ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF MICROBIAL, CELLULAR & MOLECULAR BIOLOGY

Pilot Scale Windrow Composting of Coffee Husks and Flower Residues: Biochemical and Microbiological Determination of the Composts

A thesis submitted to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Applied Microbiology

By Fekadu Shemekite

Supervisor: Fassil Assefa (Asso. Prof.)

April, 2015

Addis Ababa

Abstract

Two hundred forty thousand (240,000) tons of coffee waste and about 4000 tons of flower residue wastes are generated annually from the age-old coffee processing industries and the booming cut flower industries in Ethiopia, respectively. Most of these wastes are released to the environment without treatment, and their direct release pollutes the environment, mainly inhibiting plant growth. This necessitates the transformation of these wastes into beneficial products with a dual purpose of production of bio fertilizer and protection of the environment. To this end, a study was made to evaluate the process of composting of coffee husks and flower residues separately in order to determine their compost quality. Each material was arranged in to three different piles in a trapezoidal Windrow composting for 90 days and more, and monitored for their physico-chemical, microbiological and phyto-toxicological properties during the whole process.

Coffee husk (CH) amended with cow dung plus house hold compost (CHCD: Pile 1) and with fruit/vegetable wastes plus house hold compost (CHFVW: Pile 2) were arranged and compared against the control (CH: Pile 3) without any amendment. As a result, the two amended treatments showed similar pattern of temperature profile with the first mesophilic stage (18-42^{o}C) in the first 2 days followed by the thermophilic stage (45-70^{o}C) up to 45 days, and declined to the second mesophilic stage afterwards and remained so until the end of the experiment. In all cases, there was a steady decline of moisture content (MC %), reduction in total organic carbon (TOC%) and a rise in total Nitrogen (TN%) because of the loss of carbon in the form of CO_2 thereby decreasing the C:N ratio of Pile 1, Pile 2, and Pile 3 to 11, 13, and 18, respectively. The highest bacterial count of 9.78log^{MPN} g^{-1}dw (Pile1) and 9.45log^{MPN} g^{-1} dw (Pile 2) was seen at the start of

the process; while the highest actinobacterial counts of 9.78log^{MPN}dw (Pile 1 and Pile 2) and fungal counts of 9.78log MPN g^{-1} dw (Pile 1) and 9.45 MPN g^{-1} dw (Pile 3) were recorded at the end of the composting process, respectively. This change in number indicated a clear microbial succession along the process. The effect of the different microbial activities was reflected in the better maturation of Pile 1 with a Germination Index value >80% and C/N ratio of 11 at the end of composting compared to the control that required much more days for the material to become free from phytotoxicity. This transformation could be associated with higher volatile solid reduction (75% in Pile 1 and 65% in Pile 2), substantiating the importance of chemical parameters in coffee husk compost.

The same feed stock was also composted with cow dung (Pile 1), with fruit/vegetable wastes (Pile 2) and coffee husk alone (Pile 3) again to study their microbial diversity and enzyme activities; and samples were collected on days 0, 32 and 90. Similar changes in TOC (%) and in TN(%) were found resulting in lower C/N ratios of 11 and 13 for Pile 1 and Pile 2, respectively due to changes in temperature profile, pH and water content of the mixture. This change which directed the succession of different microbial groups was accompanied by the release of compost relevant enzymes: *hydrolases*, *phosphatases*, *peptidases* and *proteases* in Pile 1 and Pile 2 at the start; and *esterase* at the end of the process.

Furthermore, denaturing gradient gel electrophoresis (DGGE) analysis of coffee husk composting indicated distinctive community shifts during the composting process. The DGGE revealed that bacterial and fungal communities of samples from day 0 were clustered separately from communities of samples from day 32 and 90, indicating a

change in the bacterial and fungal community composition. This result attributed that microbial communities at the start were responsible in the degradation of labile organic substrates while those communities at the end involved in the stabilization of the process. Young compost of Pile 1 and 2 were clustered separately from Pile 3 as the two co-substrates introduced different and diverse microbial communities into the composting process. On the contrary, microbial community in the matured compost was grouped together showing the end compost were homogenous with well-defined microbial communities.

Principal Component Analysis (PCA) of compost communities from day 0 and day 90 composts analyzed by COMPOCHIP microarray explained 62.5% of the variations. As a result, probes KO443 and 444 (*Stenotrophomonas maltophilia*), KO609, KO610 and KO614 (*Brevundimonas/Caulobacter*), KO500 (*Derxia gummosa*) KO612, 615, 616 and 617 (*Flavobacterium/Flexibacter*), KO541 (*Pseudomonas putida*), KO252 (*Acinetobacter*) and KO342 (*Actinomyces* sp.) were found to be more influential in discriminating the samples into different composting phases. Besides, *Brevundimonas*, *Caulobacter, Chryseobacterium Sphingobacterium* were dominant during the first mesophilic phase in Piles 1 and 2. These species have broader degradation activity of complex biopolymers. On the other hand, *Flavobacteria/Flexibacter* was detected significantly in all samples, suggesting their importance in the composting process. In general, microbial diversity and numbers were lower in the mature composts as indicated by DELTA 495a, Alpha proteobacteria and Low G+C, *Xylella/Xanthomonas/Stenotrophomonas* (KO241), *Azotobacter beijerinckii* (KO277) and

the *Actinomyces* (KO342). Especially, the presence of *Actinomycetes* was an indicator of mature compost.

In flower residue, the major feed stock (flower residue) was blended with cow dung (Pile 1), with activated EM (Effective Microorganisms) and molasses (Pile 2) and compared against the control (Pile 3) without any amendment for the same period of composting and the same composting system. Changes in NH_4-N, NO_3-N, NH_4:NO_3 ratio, available P and K and concentration of micronutrients (Cu, Zn, Fe, Mn) were included in the chemical analysis in addition to TOC%, TN% and C/N ratio to better understand the efficiency of chemical parameters in maturity indices.

Consequently, the reduction in TOC (%) and the steady increase in TN(%) resulted in 10%, 16% and 24% of C/N ratios at the end of the process for Pile 1, Pile 2 and Pile 3, respectively. The use of inorganic N forms (NH_4-N, NO_3-N and NH_4-N to NO_3-N ratios) as indicators of maturity showed significant differences among the three piles. While NH_4-N reduced below 400 mg/kg in all piles which was the maximum threshold level for matured compost, NO_3-N showed an increasing trend, resulting in lower NH_4-N to NO_3-N ratio (0.12) of Pile 1, compared to Pile 2 and Pile 3 which could not reduce below 0.38 and 0.58, respectively. The analysis of available P and K also showed an increasing trend in all piles, but the concentration of available P (0.7-1%) at the end of the process was sufficient for soil nutrient supplementation. On the contrary, available K and all micronutrients were below the sufficiency level for plant production.

In general, the effect of the different microbial activities was reflected in the better maturation of Pile 1, showing a low C/N ratio (10-11) and high GI% (80-85%) at the end

of the composting; and significantly correlated with temperature, water content, pH over the 90 days of composting of both coffee husk and flower residue composts. Comparatively, Pile 2 and control of both the composting processes required more days for maturity in this respect.

In conclusion, a polyphasic approach using physico-chemical and biological parameters including temperature, C/N ratio, GI%, NH_4:NO_3 ratio, enzyme assay together with molecular tools are very essential to evaluate maturity and stability of coffee husk and flower residue composts. Bulking agents that have much amendment potential like cow dung must be encouraged in large scale composting industries to tackle the current environmental challenges; and further studies on composting of various sources of solid organic wastes with a different composting system can offer greater insight on the quality, safety and age of composting. The nutritional and economic feasibility of these compost products must be addressed in order to fully realize their benefits and to understand the implication of the end products on plant production.

Key words/phrases: *Coffee husk compost; COMPOCHIP microarray; Enzyme activity; Organic matter degradation; PCR-DGGE; Germination index*

Acknowledgement

First and for most, I would like to thank my supervisor, Dr. Fassil Assefa, for guiding me through this journey, from the moment of entry all the way through to the editing process. I thank him so much for introducing me to the fascinating world of microbial ecology and providing me an attractive topic for my study. His constant support, in the form of inspiring dialogue and willingness to so heartily undertake the task of transforming my prosaic writings into scientific manuscripts, is much appreciated. I am always inspired by his constant critics and commendable comments during the whole period of my study.

I would also like to express my heartfelt thanks to Prof. Dr. Heribert Insam, from Institute of Microbiology, University of Innsbruck, Austria for accepting me to work in his laboratory in DGGE and COMPOCHIP microarray. My especial thanks also goes to Dr. Ingrid, H. Franke-Whittle and Dr. María Gómez-Brandón from the same institute, who kindly assisted me in the molecular analysis of my samples, and providing me seeds as well as critically reviewed part of this manuscript.

I would also like to extend my thanks to Ato Alemayehu Mengistu, for his constant support and encouragement throughout the study period. I must owe him my sincere acknowledgment for his fatherhood and companionship during moments of hardships and joys.

I am so much grateful to my beloved wife, W/ro Senait G/Hiwot, for encouraging me to join this program, and bearing all family affairs, tolerating all downs that I had encountered during the study period. I must also award my appreciation to my son,

Amanuel Fekadu for tolerating loss of attention which he deserves during his critical school time.

I owe thanks to my late father Ato Shemekite Ashene, who brought me up with his unreserved love and inspiration. I also thank to my mother, W/ro Asnakech Yimer, my sister and brother, Genet and Behailu Shemekite. My thanks also go to my brother-in-law and sister-in-laws who were behind us throughout this period.

I owe due thanks to the Department of Microbial, Cellular and Molecular Biology (MCMB) of the College of Natural Sciences, Addis Ababa University for offering me the opportunity to pursue the PhD study.

I am also grateful to the Addis Ababa Environmental Protection Authority (EPA) for providing me a trial site at Gerji; and the Ethiopian Horticulture Production and Export Association (EHPEA) for funding part of the research and for getting me a trial site.

List of abbreviations

A	Actinobacteria
AAS	Atomic Absorption Spectrophotometry
ANOVAR	Repeated Measures Analysis of Variance
ATP	Adenosine Tri Phosphate
C/N	Carbon to Nitrogen
CD	Cow Dung
CFU	Colony Forming Units
CH	Coffee Husk
CP	Coffee Pulp
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic Acid
DOC	Dissolved Organic Carbon
DTPA	Diethyl Triamine Pentacetic Acid
DW	Dry Weight
EC	Electric Conductivity
EM	Effective Microorganisms
EU	European Union
F	Fungi
FR	Flower residue
FVW	Fruit Vegetable Waste
GFF	Galica Flower Farm
GI	Germination Index
HHC	House Hold Compost
HW	Horticulture Waste

IWMS	Integrated Waste Management System
MC	Moisture Content
MPN	Most Probable Number
MSW	Municipal Solid Waste
NA	Nutrient Agar
OC	Organic Carbon
OM	Organic Matter
ONRS	Oromia National Regional State
PCA	Plate Count Agar
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PLFA	Phospholipid Fatty Acid
RNA	Ribonucleic Acid
SCA	Starch Caesin Agar
SNR	Signal-to-Noise-Ratio
SPSS	Statistical Package for Social Sciences
SS	Sewage Sludge
TAB	Total Culturable Aerobic Bacteria
TN	Total Nitrogen
TOC	Total Organic Carbon
UPGMA	Un-Weighted Pair-Group Method Arithmetic Averages
VS	Volatile Solid
VS_{red}	Volatile Solid Reduction

Table of contents

List of tables

List of figures

This dissertation is prepared based on the following papers which are included in different Chapters:

1. Fekadu Shemekite and Fassil Assefa (2014). Evaluation of compost maturity and stability of the coffee husk using chemical and microbial parameters. **Submitted to Science of the Total Environment**

2. Fekadu Shemekite, Gómez-Brandón, M., Franke-Whittle, I. H. Barbara., Praehauser, B., Insam, H. and Fassil Assefa (2014). Coffee husk composting: An investigation of the process using molecular and non-molecular tools. **Waste Management 34**: 642-652. **Published**

3. Fekadu Shemekite and Fassil Assefa (2015). Maturity and stability determination of flower waste in Ethiopia: its nutritional and environmental implication. **Submitted to SINET**

Published papers was re-printed with the permission of the publishers

CHAPTER 1 Theoretical Background and Literature

1.1. General introduction

The rapid increase of urbanization and industrialization has generated large quantities of solid and liquid wastes. The total amount of municipal solid waste (MSW) generated globally reached 2.02 billion tons, and increases annually by 8 percent of which the 25 member states of EU contribute 700 million tons per year of agricultural wastes (UNEP, 2009). Likewise, the American industries generate and dispose-off approximately 7.6 billion tons of non-hazardous industrial solid wastes every year (US-EPA, 2006).

According to UNEP (2009), less than half of the solid waste is produced in developing countries of which 95 percent is indiscriminately dumped throughout the city (UNEP, 2009). In Ethiopia, the total amount of solid wastes generated in the country ranged from 2.2 to 8.8 million tons, of which 38-40% of the wastes are produced by the rural inhabitants (EPA, 2010). Most of the solid wastes (60%) produced are organic wastes originated from agriculture, agro-industry and municipal sources.

Currently, there are attempts to reduce the volume of these wastes through Integrated Waste Management System (IWMS). This is based on a 3R principles (reduce, reuse and recycle) of all wastes originated from urban, rural and industrial areas of the world (UNEP, 2009).

Composting is one of the integrated waste management strategies used for the recycling of organic materials into a useful product because of its eco-compatibility and easy operational procedures (Giglotti *et al*., 2005; Raut *et al*., 2008). It is a bio-oxidative process involving the mineralization and partial humification of any organic matter

originated from plants, animals and microbes, leading to a stabilized final product called compost (Zucconi and de Bertoldi, 1987). It is heat generating microbial driven process, and a highly active dynamic microbial system that changes its own environmental conditions (Ryckeboer *et al.*, 2003a; Steger *et al*., 2007).

The process is undertaken under controlled conditions to achieve three major objectives: 1) to overcome the phyto-toxicity of fresh non-stabilized organic matter; 2) to eliminate or reduce the presence of agents (virus, bacteria, fungi, parasites) that are pathogenic to man, animals and plants; 3) to produce an organic fertilizer or a soil conditioner from recycling of organic wastes and biomass (Insam and de Bertoldi, 2003). Consequently, application of compost to the soil-plant system increases soil nitrogen and phosphorus (Jakobssen, 1995), improves soil structure and water holding capacity (Joshua *et al*., 1998), increases microbial biomass (Insam and de Bertoldi, 2003), reduces weeds control and suppresses plant diseases (Hoitink and Boehm, 1999).

Composts differ in their quality that can be described in terms of compost stability and maturity that define the degree of decomposition and transformation of organic matter (Zmora-Nahum *et al.*, 2005). Compost stability reflects the degree of decomposition of organic matter over the course of the composting process, and is more associated to biological activity (Ceustermans *et al.*, 2010) and microbiological activity in particular (Tiquia, 2005; Zmora-Nahum *et al*., 2005).

Compost maturity, on the other hand, refers to the degree of decomposition of phytotoxic organic substances produced during the active phase of composting process with a concomitant removal of pathogens and weed seeds (Wu *et al*., 2000; Cunha-Queda *et al*.,

2007). Compost maturity is also linked to the level of nitrification (Bernal *et al*, 1998) and the degree of humification (Jouraiaphy *et al*., 2005). They, in turn, are related to agricultural values in terms of quality and marketability of the final product in relation to plant productivity (Cabañas-Vargas *et al*., 2005; Gómez-Bandón *et al*., 2008).

All taken together, compost maturity assessment is more of an art than compost stability assessment, since acceptable maturity varied depending on compost end use (Brewer and Sullivan, 2003). Apart from this, shortening of composting period with considerable reduction in the C/N ratio of the starting mixture is very important for making the composting process successful (Raut *et al*., 2008). The application of non-stabilized and immature compost to soil may cause several toxicity effects and adversely affect the environment (Butler *et al*., 2001).

A large variety of techniques have been developed for the determination of compost stability in the last few decades (Wang *et al*., 2004). Physico-chemical parameters such as temperature, pH, electric conductivity (EC), Dissolved Organic Carbon (DOC), the ratio of C to N and NH_4^+ to NO_3^- have been applied as indicators of stability (Said-Pullicino *et al*., 2007; Castaldi *et al*., 2008; Albrecht *et al*., 2008; Aslam *et al*., 2008).

Mondini *et al*. (2004) reported that microbial biomass and activity can be used as stability parameter in ligno-cellulosic waste composts for it clearly reflects the transformation of organic matter during the composting process. Variation in the composition of microbial community and total number of microorganisms at each composting phase provides valuable information about the process, for efficient and satisfactory composting process

is dependent on the presence of a high microbial diversity (Beffa *et al*., 1995; Herrmann and Shann, 1997).

Respiration (CO_2 evolution rate and/or O_2 uptake rate) has been widely used to evaluate the stability of compost (Gómez *et al*., 2006). ATP content and enzyme activity are also useful indicators of compost stability (Tiquia *et al*., 2002; Boutler-Bitzer *et al*., 2006). Knowledge on the diversity and dynamics of the microbial communities of compost is gained through the use of molecular biology tools such as community fingerprinting by amplified rDNA restriction analysis, denaturing gradient gel electrophoresis (DGGE) and hybridization techniques (Hoitink and Boehm, 1999; Alfreider *et al*., 2002; Scholass *et al*., 2005).

Nucleic acid microarrays or oligonucleotide microchips represent one of the most recent advances in molecular technologies, allowing a high-throughput format for the parallel detection of 16S rRNA genes from an environmental sample (Bodrossy and Sessitsch, 2004). The parallel detection of numerous 16SrRNA genes makes the microarray useful for environmental studies of phylogenetically diverse microbial groups. In the case of the compost environment, microarray offers a tremendous potential for process monitoring, the detection of pathogens and beneficial microbial populations (Franke-Whitle *et al*., 2005, 2009).

However, there is a strong argument on the benefits of a single parameter to measure the degree of degradation of all organic materials. Only few reports justified the advantages of application of multi-parameter that describe the stability and/or maturity of compost made from most lingo-cellulosic materials (Bernal *et al*., 2009). Besides, the addition of

high N sources at the starting material was established as a troubleshooting to balance the C/N ratio so that the requirement of the essential microbes (degraders) could be met (Ryckeboer *et al*., 2003a).

A study was, therefore, made to understand the changes that coffee husk and flower residues underwent with the composting process when they were amended with high N sources (cow dung and flower vegetable wastes) and inoculants (activated effective microorganisms and household composts). In addition, a number of parameters including physico-chemical, enzymatic, phytotoxicity, N-transformation and microbial enumeration and microbial community analysis were used to realize the significance of the amendment. As a result, significant differences have been found due to the amendments (particularly cow dung), resulting in the faster degradation of the lingo-cellulosic substrates of coffee husk and flower residue. Hence, a combination of physical, chemical and microbiological techniques formed a novel approach not only in monitoring the quantitative and qualitative fluctuations of the available substrates, but also in revealing the significant difference in composts and compost maturity.

1.2. Literature Review

1.2.1. Feedstock and events of the composting process

Composting is a complex process that is affected by several important factors that defines the direction of the process. The crucial aspect of the process lies on the nature of the starting organic materials that attract various groups of microorganisms.

