Assessment of the Bacterial and Fungal Load in Compost Manure made from Tannery Waste - Sawdust mix

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Abstract: The study aimed at enumerating bacteria and fungi in tannery waste, sawdust, soil as well as finished compost by using standard method. The bacterial isolates were species of *Bacillus*, *Eschericia coli*, *Micrococcus*, *Proteus*, *Pseudomonas*, *Streptococcus*, *Staphylococcus* and *Aspergillus*, *Mucor*, *Tricophyton*, *Rhizopus*, *Penicillium*, *Fusarium*, *Paecilomyces*, *Candida* and *Saccharomyces*. The frequencies of bacterial occurrence from the tannery waste (TW), sawdust (SD) and soil (SL) were 1.75 – 10.53%, 1.75 – 8.78% and 1.75 – 10.53% ranges respectively. *Bacillus subtilis* were most frequently encountered (26.32%) followed by *P. aeruginosa* (19.29%) and *Streptococcus faecalis* (17.54%). *S. aureus* and *Enterococcus faecalis* had the least of occurrence of (3.51%). Among the fungal isolates *A. niger* had the highest frequency of occurrence (22.22%) followed by *A. flavus* and *P. chrysogenum* with 19.05% each *Tricophyton rubrum*, *Candida albicans*, *Saccharomyces cerevisiae* and *S. cerevisiae* occurred in TW, SD and SL, while *C. albicans* occurred in TW and SD only. At the mesophilic temperature of <40 ^{o}C species of *S. aureus*, *S. faecalis*, *P. aeruginosa*, *B. subtilis*, *M. luteus*, *P. mirabilis*. *A. niger*, *A. flavus*, *P. chrysogenum*, *S. cerevisiae* and *C. albicans* predominated within 7 days of composting for both bacteria and fungi. The results obtained suggest that the bacteria and fungi associated with the production of compost from tannery waste and sawdust were mostly primary degraders of organic compounds, commonly found in the environment.

Keywords: Compost, microorganisms, tannery waste, sawdust

INTRODUCTION

Compost manures are formed from the decayed refuse as like leaves, twigs, crop residues, stubble, domestic garbage, hedge clippings, water-hyacinth, sawdust, tannery sludge and plant materials (Batham *et al*., 2013; Argun *et al.*, 2017). Compost is a dark brown, brittle and earthly smell which is rich in nutrients that support the growth of plants (Olowoake *et al.*, 2018). It is used for structural amendment of the soil, gardens, horticulture, organic farming and landscaping, with dual exceptional effects on soils, essentially on nutrient deficient soils (Olowoake *et al.*, 2018). Compost improves soil fertility, water permeability; promote plant health and crop yields (Weerasinghe and De Silva, 2017). It affects both variety as well as size of microbial population and enzyme actions (Weerasinghe and De Silva, 2017).

During composting process different microbial communities degrade organic matter into relatively stable end product (Majeed *et al*., 2021). It may be more effective when the carbon to nitrogen ratio and the moisture content are specific according to material of compost (Batham *et al*., 2013). Most widely used co-composted materials are animal waste products and agro-wastes like stalk, rice straw, sawdust, wheat straw and corn (Anwar *et al*., 2015). The addition of bulking agent for composting optimizes substrate properties such as air space, moisture content, carbon-to-nitrogen (C:N) ratio, particle density, pH and mechanical structure (Anwar *et al*., 2015). Composting has been frequently acknowledged as an environmental friendly alternative approach of recycling organic solid wastes (Ijah, 2006; Ezeagu *et al*., 2017a). Therefore, composting can be adopted for the conversion of biodegradable solid wastes into a compost material with a high agronomic value (Majeed *et al*., 2021). Adequate awareness is essential, in order to achieve sustainable management of organic waste, especially in the case of dehairing wastes, where the concentration of heavy metals is low.

Tannery waste is made up of hair waste, trimmings and fleshings (Framis, 2018). Sawdust also is a by-product of cutting, grinding, drilling, sanding, or pulverizing

wood. Therefore, sawdust is a carbonaceous organic substance which has a very rich carbon to nitrogen ratio (Betham *et al*., 2013). The study aimed at enumerating bacteria and fungi in tannery waste, sawdust, soil as well as finished compost by using standard methods.

