MICROBIAL DYNAMICS DURING COMPOSTING OF COFFEE WASTE (HUSK) IN JIMMA, SOUTHWESTERN ETHIOPIA

Seid Mohammed*1 and Diriba Muleta2

1Department of Biology, Adama Science and Technology University, Ethiopia.

2Institute of Biotechnology, Addis Ababa University, Ethiopia.

ABSTRACT
The current study is about isolation of some dominant microorganisms from compost of coffee wastes within Jimma Agricultural campus. Composting of coffee waste (husk) was conducted with cow dung, poultry manure and bone meal in the ratio of 3:1. Some physicochemical parameters temperature and pH were measured for composts of coffee husk + cow dung (A), coffee husk + poultry manure (B) and coffee husk + bone meal (C). Microbial dynamics were analyzed every five days of turning compost. Different biochemical tests such as KOH-test, motility, catalase, oxidase cytochrome test, oxidation fermentation test, Gram-staining and citrate utilization test were conducted for characterization of the obtained isolates. Data were computed using table and figure. SPSS version 16 was also used for data analyzing. Variations (p<0.05) were observed for both temperature and pH value based on type of composted materials and duration of compost. Changes in a number of microbial dynamics during composting of this coffee husk were observed. Some species of bacteria and fungi with morphological characteristics of Bacillus, Pseudomonas, Serratia, Enterobacter, Azotobacter, Penicillium, Rhizopus, Cladosporium, Aspergillus, Trichosporon, Conidiobolus, Scedosporium, Fusarium, Actinomycetes sp., Streptomycete sp and other Actinomycete sp. were presumptively isolated from coffee husk combinations. Some other Cocci, thermophilic bacteria and fungi were also isolated from compost A, B and C on the first 20 days of composting. Generally, these microbes were isolated from compost piles at different temperature and pH value.

KEYWORDS: Compost, microorganism, pH, substrate combination and Temperature.
1. INTRODUCTION

At present, the bioconversion of agriculture and industrial wastes is one of the most important techniques that is attracting the world attention. Bioconversion is a conversion of organic wastes to different useful products via a biological process involving living organisms. The conversion of biomass involves the use of enzymes, microbes, other biological agents alone or in combination form (Lasure and Zhang, 2006). Van-Weerden (1999) and Singh (1987) further noted that some of the residues (cellulosic, hemicellulosic and lignocellulosic) and waste which is not available for absorption and cultivation can be broken down by microscopic microorganisms such as yeast, bacteria, fungi, actinomycete and algae. In addition, Mane et al. (2007) explained that the use of some microorganism such as fungi for the bioconversion of lignocelluloses waste materials into food and feed that are rich in protein that offers an alternative for developing unconventional source of proteins as food or feed.

Decompositions of some waste materials brought about by several organisms including such as bacteria, actinomycete and fungi. The sole agents for decompositions of carbon rich materials are the heterotrophic microorganisms (Macdonald and Griffin, 1981). Bioremediation of coffee pulp and coffee husk carried out by fungal systems in order to minimize the contents of caffeine. Among the microbial community present in coffee waste (pulp), fungi species such as Aspergillus, Penicillium and Rhizopus are used to degrade caffeine (Yesuf, 2010).

There was succession of microbial dynamics in the composting of aerobic process that normally shifted predominantly from mesophile in the early stages to one of predominantly thermophiles at the peak of heating cycle. Mesophilous microorganisms such as Cladosporium herbarium, Aureobasidium, Alternaria alternata and Epicoccum purpurascens were used to decompose at the beginning of composting (Hudson, 1986). At the thermophilic stage of composting, microflora such as bacillus spp., trichothecium spp., methylomonas spp., and Seratia spp., At the cooling stage of composting processes, microflora of fungi species such as Aspergillus sp., Fusarium sp, Penicillium sp., Cladosporium sp., Mycotypha sp., Coprinus sp., and Cephalosporium sp. existing. At the same stage of composting processes bacterial sp. such as bacillus sp., Pseudomonas sp., Methylomonas sp., Proteus sp. and Azomonas sp. identified from municipal solid waste (Taiwo and Oso, 2004).
However, the isolation of some dominant microorganisms for coffee waste (husk) compost has been at infancy stage in Ethiopia. Nevertheless, Fan et al., (2005) isolated *Pleurotus* and *Lentenus* sp. from coffee residue (husk) in Brazil. Therefore, this study was initiated to isolate and identify dominant microorganisms (bacteria, actinomycetes and fungi) that may be involved in the composting processes of coffee wastes (husk).