These organic materials are carbohydrates, proteins, fats, hemicellulose, cellulose, lignin and mineral matter (Table 1.1). They can be either susceptible or resistant to microbial

attack. The first susceptible organic materials to microbial attack are simple sugars, starches, pectin, fatty acids, amino acids and nucleic acids, whereas the resistant ones are: hemicellulose, cellulose and lignin (Insam and de Bertoldi, 2003).

The carbohydrates serve as substrate for the microorganisms to generate energy for biosynthesis of cellular constituents. Apart from carbon source, microorganisms require other nutrients like nitrogen (N), phosphorus (P), potassium (K), and trace elements for their growth. In particular, nitrogen is the most vital element for microbial growth. If low level N occurs during the composting, the degradation process will be slow. On the contrary, excess N may be lost from the system as ammonia gas or through leaching as nitrate (Bernal *et al*., 1998).

Since composting is an aerobic process oxygen supply is essential through active mechanical aeration, convective air flow (=passive aeration) or physical turning of the compost mass. Proper aeration controls the temperature, removes excess moisture and CO_2 and provides O_2 for the biological process. Thus, the optimum O_2 concentration is between 15% and 20% irrespective of the composting system (Bernal *et al*., 2009). As a result, controlled aeration can maintain temperatures below 60–65°C, which ensures enough O_2 is supplied.

The diffusion of oxygen through the compost mass can be limited by moisture content (Haug, 1993). Excessively wet compost (>60%) limits oxygen transport, which prevents the growth of aerobic microorganisms and reduces the rate of decomposition because the free pore space may be blocked by water (Richard *et al.*, 2002). Low moisture content, on the other hand, can also restrict the movement of microorganisms and inhibit microbial

growth and activity affecting the degradation of organic compounds (Richard *et al*., 2002).

The intense microbial activity at the beginning of composting generates heat that causes the rise in temperature. Moreover, carbon dioxide and water are released as decomposition products (Ryckeboer *et al*., 2003a). One of the major objectives of composting is the effective decomposition of organic matter in the shortest time, and knowledge of the basic aspect of composting is essential to be able to control and mange the process efficiently (Steger *et al*., 2007).

1.2.2. Carbohydrates

Quantitatively, the main components of organic matter are plant origins and constituents (as presented in Table 1.1). Cellulose, hemicellulose and lignin are among the most important polymers occurring on earth.

1.2.2.1. Cellulose

All plant cell walls contain cellulose, which is mostly crystalline in native stage and is surrounded by a mixture of amorphous cellulose, hemicellulose, and lignin. Cellulose is a linear homopolymer consisting of repeating sterochemical units of glucose, or in other words repeating units of β-D-glucopyranose, which are linked together by β-1-4-glycosidic bonds (Tuomela, 2002).

The molecular mass is high, and the degree of polymerization (DP) is up to 15,000 (Insam and de Bertoldi, 2003). Crystalline cellulose is highly resistant to microbial degradation and amorphous cellulose is degraded much faster than crystalline cellulose. The cellulose degrading ability of microorganisms is dependent upon the existence of the

cellulase enzyme systems and/or the synergistic activity of microorganisms (Beguin and Aubert, 1994).

The most efficient cellulose degraders are filamentous fungi (Tuomela, 2002).Typically, the components of cellulase enzyme system, endoglucanases and cellobiohydrolases, first hydrolyse crystalline cellulose to cellobiose, and *β*–endoglucanase degrades cellobiose to glucose. *Chaetomium thermophilum*, for instance, releases glucose from native cellulose, and *Thermomyces langinosus* utilizes this sugar (Tuomela, 2002).

Table 1.1 Major natural compounds which are the substrates for decompositon

Compound	Composition	Function	P*	A*	M*	Degradability
Lignin	Polymeristates of phenylpropane derivatives, e.g. coniferyl alcohol	Structural compound	3	0	0	Very resistant mainly by fungi
Glucose ($C_6H_{12}O_6$) Polymers						
Cellulose	β-1,4 bonds	Structural compounds (Plant leaves, stems)	3	0	0	Easily, mainly by fungi but also Bacteria and Actinomycetes
Starch	Amylose: linear α-1,4-bonds; Amylopectin branched α- 1,4 bonds	Storage compounds in seeds and roots	2	0	1	Good aerobically & anaerobically (*Clostridium*)
Glycogen	α-1,4-, α-1,6-bonds	In animal muscles	0	1	0	Good
Laminarin	β-1,3-bonds	Marine algae (Phaeophyta)	2	0	0	Fair
Paramylon	β-1,3-bonds	Algae (Euglenophyta and Xanthophyta)	1	0	0	Fair
Dextran	1,6 bonds	Capsules or slime layers of Bacteria	0	0	1	Fair

Agar	Polymer of Galactose and galactouronic acid	Marine algae (Rhodophyta)	2			Resistant
Suberin, cutine	High polymeric esters of saturated and unsaturated fatty acids	Structural compound	1	0	0	Poor
Hemicellulose						
Xylan **Araban** **Mannan** **Galaktan**	Low degree of polymerization of sugar monomers (pentoses and hexoses) and uronic acids; Usually 20-100 monomers	Cell wall compound, seeds straw, wood, algae	3	0	0	Variable degradability often together with lignin
Pectin	Polymer of galactouronic acids ($3x10^4$-$5x10^5$ monomers)	Dissolved, and in cell wall in seeds, fruits, and in young wood parts	2	0	0	Easy by most microorganisms, often plant pathogens
Sucrose	Glucose-fructose disaccharide	Vacuoles	2	0	1	Very easy by most Microorganisms
Lactose	Glucose-galactose disaccharide	Milk	0	1		Easy by LAB

Hyaluronic acid	Polysaccharide of glucuronic acid and *N*-acetylglucosamine	Connective tissue	0	1	0	Easy
Chlorophyll and other pigments		Plastids	1	0	0	Easy
Alkaloids, tannins	Sugars, mainly α-D-glucose	Vacuoles	1		0	Variable
Fats and waxes	Glycerine and fatty acids	Storage compounds	1	3	1	Variable
DNS, RNS	Nucleic acids	nuclei, mitochondria	1	1	2	Easy
Poly-β-hydroxy butyric acid		Vacuoles, storage compound	0	0	2	Easy
Murein	Peptidoglycan	Cell wall bacteria	0	0	3	Easy

*P: plant; A: animals, M: microorganisms. Numbers indicate the relative importance (0…not found, 3…found in very high quantity). Adapted from Insam and de Bertoldi (2003)

1.2.2.2. Hemicellulose

Hemicellulose are mixed group of both linear and branched hetero polymers mainly comprising five monomer sugars, namely D-glucose, D-mannose, D-galactose, D-xylose, and L-arabinose (Tuomela, 2002). They are linked together mostly by β-1,4-glycosidic bonds, and occasionally β-1,3-, β-1,6-, α-1,2-, α-1,3-, and α-1,6-glycosidic bonds (Eriksson *et al*., 1990). The degree of polymerization (DP) of hemicelluloses is 100-200, which is much less than that of cellulose. The major hemicellulose of gramineous plants is xylans, which interact with lignin and cellulose probably more than any other hemicellulose. Galactoglucomannans, arabinoglucuronxylan, and arabinogalactan are the main component of softwood hemicellulose (Tuomela, 2002).

Due to a lower degree of polymerization and their amorphous nature, hemicelluloses are degraded more easily than cellulose. Nevertheless, a complex enzymatic system, such as different xylanolytic and mannan degrading enzymes, is still required because the structure of hemicellulose is variable and branched (Tuomela, 2002).

1.2.2.3. Lignin

Lignin is a natural composite material in all vascular plans, providing the plant with strength and rigidity. Lignin is an amorphous, aromatic, water-insoluble, heterogeneous, three-dimensional, and cross-linked polymer with low viscosity. The molecular mass of lignin is high and variable (600-1000kDa) (Brown, 1985). Lignin is highly reduced and its carbon content is 50% higher than that of polysaccharides, which makes it energy rich (Brown, 1985). However, lignin is the most recalcitrant polymer to microbial degradation. The degradation of lignin rather enables efficient utilization of

carbohydrates, which lignin usually makes complex with polysaccharides. Thus, microorganisms, which utilize polysaccharides, often possess lignolytic capability. Generally, lignin is finally degraded to carbon dioxide, water and humus (Tuomela, 2000). *Trichoderma* sp. *Trichurus spirilis* and *Paceilomyces fusisporous* are important fungal groups which can degrade lingo cellulosic complex in the composting process (Saha *et al.,* 2012).

1.2.3. Microbial activity and temperature in composting

Composting induces high metabolic activities of microorganisms at high density (up to 10^{12} cells g^{-1}) (Ryckeboer *et al*., 2003a). The microbial consortium present at any point of time is replaced by others in a very short interval time. This makes composting to resemble a batch culture with steady changes in substrate composition and biochemical conditions (Insam and de Bertoldi, 2007). On the contrary, continuous composting process may be regarded as a sequence of continuous cultures, each of them with their own physical (e.g. temperature), chemical (the available substrates), and biological (e.g. the microbial community composition) properties and feedback effects. Therefore, at any given time the identification of microorganisms is an important prerequisite to determine microbial diversity and metabolic activity pathways within the composting ecosystems (Steger *et al*., 2007).

Previous level of understanding of microbiota involved in composting depends largely on studies made with culture methods such as isolation and identification of bacteria, actinobacteria and fungi. Some of the first microbiological studies investigated the effect of temperature on the presence of microorganisms and on the efficiency of the compost process for composting is a self-heating biological process (Steger *et al*., 2007). Another

early study was that inoculation with thermophilic populations led to a higher decomposition rate at higher temperature than inoculation with mesophilic populations (Waksman and Cordon, 1939).

Furthermore, it was found out that bacteria, actinobacteria and thermophilic fungi were all active in compost at 50°C; however, at 65°C, fungi were rare, and at 75°C, spore-forming bacteria were the predominant organisms (Waksman *et al*., 1939). Temperature above 55°C is required to kill pathogenic microorganisms (de Bertoldi *et al*., 1983). These and other studies (Webley, 1947; Strom, 1985) showed the importance of temperature on microbial developments in the compost environment.

Overall, degradation of wastes in compost proceeds in four phases based on the development of temperature (Figure 1.1): i) first mesophilic (temperature below 45°C) ; ii) thermophilic (temperature above 45°C) ; iii) second mesophilic (cooling) ; and iv) maturing (curing) phases, which is characterized by a decrease in temperature (Insam and de Bertoldi, 2003).

i) The first mesophilic phase

At the beginning of composting, mesophilic bacteria and fungi degrade soluble and easily degradable compounds of organic matter, such as monosaccharides, starch, and lipids. Bacteria produce organic acids, and pH decreases to 5-5.5. Temperature starts to rise spontaneously as heat is released from exothermic degradation reactions. The degradation of proteins leads to release of ammonia that raises pH rapidly to 8-9. This phase lasts from a few hours to a few days.

ii) Thermophilic phase

The compost enters the thermophilic phase when the temperature reaches 45°C. Thereafter, thermophilic bacteria and fungi take over, and the degradation rate of the waste increases. If the temperature exceeds 55°–60°C, microbial activity and diversity decreases dramatically. After peak heating, the pH stabilizes to a neutral level. The thermophilic phase can last from a few days to several months (Strom, 1985).

iii) Second mesophilic (cooling phase)

After the easily degradable carbon sources have been consumed, the compost starts to cool. Second mesophilic phase during which, often dissimilar to those of the first mesophilic phase, recolonize the substrates. After cooling, the compost is stable. However, most of the species are different from the species of the first mesophilic phase.

iv) Maturing (curing phase)

Actinobacteria often grow extensively during this phase, and some protists and a wide range of macroorganisms are usually present. The biological processes are now slow, but the compost is further humified and becomes mature. The cooling and maturation phase lasts for several months or even years.

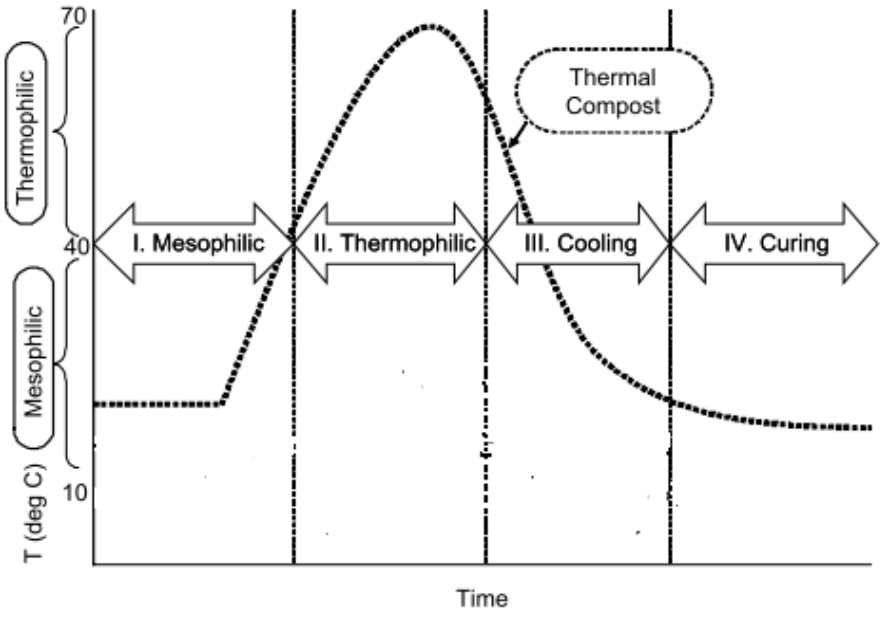


Figure1.1 Theoretical Vs time curves for the different stages of composting (from: Jack *et al.*, 2006)

These phases may have considerable overlap based on temperature gradients and differntial temperature effects on microorganisms (Figure 1.2) (Fogarty and Touvineb, 1991).

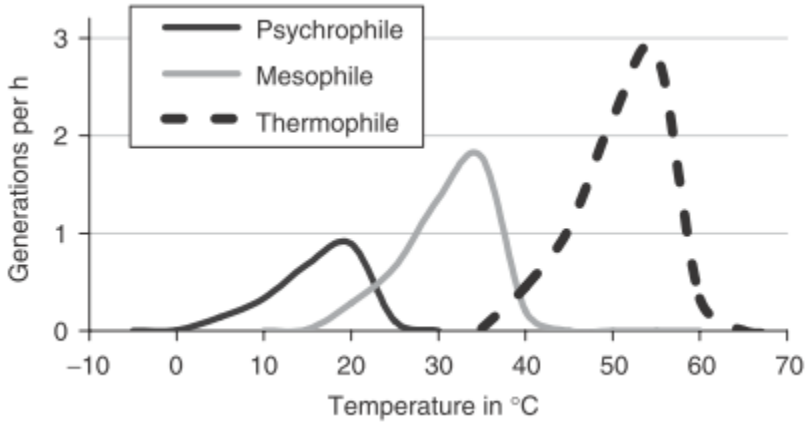


Figure 1.2 Temperature range of psychrotolerant, mesophilic and thermophilic organisms and their generation time (from: Insam and de Bertoldi, 2003)

Under optimal conditions, however, temperature distribution is not evenly reaching throughout the zones of compost piles. Thus, it is necessary to move every part of the substrate, through regular turning or tumbling, to the central hottest part of the pile (Insam and de Bertoldi, 2007). In general, four major zones could be identified within the pile from microbiological-temperature relationship point of view (Figure 1.3). These are: **Zone 1** is an outer zone, which is the coolest zone, and well supplied with oxygen; **Zone 2**, the inner zone is poorly supplied with oxygen; **Zone 3**, the lower zone is hot, and well supplied with oxygen; while **Zone 4** is the upper zone which is the hottest zone and fairly supplied with oxygen.

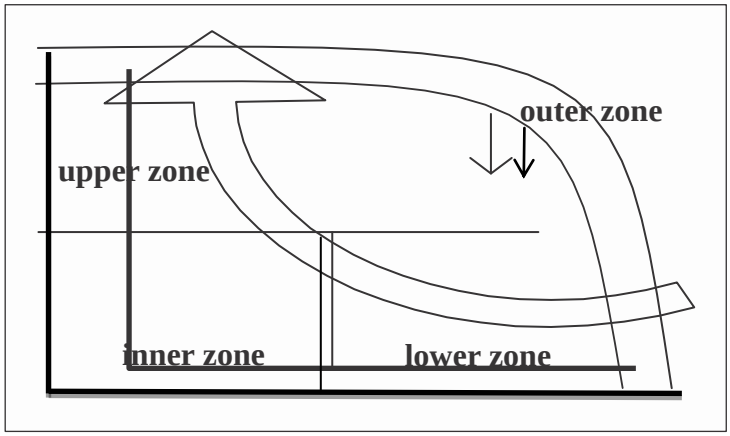


Figure 1.3. Cross section of a compost windrow, major zones and convection stream (adapted from Insam and de Bertoldi, 2007)

The general relationships of temperature as a function of composting time have been depicted in many ways and the shape of the curves varies with the organic material to be composted and the applied conditions (Steger *et al*., 2007). However, various microbial communities predominate during the various composting phases, each of which being adapted to a particular physico-chemical environment (Ryckeboer *et al*., 2003a). The

initial stage is characterized by the growth and activity of primary decomposers mainly gram negative bacteria, lactic acid bacteria, fungi and yeasts (de Bertoldi, 1998).

These organisms create a suitable physico-chemical environment for secondary organisms by producing necessary metabolites for their growth and activity. Such a condition is referred as cross feeding (Ryckeboer *et al*., 2003a). Generally, the activity of mesophilic community leads to an increase in temperature. This condition was explained by the fact that while the initial mesophilic microorganisms are inhibited due to increased temperature, the thermophilic populations have not yet developed and is below their temperature optimum (Ryckeboer *et al*., 2003a).

The second stage starts when a sufficient number of thermophiles are generated, then temperature rises again. At this time, organisms adapted to these conditions (e.g. *Bacillus* spp. *Thermus thermophiles* and *Thermoactinomyces* spp.) take over the degradation process (Strom, 1985; Blanc *et al*., 1998). At temperature exceeding 60°C, the system starts to limit itself due to the inhibitory of high temperatures (Ryckeboer *et al*., 2003a). If a good management is provided (i.e. regular aeration or frequent turning), this stage keeps on until the heat generated becomes lower than the heat dissipated. In general, high temperature helps the degradation of refractory organics such as lignocellulose (Tuomela *et al*., 2000), and elimination of pathogenic and allergenic microorganisms as well as parasites (Beffa *et al*., 1995; Herrmann and Shann., 1997).

The third stage is the cooling phase where nutrients become a limiting factor, causing a declining trend in microbial activity and heat output. The fourth stage is the maturation phases, in which the substrate quality further declines and compounds such as lignin-

humus complexes are formed that are not further degradable (Insam and de Bertildi, 2003). This stage is, therefore, characterized by a reappearance of new mesophilic communities which are often different from the intial mesophilic microflora, predominantly fungi and actinobacteria (Eiland *et al*., 2001).