MATERIALS AND METHODS

Sampling

Tannery waste was collected from local tannery at Sabon layi, Gombi Local Government Area of Adamawa State, Nigeria, and was transported in a clean polythene bags to the microbiology laboratory, Federal University of Technology, Minna, Nigeria. The sawdust was collected from Wood workshop at Mypa Road Bosso, Bosso Local Government Area, Niger State, Nigeria. Sample of tannery wastes was dried in the sun for five days, and stored in a polythene bag, prior to analysis as well as composting. Sample of fresh sawdust was air dried at room temperature for two days and then dried in the sun for three days. These wastes were packed in separate polythene bags, so as to prevent contamination with some other wastes and stored at room temperature until required.

Composting process

The composting processes were carried out in a 20 liters capacity plastic container mixed together and put in different ratios (Vich *et al.*, 2017). The experiment was small scale composting which was laid out in double replications. Treatments comprised five levels of tannery waste with sawdust mixed together in the following ratio TW:SD as 1:1, 1:5, 5:1, 1:10 and 10:1. The mixture for each treatment was composted for 42 days following the method described by Zhou *et al*. (2014) and Vich *et al*. (2017).

Microbiological analyses

Samples at each stage of composting (0, 7, 14, 21, 28, 35 and 42 days) were analyzed for total aerobic bacterial and fungal counts as described by Ahmed *et al*. (2007). A series of dilutions were prepared using sterile distilled water. One milliliter (1.0ml) of the serially diluted sample was introduced (pour plate) onto plates with nutrient agar (NA) and Sabouraud dextrose agar (SDA), for the enumeration of bacteria and fungi respectively. The NA plates were incubated at 30^{o}C for 24-48 hours, while the SDA plates were incubated at room temperature for 3-5 days. The colonies which developed were counted and expressed as colony forming units per gramme (cfu/g) of sample. Pure isolates were obtained by repeated sub-culturing on media used for the primary isolation and preserved on agar slants for further characterization and identification.

Statistical Analysis

Data acquired from the laboratory were analyzed using simple descriptive statistics involving tables, frequencies, percentages and figures.

RESULTS

The bacterial counts in tannery waste (TW), sawdust (SD) and soil (SL) were 14.0×10^6 cfu/g, 18.4×10^6 cfu/g and 10.0×10^6 cfu/g, while the fungal were 1.1×10^4 cfu/g, 1.6×10^4 cfu/g and 6.0×10^4 cfu/g respectively. Sawdust had higher bacterial counts than either the tannery waste or soil, while fungi counts were higher in soil than either tannery waste or sawdust (Table 1).

Table 1: Bacterial and fungi counts in tannery waste (TW), sawdust (SD) and soil (SL)

Sample	Bacterial counts (cfu/g)	Fungal (molds and yeasts) counts (cfu/g)
Tannery waste (TW)	14.0×10^6	1.1×10^4
Sawdust (SD)	18.4×10^6	1.6×10^4
Soil (SL)	10.0×10^6	6.0×10^4

cfu/g: colony forming units per gramme

Identification of the bacteria was carried out using colony morphology, Gram staining and biochemical tests which includes; catalase, coagulase, indole as described by (Garrity *et. al*., 2005; Cheesebrough, 2006). The bacterial isolates identified were: *Enterococcus faecalis, Micrococcus luteus, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis, Pseudomonas aeruginosa* and *Proteus mirabilis.* The frequencies of the bacterial isolates were presented in Table 2. The isolates *Pseudomonas aeruginosa* (10.53%), *Bacillus subtilis* (8.77%) and *Bacillus subtilis* (10.53%) had high frequencies of occurrence in TW, SD and SL respectively, while *Proteus mirabilis* (1.75%), *Escherichia coli* (1.75%), *Pseudomonas aeruginosa* and *Micrococcus luteus* (1.75%) had the least frequencies of occurrence in TW, SD, and SL respectively (Table 2). Generally, *B. subtilis* were more frequently encountered (26.32%) followed by *P. aeruginosa* (19.29%) and *Streptococcus faecalis* (17.54%). It was observed that *S. aureus* and *Enterococcus faecalis* had the least frequencies (3.51%) of occurrence (Table 2).