2. MATERIALS AND METHODS

2.1. Study area, Types, sources and collection of main substrates

The study was conducted at Applied Microbiology Research Laboratory, Department of Biology, in Jimma University. Four different types of substrates namely, coffee husk, bone meal, cow dung and poultry manure were used. About 1% of supplement (gypsum) was also used. Coffee husk was collected from coffee mills within Jimma town.

2.2. Substrate combination: The coffee husk was moistened for three days before piles formation. A 75% (v/v) of coffee husk was composted with different substrates (25% v/v each) such as cow dung, poultry manure and bone meal. (Table1). A 0.5% of a chopped grass was added and composted together with each of combination in order to attach each combination together.

2.3. Compost preparation: Combinations were made with each of substrate and three compost piles were made on the pure area under the tree shade. Constant moisture level was maintained during every five days of turning compost by spraying water two times a /day based on the conditions. The compost piles were turned every five days of composting. The mixed main substrate combination was covered with plastic sheets. Finally, composting was run for one month.

<table>
<thead>
<tr>
<th>Table 1. Compost formation with different substrate and supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No</strong></td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

A= Coffee husk (75%) + cow dung (25%) (v/v).
B= Coffee husk (75%) (v/v) + Poultry manure (25%) and
C= coffee husk (75%) (v/v) + Bone meal (25%) (v/v).
2.4. Determination of temperature, pH and moisture for prepared compost piles

Temperatures for compost A, B and C were continuously measured using 1 m long thermometer every five days of compost turning by inserting the thermometer approximately 25 cm deep in the piles. The value was recorded after thermometer pointers read. pH was determined according to Taiwo and Oso (2004) using pH meter (Scientific Instruments Co. (Italy) model 9000/3). Moisture was conducted according to Gosper (2003) methods.

2.5. Analysis of microbial dynamics

2.5.1. Sampling: For microbial analysis, the samples were taken from each compost pile. During turning of compost, a sample of each main substrate combination from three compost piles (compost A, B and C) were taken separately and aseptically in sterile plastic bags every five days of turning compost (six times). Each sample was labeled according to substrate combination made. The collected samples were transported to the laboratory.

2.5.2. Microbial isolation: Isolation of microbial content of samples including bacteria, fungi and actinomycetes were conducted using methods of Sundaramoorthi et al. (2011) and Nanjwade et al. (2010).

2.5.3: Microbial identification

2.5.3.1. Motility test (stab methods): A motility medium was prepared using a test tube. Purified a broth culture was taken by needle and stabbed vertically into a test tube containing motility medium (soft agar). Positive results is a spread sort of growth throughout the medium away from the stabbed point, the bacteria considered as motile. However, if the growth is restricted only to the region of stabbed, the microorganisms are non-motile.

2.5.3.2. Biochemical tests such as KOH test (Gram reaction), oxidase cytochrome test, oxidation fermentation test (o/f-test), gram-staining, citrate utilization test, catalase reaction and endospore test were conducted following the methods of Schaeffer and Fulton (1933); HPA (2008); Gregerson (1978); Quinn et al. (2004); Maria and MacWilliams (2010) and Sunduramoorth et al. (2011).

Fungal identification: The identifications of fungi were conducted using wet mount systems. A pure culture of 5 days old fungus was taken and placed in the middle of
clean slide by using needle. One drops of deionized distilled water (dH2O) was added on to pure isolates of colony that placed on the clean slide. The morphology of fungi were observed using microscope with the 10x objective.

2.6. Data Analysis
The data were entered and managed in MS Excel. During compost preparation temperature and pH such as cropping period, mushroom value were statistically analyzed for each combination using Tukey’s Honestly Significantly Different post-hoc test at p<0.05 after a one-way analysis of variance (ANOVA) using SPSS version 16.