The recolonization of a mesophilic community might be more a matter of nutritional factors than a result of temperature. Since easily utilizable compounds are exhausted in the earlier stages of composting, microorganisms present at this stage have to degrade compounds less susceptible to mineralization (Steger *et al*., 2007). It was noted that the length of the different composting phases depends on the nature of the substrate being composted and the efficiency of the process, which is determined by several factors such as starting material, O_2 supply, moisture content, active turning and outside temperature (Ryckeboer *et al*., 2003a).

1.2.4.Microbial community and Enzyme activity

As biological process, composting involves a myriad of microorganisms that are important component of the composting process (Tiquia *et al*., 2002). The number and diversity of microorganisms are indicators of specific phases of composting. However, there are cases where numbers did not show that much differences during the process. Atkinson *et al* (1996) reported that the total number of microorganisms did not differ significantly during composting while others report higher numberof microbes with regards to the mesophilic stage (Tiquia *et al*., 2002). However, there is a general consensus that the compositon of the community can vary during the different phases of the composting process (Atkinson *et al*., 1996; Hassen *et al*., 2001).

Hultman *et al*. (2009) reported that the genera *Candida*,*Pichia* and *Dipodascaceae* dominated the initial mesophilic phase, and *Thermomyces*, *Candida* and *Rhizomucor* were found at thermophilic stages during the study of municipal solid waste. But a few species were found throughout of the process. Previously, Strom(1985) and Beffa *et al*.(1995) determined the diversity of bacteria during the composting of (source separated) municipal solid wastes. During the terminal and maturation phase of compost high microbial properties of diverse functional groups of mesophilic microorganisms were identified.

Beffa *et al*.(1995) reported that nitrogen fixers, sulfur oxidizers, hydrogen oxidizers, nirifiers, and producers of extracellular polysaccharides (bacterial humin) are those of mesophilic during the maturity phase. Furthermore, *Thermus aquaticus*, *Bacillus Schlegelii*, *Hydrogenbacter* and *Bacilli* were identified during the thermophilc stages of composting. Ryckeboer *et al*. (2003b) examined diversity and population densities of prokaryotes and fungi throughout the whole composting process of soure separated household wastes, i.e. from starting material to matured compost (Table 1.2).

Table 1.2 Numbers of microorganisms during different phases of composting

Microorganisms	Stage	Number
Bacteria	Mesophilic	10^{9} to 10^{13} g^{-1} substrate
	Thermophilic	10^{8} to 10^{12} g^{-1} substrate
Actinombacteria	Mesophilic	10^{8} to 10^{12} g^{-1} substrate
	Thermophilic	10^{7} to10^{9} g^{-1} substrate
Fungi	mesophilic	10^{5} to 10^{8} g^{-1} substrate

(adapted after Ryckeboer *et al*., 2003a)

The capacity of microorganisms to assimilate organic matter depends on their ability to produce enzymes needed for the degraration of the substrate. In composting, the soluble organic matter in the starting organic material is initially assimilated by the microorganisms. Once the soluble organic matter is used up, microorganisms produce hydrolytic enzymes to depolymerize the larger compounds (i.e. lignin, cellulose and hemicellulose) to smaller fragments that are water soluble (Tiquia *et al*., 2002). The transformation process involves the succession of microbial communities that are endowed with a wide array of enzymes responsible for the changes in the physico-chemical properties of the substrate (Mondini *et al*., 2004).

Several researches were undertaken to study the relationships of different microbial groups (total aerobic heterotrophs, actinomycetes, fungi, fecal coliforms, ammonia and nitrate reducing bacteria, and denitrifying bacteria) with different extracellular enzymes (Tiquia, 2002, Cunha-Queda *et al.*, 2007, Vargas-García *et al.*, 2010). Studies on

microbial population dynamics and enzyme activities of poultry litter/yard trimmings showed that the population of fungi and actinomycetes (active in the degradation of lignin, cellulose and hemicellulose) were positively correlated with esterase, valine amino-peptidase, α-galactosidase, β-glucosidase, and lipase. Similarly, β-galactosidase (enzyme involved in the hydrolysis of lactose) showed the most significant correlation with total heterotrophs, ammonium- and nitrate-oxidizing bacteria, denitrifying bacteria and fecal coliforms (Tiquia, 2002).

Given the difficulties to quantify the activities of such a large number of enzymes in nature, few key enzymes were singled out as important indicators of high microbial activity in the composting process (Sánchez-Mondero *et al*., 2001; Mondini *et al*., 2004; Cunha-Queda *et al*., 2007; Liu *et al*., 2011). Mostly, the measure of dehydrogenase activity was considered as a general index of microbial activity on account of its role on the oxidative phosphorylation process, in the respiratory metabolism of microorganisms (Vargas-García *et al.*, 2010), whereas the other enzymes such as β-glucosidase, phosphatase, protease and urease are a measure of specific activity (Vargas-Garcia *et al.*, 2010).

Enzymes in composts can be fundamentaly classified into two distinct groups: enzymes inside viable cells (intracellular enzymes) and enzymes outside viable cells (exo or extracellular enzymes). It is not possible to distinguish between the intracellular and extracellular activities of enzymes in whole compost suspensions (Vuorenin, 2000). However, after a brief incubation period, a large proportion of the enzymes in compost belong to extracellular groups of enzymes are detected (Vuorenin, 2000).

Some of the enzymes are related to the mineralization of C, N and P. For example, the mineralization of N during composting involves the release of N from non peptide C–N bonds in amino acids and urea mediated by amidohydrolase and dehydrogenase enzymes (Tabatabai, 1994). Alkaline and acid phosphatases are important enzymes in organic P mineralization (Nannipieri *et al*., 2012). The mineralization of C from glucose is mediated by the activity of β-glucosidase enzyme (Nannipieri *et al*., 2012).

The enzyme activities varied during the different ages of composting. For example, the evolution and activities of alkaline phosphomonoesterase was higher during the early mesophilic periods in cattle manure compoting (Godden *et al* ., 1996). Castaldi *et al*.(2008) also reported that *protease, urease, cellulase, β-glucosidase* and *dehydrogenase* enzymes were characterized by significant changes during the first two weeks of MSW composting but they gently decreased after the fourth weeks (maturity phase) of the process. This implies that the evolution of enzymatic activities could be a suitable indicator of the state and age of the organic matter. This is the common trend of well managed composting system (Vuorenin,2000).

It is also important to note that the kinetic properties of enzymes, particularly phosphomonoesterase (PME), differ significantly in different kinds of manure (Dick and Tabatabi, 1984) and composted manure (Vuorenin, 2000). Garcia *et al.* (1992) detected more PME and β-glucosidase activities from cattle manure than sheep manure before and after vermicomposting.

1.2.5. Factors affecting compost stability and maturity

Determination of compost stability and/or maturity is an important procedure to evaluate compost quality. Maturity parameters are based on different physical, chemical and biological, including microbial activities (Cooperband *et al*., 2003; Brewer and Sullivan, 2003 and Bernal *et al*., 2009).

The factors affecting the composting process can be divided into two groups: those depending on the formulation of the composting mix, such as nutrient balance, pH, particle size, porosity and moisture; and those dependent on the process management, such as O_2 concentration, temperature, and water content (Bernal *et al*., 2009). Based on these factors, a number of criteria and parameter have been proposed for testing compost maturity. These parameters with various technical complications and degree of reliability are employed for the evaluation of compost stabilization management and the composting system (Lasardi and Stentiford, 1998).

1.2.5.1. Temperature

The temperature pattern shows the microbial activity and the occurrence of the composting process. The optimum temperature range for composting is 40-65°C. de Bertoldi *et al*., (1983) proposed that the range of 52-60°C is the most favorable for decomposition. Temperatures above 55°C are required to kill pathogenic microorganisms. But if the temperature exceeds the tolerance range of the thermophilic decomposers, the effect is damaging for composting, for microbial activity declines rapidly.

Temperature regulation is required for controlled composting and excess heat can be removed through several methods. Some of these are control the size and shape of the composting mass; frequent turning and redistribution that could remove heat through temperature feedback-controlled ventilation (i.e. Rutegers strategy).

1.2.5.2. C/N ratio

The two most vital elements required by microorganisms are carbon (degradable C) for energy source and N compound for biosynthesis. The nutritional balance (C/N ratio) is essential at the start of composting. The C/N ratio in the range 25-35 is adequate for the starting mixture because it is considered that the microorganisms require 30 parts of C per unit of N (Ryckeboer *et al*., 2003a). High C/N ratios make the process very slow as there is an excess of degradable carbohydrate for the microorganisms. On the other hand, with low C/N there is an excess of N per degradable-C and inorganic N is produced in excess that can be lost by ammonia volatilization or by leaching from the composting mass (Eiland *et al*., 2001).

According to Bernal *et al*. (1998), C/N ratio defines the nutritional balance of compost and is correlated with many chemical characteristics of the material. In general, the C/N ratio of matured compost lay in the range 10 to 15 so that the age of compost should be suitable for soil amelioration (Insam and de Bertoldi, 2003).

1.2.5.3. pH

Different organic matter with a wide range of pH (from 3 to 11) can be composted (de Bertoldi *et al*., 1985). A pH of 6.7-9.0 supports good microbial activity during

composting. Optimum values are between 5.5 and 8.0 as depicted in Figure 1.4. (de Bertoldi *et al*., 1985).

pH declines at the start of composting due to organic acid formation under temporary anaerobic conditions caused by acid forming bacteria. The low pH is caused by a short chain acids, mainly lactic and acetic acids (Eklind *et al*., 1997). This pH value, however, lasts for a few days and the intermediate metabolites are completely mineralized because of short chain acids are consumed by mesophilic microbes and nitrogen is mineralized (Beck–Friis *et al*., 2003).

In the process, ammonia could be released just offsetting the acidic phase, from the catabolic reaction of proteinaceous matter, reaching to high alkaline pH (i.e. 9.0-to 9.5). When the ammonification phases are over, pH drops to around 8.0 to 8.5 at the end of the process (Diaz and Savage, 2007). However, compost pH is also influenced by other environmental factors such as aeration and temperature where higher oxygen concentration reduces organic acids with a faster rise in pH (Beck-Friis *et al*., 2003).

The pH factor is very relevant for controlling N-losses by ammonia volatilization when it is greater than 7.5. Elemental sulphur (S°) has been used as an amendment for modulation of high pH values during composting. Addition of lime (calcium phosphate) to regulate high pH needs to be avoided for addition of lime to compost mass results in ammonium nitrogen loss at the later stages of composting (Diaz and Savage, 2007).

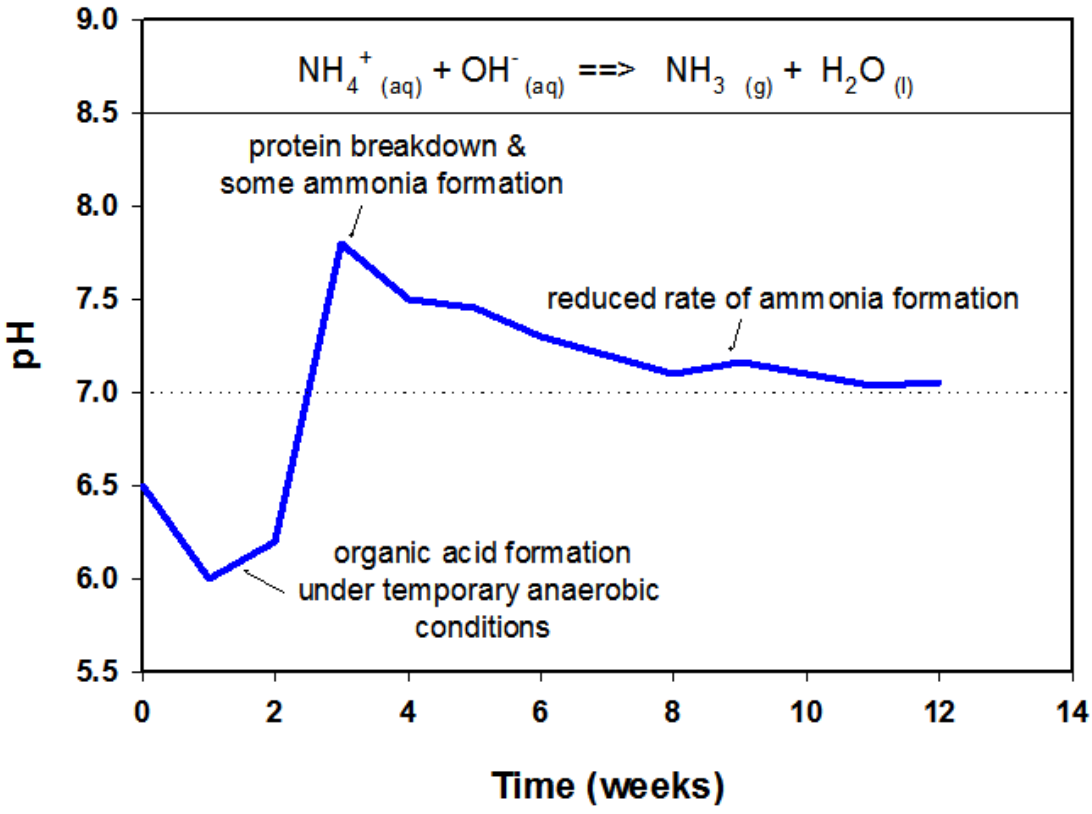


Figure 1.4 Changes in pH during composting of solid organic wastes (de Bertoldi *et al*., 1985)

1.2.5.4. Moisture

Another important environmental factor is moisture content of the mixture and water should be present in appropriate amount throughout the composting process. Water stress is the most common limiting factor for microbial activity on solid substrates.

According to Richard *et al*. (2002), moisture management requires a balance between the process of stimulating microbial activity and permitting sufficient oxygen supply within the compost matrix. 60% moisture content in the starting material is satisfactory whereas lower limit of moisture content was set to be 40% (Diaz and Savage, 2007). However,

the maximum permissible moisture content also depends on the degree of the resistance of individual particles to compression.

During composting process, matrix structure of the material and water content are not static for they drastically change over time reducing particle size, leading to a reduction in total porosity (Richard *et al*., 2002). Despite the fact that 0.5 to 0.6g H_2O of metabolic water can be produced per gram of volatile solid decomposed, the heat and air flow generated during composting evaporate more water than is produced and tend to dry the material out (Choi *et al*., 2001). Consequently, materials such as wood chips, straw, hay, coffee husk, rice hulls and corn stover have greater structural strength which requires higher permissible moisture content as high as 75-80%.

On the other hand, for those materials structurally weak (e.g. paper) and become deformed upon compression, the upper permissible moisture content is 55-60% (Diaz and Savage, 2007). Other materials such as fruit waste, cannery wastes, sludge and animal manure are devoid of definite shapes. The maximum permissible moisture content in composting of those materials together with a bulking agent should not exceed above 60%.

Too little moisture means early dehydration of the mass which stops the biological process; whereas excess moisture limit oxygen transport, leading to slower decomposition and increased emission of anaerobic odors (Richard *et al*., 2002). In general, a 40-60% of moisture content is satisfactory for proper composting (Diaz and Savage, 2007).

1.2.5.5. Electric conductivity (EC)

The EC can affect the quality of composts because it reflects the salinity and suitability for crop growth. According to Gómez-Brandón *et al*. (2008), the EC increased after the active phase (day15) of composting of cattle manure and ended at EC value of 3mS cm^{-1}, probably due to the release of soluble salts such as ammonium, phosphate resulting from the decomposition of easily biodegradable organic substrates. The EC values of finished compost greater than 3mS cm^{-1} indicates that the material cannot be safely applied to soil (Soumarė *et al.*, 2002).

1.2.5.6. Inorganic Nitrogen (NH_4-N and NO_3-N)

The N mineralization was also an important parameter for evaluating compost maturity. Finstein and Morris (1975) reported that when the NH_4^+ concentration decreases and NO_3^- appears in the composting material it is ready to be used as compost. A high level of NH_4^+ points to unstabilized material, leading Zucconi and de Bertoldi (1987) to establish a limit of 0.04% for mature city refuse compost. Bernal *et al*. (1998) established a NH_4/NO_3 ratio of less than 0.16 as critical index for compost maturity and stability of compost and recommended for soil application.

1.2.5.7. Microbial activity

The existence of different groups of microorganisms in the different phases of composting is another factor to evaluate the process and the final product. The microorganisms involved in composting develop according to the type of utilizable organic matter and temperature of the mass, which defines the different stages of the process. Bacteria predominate early in composting; fungi are persistent during all the process but predominate at water below 35% and are not active at temperature greater

than 60°C. Actinobacteria predominate during stabilization and curing and together with fungi are able to degrade resistant polymers (Bernal *et al*., 2009).

Microbial succession during the various composting time is, therefore, considered as indicators of the composting process (Liu *et al*., 2011) because the appearance of specific microorganisms reflects the degree of maturity of compost (Ishii *et al*., 2000). Various methods were developed to evaluate microbial succession. Among these, microbial biomass count (Tiquia *et al*., 2002; Liu *et al*., 2011) and analysis of phospholipid fatty acid (PLFA) profile are used to describe the microbial succession in different compost system (Eiland *et al*., 2001; Steger *et al*., 2007).

Shifts in the microbial community have also been investigated by methods like the measurement of enzyme activity (Tiquia *et al*., 2002; Mondin *et al*., 2004; Castaldi *et al*., 2008; Vargas-García *et al*., 2010; Liu *et al*. 2011). Several studies also showed the appearance of different enzymes along the course of the composting process (Cunha-Queda *et al*., 2007; Mondini *et al*., 2004) was an indication of the stage of the degradation and in turn, define the level of maturity. Enzyme testing using API ZYM^{TM} kit showed potential usefulness as indices of the course of the co-composting of poultry manure and yard trimmings (Tiquia, 2002). API ZYM^{TM} test can be used to monitor quantitative and qualitative fluctuation of substrate, and to reveal differences in composts and compost maturity.

The monitoring of hydrolytic extracellular enzyme in compost extract throughout the process provide useful information to understand the transformation occurring during composting in general, and that of the dynamics of important elements such as C and N

in particular (Vargas-García *et al*., 2010). These authors reported that the nature of the raw material determined the evolution of enzyme activity during the comparison of horticulture waste (HW), sewage sludge (SS) and municipal solid waste (MSW). Mondini *et al*. (2004) found out that alkaline phosphatase and arylsulphate seemed to be the most reliable index of compost stability for yard waste and cotton waste composts. The stage of composting also determined the maximum value of the enzyme activity. According to Cuna-Queda *et al*. (2007) maximum values of enzyme activities were observed during both the thermophilic and the mesophilic (<40°C) phases.

Apart from enzyme assay, PCR-based methods such as denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al*., 1993; Pedro *et al*., 2001) can be used to monitor and identify microbial groups associated with shifts in microbial community structure, including non culturable microorganisms (Steger *et al*., 2007).

The use of nucleic acid microarray, or oligonucleotide microchips, represents one of the most recent advances in molecular technology, allowing a high throughput format for the parallel detection of 16S rRNA genes from compost and other environmental samples (Franke-Whittle *et al*., 2005). The DNA micorarray technology offers the possibility to analyze the entire array of microorganisms from compost samples to ensure whether target microorganisms are present or absent at a particular time of the process. Thus, the technology provides a tremendous potential for process monitoring, detection of pathogens, and beneficial microbial population (Franke-Whittle *et al*., 2005).

