Figure 3: Pure cultures of fungal isolates on potato dextrose agar plates

3.3 Screening for Laccase production

Five of the 10 isolates, were observed to have caused a color change in the tannic acid supplemented media giving a brownish black coloration (Table 3).

Table 3: Laccase-screening of Isolates using Tannic Acid

Isolates	Laccase production
ML1	**-**

ML2	+
ML3	-
ML4	-
ML5	+
OL1	-
OL2	+
IL1	+
IL2	+
IL3	-

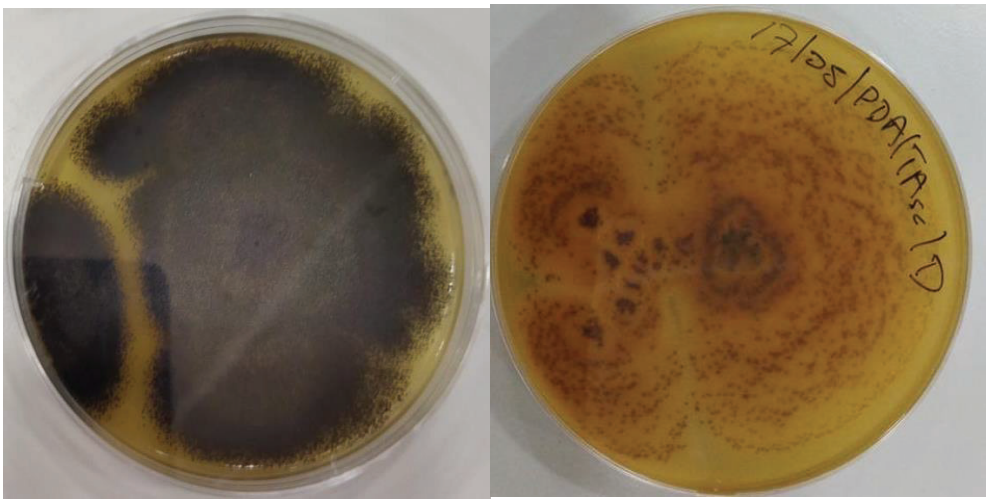

Figure 4: Brownish-black coloration on plates from tannic acid screening

4. Discussion

Studies reveal that soil organic carbon concentrates mostly at the topsoil [18]. Topsoil organic carbon ranges from 0.5% to 3.0% for most upland soils [19]. The results above show the beneficial effects of sawdust on soil properties. The carbon content of sawdust which is usually in a range of 60-80% when added to the soil brings about a substantial increase in the soil carbon content by at least 40%; with the soil nitrogen content also affected. The microorganisms present in the soil use up more nitrogen in addition to that of the sawdust for the production of lignolytic enzymes in order to be able to adapt to the sawdust-polluted environment [6, 21]. This results in a depletion in the nitrogen present within soil; and may prove useful in supporting the growth of beneficial microorganisms that improve soil quality by breaking down nutrients and making them available for beneficial purposes such as remediation and fertilization. In a study by Tanee and Albert [26], it was observed that adding sawdust as a biostimulation material to crude oil polluted soil affected soil parameters as well as cassava (*Manihot esculenta*; Crantz) development and yield. Also, sawdust ash has been reported to be an effective limiting material and rich source of Mg, K, P, N and Ca [27]. Low phenol content in the soil under study is attributable to the presence of phenol-degrading microbes as a result of the presence of lignin-containing sawdust. The lignin present in the sawdust aids synthesis and release of such phenol-degrading enzymes as laccases, and lignin and manganese peroxidases.

The results corroborate the reports of Abd El-Halim and El Baroudy [28] on effects of pinewood sawdust on various hydro-physical characteristics of expansive soil obtained from Egypt's middle Nile Delta. Sawdust addition was shown to reduce clay cracking breadth, linear shrinkage, plasticity index, and size fraction while considerably increasing falling-head permeability, indicating an improvement in soil hydro-physical characteristics. Khan and Khan [29] observed that mechanical parameters of soil were greatly improved by the addition of sawdust ash with up to 22% increase in friction angle upon sawdust addition.