Table 2: Frequency of occurrence of bacteria in tannery waste, sawdust and soil

Bacteria	Tannery waste (TW)	Sawdust (SD)	Soil (SL)	Total
Bacillus subtilis	4 (7.02)	5 (8.77)	6 (10.53)	15 (26.32)
Escherichia coli	4 (7.02)	1 (1.75)	2 (3.51)	7 (12.28)
Enterococcus faecalis	2 (3.51)	0 (0.00)	0 (0.00)	2 (3.51)
Micrococcus luteus	2 (3.51)	3 (5.26)	1 (1.75)	6 (10.52)
Staphylococcus aureus	2 (3.51)	0 (0.00)	0 (0.00)	2 (3.51)
Streptococcus faecalis	2 (3.51)	5 (8.77)	3 (5.26)	10 (17.54)
Pseudomonas aeruginosa	6 (10.53)	4 (7.02)	1 (1.75)	11 (19.29)
Proteus mirabilis	1 (1.75)	3 (5.26)	0 (0.00)	4 (7.01)
Total	23 (40.36)	21 (36.83)	13 (22.80)	57 (100)

Number in parenthesis is percentage (%) frequency of occurrence

The fungal identification was done as described by (Barnett *et. al.*1990; Watanabe, 2010). The fungi identified and their frequencies of occurrence were presented in Table 3. *Aspergillus niger* had the highest frequencies of occurrences in TW, SD and SL ranging from 6.35% to 7.94%. The lowest frequency of occurrence was recorded with *Tricophyton rubrum* (1.59%) in tannery waste. Generally, *A. niger* had the highest frequency of occurrence (22.22%) followed by *A. flavus* and *P. chrysogenum* with 19.05% each. The fungus *Tricophyton rubrum* had the lowest frequency of occurrence (1.59%). The yeasts, *Candida albicans* had a total frequency of 6.34% while *Saccharomyces cerevisiae* had 9.52%. *S. cerevisiae* occurred in TW, SD and SL, while *C. albicans* occurred in TW and SD only (Table 3).

Table 3: Frequency of occurrence of fungi in tannery waste, sawdust and soil

Fungi	Tannery waste (TW)	Sawdust (SD)	Soil (SL)	Total
Aspergillus niger	5 (7.94)	5 (7.94)	4 (6.35)	14 (22.22)
Aspergillus flavus	3 (4.76)	4 (6.35)	5 (7.94)	12 (19.05)
Aspergillus fumigatus	0 (0.00)	0 (0.00)	3 (4.76)	3 (4.76)
Tricophyton rubrum	1(1.59)	0 (0.00)	0 (0.00)	1 (1.59)
Saccharomyces cerevisiae	2 (3.17)	3 (4.76)	1 (1.59)	6 (9.52)
Candida albicans	2 (3.17)	2 (3.17)	0 (0.00)	4 (6.34)
Mucor mucedo	0 (0.00)	1 (1.59)	2 (3.17)	3 (4.76)
Rhizopus microspores	0 (0.00)	1 (1.59)	2(3.17)	3 (4.76)
Penicillium chrysogenum	2 (3.17)	4 (6.35)	6 (9.52)	12 (19.05)
Penicillium notatum	1 (1.59)	2 (3.17)	2 (3.17)	5 (7.94)
Total	16 (25.40)	22 (34.92)	25 (39.68)	63 (100)

Number in parenthesis is percentage (%) frequency of occurrence

The bacterial counts recorded during tannery waste with sawdust (TW/SD) composting ranged from 54 × 10^6 cfu/g to 164 × 10^6 cfu/g for TW/SD 1:1 52× 10^6 cfu/g - 180 × 10^6 cfu/g for TW/SD 1:5 over a period of 42 days. For TW/SD 5:1, TW/SD 1:10 and TW/SD 10:1, the bacterial counts ranged from 62 × 10^6 cfu/g to 160 × 10^6 cfu/g, 44 × 10^6 cfu/g - 140 × 10^6 cfu/g, and 32 × 10^6 cfu/g - 162 × 10^6 cfu/g respectively over the same period. Generally, it was observed that the bacterial counts increased within 7 days after which counts decreased gradually till the end of the composting process, that is, after 42 days (Fig. 1). TW/SD 1:5 supported the highest bacterial counts trailed by TW/SD 5:1, TW/SD 10:1, TW/SD 1:1, and TW/SD 1:10 in that order (Fig. 1).