1.2.5.8. Phytotoxicity

The other important factor in determining the agricultural value of compost is the estimation of maturity based on phytotoxicity tests. Plant tests used in research and in quality standards can be divided into three based broad categories: germination tests (including root assessments), growth tests (assessment of top growth and sometimes root mass), combinations of germination and root growth.

According to Zucconi *et al*. (1985) a germination index below 50% characterizes immature compost. Zucconi and de Bertoldi (1987) discussed the difference between germination and growth tests. Germination tests provide an instant picture of phytotoxicity, whereas growing tests can show changes in the stability or maturity of the compost tested: there may be damaging effects on growth in the earlier stages, but beneficial effects later on, with different conclusions depending on the time of assessment.

García-Gómez *et al*. (2003) also studied germination index and pot experiment together on ryegrass showing phytotoxic effects from immature compost even when the germination index was above 87%. The relationship between the CO_2 respiration and phytotoxicity of immature compost was studied by García-Gomez *et al*. (2003), using the CO_2-C production by OM mineralization and plant growth. The CO_2-C evolved correlated with plant growth and immature compost caused N-mineralization in the soil, leading plant N-deficiency.

1.2.6. Bio-augmentation of selected (active) microorganisms in compost

There are two methods of increasing the rate of biodegradation: bio-stimulation and bio-augmentation. Bio-stimulation refers to the stimulation of indigenous population by supplying the required factors (e.g. nutrients). Bio-augmentation is the process of introducing microorganisms selected to perform a desired task such as degrading big polymers (Walter, 1997). It involves the introduction of large numbers of non-indigenous microorganisms into the environment. One of these technologies is the introduction of effective microorganisms to composting. Effective Microorganisms (EM) are developed and applied (Bioaugmentation) to compost industry with the intention of enhancing the process and producing quality end products (Higa, 1994). EM is a solution containing over 80 species of co-existing microorganisms selected from thousands of microbial species found in the environments, including the food and fermentation industries. EM was developed from three principal microorganisms; namely photosynthetic bacteria, lactic acid bacteria and yeasts (Higa, 1994).

The introduction of EM technology into composting system was based on the belief that the inoculation of the organic material with EM could shift the microbiological equilibrium. It is believed that they played significant role in decomposing larger organic compounds into low molecular organic compounds: amino acids, sugars, vitamins and other bioactive ingredients that stimulate the natural flora.

The addition of lignocellulolytic microbial consortium, *Aspergilus niger* and *Trichoderma viride*, enhanced the rate of composting under natural pH during composting of rice straw based on the study of the physicochemical properties of pH amended and natural conditions. The bioconversion was maximum in

Trichoderma/Aspergillus inoculated treatments within 14-21 days as indicated by the profiles of electric conductivity, bulk density, total carbon and nitrogen ratio, and germination index, showing 74.5 % GI after 21 days of composting for the treatment without pH amendment.

1.2.7. Classification of composting system

In general, the composting system is currently classified into two broad categories. i.e. "windrow" and "in vessel" systems. According to Diaz *et al* (2007), windrow system can be explicitly defined as the piling up of substrates into piles. Typically, the height of the pile can range between 1.5 and 2.5 m, usually with elongated windrows depending on land availability and volume of the source material. With in-vessel systems, however, all or part of the composting can take place in a reactor (Dziejowski and Kazanoska, 2002). In fact, the in-vessel systems currently used worldwide involve the use of windrows for curing and maturation.

Windrow system may be further subdivided into the "turned windrow" and the "forced air windrow" (static pile or stationary windrow) system, based on the method of aeration (Diaz *et al*., 2007).

1.2.7.1. Forced air windrow

In the forced air windrow system, air is either forced upwards through the composting mass or is pulled downwards and that is why the alternative designation of "forced aeration" is applied. In both instances, the composting mass is not disturbed. This is because the forced aeration system involves an initial period of drawing air into the pile, followed by a period of forcing it upward through the pile. In the pulling or "suction"

stage, the air that leaves the system either is discharged directly into the environment, or is forced through a pile of finished compost or other "stable" organic matter (a biofilter). The rationale behind this procedure is to deodorize the effluent air stream. It has been mostly verified that finished compost and other organic materials can serve as an odor filter (Schlegelmilch *et al*., 2005). In general, the basic arrangement of an aerated static pile follows six steps:

1. mixing of bulking agents with the waste to be composted
2. construction of windrow,
3. composting process,
4. screening of the composted mixture to remove reusable bulking agent,
5. curing, and
6. storage

The construction of the windrow requires a series of perforated pipes that is placed on the compost pad. The diameter of the pipes must be in the range 10.2-15.5cm. The pipes are oriented longitudinally and parallel to what would be the ridge of the windrow. Short-circulating of air is avoided by ending the pipes 1.5-2.7m from the edges of the windrow. The perforated pipes are connected to a blower through a length of non-perforated pipe. After the network of piping is in place, it is covered with a layer of bulking agent or finished compost that extends over the area to be covered by the windrow of material to be composted. This foundation layer is provided to facilitate the movement and uniform distribution of air during composting. It also absorbs excess moisture and thereby minimizes seepage from the pile (Diaz *et al*., 2007).

The material to be composted is then stacked on the piping and bed of bulking material to form a windrow. Then the finished pile should be about 20-30 m long, about 3-6 m wide and about 1.5-2.5 m high. Finally, the entire composting material may be covered with a layer of matured compost that is about 15cm thick if the covering compost is screened, and about 20cm thick if unscreened. The covering serves to absorb objectionable odors from the composting mass and ensures the occurrence of high temperature levels throughout the composting material. There are also synthetic materials that can be placed on the windrow to essentially achieve the same results (Diaz *et al*., 2007).

Despite this, the static pile method is not suitable for all types of raw materials and under all conditions. The method, for instance, works best and perhaps only with a material having a particle size that does not exceed 3.5-5cm in any dimension. A mixture of particles that are too large and that exhibit a wide spectrum of particle sizes can easily result in uneven distribution and movement of air through the pile. Uneven distribution of air through the pile promotes short circulating and the development of anaerobic pockets of decomposing materials.

1.2.7.2. Turned windrow system

The term “turning” is associated with the method of aeration. In essence, turning consists of operations in tearing down and reconstructing the mixture. Turning has advantages of not only aeration, but also encourages uniformity of decomposition by exposing all the materials at one time or another to the particular active interior zone of a pile. It also serves to assist the reduction of particles in size and advances in the loss of water from the composting matrix if the moisture level is too high. In contrast, if water level is too

low, turning may be a disadvantage since it dries out. Hence, the appropriate time to moisture adjustment is during the turning process (Diaz *et al*., 2007).

In windrow, piles should be constructed in the form of a windrow and roughly conical in cross section. However, depending on the weather condition the shape of the pile has to be adjusted. In dry, windy periods, a pile in the shape of a loaf tending toward a flattened top would be appropriate because the ratio of exposed surface to volume is lower with such a configuration. Furthermore, the volume of the overall hot zone is greater than with a triangular or conical cross section. Contrariwise, during wet weather the flattened top is a disadvantage because water is absorbed into the composting mass rather than shed. In mechanically operating method of turning, the pile configuration that results will be the one imparted by the machine.

Normally, the height of the windrow should be roughly that of the average laborer which can be easily reached with the normal pitch of the equipment used in turning. In practice, the windrow should never exceed above the range of 1.5 to 2m high. The height of mechanical turning, however, depends on the design of the turning equipment, which is generally between 1.5 to 3.0 m high. The breadth of the pile, on the other hand, is a function of convenience and expediency. In general, the width of a windrow should be about 2.5m with manual turning whereas in mechanical turning, the width depends upon the design of the machine. But it is usually from 3.0 to 4.0m. As regards to length, it is merely indeterminate. For example, according to Diaz *et al* (2007), the length of a 180tone windrow (conical shape) of a material at a height of 1.8m and width of 2.5m would be about 46.0m.

Arrangement of windrow basically depends on two key factors: availability of land an accessibility of equipment. In general, the total area requirment in manual turning is at least two times and more likely 2.5 times that of the original windrow. This is due to the process used for turning. When the secod turning takes place, the material is returned to its origional place. The area required in mechanical turning with type of machine. Some machines accomplish turning in such a manner that as the original windrow is torn down, the new windrow is reconstruced directly behind the machine. This is done either by designing the machine such that it straddles the windrow, or that it has a mechanism for tearing down a windrow and passing the material over the cab to the rear of the machine as it moves forward. The area required by such a machnie is little more than that of the original windrow, including only enough added space to permit the maeuvering of the machine. Other types of machines rebuild the windrow adjacent to its original position. The area requirment, therefore, is comparable to that described for manual turning.

Turning is conveniently carried out by the use of pithchfork, which normally have four or five tines. Ideally, in the process of rebuilding the pile, material from the outside layers of the original windrow should be placed in the interior of the rebuilt windrow. If this ideal situation is not practically attained, the deficiency can be compensated by increasing the frequency of turnng.

Essentially, the frequency of turning depends on the ratio of available oxygen-to- oxygen requirments. Structural strength, moisture content of the material, pathogen destruction and uniformity of decomposition are some of the most important characteristics in determining the frequency of turning.

1.2.8. Management of agricultural solid wastes in Ethiopia

In Ethiopia, though municipality wastes are dumped into landfills or are scattered as litters without proper management, most of agricultural residues from low-input small holder farming are used for energy for households, burned or left in the farm as organic input for crop production. However, the recent industrial level of coffee production and horticultural products near urban centers are beginning to produce huge amount of plant residues. One of the environmental problems in coffee processing is the direct release of coffee wastes (coffee husk and coffee pulp) to the environment (Pandey *et al*., 2000). According to CTA (1999) more than 240,000 tons of coffee husk is released into the environment annually.

Horticultural products of rose in Ethiopia also generate daily up to ten tones of 'Fresh Organic Wastes' (EHPEA, annual report, 2014). These wastes arise from pruning and crop hygiene, processing in the pack house, (rejects, trimmings and leaves) and occasionally processed flowers damped as waste when prices are low or flights very delayed. Additional waste is also generated in large volumes when the rose bushes are removed at the end of their useful crop life (EHPEA Annual report, 2014). These wastes are usually dumped freely (heaps) around the farm.

Few studies showed that burning and dumping of huge amounts of organic wastes of coffee husks and flower residues are the methods of removal of wastes by this agribusiness (CTA, 1999; Tenaw *et al*., 2006; EHPEA annual report, 2014).

1.2.9. Research in Ethiopia

So far, there are few reports in Ethiopia on the use of different agricultural wastes as soil amendments. Lulu and Insam (2000) investigated the effects of application of fresh and composted mustard meal on wheat yield at different rates (equivalent to 120, 240 and 360 kg N ha^{-1}) on humid tropical vertisol for 7 years at Ginchi research station. They showed that application of composted mustard meal induced shifts in microbial community structure (bacteria to fungi) from r to K strategists, and had positive medium-term effects on biological qualities of the vertisol.

Tenaw and Kelsa (1998) showed the potential of coffee residue in increasing crop yield by replenishing soil organic matter, enhancing nutrient cycling, and maintaining soil physical quality. Tenaw *et al*. (2006) also evaluated mineralization of N from decomposing coffee residue applied as surface mulch on soil over time, and showed that the coffee residue is low in quality to improve soil fertility. The low quality of the residue was attributed to high lignin and C/N ratio, and low N concentration as well as high immobilization of N. Sharma *et al*. (1997) also investigated effects of organic residue supplement on the microbial communities of four tropical soils (Jima Awassa, Holeta and Ginchi).

Despite this, there is always a direct and continuous release of these two important agricultural wastes (coffee husks and flower residues) to the environment. This practice, however, resulted in environmental pollution besides the loss of valuable plant nutrients (Tenaw *et al*., 2006). According to Murthy and Naidu (2012), coffee wastes such as coffee husk (CH), coffee pulp (CP) and other by-products constitute a source of severe

contamination and pose serious environmental problem. As a result, coffee processing units that are located in almost each coffee estate pose threat to the environment because of unsafe disposal of Coffee Husk, Pulp and effluents leading to pollution of water and land around the processing areas (Murthy and Naidu, 2012). Moreover, large-scale utilization and management of CH around the world still remains a challenge due to its content of caffeine (1%), free phenols (≈1%), tannins (5%), chlorogenic acids (2.5%), which are known to be very toxic to many life processes (Fan *et al*., 2003). Traditionally, Coffee Husk has found only limited application as fertilizer and livestock feed (Murthy and Naidu, 2012). Nonetheless, it was found suitable for composting since it contains high contents of cellulose and hemicellulose besides potash and lignin. Though little effort have been made on the utilization of CH as source of compost feedstock in Ethiopia, reports elsewhere (Sathyanarayana and Kahan, 2008; Adi and Noor, 2009) indicated that composting, which is cost effective technology and can be used at industrial level for recycling of this waste are widely known to enhance soil nutrients, provide better plant growth and possess commercial appreciation (Murthy and Naidu, 2012).

Indeed, the application of unstable materials originated from any agricultural wastes including flower residues did also produce a competition for oxygen between microbial biomass and plant roots/seeds (Gómez-Brandón et al., 2008). This fact can deprive plant roots/seeds of oxygen, and lead to the production of hydrogen sulfide (H_2S) and Nitrite ion (NO_2^-) (Mathur *et al*., 1993). Another problem associated with the direct release of these two wastes into the soil ecosystem is nitrogen starvation of plants as soil microbes are scavenging for soil nitrogen to make up for their deficit resulting from the application

of these unstable wastes with a high C to N ratio. The phytotoxicity of unstable composts represents another major problem; this is due to the emission of ammonia and the presence of phytotoxic substances like phenolic compounds, ethylene oxide, chlorogenic acids, tannins that may be released during the decomposition of unstable compost in soil. Low molecular acids (i.e. acetic, propionic and butric acids) produced by the anaerobic digestion of the organic matrix are also responsible for compost phytotoxicity (Fuchs, 2002).

Composting has been demonstrated to be a valuable strategy for recycling of these two wastes, allowing the recovery of denuded land and sustainable management of agricultural/horticultural lands (Sànchez-Monedero *et al*., 2001). Although the use of these two compostable organic matrices is significant, their high C/N ratio can make the process very slow. They can be, however, significantly enhanced by the addition of N-rich residues such as cow dung that have experimentally been proved to decrease the initial C/N ratio of the mixtures and improve the composting process (Li *et al*., 2008). In addition, application of activated effective microorganisms (EM) and House Hold Compost (HHC) to the lingo-cellulosic material were reported to enhance degradation (Higa, 1994). To this effect, no information is available regarding the use of N-rich animal waste (cow dung), EM and HHC for composting with lingo-cellulosic residues which might become a valuable strategy for their disposal. Besides, there is dearth of information and knowledge on process monitoring of composting and quality compost production from coffee husk and flower residues.

1.3. Objective of the Study

General objective

The general objective of this study is to monitor the composting process of two agro-industrial wastes, coffee husks and flower solid residues, and evaluate the compost quality (compost maturity and stability) using different physico-chemical and biological (microbial load, enzyme, molecular, germination) parameters.

Specific objectives

The specific objectives of this thesis were to:

1) Evaluate the physico-chemical factors of agro-industrial composting
2) Establish the microbial communities during coffee husk composting
3) Establish the extracellular enzyme profiles during composting of coffee husk
4) Evaluate compost stability and maturity of flower residue and coffee husks and their potential as soil stabilization.

Chapter 2 Evaluation of compost maturity and stability of the coffee husk using chemical and microbial parameters

Fekadu Shemekite*[1] and Fassil Assefa[1]

[1]Addis Ababa University, Department of Microbial, Cellular and Molecular Biology (MCMB)

*[1] Addis Ababa University, Department of Microbial, Cellular and Molecular Biology (MCMB)

e-mail: fshemekite@gmail.com

Abstract

Coffee waste is one of the voluminous agro-industry wastes generated in Ethiopia. The direct release of this waste pollutes the environment and cause ecological damage that inhibits plant growth. This necessitates the transformation of this waste into a useful product with a dual purpose of fertilizer production and protection of the environment. This preliminary study was, therefore, made to understand the process of coffee husk composting in a Windrow composting system for 90 days by measuring various parameters of compost stability and maturity. The process included three treatments: coffee husk amended with cow dung and house hold compost (Pile 1), the same feedstock with fruit/vegetable wastes and house hold compost (Pile 2), and coffee husk alone (Pile 3) as control. Samples were collected on days 1, 10, 24, 32, 48, 56, 66, and 90 to measure chemical and microbial parameters. As a result, C/N ratios of Piles 1 and 2 decreased significantly to 10-12% over the 90 days. The bacterial counts was highest ($9.58\log^{MPN}$ g^{-1}dw, Pile 1) at the start of the process, likewise the highest actinobacterial ($9.54\log^{MPN}$ g^{-1} dw, Pile 1) and fungal counts ($9.58\log^{MPN}$ g^{-1} dw, Pile 1) was recorded at the end of the process. The effect of the different microbial activities was reflected in the better maturation of Pile1 with GI (>80%) at the end of composting compared to the control that required much more days for the material to become free from phyto-toxicity. This transformation could be associated with higher volatile solid reduction in Pile 1 (75%)

and Pile 2 (65%) substantiating the importance of both chemical parameters and germination index together with microbial count to determine the quality of coffee husk compost.

Keywords/phrases: *C/N ratio, Germination index, mesophilic, phytotoxicity, thermophilic*

Introduction

Coffee production is an important sector of the agro-industry in Ethiopia, accounting for up to 65% of the total exports from the country (Tenaw *et al.*, 2006). Industrial coffee production involves either a dry or a wet processing method for the removal of the shell and mucilaginous parts from the cherries (Pandey *et al*., 2000), resulting in the production of coffee husk and pulp, respectively. According to Murthy and Naidu (2012), around two tons of such ligno-cellulosic residues are obtained after the de-hulling of 2-6 tons coffee cherries. In Ethiopia, coffee cherries are mostly processed using the dry method, thereby leading to an annual release of more than 240,000 tons of coffee husk into the environment (CTA, 1999).

This represents a serious environmental problem for the waste contains high content of tannins, phenolic compounds and chlorogenic acids (Fan *et al*., 2003) that could inhibit plant root growth and lead in greenhouse gas emissions through anaerobic decomposition that necessitates the proper use and disposal of coffee waste (Murthy and Naidu, 2012).

Composting is one of the biological methods of solid waste treatment and is widely known and accepted as a technology for recycling of agricultural waste materials under aerobic conditions (Murthy and Naidu, 2012). It transforms waste materials into a high quality amendment/fertilizer, rich in organic matter and nutrients (Insam and de Bertoldi, 2003).

Application of un-decomposed or immature composts to soil can lead to immobilization of plant nutrients and inhibit plant growth (phytotoxicity) due to emission of ammonia, production of phenolic compounds and byproducts such as ethylene oxide, low molecular

weight organic acids such as acetic, propionic and butyric acids that are synthesized during the decomposition of unstable compost (Butler *et al*., 2001; Fuchs, 2002; Gómez-Brandón *et al*., 2008). This shows that management of the composting process must include the potential agronomic value of the end product and its suitability for plant growth by evaluating its degree of maturity using seed germination test (Cooperband *et al*., 2003).