Due to their considerable flexibility and capacity to assume a variety of morphologies in response to challenging or unfavorable circumstances, fungi are reportedly very successful soil inhabitants [30]. Also, their ability to secrete an array of extracellular enzymes, lends the capacity to breakdown diverse forms of organic materials and decomposing soil components; thus, contributing in the preservation of carbon and nutrients balance [31]. However, fungal diversity and activity is influenced by a number of biotic [32] and abiotic (temperature, structure, salinity, moisture, and soil pH) factors [33, 34]. In this study, ten (10) fungal isolates were cultured from sawdust-polluted soil and five (5) of the organisms tested positive for laccase production upon screening with tannic acid. This ability of sawdust to spur up the growth of ligninolytic fungi abound in literature [35, 36, 37]. The fungal species identified in these studies include *Aspergillus niger*, *A. flavus*, *Penicillium spp*, *Candida sp, Saccharomyces cerevisiae,* and *Rhizopus sp*. In this study, positive reaction for laccase production was observed in *Aspergillus niger*, *Penicillium spp*, *Candida sp, Saccharomyces cerevisiae,* and *Saccharomyces sp*. The growth of these fungi can be attributed to their ability to produce enzymes capable of degrading lignin such as laccase and lignin peroxidase as well as other enzymes that aid microbial degradation of sawdust in the soil such as carboxymethyl cellulase and xylanase. This corroborates the reports of Godliving and Yoshitoshi [38] and Zhang et al. [36] that fungal and bacterial sawdust decomposition is driven by secretion of such enzymes as laccase, xylanase, cellulose, carboxymethyl cellulose, and lignin peroxidase. The enzymes are stimulated by the presence of cellulose, hemicellulose, and lignin present within the sawdust. The presence and utilization of these enzymes are very important for the mechanical breakdown of sawdust components to aid mycelial expansion and maximization of nutrients. Lignin or ligninocellulosic properties are the major stimulants for the synthesis of laccase in growing fungi on sawdust [39].

The growth of indigenous microorganisms has shown to be essential for the bioremediation of contaminated sites [40]. Sawdust in the soil is a stimulant for the growth of useful fungi capable of degrading recalcitrant substances within the environment. Nwinyi and Ikhine [1] reported the ability of soil fungal species of *Penicillium, Aspergillus, Mucor* and *Rhizopus* to degrade crude oil contamination in soil. These organisms were isolated from the contaminated soil after biostimulation with sawdust. Onwudike et al. [41] established that the activity of indigenous microbes and various biostimulants promote the degradation of hydrocarbons in crude oil-contaminated soils, with sawdust presenting a 62.61% degradation. Likewise, laccase has been recorded to be very useful in the biodegradation of a wide range of soil pollutants like polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), toluene, xylene (BTEX), ethyl-benzene, 1,1,1-trichloro2,2-bis (4-chlorophenyl), ethane (DDT), trinitrotoluene (TNT) and pentachlorophenol (PCP). Sawdust represents a suitable substrate for the production of useful metabolites [37]; and its use as a source of carbon for fungal growth, thus aiding the production of useful compounds such as 2,2-Dimethyl-2-ethylhexyl ester, propanoic acid, furfural, acetic acid, Glycine N-Cyclopropylcarbonyl-methyl ester and Methylene cyclo-propane carboxylic acid [33, 37]. In a study by Daasi et al. [42], sawdust was compared to other materials for application as support-substrate including olive leaves, wood, filter paper, the tip of palm, straw, oatmeal, etc for the production of laccase by *Coriolopsis gallica* and was found to be the best support-substrate with its culture having the highest laccase activity of the lot.

5. Conclusion

As shown in this study, sawdust possesses 85% carbon, in addition to other properties useful for microbial growth of fungal species of *Aspergillus, Penicillium, Saccharomyces,* and *Candida*. The presence of lignin in sawdust stimulates the production of the ligninolytic laccase enzyme. These isolates' capacity to generate laccase and other ligninolytic enzymes enhances their survival in diverse conditions. Thus, adding economic value while also providing sustainable solutions to waste disposal costs and pollution challenges associated with agro-industrial wastes like sawdust.

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7. Conflict of Interest
The authors declare no conflict of interest.

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