3. RESULTS AND DISCUSSION
3.1. Mean value of Temperature
Different values of temperatures were recorded during compost preparation for all piles (Fig 1). At the beginning of compost preparation, the mean values of temperatures were small for all three compost piles (on Day-1, 5 and 10). However, the highest values were occurred on Day-15 for all compost piles (Figure 1). High temperatures were also recorded on Day-20 (A=51.0 °C, B=51.33 °C and C=50.67 °C, (Figure 1). However, as the compost maturity increased, the mean values of temperatures were declined for all compost piles on Day-25 and 30 (A, B and C) (Fig 4). There was no significant variation between Day-1 and Day-30 (p=0.981) and Day-10 and Day-20 (p=0.803). However great variations (p<0.05) were obtained among the rest days of composting temperature. Generally, there was no variation observed between compost A and C (p=0.764). However, variations were detected between compost A & B (p=0.001) and compost B & C (p=0.00).

![Figure 1. Mean values of temperature recorded for each of compost piles on different composting days.](image-url)
Variations in temperatures were based on the kind of organic waste used for composting and the days of composting. Initially, temperatures for compost A, B and C were lower. However, after five days of turning compost, temperatures raised and attained maximum value on the 15 days of composting (Fig 1). The raising of temperatures may be due to greater activity of fermenting microorganisms that actively involved in decomposition and generate heat energy in all compost combinations. In addition, mixing of materials and turning of compost may supply enough oxygen for microorganisms that participated in degradation of coffee husk. In line with present study, Tiquia et al. (1996) stated the rise and fall of temperature to be correlated with the rise and fall of microbial activities. The temperature of the composting waste was normally raised from predominantly mesophile to thermophiles at the peak of the heating cycle in the early stages of thermogenesis due to microbial succession in the aerobic processes (Hudson, 1986). Adegunloye et al., (2007) reported a comparable result that the maximum temperature for maximum biodegradation in the compost preparation to be kept at 45-55 °C.

In the present study, the mean temperatures of the compost decrease in the final stages of composting (Fig 4). The decreasing of temperature may be due to decreasing of some thermophilic microbes from compost which used to rise temperature after biodegradation and releasing of heat in the form of energy. Hemophilic microorganisms stop their activity due to exhaustion of substrates which results in increasing of temperature. During this period, mesophilic organisms recolonize the substrate from surviving spores or external environments (Ryckeboer et al., 2003).

In this study, fluctuation of compost temperature in terms of composting days might be contributed to selective growth of only the same kind of microorganisms such as Bacillus spp. which probably used for decomposition of this coffee husk (Table 2). In line with this study, Thompson (2007) reported that high temperature is an advantageous for dominance of Bacillus stearothermophilus pure culture. The same temperatures are not recorded in all zones of compost piles. It was changed by regular turning or every part of the substrate is moved to the central which is the hottest part of the pile. The composting process followed a general pattern of an initial rise in compost temperature to maximum of 63-66 °C and a gradual decline in temperature to 18-29 °C (Nair and Markham, 2008) which is a similar result with our finding.
Nutritional values of compost are also used to initiate microbial succession during composting processes. This increases the temperature and this can be the reason for highest mean value of temperature was recorded for compost B that contains poultry manure which is followed by cow dung combination (Figure 4). For explanation the Microbial succession may occur due to nutritional value of poultry manure such as protein, nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) that stated by Chastain et al. (1999) and parness (1990). Microorganisms may also use organic substance found in cow dung and coffee husk such as carbon and nitrogen source which stated by Perth et al. (2007).

Temperatures fall at final stage of composting could be due to low activity of microbes that participate in decomposition of organic wastes. Adegunloye et al. (2007) recorded a minimum level of temperature (28-31 °C) from cow dung compost in the later stage of compost which probably due to size of piles, moisture contents, ambient temperatures, degree of aeration and characters of composting materials.