A large variety of physicochemical techniques have also been reported for the determination of compost stability (Wang *et al*., 2004).Chemical parameters such as pH, electrical conductivity (EC), changes in total organic carbon (TOC), total nitrogen (TN) and the ratio of C to N have been applied as indicators of stability (Eiland *et al*., 2001).

Temperature profile of composts is also used to monitor the process of composting. The changes in temperature and mineralization of organic matter are also related to a large variety of mesophilic, thermotolerant and thermophilic aerobic microorganisms, including bacteria, actinobacteria and fungi (Beffa *et al*., 1995; Hassen *et al*., 2001., Liu *et al*., 2011). The use of different parameters provides valuable information to evaluate the maturity and/or stability of composts (age of composts) in order to determine the quality of compost to apply for agricultural/horticultural and landscaping activities.

The main objective of this study was, therefore, to monitor the physico-chemical changes during the composting of coffee husks, and to determine compost maturity by measuring the physic-chemical and microbial succession of the process, and to develop efficient strategies for the management and valorisation of coffee waste.

Material and methods

Arrangement of compost piles and sampling techniques

Coffee husks (CH) were collected from Bebeka and Mizan Teferi (Southeastern, Ethiopia). Fruit/vegetable wastes (FVW) and cow dung (CD) were collected from the market center and dairy enterprises of Addis Ababa City center, respectively. They were used as bulking agents during composting of coffee husk. Matured household compost (HHC) obtained from Gerji compost demonstration site was used as inoculum to hasten the degradation process.

In this experiment, three different piles were prepared in Windrow method for coffee husk composting following the procedure of Díaz *et al.* (2002). The first pile was composed of coffee husk, cow dung and household compost at a ratio of 4:1:0.36, whereas the second pile constituted of coffee husk, fruit/vegetable waste and household compost at a rate of 3:1:0.04. The third pile was left as control without amendments of any organic matter. The composting trial was carried out at Gerji (Bole sub-city, Addis Ababa) composting research site of the Addis Ababa Environmental Protection Authority (EPA). Physical and chemical analyses of the feed stocks are tabulated on Table 2.1. The C/N ratio of each feedstock was adjusted according to formula of Cornell Composting Science and Engineering (Richard *et al*., 2002).

$$R=\frac{Q_1(C_1X(100-M_1)+Q_2(C_2X(100-M_2)+Q_3(C_3X(100-M_3)+...}{Q_1(N_1X(100-M_1)+Q_2(N_2X(100-M_2)+Q_3(N_3X(100-M_3)+...}$$

In which,

R=C/N ratio of compost mixture

Q_n=mass of material n

C_n= Carbon (%) of material n

N_n= Nitrogen (%) of material n

M_n= Moisture content (%) of material n

Table 2.1 Phyisco-chemical characteristics of the feedstocks of CH composting

Parameter	**CH**	**FVW**	**CD**	**HHC**
T (°C)	17.2(0.2)	15.3(0.3)	18.5(0.1)	16(0.1)
MC (%)	13(0.1)	74(0.6)	64(0.3)	35.3(0.4)
pH (1:10),DH_2O	5.3(0.1)	6.6(0.1)	7.7(0.1)	7.9(0.1)
Organic C (%)	54.5(0.6)	40.1(0.4)	23(0.6)	20(0.3)
Total N (%)	1.9(0.1)	1.32(0.02)	1.8(0.02)	1.85(0.03)
C:N ratio	28.4(0.6)	30.3(1.6)	12.8(0.1)	10.5(0.01)

Values are expressed as means and standard error of triplicate samples.

Each pile was arranged in a trapezoidal shape with a volume of $1.5m^3$ (1mx1mx1.5m). All piles were covered with grass materials after adjusting their moisture contents to 60% by sprinkling of water to the piles (Díaz and Savage, 2003). A total of seventy-two samples, representing each pile (around 500 g each), were collected separately on days 1, 10, 24, 32, 48, 56, 66 and 90 of sampling and transported to the microbiology laboratory, Addis Ababa University (AAU) for both microbiological and physic-chemical analysis. All analyses were carried out in triplicates.

Physical and chemical analysis

Temperature of each pile was measured on the spot at different points (30cm, 50cm and 80cm depth from top to bottom) before turning the composts using a digital thermometer

(model H-9283). The moisture content of composts was determined after having taken 10g of the samples and oven-drying at105 $^{\circ}$C for 24 h. Total C and Volatile solids were determined by dry combustion at 550 $^{\circ}$C (as the loss on ignition) (Haug, 1993)

Total N content (TN) was measured by the Kjeldahl digestion analysis. pH and electrical conductivity (EC) were measured from the aqueous extracts (1:10, w/v) of composts using a pH meter (HD8602, Italy) and a conductivity meter (CC401 ELMEIRON, Poland), respectively. Volatile solids (VS, % dw) content was calculated according to the following equation (Chroni *et al.*, 2009).

$$VS_{red}=100*\left[1-\frac{VSf*(100-VSi)}{VSi*(100-VSf)}\right]$$

Where VS_i: the initial volatile solid contents, (%dw) and VS_f: the final volatile solid contents of sample f, (% dw)

Enumeration of total aerobic heterotrophs, actinobacteria and fungi

For sample processing, compost samples were ground to 0.25 mm, serially diluted to appropriate proportion in sterile water and inoculated onto their respective media using the plate frequency technique. Counts of aerobic heterotrophs, actinobacteria and fungi were determined by direct plating on appropriate media (Tiquia *et al*., 2002). Total aerobic mesophilic bacteria was made on plate count agar (PCA, Oxoid, England), and the number of actinobacteria and fungi was counted on Starch Casein Agar (SCA) and Potato Dextrose Agar (PDA), respectively.

Plates were incubated for three days at 30 ± 2 $^{\circ}$C for the growth of bacteria and fungi, and between five and seven days for actinobacteria. After incubation, the population of the

different microbial groups was estimated using the Most Probable Number (MPN) method (Tiquia *et al,* 2002).

Enumeration of endospore forming bacteria and thermophilic microbes

Enumeration of spore forming bacteria was made using the protocol of Hassen *et al*. (2001). It was similar with the enumeration of mesophilic bacteria as before, except that diluted extracts were subjected to treatment for 10 min at temperature of 80°C. For the enumeration of thermophilic or thermotolerant microbial groups (bacteria, actinobacteria and fungi), plates were incubated at 55°C for 3days (Beffa *et al*., 1995).

Phytotoxicity test

The phytotoxicity study of the compost samples was determined following the method of Zucconi and de Bertoldi (1987). Aqueous extracts were prepared (10:50, w/v), centrifuged at 10,000 rpm (Wagtech International, 3000 system) and filtered through a 0.45 µm membrane filter. Ten seeds of garden cress (*Lepidium sativum* L.) were evenly distributed on a filter paper in a Petri dish and watered with 1 ml of the supernatant. A control treatment consisting of ten seeds watered with 1 mL of distilled water was also included. All Petri dishes were incubated at 22° C for 24 h in the dark and then 1 ml of ethanol was added to stop germination. The number of germinated seeds with at least 1 mm was counted and the length of the germination root was also measured. Then germination index was calculated following the formula given by Zucconi and de Bertoldi (1987).

$$GI\ (\%) = \frac{Ns * Rs}{Nb * Rb} * 100$$

Where, GI germination index

Ns number of germinated seeds with sample extracts

Nb number of germinated seeds in the control

Rs root length of germinated seeds watered with sample extracts

Rb root length of germinated seeds watered with control extracts

Data Analysis

Biochemical and microbiological data were analyzed by repeated measures analysis of variance (ANOVAR) in which piles represented the subjects, and the composition of parent material (CHCDHH, CHFVWHH and CH) was fixed as the between-subject factor, and the composting time (1,,10,24,32,48,56,66,90) was fixed as the within subject factor. All the variables met the sphercity condition (Mauchly's test).

When the sphercity assumption violated, correction was made by the Geisser-Greenhouse (G-G) procedure (Potvin *et al.*, 1990). Significant differences were further analyzed by paired comparison with the Bonferroni test. To demonstrate the relationship between certain chemical parameters and two maturity indices (C/N ratio and GI) of all coffee husk composts, Pearson product moment correlation (r^2) was performed.

Data of all experiments were taken in triplicate and analyzed by statistical computing packages (SPSS version 21). Values were presented as the average of three replicates and means were separated by the least significant difference according to the Bonferroni test.

Result and Discussion

Effect of temperature on microbial population

The temperature profile of the three coffee husk compost piles showed four successive phases: initial mesophilic phase (18-42°C) which lasted for two days in Pile 1 and Pile 2 but for one week in Pile 3 (Figure 2.1). The thermpophilic phase (45-70°C) lasted for 45 days in Pile 1 and Pile 2, but for 15 days in Pile 3.The second mesophilic phase (18-42°C) for Pile 1 and Pile 2 was 22 and 30 days, respectively. On the contrary, the second mesophilic phase for pile 3 took 60 days. After 66 days**,** all piles went through maturation (curing) phase (data not shown). However, significant variations were observed among the three piles with regard to temperature changes as a function of time (ANOVAR $F_{16.48}$=5.64, P=0.05).

In all piles, there was a decrease in temperature after 40 days which could be attributed to the depletion of easily degradable organic materials (Caceres *et al*., 2006). This result may indicate that the longer thermophilic temperature in cow dung amended compost ensures sanitization of the end product (Lasardi and Stentiford, 1998); and co-composting with cow dung, fruit/vegetable waste and household compost accelerated the process (Díaz *et al*., 2002). The instant temperature increase and longer maintenance at thermophilic temperature in co-composted piles (Pile and Pile 2) reflected the better

performance of biological activities than slower initial temperature and immediate fall of the temperature during the thermophilic stage of the control (Pile 3).

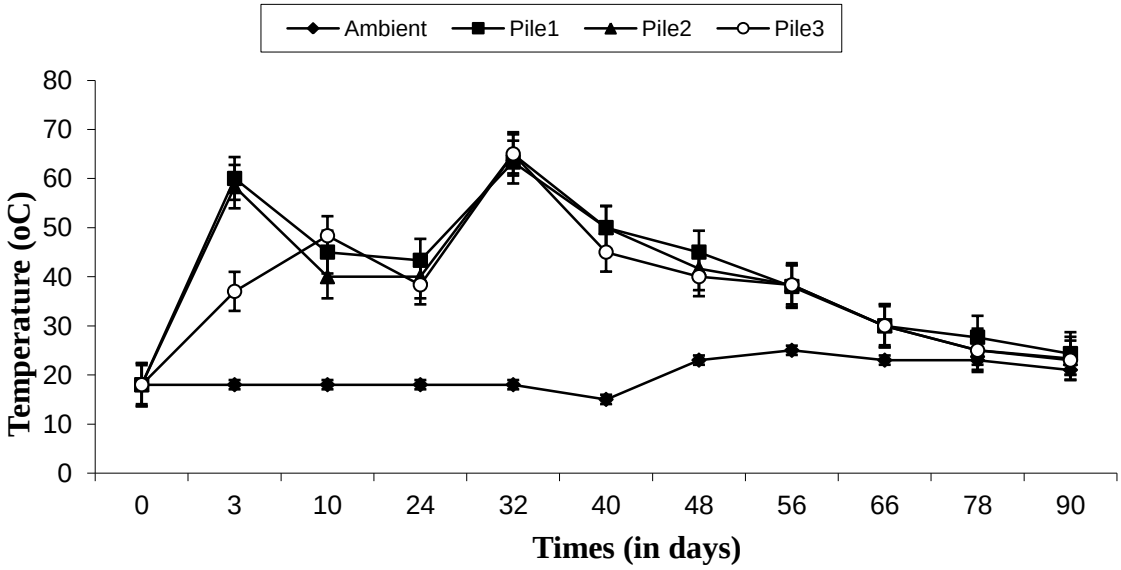


Figure 2.1 Temperature changes during composting of three different piles. Values are expressed as means and standard errors of triplicates samples

Effect of moisture on composting process

The Moisture adjustment made on all piles during the experiment was within the recommended range of 40-70% (Table 2.2). The adjustment was made to compensate the moisture loss that could be occurred as a result of increased heat and enhanced desiccation. However, there was a significant difference in moisture content at the start, the active and the end stage of the process (ANOVAR $F_{7,14}$=25.52, p=0.0001). At the beginning of composting, the moisture content of all piles was above 50%., and remained so for about 24 days. As the process continued, the moisture percentage was increased by

nearly 10% until day 56, and declined gradually to 45-47% which was still above the recommended range for matured compost (i.e. 30%) (Díaz and Savage, 2007).

pH and electric conductivity

In this study, the pH for the first 3-10 days was slightly acidic for Pile 1 (6.37) and Pile 2 (5.89) but more acidic for Pile 3 (5.23). However, the pH increased and reached to basic pH levels (8.75-8.89) afterwards in Pile 1 and Pile 2, whereas in pile 3 the pH reached 7.3 after 24 days (table 2.2). The pH in all piles continued to increases until it was stabilized to pH (7.5-7.8) at the end of the composting process. In general, the difference in pH values were significant as a function of time (Compost time ANOVAR $F_{14,42}$=138.974, p=0.0001). The increase in pH values over time can be attributed to a release of ammonia associated with protein mineralization (Gómez-Brandón *et al*., 2008).

The electrical conductivity of the three compost piles showed similar pattern in that they showed a sharp and higher increase in EC (9-25%) between the 32ndday and 90th day of composting, with the highest increase in Pile 1(25%), followed by Pile 2 (23%) without showing significant difference among the piles over compost time (Table 2.2 pile x time ANOVAR, $F_{14.42}$=0.974, P>0.05). The low EC content of the three coffee husk composts obtained at the 90 days did not exceed the limit value 3dSm^{-1} indicating a material to be safely applied for soil amelioration (Soumaré *et al.,* 2002). This result was different from previous findings where high EC values (>3dSm^{-1}) were obtained before 90 days compost in the study of manure compost (Gómez Brandón *et al*, 2008) and three dairy manure amended rice chaff compost piles (Liu *et al.*, 2011).

Table 2.2 Changes in moisture, pH and EC during composting of three coffee husk composts

Parameter	Piles	Time							
		1	10	24	32	48	56	66	90
Moisture(%)	1	53.3	49.0	57.0	64.7	65.0	64.0	57.0	48.0
		(1.7)^{b}	(3.6)^{bc}	(1.8)^{b}	(1.4)^{a}	(3.0)^{a}	(1.6)^{a}	(2.6)^{b}	(1.7)^{c}
	2	53.3	54.0	58.3	65.3	64.7	64.7	66.0	48.0
		(1.7)^{b}	(3.6)^{b}	(1.8)^{b}	(1.4)^{a}	(2.9)^{a}	(1.6)^{a}	(2.6)^{a}	(1.7)^{c}
	3	53.3	48.7	56.3	61.0	66.3	65.7	52.3	44.7
		(1.7)^{b}	(3.6)^{b}	(1.8)^{b}	(1.4)^{ab}	(2.9)^{b}	(1.6)^{a}	(2.6)^{b}	(1.7)^{c}
pH(1:10)	1	6.37	8.12	8.35	8.16	8.35	8.19	8.36	7.75
		(0.11)^{c}	(0.31)^{a}	(0.03)^{a}	(0.04)^{a}	(0.03)^{a}	(0.03)^{a}	(0.04)^{a}	(0.06)^{ab}
	2	5.89	8.07	8.39	8.16	8.36	8.23	7.38	7.65
		(0.11)^{d}	(0.31)^{a}	(0.03)^{a}	(0.04)^{a}	(0.03)^{a}	(0.03)^{a}	(0.04)^{c}	(0.06)^{b}
	3	5.23	7.15	8.31	8.22	8.32	8.25	7.27	7.46
		(0.11)^{d}	(0.31)^{bc}	(0.03)^{a}	(0.04)^{a}	(0.03)^{a}	(0.03)^{a}	(0.04)^{c}	(0.06)^{b}
EC(dS m^{-1})	1	1.23	1.25	1.39	1.54	1.62	1.77	1.89	1.92
		(0.04)^{c}	(0.1)^{c}	(0.1)^{c}	(0.17)^{b}	(0.13)^{b}	(0.18)^{ab}	(0.04)^{a}	(0.06)^{a}
	2	1.42	1.45	1.40	1.53	1.65	1.76	1.83	1.88
		(0.04)^{c}	(0.1)^{c}	(0.1)^{c}	(0.17)^{bc}	(0.13)^{b}	(0.18)^{a}	(0.04)^{a}	(0.06)^{a}
	3	1.25	1.21	1.32	1.43	1.46	1.52	1.52	1.56
		(0.04)^{c}	(0.1)^{c}	(0.1)^{bc}	(0.17)^{b}	(0.13)^{b}	(0.18)^{ab}	(0.04)^{a}	(0.06)^{a}

Values are means (n=3) with standard error in brackets. For each parameter values followed by the same letters are not significantly different at Bonferroni (α=0.0001).

Volatile solid reduction

With regard to the Volatile solids (Vs), the different compost piles decreased significantly over the composting time (Table 2.3. ANOVAR $F_{7,14=}$192.459, P=0.0001). Accordingly, in Pile 1, the volatile solids (VS) content was 91.1% at the start of the process, which decreased to 72.2% over time corresponding to a volatile solid reduction of nearly 75%; whereas, in Pile 2, the decrease in volatile solids content was from 89% to 73.4%. This corresponded to an increase of 65% in volatile solid reduction. In contrast, the control (Pile 3) slightly decreased its volatile solids content from 91.1% to 83.3% with concomitant increase of only 55% of the volatile solid reduction.

In general, over 60% of the volatile solid reduction occurred by the end of the 32nd day in Pile 1 as compared to 56th day of reduction, at the end of the thermophilic stage in Pile 2, This study was different from Chroni *et al.* (2011) who reported that over 50% volatile solids reduction had occurred by the end of the 58th day for source separated food waste and grass clipping composts, probably due to the lower thermophilic temperature (maximum 48°C). In the present study, temperature was positively correlated with volatile solids (VS) content, suggesting that higher temperature could drive the largest degradation of the organic materials in compost mixture.