The fungal counts recorded during TW/SD mix composting ranged from 4 × 10^4 cfu/g to 36 × 10^4 cfu/g for TW/SD 1:1, 7× 10^4 cfu/g - 40 × 10^4 cfu/g for TW/SD 1:5 over a period of 42 days. For TW/SD 5:1, TW/SD 1:10 and TW/SD 10:1, the fungal counts ranged from 4 × 10^4 cfu/g to 20 × 10^4 cfu/g, 6 × 10^4 cfu/g - 24 × 10^4 cfu/g, and 2 × 10^4 cfu/g - 18 × 10^4 cfu/g respectively over the same period. Typically, it was observed that the fungal counts increased between 0 and 7 days after which counts decreased steadily till the end of the composting process, that is, after 42 days (Fig. 2). TW/SD 1:5 supported the highest fungal counts followed by TW/SD 1:1, TW/SD 1:10, TW/SD 5:1, and TW/SD 10:1, in that order (Fig. 2).

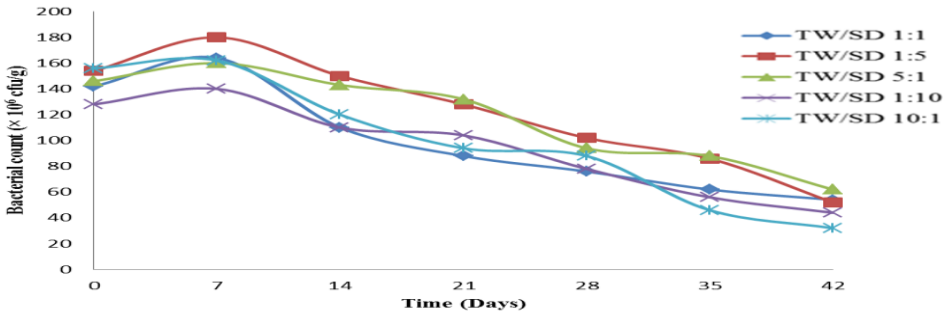

Figure 1: Bacterial counts during composting of tannery waste (TW) with sawdust (SD)

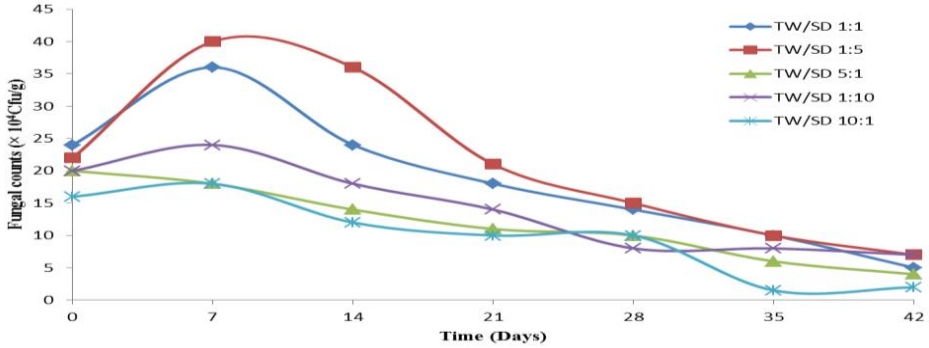

Figure 2: Fungal counts during composting of tannery waste (TW) with sawdust (SD)

DISCUSSION

The bacterial counts in TW, SD and SL were in the ranged 10.0 × 10^6 cfu/g – 18.4 × 10^6 cfu/g, while the fungal counts ranged from 1.1 × 10^4 cfu/g - 6.0 × 10^4 cfu/g. Many researchers have reported the microbial counts of tannery wastes, sawdust and soil. Das *et al.* (2017) reported that the microbial load in tannery waste samples contained massive counts of bacteria and fungi in the average 10^8 cfu/g. Higher bacterial and fungal counts for sawdust waste was also reported by Haseena *et al.* (2016) and Idu *et al.* (2019). The higher microbial counts might be due to the available nutrients (carbon, nitrogen or energy) present in the wastes, which are required for proliferation and survival of microorganisms.

Adebola *et al.* (2019) evaluated the microbial loads of the soil (from fadama, hydromorphic and uncultivated field) of National Cereal Research Institute rice field, Badeggi, Niger State, Nigeria; and found that some bacterial and fungal species were higher in hydromorphic field than the uncultivated soil. Wani *et al.* (2018) reported that higher microbial counts were observed in forest soils and lower in agricultural soils of North western zone of Kashmir, possibly because of the fact that greater carbon source in the form of organic matter existed in the forest soils as compared to other land use systems.