3.2. Mean values of pH

The mean values of pH for coffee husk only (CH) and other three compost piles were varied based on the kind of substrate combination used and duration of compost piles (Figure 2). The highest mean value of pH (9.48) was detected in the combinations of coffee husk + poultry manure (B) on Day-25. However, the lowest mean value of pH was recorded for CH which was ranged between 5.4-5.97. From composted piles, the least mean (6.16) value of pH was recorded for combination An on Day-1 composting (Fig 2). There was no significant difference between Day-5 & Day-15 (p=0.145), Day-5 andDay-20 (p=0.62), Day-5 & Day-30 (P=0.903), Day-10 and Day-15 (p=0.144), Day-10 & Day-20 (p=0.296), Day-15 and Day-20 (p=0.244), Day-15 andDay-25 (p=0.079) and Day-15 and Day-30 (p=0.181). However, among the rest days of composting, there was a variation (p<0.05). There was also significance variation amongst compost A, B, C and untreated CH only (p<0.05).
Figure 2. Mean value of pH for coffee husk alone (CH) and other compost pile of substrate combination.

The pH of compost showed different pattern from the beginning of composting until compost proceeded (Fig 2). The highest (9.48) mean pH value was recorded for compost B. This could be due to ammonia production after decomposition of these wastes by some fermenting microorganisms.

Nutritional contents of composts may also play significance role for increasing of pH-value since the highest value of pH was recorded for poultry manure compost. The nutritional combination may have relatively the high nitrogen content and posed the highest pH value. Parnes (1990) reported that poultry manure contains a higher total N concentration and lower C: N ratio. It raises pH-value and organic compound contents. There is a positive linear relationship between application rate of poultry manure and pH-value. Moreover, surface application of poultry manure also effectively increases soil pH (Mian and Rodriguez-Kabana, 1982; Cheung and Wong, 1983; Hue and Licudine, 1999). Preeth et al. (2007) revealed that the pH of the compost was 8.95 on 30 days of composting which slightly decreased with the increase in the composting periods.

The finally reached pH-value of 7.41 on 150 day of composting. The authors further noted that the decreasing of pH-value after 15 days might be due to production of organic acids, phenolic compounds during decomposition of organic materials and further increase in pH due to the formation of ammonia during decomposition. Similarly the result observed by Adegunloye et al. (2007) and Amanullah (2007) also indicated that the pH level increased with the progress of decomposition processes and the pH decreases slightly.
and reached minimum value in the first 3 weeks of composting of beans husk, cassava peels, plantain peels and cow dung.

From this finding, the reduction of pH-value for CH (Fig 2) could be due to phenolic compound that naturally exists in the coffee waste (coffee husk). However, the current result revealed that after fermentation, the pH values for all composted substrates were sharply and uniformly raised. In agreement with this study, Preethu et al. (2007) recorded pH-value of both coffee pulp (6.8) and husk (5.3) which was low in reaction compared to compost of cow dung (7.8) that was slightly alkaline. Our results are agree with those of Adegunloye, et al., (2007) who stated that the pH decreases slightly and reached minimum volume in the first 3 weeks of composting of beans husk, cassava peels, plantain peels and cow dung. The highest reduction of phenolic compound (especially, caffeine) was taking place during substrate preparation by aerobic fermentation and pasteurization or immersion in hot water at 70°C for 15 min (Martinez-Carrera et al., 2000).

3.3. Microbial dynamics within the compost piles
Some microorganisms were isolated from compost A, B and C beginning from compost preparation to maturity stage. The populations of microorganisms were bacteria, actinomycete and fungi. These microorganisms dominated in the compost piles at different mean values of temperature and pH. The isolated bacterial strains are presented in Table 2 and actinomycetes in Table 3. Some isolates of fungi are also presented in Table 4. Few colonies of bacteria and Actinomycete are shown in plates (Fig 3). The highest number of gram-positive rod shape bacteria and a few numbers of gram positive cocci (less frequent) were also isolated from compost (Table 2). Some cultural features of gram positive rod shaped bacteria are shown in plates (Fig 4). Some gram-negative rod shaped bacteria also isolated from composts (Table 2). Morphological study (gram staining) showed the presence of negative rod shape (dominant) (Fig 5). Some species of actinomycetes were also isolated from composts on the first 5, 10, 15, 20, 25 and 30 days of composting (Table 3). The morphology of all isolated actinomycetes almost similar and they were gram positive, rod-shaped, filamentous and fragmented (Fig 6).
### Table 2. Biochemical characteristics of bacterial genera from compost piles and their presumptive identity