Table 2.3 Changes in volatile solids during composting of three coffee husk piles

Parameter	Piles	Time (in days)							
		1	10	24	32	48	56	66	90
VS(%)	1	91.1	85.1	81.5	79.2	77.4	76.3	75.7	72.2
		(0.9)[a]	(0.8)[b]	(1.1)[c]	(1.5)[d]	(1.5)[e]	(1.3)[e]	(1.1)[ef]	(0.9)[f]
VS_{red}(%)	1	0	41.8	57.2	62.5	66.5	68.6	69.7	74.7
			(1.5)[a]	(2.4)[b]	(1.2)[c]	(1.3)[d]	(1.6)[d]	(1.7)[d]	(2.0)[d]
VS(%)	2	89.0	86.0	82.4	79.5	77.1	75.3	74.8	73.8
		(0.9)[a]	(0.8)[b]	(1.1)[c]	(1.5)[d]	(1.5)[e]	(1.3)[e]	(1.1)[ef]	(0.9)[f]
Vs_{red}(%)	2	0	18.7	42.1	52.0	58.5	62.5	63.5	65.4
			(1.4)[a]	(2.4)[b]	(1.2)[c]	(1.3)[d]	(1.6)[d]	(1.7)[d]	(2.0)[d]
VS(%)	3	91.9	90.9	87.8	85.5	83.7	83.5	83.7	83.3
		(0.9)[a]	(0.8)[b]	(1.1)[c]	(1.5)[d]	(1.5)[e]	(1.3)[e]	(1.1)[ef]	(0.9)[f]
Vs_{red}(%)	3	0	11.1	36.4	47.2	54.5	55.0	57.0	54.5
			(1.5)[a]	(2.4)[b]	(1.2)[c]	(1.3)[d]	(1.6)[d]	(1.7)[d]	(2.0)[d]

Values are means (n=3) with standard error in brackets. For each parameter values followed by the same letters are not significantly different at Bonferroni (α=0.0001). Vs=Volatile solid; Vs_{red}=Volatile solid reduction

Changes in TOC, TN, C/N ratio and GI

Table 2.4 shows the changes in some of the chemical properties of the compost during the process. The fruit/vegetable amended compost (Pile 2) and control (Pile 3) had higher initial organic carbon than Pile 1 (Table 2.4). The highest total organic carbon reductions were found in Pile 2 (about 38%) as a function of time, followed by Pile 1 (about 30%), and Pile 3 (23%) reflecting a good mineralization of organic matter from the start up to the end of composting in the amended composts. At the end of the process, the lowest TOC was obtained in Pile 1, and the highest in Pile 3. The high TOC in the end of the process mainly came from the higher concentration of more recalcitrant compounds present in Pile 3 compared to the other two co-composted piles that could fuel better kinetic process. In general, total organic carbon had significant variations among the three piles (Table 2.4 ANOVAR, $F_{7,\ 14}$=195.076, p=0.0001). This implies that better organic matter degradation has occurred in the two co-composted piles than the control.

There was a significant difference in total nitrogen (TN%) among the three piles (Table 2.4 ANOVAR, $F_{7,14}$=33.11 p=0.0001). But the variation between the two co-composted organic materials and the control pile was highly significant at all time of composting. As a result, the C to N ratio of these piles (Pile 1 and Pile 2) decreased steadily and reached within the maximum permissible maturity range of C:N ratio (15) before day 48 for Pile 1 and on day 48 for Pile 2 (Díaz and Savage, 2007). However, the control (Pile 3) never attained this level even after day 90.

Table 2.4 Changes in chemical components and GI of three piles of coffee husk

Parameters	Piles	Time							
		1	10	24	32	48	56	66	90
OC%	1	48.1 (0.3)[a]	45.6 (0.8)[b]	44.3 (0.7)[c]	41.4 (0.9)[cd]	41.5 (2.1)[d]	37.6 (0.8)[e]	35.4 (0.7)[e]	33.9 (0.6)[e]
	2	57.5 (2.3)[a]	51.3 (0.5)[b]	45.3 (1.0)[c]	43.0 (0.6)[cd]	44.0 (0.5)[d]	39.3 (0.2)[e]	37.1 (0.4)[e]	36.3 (0.8)[e]
	3	54.4 (0.1)[a]	53.3 (0.2)[b]	48.9 (0.6)[c]	43.2 (1.2)[cd]	46.1 (1.4)[d]	40.0 (0.4)[e]	39.8 (1.0)[e]	41.7 (0.3)[e]
TN%	1	2.8 (0.1)[a]	3.0 (0.1)[a]	3.2 (0.1)[a]	2.9 (0.03)[a]	3.3 (0.03)[a]	3.1 (0.1)[a]	3.2 (0.03)[a]	3.1 (0.1)[a]
	2	2.4 (0.03)[b]	3.1 (0.1)[a]	2.9 (0.1)[b]	2.8 (0.1)[a]	3.3 (0.03)[a]	2.8 (0.03)[b]	2.6 (0.03)[b]	2.8 (0.03)[b]
	3	1.8 (0.03)[c]	2.0 (0.03)[b]	1.9 (0.03)[c]	2.0 (0.03)[b]	2.1 (0.03)[b]	1.9 (0.03)[c]	1.9 (0.03)[c]	2.3 (0.1)[c]
C:N	1	17.5 (0.4)[a]	15.1 (0.2)[a]	14.0 (0.1)[a]	14.2 (0.3)[a]	12.7 (0.7)[a]	12.1 (0.1)[a]	11.7 (0.1)[a]	11.1 (0.3)[a]
	2	24.4 (0.1)[b]	16.4 (0.2)[b]	15.4 (0.3)[a]	15.4 (0.5)[b]	13.5 (0.8)[a]	14.1 (0.3)[a]	14.0 (0.2)[b]	12.7 (0.03)[b]
	3	29.7 (0.3)[c]	27.1 (0.5)[c]	25.8 (0.4)[b]	21.7 (0.3)[c]	21.6 (0.5)[b]	20.7 (1.0)[b]	20.6 (0.4)[c]	18.2 (0.8)[c]
GI%	1	0	33.58 (2.05)[e]	42.16 (1.63)[e]	48.90 (1.57)[d]	53.94 (5.33)[cd]	65.65.5 (2.16)[c]	80.61 (2.41)[b]	85.25 (1.97)[a]
	2	0	13.41 (2.05)[e]	26.18 (1.63)[e]	24.61 (1.57)[d]	36.44 (5.33)[cd]	55.29 (2.16)[c]	65.70 (2.41)[b]	74.69 (1.97)[a]
	3	0	2.39 (2.05)[e]	4.75 (1.63)[e]	19.46 (1.57)[d]	30.49 (5.33)[cd]	38.52 (2.16)[c]	54.33 (2.41)[b]	46.69 (1.97)[a]

Values are means (n=3) with standard error in brackets. Values followed by different letters showed that there is significant differences within piles (Tukey HSD test α=0.05)

The decline in C to N ratio of Pile 1 was more pronounced reaching at a C to N ratio of 11 at day 90. Gómez Brandón *et al*. (2008) also reported similar changes in cattle manure composting in Spain suggesting that depletion of easily degradable carbon compounds was the main driving force to bring early maturity as N could be slightly changed. The C to N ratio generally decreases throughout the composting process due to the release of CO_2 and then stabilized within the range of 10-15 when organic waste is composted (Insam and de Bertoldi, 2003). Overall, the total carbon and C:N ratio had an inverse relationship with compost maturity and stability (Cooperband *et al.*, 2003), resulting in earlier maturity time for the two amended composts because the C:N ratio had come down to 11% and 12% for Pile 1 and Pile 2, respectively. In contrast, the control pile remained high even after day 90.

The germination indices (GI) of all piles reached a value of 34% in pile1 and 13% in Pile 2 during the initial active phase at day10 (Table 2.4) whereas germination index was very low (2.3%) in Pile 3 at the same day. At maturation stage, a GI of 86% and 75% was recorded for Pile 1 and Pile 2, respectively. In contrast, the control (Pile 3) exhibited a GI value of 46% at the same day suggesting that this pile required longer time to overcome the threshold limit of 80% stated by Zucconi and de Bertoldi (1987). Besides, a GI value of greater than 80% in cow dung amended compost was an indication of the disappearance of phytotoxicity and the maturity of compost (Tiquia *et al*., 1996). Therefore, composting time had significant effect on germination index in those piles as indicated by GI% (Table 2.4. composting time ANOVAR $F_{7, 14}$=253.6, p=0.0001).

Overall, pile1 matured earlier than the other piles implying that addition of high value organic matter such as cow dung as well as inoculation with mature household compost

facilitated the faster degradation of phytotoxins released in the process; whereas, the two piles remains unsafe for the application of the material to plant and soil system.

Microbial profile of coffee-husk composting

The total mesophilic counts of bacteria, actinobacteria and fungi in the three piles showed significant variations and fluctuations across the composting time (Figure 2.2). The variation showed similar pattern in Pile1 and Pile2, which was significantly different from pile3. In Pile1, total count of aerobic mesophilic bacteria was highest at the start of composting (9.58log^{MPN} g^{-1} dw), and lowest at the peak thermophilic phase (day32, 6.30log ^{MPN} g^{-1} dw) and gradually increased thereafter reaching 8.99 log^{MPN} g^{-1} dw at the end of composting (day90).

Actinobacteria was lower in number on day1 (6.29log^{MPN} g^{-1} dw), then increased gradually by one unit. After 56 days of composting, the number increased by two units reaching maximum counts (i.e. 9.54log^{MPN} g^{-1} dw) on day 90. In contrast, most of the fungi were eliminated at temperatures above 50°C (day32) but were recovered later when temperature was moderate (<45°C) due to the inability of most fungi to function at higher temperature (Ryckeboer *et al*., 2003a). Population of actinobacteria and fungi increased in number at the latter phase of composting, indicating that temperature changes typically affected the distribution (population density) of total aerobic mesophilic, actinobacteria and fungi.

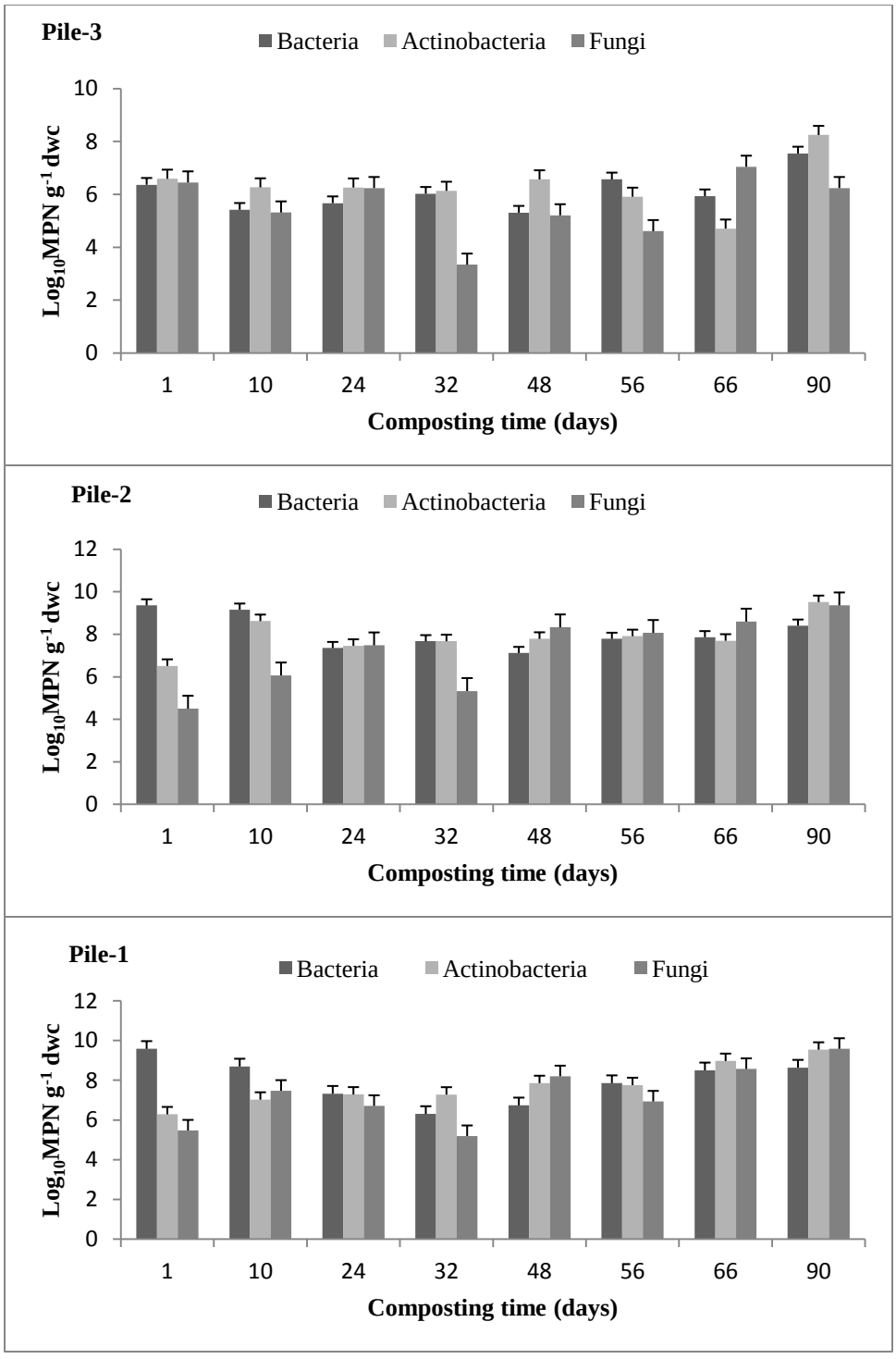


Figure 2.2 Number of total aerobic bacteria, actinobacteria and fungi at the different composting time in Pile-1 (CH+CD+HHC), Pile-2 (CH+FVW+HHC), and Pile-3 (CH). Data are expressed as log ^{MPN} g^{-1} dry weight (dw). Values are means (n=3) with standard error.

In pile2, bacterial number was the highest (9.36log^{MPN} g^{-1} dw) at the start of composting. But this number was gradually dropped by two units after day24 (7.46log^{MPN} g^{-1} dw) until it showed a slight increase (8.41log^{MPN} g^{-1} dw) by day90. The highest population of actinobacteria and fungi were obtained by the end of composting (i.e. 9.52log^{MPN} g^{-1} dw for actinobacteria and 9.36 log^{MPN} g^{-1} dw for fungi). Although the increase and decrease of the different microbial groups in pile3 followed the temperature pattern of composting, they showed relatively lowest numbers of counts in Pile 3 compared to Pile1 and Pile2. The number varied from 5.41 to 7.54 log^{MPN} g^{-1} dw for mesophilic bacteria, from 4.70 to 8.25 log ^{MPN} g^{-1} dw for actinobacteria and from 3.34 to 7.04log ^{MPN} g^{-1} dw fungi.

In general, the population of bacteria, actinobacteria and fungi in this study was much lower than the report of Ryckeboer *et al*. (2003b), probably due to the use of selective media and different colony counting technique (CFU). According to these authors, approximate numbers of bacteria at mesophilic stage ranged from 10^9 to10^{13} g^{-1} substrates, numbers of actinomycetes ranged from 10^8 to 10^{12} g^{-1} substrates, and that of fungi from 10^5 to 10^8 g^{-1} substrates. On the other hand, thermophilic bacteria and actinobacteria ranged from 10^8 to 10^{12} g^{-1} substrates and $10^7$to 10^9 g^{-1} substrates.

Tiquia *et al*. (2002) reported that actinobacteria exploit the compost environment immediately after the piles cool from heat phase, and reached highest degree of activity after one month of composting in lignin rich organic matters. Pandey *et al*. (2000) also showed the dominance of actinobacteria in the degradation of lignin in coffee husk. In general, the microbial profile of the composts showed a pattern where the higher number of total aerobic bacteria occurred at the start of composting, whereas the actinobacteria and fungi dominated at the end of composting depending upon the available

biodegradable fractions available through several stages of composting (Beffa *et al*., 1995; Ryckeboer *et al*., 2003b).

Table 2.5 shows the population of thermophilic/thermotolerant bacteria and actinobacteria in the three piles. In all piles, the change in temperature to thermophilic stages elucidated the presence or absence of the three groups of microorganisms. Thermophilic fungi that usually develop within 40-53°C (Finstein and Morris, 1975) were totally absent in the present study.

On the other hand, thermophilic bacteria were very active and larger in number at temperature between 45-60°C in pile1; but their numbers were significantly reduced to 1 and 2 units when temperature was above 60°C. Similarly, in Pile 2 and Pile 3 the number of thermophilic bacteria was higher at temperature range 40-60°C. Bacterial numbers ranged from 7.94 to 8.85 $\log^{MPN} g^{-1}$dw in pile2 and from 7.42 to 8.20 $\log^{MPN} g^{-1}$dw) in Pile 3; but lower in Pile 2 (7.24$\log^{MPN} g^{-1}$ dw) and absent in Pile 3 when temperature went above 60°C.

Table 2.5 Number of thermophilic microbes (Log^{MPN}g-1 dw) in coffee husk compost

Temperature	Pile-1		Pile-2		Pile-3	
	TAB	Act	TAB	Act	TAB	Act
50-60°C	8.15 (0.49)^{ab}	8.16 (0.50)^{a}	7.94 (0.34)^{a}	7.44 (0.86)^{a}	7.95 (0.52)^{a}	6.18 (0.12)^{a}
45-48°C	8.82 (0.33)^{b}	8.62 (0.49)^{a}	8.85 (0.47)^{a}	6.91 (0.86)^{a}	8.20 (0.40)^{a}	8.66 (0.44)^{b}
45°C	7.23 (0.42)^{ab}	7.63 (0.97)^{a}	8.14 (0.63)^{a}	7.58 (0.31)^{a}	7.42 (0.03)^{a}	7.92 (0.70)^{ab}
63-65°C	6.64 (0.28)^{a}	7.18 (0.31)^{a}	7.24 (0.47)^{a}	na	na	na

Values are expressed as means (n=3) with standard error. Different letters along each column indicate that there was significant difference between sampling time (Tukey HSD test α=0.005). TAB: total aerobic bacteria, Act: Actinobacteria; na=not available

In general thermophilic bacteria were extensively active at 45-60°C, and at temperatures above 60°C the degradation process was performed essentially by these microorganisms as seen by previous reports (Nakasaki *et al.*, 1985; Strom, 1985; Beffa *et al*., 1995).

In the present study, thermophilic actinobacteria were present in higher numbers at temperature 45-60°C. Their number varied from 7.63 to 8.62 log ^{MPN} g^{-1} dw (Pile 1) from 6.91 to 7.58 log ^{MPN} g^{-1} dw (Pile 2); and from 6.18 to 8.66 log^{MPN} g^{-1} dw (Pile 3); whereas no actinobacteria could be detected in all piles when the temperature rose to 65°C except in pile1 (7.18 log ^{MPN} g^{-1} dw).

In the present study, changes in organic carbon was higher in Pile 2 (25%) and pile3 (21%) at day 32nd compared to 16% and 4% removal of carbon by 90th day (Table 2.3), reflecting that thermophilc microbes played much more enhanced degradation process than mesophilic microbes.

Spore forming bacterial number involved only during thermophilic temperature phase (Table 2.6). During the first peak temperature (50-60°C) by day3, large numbers of spores ($2.05X10^7$ to $5.4X10^7$) were found in Pile 1 and Pile 2. These numbers, however, slightly decreased when the temperature increased to 63-65°C. In Pile 3, highest number of spores was found when the temperature rose to 65°C and dropped by day40 when temperature declined below 45°C.

The absence of spores at the end of composting could be explained by an increase of total microorganisms during the phase of cooling as a result of germination and multiplication of the spore forming bacteria such as the genus *Bacillus* (Hassen *et al*., 2001). Beffa *et al*., (1995) reported that thermophilic bacterial count of 10^4 to10^6 cells/g compost dry weight (cdw) at temperature 60–80°C; and 10^5 to 10^7 cells/g cdw at temperature 55–75°C; 10^3 to 10^{10} cells/g cdw at T 40–80°C occurred.