The study showed that the isolated bacteria from the tannery waste were *Bacillus subtilis, Escherichia coli, Micrococcus luteus, Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeruginosa* and *Proteus mirabilis*, while the fungal (moulds and yeasts) species isolated were *Aspergillus niger, Aspergillus flavus, Penicillium notatum, Penicillium chrysogenum, Trichophyton rubrum, Mucor mucedo, Rhizospus microsporum, Saccharomyces cerevisiae* and *Candida albicans*. Different studies have shown that tannery wastes habour common indigenous microorganisms present in the soil. These include: *Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa, Aspergillus niger* and *Penicillium chrysogenum* (Emmanuel *et al.*, 2017b; Mohammed *et al*., 2017; Adebola *et al*. 2019). Lennox *et al.* (2010) reported that these indigenous bacterial and fungal isolates played significant role in the degradation of sawdust. The microbial species such as *Bacillus, Pseudomonas, Aspergillus, Mucor, Saccharomyces,* have strong decomposing ability to solid wastes and use it for carbon and energy generation. The high levels of nitrogen and proteins available on animal skins might favour the growth of microorganisms. Emmanuel *et al*. (2017a) reported similar microbial population from dumpsites in Abakaliki Metropolis, Nigeria, with the strong biodegradation ability.

Soil is a common reservoir for microorganisms as saprophytes or pathogens. Similar, microbial isolates were observed in this study. Different microbial populations are maintained by soil thus the organisms play a vital function in ecosystem level processes such as, nutrient cycling as well as decomposition of organic matter (Wani *et al*., 2018). Akpomie (2013) observed *Saccharomyces cerevisiae* from tannery effluent sample. Gbolagunte and Silas (2016) also isolated *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger,* from landfills and tannery waste and most of these fungi are waste degraders. Similarly, Umar *et al.* (2017) reported the occurrence of similar bacterial isolates (*Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis,* and *Streptococcus faecalis*) in tannery effluent. The detection of *Escherichia coli* in tannery waste (TW) could be due to contact with faecal material, while *Staphylococcus aureus* could be as a result of insanitary condition of the tanning surrounding/environment.

The frequency of occurrence of bacterial isolates revealed that *Bacillus subtilis* had (26.32%), *Pseudomonas aeruginosa* had (19.29%), *Streptococcus faecalis* (17.54%), and *E. coli* (12.28%). The bacteria, *Staphylococcus aureus* and *Enterococcus faecalis* had the lowest frequency of occurrence (3.51% each). This agrees with the results of Chukwuemeka *et al.* (2013) and Akinnibosun and Ayejuyoni (2015). These bacterial species (*Bacillus subtilis, E. coli, Streptococcus faecalis, Pseudomonas aeruginosa*) have been engaged in rapid decomposition organic and inorganic compounds which the organisms use for growth (as sole source of carbon and energy). Mohammed *et al.* (2017) also reported higher occurrence of some bacterial species in tannery effluents, and these bacteria participate in the breakdown of organic and inorganic compounds. However, these results differed from the study of Adebola *et al.* (2019) who reported highest percentage frequency occurrence of *Micrococcus luteus* (24.99%), *P. aeruginosa*

(23.79%), and the least percentage frequency of occurrence of *Staphylococcus aureus* (5.84%) and *Escherichia coli* (4.32%) in rice field soil. The difference in results might be attributed to high organic matter presence favouring their growth and proliferation.

The frequencies of occurrence of fungi in TW (25.40%), SD (34.92%) and SL (39.68%) were high. *Aspergillus niger* had (22.22%), followed by *Aspergillus flavus* and *Penicillium chrysogenum* (19.05%), while *Tricophyton rubrum* had the lowest frequency of occurrence (1.59%). Adebola *et al.* (2019) reported the percentage frequency occurrence of fungal species in rice field soils of Badeggi, Niger State, Nigeria, which include *Aspergillus niger* (24.28%), *Aspergillus flavus* (23.33%), *Mucor* sp. (4.47%), and attributed the highest frequency of *Aspergillus* sp., to farming activities observed on the soils as well as the usage of fertilizers. Akpomie and Ejechi (2016) reported the occurrence of *Aspergillus niger* and *Penicillium chrysogenum* as the major isolates of tannery samples. Bello *et al.* (2020) observed the percentage occurrences of fungal species of *Aspergillus* and *Penicillium* with (20%), and (10%) respectively in soil polluted with tannery waste, whereas in an unpolluted soil fungal isolates obtained were *Aspergillus* (30%), *Fusarium* (10%), *Mucor* (10%), *Rhizopus* (10%) and *Penicillium* (10%). Microbes that possessed hydrolyzing activities tend to prevail.