<table>
<thead>
<tr>
<th>Day of composting</th>
<th>Compost piles</th>
<th>Biochemical test</th>
<th>Microscopic Morphology</th>
<th>Presumptive identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>A, B, and C</td>
<td>+ve +ve +ve +ve +ve</td>
<td>Long rod shaped</td>
<td>Pseudomonas sp.</td>
</tr>
<tr>
<td></td>
<td>- A and C</td>
<td>-ve +ve +ve +ve +ve</td>
<td></td>
<td>Aspergillus sp.</td>
</tr>
<tr>
<td></td>
<td>- B and C</td>
<td>-ve +ve +ve +ve +ve</td>
<td>Rod shaped and slightly curved; occur in single as well as sometimes in pairs</td>
<td>Bacillus sp.</td>
</tr>
<tr>
<td>Day 5</td>
<td>B and C</td>
<td>+ve +ve +ve +ve +ve</td>
<td>Rod pigmented colony and short rod bacillus</td>
<td>Serratia sp.</td>
</tr>
<tr>
<td></td>
<td>- A and C</td>
<td>+ve +ve +ve +ve +ve</td>
<td>Short rod bacillus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- B</td>
<td>+ve +ve +ve +ve +ve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 10</td>
<td>A and C</td>
<td>+ve +ve +ve +ve +ve</td>
<td>Rod shaped; some occurred in pairs and few in size</td>
<td>Listeria sp.</td>
</tr>
<tr>
<td></td>
<td>- A and C</td>
<td>+ve +ve +ve +ve +ve</td>
<td>Rod shaped and slightly curved; occur in single as well as sometimes in pairs</td>
<td>Bacillus sp.</td>
</tr>
<tr>
<td></td>
<td>- B</td>
<td>+ve +ve +ve +ve +ve</td>
<td>Rod shaped and slightly curved; occur in single as well as sometimes in pairs</td>
<td>Listeria sp.</td>
</tr>
<tr>
<td>Day 15</td>
<td>B and C</td>
<td>+ve +ve +ve +ve +ve</td>
<td>Rod shaped and slightly curved; occur in single as well as sometimes in pairs</td>
<td>Listeria sp.</td>
</tr>
<tr>
<td></td>
<td>- A and C</td>
<td>+ve +ve +ve +ve +ve</td>
<td>Rod shaped and slightly curved; occur in single as well as sometimes in pairs</td>
<td>Listeria sp.</td>
</tr>
<tr>
<td></td>
<td>- B</td>
<td>+ve +ve +ve +ve +ve</td>
<td>Rod shaped and slightly curved; occur in single as well as sometimes in pairs</td>
<td>Listeria sp.</td>
</tr>
<tr>
<td></td>
<td>- A and B</td>
<td>+ve +ve +ve +ve +ve</td>
<td>Rod shaped and slightly curved; occur in single as well as sometimes in pairs</td>
<td>Listeria sp.</td>
</tr>
<tr>
<td></td>
<td>- C</td>
<td>+ve +ve +ve +ve +ve</td>
<td>Rod shaped and slightly curved; occur in single as well as sometimes in pairs</td>
<td>Listeria sp.</td>
</tr>
</tbody>
</table>