Statistically, however, no difference could be observed across the composting time with respect to spore density in Pile 1 and Pile 2, whereas, significant difference was seen in Pile 3. This difference was an indicator of the relationship between temperature and spore forming bacteria. This was evident that certain microbes produce resistant forms against stresses, notably by the development of spores.

Table 2.6 Number of endospore bacteria (spores g^{-1} DW) during composting

Temperature	Pile1	Pile2	Pile3
50-60°C	$2.05X10^{7}(0.24)^{a}$	$5.4X10^{7}(2.25)^{a}$	$1.23X10^{4}(0.68)^{a}$
63-65°C	$1.91X10^{7}(0.52)^{a}$	$4.52X10^{7}(1.37)^{a}$	$1.36X10^{8}(4.28)^{b}$
40-45°C	$1.23X10^{7}(0.68)^{a}$	$4.62X10^{7}(0.46)^{a}$	$7.71X10^{6}(6.07)^{ab}$

Values are expressed as means plus standard error of three triplicate samples. Different letters along the column of each pile indicated that there was significant difference between samples (Tukey HSD α=0.05).

Relationship between C:N ratios/germination index and other chemicals

In the present investigation, correlation analysis was performed using C/N ration and germination index (GI%) **as maturity index** and other chemical parameters for the eight composting times (1, 10, 24, 32, 48, 56, 66, 90). Results indicated that C/N ratio and GI% of the three piles were positively correlated with a number chemical parameters of the compost (Table 2.7).

In Pile 1, GI was correlated significantly with volatile solid reduction (r=0.90), pH (r=0.57) and TN (r=0.32). Similarly, GI was significantly correlated with volatile solid reduction (r=0.85), total organic nitrogen (r=0.13) and pH (r=0.27) in Pile 2. The same trend was also found in Pile 3 where GI showed significant correlation with pH (r=0.27), volatile solid reduction (r=0.79) and TN (r=0.29).

In all piles, C/N ratio was positively correlated with total organic carbon (i.e. Pile 1: r=0.88; Pile 2: r=0.85; Pile 3: r=0.927). Therefore, this study revealed that GI and C/N

ratio of the coffee husk compost were influenced by pH and total nitrogen, probably due to the release of simple organic acids at the start and ammonium in the active phase of the process. The positive correlation between organic carbon and C/N ratio reflected the entire degradation of the organic matter converting into CO_2 as a result of microbial degradation.

Table 2.7 Pearson correlations (r^2) between different chemical properties in cow dung plus, fruit/ vegetable plus house hold compost amended coffee husk piles (Pile1: CHCD; Pile2: CHFVW; Pile3:CH)

	Pile-1						Pile-2						Pile-3					
	pH	GI	VS_r	OC	TN	C/N	pH	GI	VS_r	OC	TN	C/N	pH	GI	VS_r	OC	TN	C/N
pH	1						1						1					
GI	0.571**	1					0.275	1					0.274	1				
VS_r	0.808**	0.898**	1				0.588**	0.851**	1				0.733**	0.794**	1			
OC	-0.313	-0.880**	-0.708**	1			-0.351	- 0.875**	-0.937**	1			-0.534**	-0.854**	0.898**	1		
TN	0 .728**	0.318	0 .534**	0.009	1		0.679**	0.132	0.261-	0.114	1		0.554**	0.293	-0.499*	-0.296	1	
C/N	-0.652**	-0.932**	-0.894**	0.868**	-0.483**	1	-0.803**	-0.718**	-0.867**	0.846**	-0.619**	1	-0.665**	-0.812*	-0.94**	0.93**	-0.63**	1

****Correlation is significant at the 0.01 level (1-tailed); *Correlation is significant at the 0.05 level (1-tailed). Correlation was performed for three piles (72 data). pH; GI=germination index; VS_r= volatile solid reduction; OC=total organic carbon; TN=total nitrogen; C/N=carbon to nitrogen**

In general, this study revealed that the highest number of bacteria at the start and the highest count of actinobacteria and fungi indicated typical microbial succession along the composting process. The pattern of microbial succession resulted in higher germination index (GI=80%) and C/N ratio (about 11) which is an indication of better maturation in Pile 1 compared to Pile 2 and 3. These maturity indices (GI% and C/N ratio) have been influenced by different physicochemical properties of compost. i.e. pH, organic carbon (OC), total nitrogen (TN) and volatile solid reduction (VS_r).

Chapter 3.Coffee husk composting: An investigation of the process using molecular and non-molecular tools

Fekadu Shemekite[a],*, María Gómez-Brandón[b], Ingrid H. Franke-Whittle[b], Barbara Praehauser[b], Heribert Insam[b], Fassil Assefa [a]

a Department of Microbial, Cellular and Molecular Biology, Addis Ababa University

b Institute of Microbiology, University of Innsbruck, Technikerstraβe 25d, A-6020 Innsbruck, Austria

Abstract

Composting is an intense microbial activity on organic rich substrates that are mineralized into inorganic matter. The process is complex and involves different types of microorganisms in succession; and produce a stable final product that are valuable and inexpensive soil conditioner. Control of composting process can be achieved by either adjusting process parameters such as moisture, temperature or oxygen level or by altering the starting conditions (C/N ratio). To this end, various parameters were measured during a 90 day composting process of coffee husk with cow dung (Pile 1), with fruit/vegetable wastes (Pile 2) and coffee husk (Pile 3). Samples were collected on days 0, 32 and 90 for chemical and microbiological analyses. C/N ratios of Piles 1 and 2 decreased significantly over the 90 days. The highest bacterial counts at the start and highest actinobacterial counts at the end of the process (Piles 1 and 2) indicated microbial succession with concomitant production of compost relevant enzymes. Denaturing gradient gel electrophoresis of rDNA and COMPOCHIP microarray analysis indicated distinctive community shifts during the composting process, with day 0 samples clustering separately from the 32 and 90 day samples. This study, using a multi parameter approach, has revealed differences in quality and species diversity of the three composts.

Key words/phrases: *COMPOCHIP microarray; Enzyme activity; Organic matter degradation; PCR-DGGE*

Introduction

Coffee production is the mainstay for more than 15 million people (20% of the population) and constitutes an important sector of the agro-industry in Ethiopia, (Wiersum *et al.*, 2008). It is estimated that industrial coffee processing produces and releases more than 240,000 tons of coffee waste (coffee husk and pulp) into the environment (CTA, 1999). The direct application of these organic matters as organic supplement may cause serious root damage by the production of simple organic acids and competition for nitrogen starving the soil microorganisms for this important element (Gómez-Brandón *et al*., 2008).

The hitherto works on coffee waste management in Ethiopia showed that its application as mulch can improve soil fertility and increase yield (Tenaw and Kelsa, 1998). This indicates that if proper management of coffee waste through oxygen-driven biological methods, such as composting, is used, it can serve a dual purpose of environmental protection and fertiliser production (Murthy and Naidu, 2012).

Composting is one of the most widely-known and accepted technologies for recycling of agricultural waste materials under aerobic conditions (Insam and de Bertoldi, 2003). The transformation process involves the succession of specialized microbial communities that express a wide array of enzymes responsible for the changes in the physico-chemical properties of the substrate. The presence and activities of specific intracellular and/or extracellular enzymes during composting, therefore, is a very good indicator for the proper monitoring of the process of biodegradation and the quality of compost (fertilizer) produced (Vargas-Garcia *et al*., 2010).

Apart from enzymes, molecular methods are used for profiling of complex microbial communities associated with compost processes. To this effect, Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S and 18S rRNA gene fragments are used as a reliable and fast fingerprinting technique for determining the bacterial and fungal communities from environmental samples (Muyzer and Smalla, 1998).

In addition, specific compost-targeted microarrays have also been used to investigate bacterial and fungal communities present in composts (Franke-Whittle *et al*., 2005, 2009; Hultman *et al.,* 2010). The COMPOCHIP contained several oligonucleotide probes that are used for the detection of compost degrading bacteria (Franke-Whittle *et al*., 2005, 2009). The combined use of both DGGE and the COMPOCHIP microarray offers a deeper insight into the microbial communities during organic waste composting (Danon *et al.,* 2008; Fernández-Gómez *et al*.,2012).

The objectives of this study was to monitor the physico-chemical changes during the composting of coffee husks, with a special emphasis on extracellular enzymatic activities, and investigate the microbial community dynamics throughout the process, using both culture-dependent and culture-independent approaches.

Materials and Methods

Composting process and sample collection

Two different composts were prepared by mixing coffee husks with cow dung and coffee husk with fruit/vegetable wastes in proportions of 4:1 and 2:1 (on fresh weight basis), respectively, taking into account their C to N ratios (Table 3.1).Coffee husks were collected from coffee processing areas: Bebeka and MizanTeferi towns (Southwestern,

Ethiopia). The fruit/vegetable wastes and the cow dung were collected from the market center and dairy enterprise in Addis Ababa, respectively. The physico-chemical properties of the materials are shown in Table 3.1.

Composting was conducted at the Gerji compost demonstration site owned by Addis Ababa Environmental Protection Authority (EPA). About 2000 kg of each mixture was composted in roughly trapezoidal piles with a volume of $1.5m^3$: coffee husk + cow dung (CHCD, Pile 1); coffee husk + fruit/vegetable wastes (CHFVW, Pile 2). A pile containing only coffee husk (Pile 3, CH) was also included in the experimental set-up. All three piles were composted following the aerobic windrow method (Diaz *et al*., 2002) and subjected to manual turning every day during the first week and once per week until the end of the process.

Moisture content in the piles was adjusted by adding water to keep the level at approximately 60%, and piles were covered with perforated plastic materials in order to prevent excessive moisture loss. The temperature was measured in triplicate on days 0, 32 and 90 of composting using a digital thermometer. A total of twenty-seven samples, representing each pile (around 500 g each), were collected separately on days 0, 32 and 90 of sampling. Samples were immediately stored at -20 °C upon arrival at the laboratory for molecular analyses and at 4 °C for the remaining analyses. All analyses were carried out in triplicates.

Physico-chemical analysis

All physico-chemical analyses were undertaken in triplicates. The moisture content of composts was determined after oven-drying of 10 g fresh weight samples at 105 $^{\circ}$C for

24 h. Total C was determined by dry combustion at 550 °C and total N content (TN) by the Kjeldahl digestion analysis (Keenly and Nelson, 1982). pH and electrical conductivity (EC) were measured in aqueous extracts (1:10, w/v) using a pH meter (HD8602, Italy) and a conductivity meter (CC401 ELMEIRON, Poland), respectively.

Enumeration of total aerobic heterotrophs, actinobacteria and fungi

Total aerobic heterotrophs, actinobacteria and fungi were detected from samples using direct plating on appropriate media (Tiquia *et al.,* 2002). For the enumeration of total aerobic heterotrophs, plate count agar (PCA, Oxoid, England) was used, whereas actinobacteria and fungi were counted on starch casein agar (SCA) and potato dextrose agar (PDA), respectively. Compost samples were ground to 0.25 mm and prepared in appropriate dilution, and inoculated onto their respective media using the plate frequency technique. Plates were incubated at 30 ± 2 °C for three days for bacteria and fungi, and between five and seven days for actinobacteria. After incubation, any observed visible growth was scored positive and the populations of the different groups in the compost samples were estimated using the Most Probable Number (MPN) method (Tiquia *et al.,* 2002).

Enzyme activity using API ZYM™ kit

The relative enzymatic activity in the compost samples was determined by using the API ZYM™ kit according to the manufacturer´s protocol. Each strip contained 20 microcupules containing dehydrated chromogenic substrates for different enzymes and a control. For this study, compost samples (5 g, fresh weight) were mixed with 50 ml of sterile water, homogenized in a horizontal shaker for 10 min, allowed to settle for another 10 min and the supernatants were analyzed for enzymatic activities. An aliquot (65µl) of

the supernatant was dispensed into each of the twenty microcupules. The API ZYM™ strips were covered and incubated at 37 °C for 4.5 h. Then, 30 µl of each reagent (ZYM A and ZYM B) was added to each microcupule. After 5 min, color development was evaluated and a numerical value ranging from 1 to 5 (1=5 nM; 2=10 nM; 3=20 nM; 4=30 nM; 5=40 nM) was assigned according to the color chart provided by the manufacturer. The results were recorded as low intensity [1], moderate intensity [2–3], and high intensity [4–5], as described by Tiquia *et al.* (2002).

DNA extraction and PCR-DGGE analysis of microbial community

Total DNA was extracted in triplicate from 0.25 g of each sample using the PowerSoil™ DNA Isolation Kit (MO Bio Laboratories Inc., Carlsbad, USA) according to the manufacturer's protocol in order to improve the DNA yield. After an abbreviated bead beating step (10 min), samples were frozen at -80°C for 30 min and then heated for 10 min at 60°C. This freeze-thaw step was repeated once, before continuing with the standard protocol.

The PCR amplification of bacterial and fungal communities was performed as described by Fernández-Gómez *et al.* (2010). Proper sizes of amplification products were verified by electrophoresis in 1% agarose gels, and PCR product concentration was determined with PicoGreen dsDNA quantification reagent (Invitrogen, Carlsbad, USA). Fluorescence was measured using an Anthos Zenyth 3100 multimode reader (Anthos Labtec, Austria) and the Software for Anthos Multimode Detectors (Version 2.0.0.13).

The denaturing gradient gel electrophoresis (DGGE) of bacterial and fungal communities was performed by loading 60 ng of PCR products in a 7% (w/v) polyacrylamide gel in 1

X TAE (20 mMTris–HCl, 10 mM acetate, 0.5 mM Na_2EDTA) containing a denaturing gradient of 40 to 70% and 30 to 60%, respectively (100% denaturants consisting of 7 mol/L urea and 40% formamide). A 100 bp DNA ladder (Genecraft®, Germany) served as marker. Gels were run in an INGENYphorU System (Ingeny International BV, The Netherlands) at 60°C for 16 h at 100 V. Gels were stained with silver nitrate using the Hoefer Automated Gel Stainer (Amersham Pharmacia Biotech, Germany), air dried and scanned for subsequent image analysis.

Microarray analysis

The COMPOCHIP microarray, spotted with 414 probes targeting compost-relevant microorganisms, including plant, animal and human pathogens, and bacteria related to plant disease suppression was used. All the probes included on the COMPOCHIP microarray were designed so to have similar melting temperatures, and probe sequences ranged in length from 17 to 25 nucleotides. Fluorescence labelling of target DNA by PCR using the 8F and 1492R primers, hybridization, scanning of arrays and image analysis were conducted as described by Franke-Whittle *et al.* (2009).

Data analysis

Chemical and microbiological data were analysed by repeated analysis of variance (ANOVAR) in which piles represented the subjects. In addition, the composition of parent material (CHCD, CHFVW and CH) was fixed as the between- subject factor, and the composting time (0, 32 and 90 d) was fixed as the within-subject factor**.** All the variables met the sphericity condition (Mauchly´s test), except for pH, EC and organic C. In these cases the sphericity violation was corrected with the Geisser-Greenhouse (G-G) procedure (Potvin *et al*., 1990). Significant differences in the main effects were further

analyzed by paired comparisons with the Tukey HSD test. Pearson product moment correlation (r^2) was used to calculate general correlation between enzyme activities and microbial MPN counts. The statistical analyses were performed using SPSS v16.0. A principal component analysis (PCA) from the enzyme activities was also performed using Statisticav9 software.

A comparison of bacterial and fungal DGGE patterns was made with the GelCompar II software package (Applied Math, Belgium). DGGE bands were normalized using the reference position defined by the molecular-weight marker in order to align the bands for proper comparison. A cluster analysis was performed using Dice correlation coefficients and the unweighted-pair-group method with arithmetic averages (UPGMA) clustering algorithm. The program settings were at 1.0% optimization and 1.0% position tolerance. Principal component analysis (PCA) of the total signal-to-noise ratio (SNR) from microarray data of the study was conducted, using CANOCO for windows 4.5 (Ter Braak and Smilauer, 2002).

Results and Discussion

Changes in physico-chemical parameters during composting

In this study, four temperature phases were seen for the different compost piles, as shown in Fig. 1. An initial mesophilic phase (18-42 °C) was followed by a thermophilic phase (45-70°C), a second mesophilic (42-25 °C) and finally a cooling phase ($\leq$25 °C). However, significant differences were evident in the temperature changes among the three piles as a function of composting time (compost pile x time ANOVAR $F_{27,72}$=27.78, P=0.00001). Piles 1 and 2 exhibited a short initial mesophilic phase and self-heated to 60

°C within three days (Figure 3.1). In contrast, Pile 3 (composed of coffee husk alone) showed a much longer initial mesophilic phase which lasted for almost three weeks, prior to the start of the thermophilic stage which peaked on day 32, and lasted for only one week.

The second mesophilic phase followed and continued until day 78 when the cooling phase started. These results suggest that co-composting of coffee husk with either cow dung or fruit/vegetable wastes accelerated the composting process (Díaz *et al*., 2002). These findings are in agreement with those of Boulter-Bitzer *et al*. (2006), who produced four composts from mixtures of different feedstocks (horse, chicken and paunch manure, bone meal ash, bark mix, soybean meal, and milogranite), and reported increases in temperature within the first 10 days from ambient to 55 to 70 °C, and a second increase in temperature after 31 days, prior to a cooling phase. The decrease in temperature is a result of the exhaustion of easily degradable organic materials.

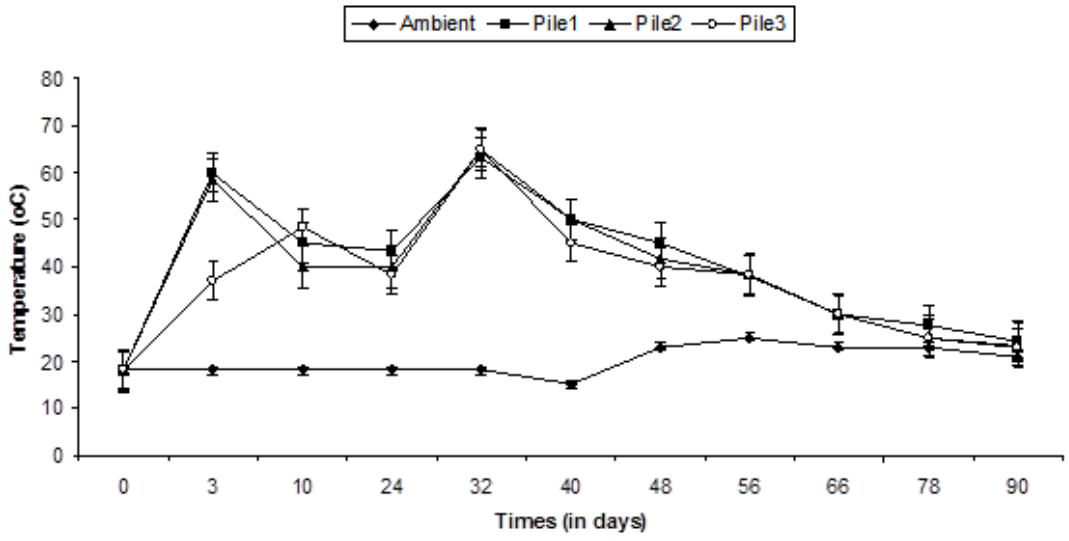


Figure 3.1 Temperature changes during composting of three different piles. Values are expressed as means and standard errors of triplicates of ambient T in Piles (coffee husk+cow dung), Pile2 (coffee husk+fruit/vegetable wastes), and Pile3 (coffee husk).