Microbiological properties of the compost have been studied. The common mesophilic microbial species identified in the mesophilic stage were *Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus niger, Mucor mucedo* and *Penicillium chrysogenum.* The most dominant species identified in this study were the most probable compost microbes reported by different researchers. This finding is in agreement with Mehta and Sirari (2018) who reported the occurrence of such microbes during mesophilic stage of composting (temperature between 20 and 40^{o}C). Similarly, Chinakwe *et al.* (2019) reported microbial isolates such as *Bacillus, E. coli, Micrococcus, Proteus, Pseudomonas, Staphylococcus, Streptococcus, Aspergillus, Candida albicans*, *Fusarium*, *Mucor*, *Rhizopus* and *Saccharomyces* species during composting of some organic wastes in greenhouse. Mesophilic microbes are known to be the most prevalent degraders of different organic waste materials, and their occurrence in the compost relied on the type of organic waste involved, pH and the temperature of the composting materials. Ezeagu *et al.* (2017b) also observed similar species of microbes in a study conducted on enhanced biodegradation of organic municipal solid wastes for organic fertilizer production.

Yeasts were also observed during the composting period probably due to the degradation role they played on the organic waste. This corroborates with the work of Ezeagu *et al.* (2017a) who also reported that the yeast *Saccharomyces cerevisiae* aided the waste degradation. Fungi are the major constituents of the microbial biomass, and their comparative significance varies greatly with the degradation of organic matter content of the composting mixture. In this study, the numbers of fungi isolates observed during the composting period were many with tannery waste to sawdust ratios (TW/SD 1:5 and TW/SD 1:10 each) samples were plentiful. This might be connected to the presence of cellulose material and the acidity (pH) of the sawdust which favoured the heavy growth of fungi, as they like acidic growth medium. Ezeagu *et al.* (2017a) also observed that the fungi *Aspergillus niger* had the ability to degrade cellulose by enzymatic (cellulase) hydrolysis of sawdust. The fungal counts were more at the initial days of composting (acidic condition) than towards the end of the process when the composted mixtures (TW/SD) turned alkaline. This result is in agreement with the report of Ezeagu *et al.* (2017a) who revealed that acidic pH value favoured the growth of moulds and yeasts.

Fatunla *et al.* (2016) conducted microbial counts of the fresh mixture (sewage sludge/sawdust) which showed that bacterial and fungal counts were higher at the beginning of composting and the values significantly decreased after 40 days of in-vessel composting.

During the thermophilic stage of composting the occurrence of *Bacillus, Micrococcus, Pseudomonas, Staphylococcus* and *Aspergillus* were observed. Chinakwe *et al.* (2019) also reported similar microbes but with the exception *Staphylococcus.* The decline in microbial counts might be as a result of depletion of nutrients within the compost. It was observed that the numbers of *Aspergillus* species in this study were higher than the other mould isolates. This study corroborates with Haas *et al*. (2016) who also found the persistence of *Aspergillus* species within the compost during composting. This might be because thermophilic fungi grow and persevere during the rotting process due to generation of heat. In addition, Escobar and Solarte (2015) reported the domination of the genera *Aspergillus* and *Penicillium* associated with organic manure obtained by composting of agricultural waste.

CONCLUSION

The study revealed that TW, SD and SL harboured different microbial communities including species of *Micrococcus, Escherichia, Staphylococcus, Bacillus, Pseudomonas, Proteus. Aspergillus, Paecilomyces, Fusarium, Mucor, Tricophyton, Rhizospus, Candida* and *Saccharomyces*. *Bacillus subtilis* was found to be the most encountered bacteria (26.32%), while *Aspergillus niger* had the highest (22.22%) as fungal isolate. The resultant compost matured after 42 days of composting. This indicates that microbes are essential in the breakdown of organic wastes at various stages of composting resulting to the production of compost. Besides, some of the microbes might have bio-control potentials.

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