*Note: High risk*

### Table 4. Identification of fungal genera from compost piles and their presumptive identity

<table>
<thead>
<tr>
<th>Day of composting</th>
<th>Compost piles</th>
<th>Colony and Microscopic morphology</th>
<th>Presumptive identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>A, B, and C</td>
<td>Red colony on the plates, grey powdery of sporangia, non-septate, single sporangiospores and conidial arrangement on the conidiophores</td>
<td>Unidentified sp.</td>
</tr>
<tr>
<td></td>
<td>- A and C</td>
<td>Yellow color on plate with budding and yeast-like cell</td>
<td>Rhizopus sp.</td>
</tr>
<tr>
<td></td>
<td>- B and C</td>
<td>Black powder sporangia, non-septate, single and unbranched sporangiospores that occur from stolons.</td>
<td>Aspergillus sp.</td>
</tr>
<tr>
<td></td>
<td>- A</td>
<td>White and separate colony on the plate, microscopically with spores.</td>
<td>Candida sp.</td>
</tr>
<tr>
<td>Day 3</td>
<td>A and B</td>
<td>- Yellow color on plate, septate, with branched and chlorid conidia</td>
<td>Trichoderma sp.</td>
</tr>
<tr>
<td></td>
<td>- A and C</td>
<td>- Black powder sporangia, non-septate, single and unbranched sporangiospores that occur from stolons.</td>
<td>Cladosporium sp.</td>
</tr>
<tr>
<td></td>
<td>- A and B</td>
<td>- Black powder sporangia, non-septate, single and unbranched sporangiospores that occur from stolons.</td>
<td>Aspergillus sp.</td>
</tr>
<tr>
<td></td>
<td>- A</td>
<td>- Red colony on the plate and small terminal conidia that were formed in simple and or branching chains.</td>
<td>Cladosporium sp.</td>
</tr>
<tr>
<td>Day 10</td>
<td>A and C</td>
<td>- Green colony on plates, septate, branched sporangiospores and conidial conidia.</td>
<td>Paecilomyces sp.</td>
</tr>
<tr>
<td></td>
<td>- A and B</td>
<td>- Black colony on the plate and small terminal conidia that were formed in simple and or branching chains.</td>
<td>Cladosporium sp.</td>
</tr>
<tr>
<td>Day 15 and 20</td>
<td>A and C</td>
<td>- Long sporangiospore that arise from stolons, septate, and conidial conidial arrangements on sporangiospore.</td>
<td>Thermophilic fungi sp.</td>
</tr>
<tr>
<td>Day 20</td>
<td>A and C</td>
<td>- Small round colony and white in color</td>
<td>Unidentified sp.</td>
</tr>
<tr>
<td></td>
<td>- A and C</td>
<td>- Green colony on plates, septate, branched sporangiospores and conidial conidia.</td>
<td>Paecilomyces sp.</td>
</tr>
<tr>
<td></td>
<td>- A</td>
<td>- Black colony on the plate and small terminal conidia that were formed in simple and or branching chains.</td>
<td>Cladosporium sp.</td>
</tr>
<tr>
<td>Day 30</td>
<td>A and C</td>
<td>- Black powder sporangia, non-septate, single and unbranched sporangiospores that occur from stolons.</td>
<td>Rhizopus sp.</td>
</tr>
<tr>
<td></td>
<td>- A</td>
<td>- Black colony on the plate and small terminal conidia that were formed in simple and or branching chains.</td>
<td>Cladosporium sp.</td>
</tr>
<tr>
<td></td>
<td>- A and B</td>
<td>- White to grey color colony and dispersed conidia</td>
<td>Cladosporium sp.</td>
</tr>
</tbody>
</table>
Figure 3. Bacterial colonial morphology:
(a) A red pigmented pure bacteria (*Serratia* sp.) isolated from compost B on the five days of composting,
(b) Unfolded and white (regular and irregular) pigmented colony of *actinomycete* sp. from compost A on the Day-25,
(c) Yellow pigmented colony of Actinomycete (*Nocardia* sp.) from compost C on 25 days of composting
(d) White colors of actinomycete colony (*striptomycte* sp.) from compost A on the Day-25 of composting.
Figure 4. stained film(1000x):
(a) Gram positive rod shaped bacteria isolated from compost A on the first five days of composting.
(b) Gram positive rod shaped bacteria (*Bacillus* sp.) isolated from compost B on the tenth days of composting.
(c) Gram-positive rod with endospore formers (*Bacillus* sp.) isolated from compost A on Day-20 of composting,
(d) Gram-positive rod shaped (*Bacillus* sp.) isolated from compost A on the thirty days of composting (Day-30).
(e) Gram-positive rod with slightly round end bacteria (unidentified bacterial spp) isolated from compost B on the 25 days of composting.

Figure 5. stained film(1000x):
(a) Gram negative rod shaped bacteria (*Serratia* sp.) that isolated from compost B on Day-5 of composting.
(b) Gram-negative and rod shaped isolated (*Enterobacter* sp.) from compost C on the first five days of composting.
(c) Gram negative rod (*Azotobacter* sp.) isolated from compost B on Day-5 of composting.

(d) Gram-negative and long rod shaped (*pseudomonas* sp.) isolated from compost B on the ten days of composting (on Day-10).

![Image](image_url)

Figure 6.: stained film(1000x):

(a) Gram-positive long chain and rod shaped actinomycete (*Actinomyces* sp.) isolated from compost A on Day-5.

(b) Gram-positive rod shaped and intertwined mass of actinomycete isolated from compost C on Day-15 of composting.

(c) Gram positive mass of branched intertwined filamentous actinomycete (*Nocardia* sp.) isolated from compost C on the Day-25.

(d) Gram positive and cocci actinomycete (*Nocardia* sp.) that isolated from compost A on the Day-25 and

(e) Gram-positive rod shape and branched actinomycete sp. (*Streptomyces* sp.) isolated from compost B on the 20 days of composting.

Microorganisms isolated in the compost of coffee husk consist presumptively of *Pseudomonas* spp., *Serratia* sp., *Enterobacter* sp. and *Azotobacter* sp. They were generally isolated from compost in the early stage of composting at a different temperature and pH value (Table 2). Result indicated that they can survive in the high temperature and slightly acidic to alkaline compost. These microorganisms can tolerate unfavorable environment due to temperature and pH using their own mechanisms. *Pseudomonas* were isolated at a very high temperature and have the capacity to tolerate this temperature...
probably due to structural modification. They may exist in this temperature only for short period of time.

The bacterial genera such as Bacillus sp., Pseudomonas sp., Enterobacter sp., Klebsiella sp. Escherichia Acinetobacter sp and Serratia spp can survive in the compost at the early stage of time (days 0 and 8) at 45 °C and 4.5-8 pH. The presences of some bacterila sp. are probably due to water source mixing with compost (Taiwo and Oso, 2004; Dahiya et al., 2005; Gbolagade, 2006; Silva et al., 2009). Zaved et al., (2008) stated that a numbers of genera such as Pseudomonas, Achromobacter and Bacillus can be survived in most aerobic soils of organic manure at pH 5.1-10.2. Pseudomonas spp have the capacity to grow at 50 to 55°C in the compost. They able to survive in cow dung compost in the first two weeks of composting and after which they were eliminated probably due to unfavorable environmental conditions at the later period of composting (Hayes, 1968; Adegunloye et al., 2007). In contrast, Stainer et al. (1998) stated that Pseudomonas species are aerobic nutritionally versatile microbes that have the capacity to release nutrient after degradation of many natural and synthetic organic compounds such as humic acids and pesticides.

Bacteria such as Pseudomonas putida, Bacillus megatarium, Bacillus pumilis, Azotobacter vinelandii and Serratia marcescens seem to play a leading role in decomposing, low-molecular-weight of phenolic, phytotoxicity of olive mill waste water (OMWW), lignin and compost in the aerobic conditions. For instance, Serratia marcescens can produce laccase enzyme and positively correlated with lignin mineralization and solublization in the compost. However, the contribution of bacteria to the complete biodegradation of lignin in natural environment where fungi are also present is not well known (Morii et al., 1995; perestelo et al., 1996; Ramos- Cormenzana et al., 1996; Daniel and Nilsson, 1998; Ehaliotis et al., 1999; Rebollido et al., 2008).

Actinomycete spp. were mostly isolated at the maturate stage of composting. In the early stage of composting, members of actinomycete were not isolated from compost which could be probably due to slightly acidity of composts (pH 6.16-7.22) (Figure 2). Actinomycetes have also the capacity to survive in the high temperature and slightly alkaline compost. Environmental factors such as compost type, moisture, pH and temperatures may also influence their occurrence. Compost A and B relatively support
the larger isolates of actinomycetes than compost C which can be attributed to nutritional value of compost type. At these temperature and slightly alkaline compost of coffee husk, some *Actinomyces* spp can thrive and might have been assisting decomposition of compost. Zaved *et al.* (2008) isolated *thermoactinomycete* that are able to grow at temperature of 50 and 60 °C.

Actinomycete spp. are generally more tolerant than fungi to moderately high temperatures and their number and species diversity increase surprisingly at 40-60°C. Actinomycetes are mostly dominant in the neutral and alkaline soil (Sakai *et al.*, 1994; Peterova and vlahov, 2007; Jeffrey, 2008; Basavarj *et al.*, 2010).

The occurrence of actinomycetes spp. are associated with the presence of stable compound compared to easily degradable compounds which are degraded by other microorganisms during the early phase of composting process. These members are high in the lateral stage of composting, especially after 65 days of composting and utilize complex organic waste. For instance, microorganisms such as *Micromonospora* spp, *Streptomyces* spp, *Nocardia* spp. *Dactylosporangium* and *Kibdelosporangium* are isolated from compost on the different days of composting. Their appearance as a grey- white growth at the surface of the composting material is an indication of maturation of compost (Nishad *et al.*, 1997; Gazi *et al.*, 2007; Arslan *et al.*, 2008; Silva *et al.*, 2009; Rakshanya *et al.*, 2011). In this study, the presence of these actinomycetes spp. in the compost at a high temperature may minimize some lignocellulosic compound of composted coffee husk.

The recorded diverse members of fungi dominated compost A, B and C at different temperature, pH and days of composting. During the initial and final composting processes when compost temperatures were lower some fungal genera such as *Rhizopus, Penicillium, Cladosporium, Aspergillus* and *Fusarium* are highly dominated the compost piles. In agreement with this finding Vajna *et al.* (2010) demonstrated that in the initial phase of composting, different *Penicillium, Fusarium, Aspergillus, Cladosporium, Trichoderma, Alternaria* and *Mucor* species were detected most frequently. Compost A and B relatively supported high number of isolates of fungi. Nevertheless, compost C supports only a few isolates of fungi. This may be because of unfavorable nutritional contents of compost C which contain bone meal. In addition to this, the result showed that, as the composting day (Day-15 at 57.33 °C and 8.39 pH) increases, a
compost C develops a bad smell and odor. This may be due to ammonia formation. This finding was in agreement with Inoko (1982) and Haga (1990), who reported Phosphocompost and a dried manure mixture such as bone meal could both greatly increase the pH and reduce the N contents probably because the ammonium volatilized at high temperatures.

Diversity of fungi at thermophilic stage is markedly reduced. Only few thermophilic fungi such as Aspergillus, Trichotheicum, Scopulariopsis, Mycotypha and Humicola are isolated at 40- 60 °C temperature and at slightly alkaline compost. The existence of these fungi genera in the compost with high temperature is probably due to the short duration of the exposure (Sakai et al, 1994; Thambirajah et al., 1995; Taiwo and Oso, 2004).

Fungi genera such as Trichosporon, Conidiobolus and Scedosporium were detected at the early stage of composting. These fungi may degrade lignocellulosic compound and contribute to the maturity of compost and there by encourage other microbial succession. In line with this study, some fungi sp. such as Penicillium rouquifortii, P. curtosum Trichosporon multisporum and other microorganisms are used to degrade caffeine and phenolic compound in the coffee wastes. Conidiobolus spp. is also isolated from compost of leaf litter or decomposed terrestrial plant materials at 40 °C (Aquiahuatl, et al., 1988; Pandey, et al., 2000; Waingankar, 2008; Derito and Madsen, 2009).

The number and diversity of microorganisms are more when combinations of wastes are used for composting. Therefore, the presence of relatively large populations of fungi in the compost of coffee husk combination might have been facilitating the degradation of phenolic compound (i.e. caffeine and tannin) as reported by Preethu et al. (2007).

4. CONCLUSION
Temperature was an important factor in composting efficiency since it may be influence activity and diversity of microorganisms that come to dominate it. Change in temperature of compost was also occurred. Finally, for all compost the temperature was uniformly declined and enters the cooling phase.
pH-value of compost was showed variation (p<0.05) based on the type of substrate utilized and duration of compost. Compost support the highest number of bacterial isolates than actinomycete and fungal which showing that the probability of their better adaptation to partly extreme conditions with high temperatures and pH-value.

In this study, bacteria, Actinomycete and fungi species isolated from the composted agro-wastes (combination of coffee husk) might be used for degradation of lignocellulosic and phenolic compound (i.e. caffeine, tannins and cellulose) in the organic coffee husk.

ACKNOWLEDGMENTS
We would like gratefully to thank Jimma University, School of Graduate Studies for financial support during our study. Lastly, it is our pleasure to acknowledge both Biology and Chemistry Department laboratory technicians for their unlimited assisting during study.

6. REFERENCES