The key physico-chemical properties of the composts at the start, after 32 and after 90 days of composting are shown in Table 3.1. Overall, the moisture content was in the range 54% to 65% (mass/mass) for the three piles, although significant differences were found over time (ANOVAR $F_{2,12}$=4.27, P=0.04). In the two co-composted piles, highest moisture levels were seen in the active phase, with approximately 10% moisture losses occurring afterwards. In contrast, moisture levels increased during the process in Pile 3. The reduction in moisture in Piles 1 and 2 can be explained by microbial heat generation causing enhanced desiccation (Rebollido *et al*., 2008). Moisture loss during the composting process can be considered as an indicator of the decomposition rate because the heat generation that accompanies decomposition drives evaporation.

The pH values of the different compost piles increased from acidic values at the start of the experiment to alkaline values on day 32. This pH rise over time was more pronounced for Pile 3 than for the two co-composted piles (compost pile x time ANOVAR $F_{4,12}$=27.32, P=0.00001). Between days 32 and 90, pH values were found to decrease minimally (Table 3.1). The increase in pH during the composting process can be attributed to a release of ammonia associated with protein degradation, and its subsequent decrease to a volatilisation of ammoniacal nitrogen and H^+ release resulting from a microbial nitrification process (Gómez-Brandón *et al*., 2008). The pH values of the composts at maturation indicate the suitability of the composts for agricultural application (Bernal *et al*., 2009).

Electrical conductivity increased significantly in all piles from the start of the experiment (Table 3.1; ANOVAR $F_{2,12}$=60.36, P=0.00001), probably due to the release of ions, such

as phosphate, ammonium and potassium throughout the composting process (Bernal *et al*., 2009). EC decreased after 32 d (Table 3.1), and after 90 d, composts had EC values suitable for soil application (< threshold value of 3 mS cm^{-1}; Gómez-Brandón *et al*., 2008).

The total organic carbon (TOC) content in all composts dramatically decreased during the composting process (Table 3.1), reflecting a notable mineralization of organic matter. The highest TOC reductions were found in Pile 2 (about 37 %), followed by Pile 1 (26 %), and Pile 3 (23 %), resulting in a significant interaction between compost pile and time (ANOVAR $F_{4,12}$=7.01, P=0.002). In our study, the control pile (coffee husk compost) had the lowest CO_2 loss, probably because of the higher concentration of recalcitrant compounds. This pile contained no additional organic matter that could fuel the kinetic process; hence, its decomposition occurred more slowly.

Total nitrogen (TN) percentage showed an increasing trend with composting duration (Table 3.1; ANOVAR $F_{2, 12}$=46.60, P=0.00001) which is due to the concentration effect caused by carbon loss associated with mineralization of the organic matter. The C/N ratio decreased significantly over time (Table 3.1), especially in the co-composted piles (compost pile x time ANOVAR $F_{4,12}$=27.16, P=0.00001),as C was lost in the form of CO_2 through microbial respiration and N was recycled (Ryckeboer *et al*., 2003b). A higher C/N ratio at the end of the composting process for the coffee husk compost (Pile 3) was found, indicating a slow kinetic process in this compost. The addition of cow dung and fruit/vegetable wastes to coffee husk, however, significantly contributed to the decomposition of the ligno-cellulosic compounds of the husk, resulting in higher N losses in these mixed composts corroborating the data of Sánchez-Monedero *et al*. (2001).

Table 3.1 Physico-chemical properties of the raw materials and compost during the composting on days 0, 32 and 90

Parameters	Raw Materials			Pile1			Pile2			Pile3		
	CH	FVW	CD	0	32	90	0	32	90	0	32	90
Moisture (%)	13.03	74.3	64.33	54.33	64.33	57.67	56.33	65.33	58.33	58.67	61.00	65.33
	(0.6)	(0.99)	(0.12)	(4.84)^{a}	(0.67)^{b}	(1.20)^{a}	(6.69)^{a}	(0.67)^{b}	(0.88)^{a}	(0.88)^{b}	(2.08)^{ab}	(0.88)^{a}
pH (1:10)	5.32	6.21	7.8	6.37	8.16	7.89	6.07	8.16	7.79	5.23	8.21	7.46
	(0.05)	(0.18)	(0.01)	(0.09)^{c}	(0.03)^{a}	(0.05)^{b}	(0.09)^{c}	(0.03)^{a}	(0.06)^{b}	(0.09)^{c}	(0.06)^{a}	(0.02)^{b}
EC (dS m^{-1})	2.24	2.25	0.85	1.23	1.84	1.77	1.40	1.99	1.75	1.25	1.93	1.57
	(0.15)	(0.15)	(0.01)	(0.01)^{b}	(0.09)^{a}	(0.01)^{a}	(0.05)^{b}	(0.09)^{a}	(0.02)^{a}	(0.03)^{b}	(0.16)^{a}	0.04)^{ab}
Organic C(%)	54.5	40.1	23.0	48.09	41.46	35.38	57.48	43.00	36.24	54.46	43.16	41.72
	(0.35)	(0.08)	(0.12)	(0.32)^{a}	(0.89)^{b}	(0.73)^{c}	(0.86)^{a}	(1.01)^{b}	(0.36)^{c}	(0.06)^{a}	(1.15)^{b}	(0.33)^{b}
Total N(%)	1.83	1.32	1.82	2.76	2.92	3.19	2.37	2.80	2.84	1.84	1.99	2.31
	(0.03)	(0.01)	(0.03)	(0.07)^{b}	(0.03)^{b}	(0.03)^{a}	(0.02)^{b}	(0.13)^{a}	(0.03)^{a}	(0.01)^{c}	(0.01)^{b}	(0.06)^{a}
C to N ratio	29.77	30.46	12.64	17.51	14.21	11.07	24.38	15.44	12.73	29.54	22.74	18.11
	(0.69)	(0.15)	(0.12)	(0.34)^{a}	(0.32)^{b}	(0.22)^{c}	(0.18)^{a}	(0.77)^{b}	(0.05)^{c}	(0.16)^{a}	(0.54)^{b}	(0.34)^{c}

Values are means (n=3) with standard error in brackets. CH=coffee husk. FVW=fruit/vegetable wastes. CD=cow dung. Pile 1: coffee husk + cow dung; Pile 2: coffee husk + vegetable/fruit waste; Pile 3: coffee husks. EC: electrical conductivity. For each pile different letters indicate significant differences between samples (Tukey HSD test; α = 0.05).

Changes in microbial numbers during composting

Total mesophilic counts of bacteria, actinobacteria and fungi in the three different compost piles indicated significant variations in relation to temperature changes during the composting process (Table 3.2). At the start of the process, high numbers of bacteria were obtained in Pile1 (9.58 log ^{MPN} g $^{-1}$dw) and Pile 2 (9.36log ^{MPN}g^{-1}dw). Yet, both piles were found to contain significantly lower numbers of bacteria (6.37 log^{MPN}g^{-1}dw, Pile 1; 6.79 log ^{MPN}g^{-1}dw, Pile 2) during the active phase of composting as the temperatures peaked. Bacterial numbers increased again in the maturation phase (Table 3.2). These findings were expected, based on the findings of others (Hassen *et al.*, 2001). In contrast, bacterial numbers in Pile 3 did not differ significantly for the three sampling times (Table 3.2), thereby resulting in a significant interaction between compost pile and time (ANOVAR $F_{4, 12}$=29.03, P<0.001).

As occurred with mesophilic bacteria, actinobacterial numbers varied significantly during composting only for Piles 1 and 2 (Table 3.2; ANOVAR $F_{4,12}$=9.39, P=0.001). Specifically, the highest number of actinobacteria was found after 90 d of composting in both co-composted piles. Indeed, this group of organisms is able to utilize big polymers and accounts for a majority of the microorganisms present in mature composts (Steger *et al*., 2007). Actinobacteria are also considered important for compost hygienization.

In all piles, fungal numbers were significantly reduced at temperatures above 50 °C and increased later when temperatures declined (Table 3.2; ANOVAR $F_{2,12}$= 499,31, P=0.000001). This is because most fungi are unable to thrive at temperatures above 50 °C (Tiquia *et al*., 2002; Ryckeboer *et al*., 2003b). Although the exact role of fungi may not be clear (Tiquia *et al*., 2002), the return of fungal communities at the end of composting

probably can be explained as a result not only of the reduced temperatures, but also of the less aggressive environmental factors prevailing and the availability of cellulose and lignin in the remaining piles (Hassen *et al*., 2001).

Evolution of extracellular enzymes during composting

Analysis of API ZYM ^{TM} testing of the three different compost piles showed varying diversity and relative abundance of enzymes as the composting process proceeded (Table 3. 3). In most cases, the activity of enzymes was highest at d 32, during the active phase of composting, and decreased significantly to a negligible level at the end of the process, indicating the stabilization of the pile (Mondini *et al*., 2004). In Pile1, phosphatases (alkaline, acid and naphthol-phosphohydrolase), esterases (esterase and esterase-lipase), β-galactosidase and β-glucosidase were the highest in the active phase of composting. In Pile2, phosphatase and esterase groups showed increasing activity from low to high and moderate level of concentration as composting process advanced, respectively. On the other hand, the protease enzymes decreased in activity level with time of composting. Synthesis of phosphatases which are induced by phosphohydrolate compounds, are considered to be indicators of microbial presence; and protease which converts polypeptides into amino groups can be considered as good indicator of organic matter decomposition. Increased activities of amino peptidase and protease enzymes in Pile 1 and 2 are a sign of high concentration of protein macromolecules at the beginning and thermophilic phases (Vargas-Garcia *et al*., 2010).

Table 3.2 Enumeration of total aerobic heterotrophic bacteria, actinobacteria and fungi in compost samples collected from the three different piles on days 0, 32 and 90. Data are expressed as log (MPN g-1dw).

Microbial groups	Pile 1			Pile 2			Pile 3		
	0	32	90	0	32	90	0	32	90
Total aerobic bacteria	9.58	6.37	8.61	9.36	6.79	8.21	7.89	7.85	7.59
	(0.10)^{a}	(0.08)^{c}	(0.29)^{b}	(0.08)^{a}	(0.13)^{c}	(0.08)^{b}	(0.03)^{a}	(0.20)^{a}	(0.26)^{a}
Actinobacteria	8.28	7.93	9.71	8.57	7.57	9.48	8.41	8.20	7.94
	(0.05)^{b}	(0.34)^{b}	(0.09)^{a}	(0.15)^{b}	(0.19)^{c}	(0.14)^{a}	(0.02)^{a}	(0.13)^{a}	(0.45)^{a}
Fungi	7.46	5.20	9.22	8.51	5.34	8.21	7.89	5.35	6.25
	(0.05)^{b}	(0.08)^{c}	(0.03)^{a}	(0.20)^{a}	(0.14)^{b}	(0.08)^{a}	(0.18)^{a}	(0.14)^{c}	(0.09)^{b}

Values are means (n=3) with standard error in brackets. Pile 1: coffee husk + cow dung; Pile 2: coffee husk + fruit/vegetable waste; Pile 3: coffee husk.For each pile different letters indicate significant differences between samples (Tukey HSD test; $\alpha = 0.05$)

Pile 1 and Pile 2 expressed a maximum and moderate activity of the enzymes β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase and N-acetyl β-glucosaminidase at day 32, respectively. Maximum activity of β-glucosidase can be associated with higher presence of readily metabolisable substrates, important in the hydrolysis of β-glucosides, such as cellobiose (Vargas-Garcia *et al.*, 2010). Accordingly, maximum activity of this enzyme was observed in Pile1 at the bio-oxidative stage.

In Pile 3, the relative abundance of many of these enzymes showed significantly varying levels of activities at day 32, but negligible at the beginning and end of the process probably because of the occurrence of non-aggressive environmental factors such as medium temperature and moisture and slight alkalinization (pH 8.16-8.21)(Hassen *et al*., 2001).

In general, key enzymes of agronomic value that gave valuable information related to the dynamics of important nutritional elements such as C, N and P; and relevant for the characterization of the composting processes showed similar trends regarding presence and compost age, especially for the two co-composted piles. Similar findings were also reported by Vargas-Garcia *et al*. (2010). The difference in the activities of various extracellular hydrolytic enzymes can be attributed to the substrates used for composting. Indeed, the degradation of larger polymers such as cellulose, hemicelluloses and lignin in compost is the result of the combined and successive actions of many microorganisms (Tiquia *et al*., 2002), and as such, the differing activity levels of the various enzymes during the composting process is not unexpected.

Table 3.3 Relative activity of extracellular enzymes extracted from different compost piles on days 0, 32 and 90

Enzymes	Pile1			Pile2			Pile3		
	0	32	90	0	32	90	0	32	90
Alkaline phosphatase	1	5	3	1	4	3	0	3	1
Acid phosphatase	1	5	3	2	4	3	0	4	0
Naphtholphosphohydrolase	1	5	3	1	4	3	0	5	0
Esterase	1	5	3	1	2	2	0	4	1
Esterase lipase	2	5	1	1	3	2	0	1	1
Lipase	2	1	1	1	2	3	0	1	1
Leucine-arylamidase	4	5	1	3	4	1	0	3	1
Valine-arylamidase	4	3	1	3	3	1	1	2	1
Cysteine-arylamidase	4	2	1	4	3	1	1	2	1
Trypsin	4	2	1	4	1	2	1	1	0
α-Chymotrypsin	4	2	1	4	1	2	1	1	1
α-galactosidase	1	1	0	0	0	1	0	1	0
β-galactosidase	1	5	0	0	3	1	0	3	0
β-glucouronidase	1	2	0	0	2	0	0	2	0
α-glucosidase	1	4	0	0	2	0	0	0	1
β-glucosidase	1	5	0	0	2	0	0	4	0
N-acetyl- β-glucosaminidase	3	4	0	0	2	0	0	4	1
α-mannosidase	1	1	0	0	0	0	0	0	0
α-fucosidase	0	0	0	0	0	0	0	0	0

Averages of triplicate values are expressed as intensity of enzymes (1-poor, 2-4-moderate, 5-excellent)

In the present investigation, correlation analysis was performed using microbial count and enzyme activity for the three sampling times (0, 32 and 90). Results indicated that total aerobic heterotrophs, actinobacteria and fungi in the coffee husk plus cow dung and coffee husk with fruit/vegetable waste composts were positively correlated with a number of enzymes (Table 3. 4). In Pile1, a positive correlation was seen for actinobacteria with three phosphatase enzymes that are involved in the release of phosphate from organic compound. Fungal and actinobacterial numbers in Pile1 were also significantly positively correlated with β-galactosidase (r =0.92 and r=0.93, respectively), β-glucuronidase(r =0.99 and r =0.88), α-glucosidase and β-glucosidase (r =0.92 and r =0.93). These enzymes are very important in the hydrolysis of lactose and cellobiose. In this study, fungal and actinobacterial abundance were positively correlated with esterase-lipase (r =0.93). However, total aerobic heterotrophs were significantly correlated with lipase (r =0.94), valine arylamidase (r =0.88), cysteine arylamidase (r =0.87), trypsine (r =0.87), chymotrypsine(r =0.86) and α galactosidase (r =0.94).

In Pile2, total aerobic heterotrophs showed significantly positive correlation with trypsin and chymotrypsine (r =0.82) whereas actinobacteria had significant correlations with a number of glycosyl-hydrolase enzymes such as α-galactosidase(r =0.82), β-galactosidase (r =0.79), β-glucuronidase (r =0.82), α-glucosidase and β-glucosidase (r =0.77). In Pile3, however, only fungi were highly significantly correlated with phosphohydrolase (r =0.88) and β-glucosidase (r =0.91).

Table 3.4 Pearson correlation (r^2) between enzymatic activities and microbial populations in the different compost piles

	Pile1			Pile 2			Pile 3		
	TAB	A	F	TAB	A	F	TAB	A	F
Acid phosphatase	-0.29	0.71*	0.43	-0.39	0.35	0.11	-0.03	0.11	0.74*
Alkaline phosphatase	-0,28	0.70*	0.43	-0.49	0.21	-0.48	-0.54	-0.48	0.74*
Phosphohydrolase	-0.29	0.71*	0.43	-0.59	0.15	-0.14	-0.45	-0.37	0.88*
Esrerase	-0.37	0.61	0.32	-0.82**	-0.25	-0.52	-0.23	-0.04	0.79*
Esterase-Lipase	0.44	0.93**	0.93**	-0.24	0.51	0.31	-0.43	-0.66	0.79*
Lipase	0.94**	0.61	0.90**	-0.51	0.36	0.00	0.52	-0.44	0.38
Leucine-Arylamidase	0.77*	0.80*	0.98**	-0.25	-0.82**	-0.60	-0.36	-0.52	0.66
Valine-Arylamidase	0.88**	0.23	0.60	-0.09	-0.78**	-0.42	0.38	0.51	0.39
Cysteine-Arylamydase	0.87**	0.04	0.43	0.13	-0.71	-0.42	0.34	0.60	0.39
Trypsin	0.87**	0.04	0.43	0.82**	0.14	0.46	0.31	0.49	-0.24
Chymotrypsin	0.86**	0.04	0.43	0.82**	0.14	0.46	0.22	0.52	0.14
α-galactosidase	0.94**	0.61	0.90**	0.06	0.82**	0.59	0.46	0.39	0.39
β-galactosidase	0.40	0.93**	0.92**	0.06	0.79*	0.59	-0.59	-0.70*	0.80*
β-glucuronidase	0.67*	0.88**	0.99**	0.06	0.82**	0.58	-0.30	-0.63	0.46
α-glucosidase	0.44	0.93**	0.92**	0.06	0.77*	0.58	-0.31	-0.34	0.91**
N-acetyl β-glucosamindase	0.77*	0.80*	0.98**	0.04	0.83**	0.59	-0.40	-0.22	0.79*
α-Mannosidase	-0.22	-0.92	0.83**	0.00	0.00	0.00	0.00	0.00	0.00
α-Fucosidase	-0.72	-0.31	0.07	0.00	0.00	0.00	0,00	0.00	0.00

*Correlation is significant at 0.05 level. ** Correlation is significant at 0.01 level. Pile 1: Coffee husk + cow dung; Pile 2: Coffee husk + fruit/vegetable waste; Pile 3: coffee husk alone.

Figure 3.2 shows the loading plot of PCA for the three composts collected on days 0, 32 and 90. Overall, PCA grouped all samples collected on day 0 differently from samples collected on days 32 and 90. Also, the younger composts (Piles 1 and 2) grouped distant from those of Pile 3 (higher PC2 scores). Therefore, we can conclude that a certain degree of decomposition had occurred in the dung and fruit/vegetable wastes before they were used in co-composting, as a considerable number of the enzymes investigated were active on day 0. The day 90 composts from Piles 1 and 2 clustered close together indicating that the enzyme profiles in the mature composts were similar. Furthermore, the composts collected in the active phase were grouped loosely along the PC1. This is consistent with the correlation analysis (Table 3. 4) that indicated that the important enzymes in composting included phosphatases, esterase-lipases, proteases (trypsin and chymotrypsin), β-glucosidases and β-galactosidases.

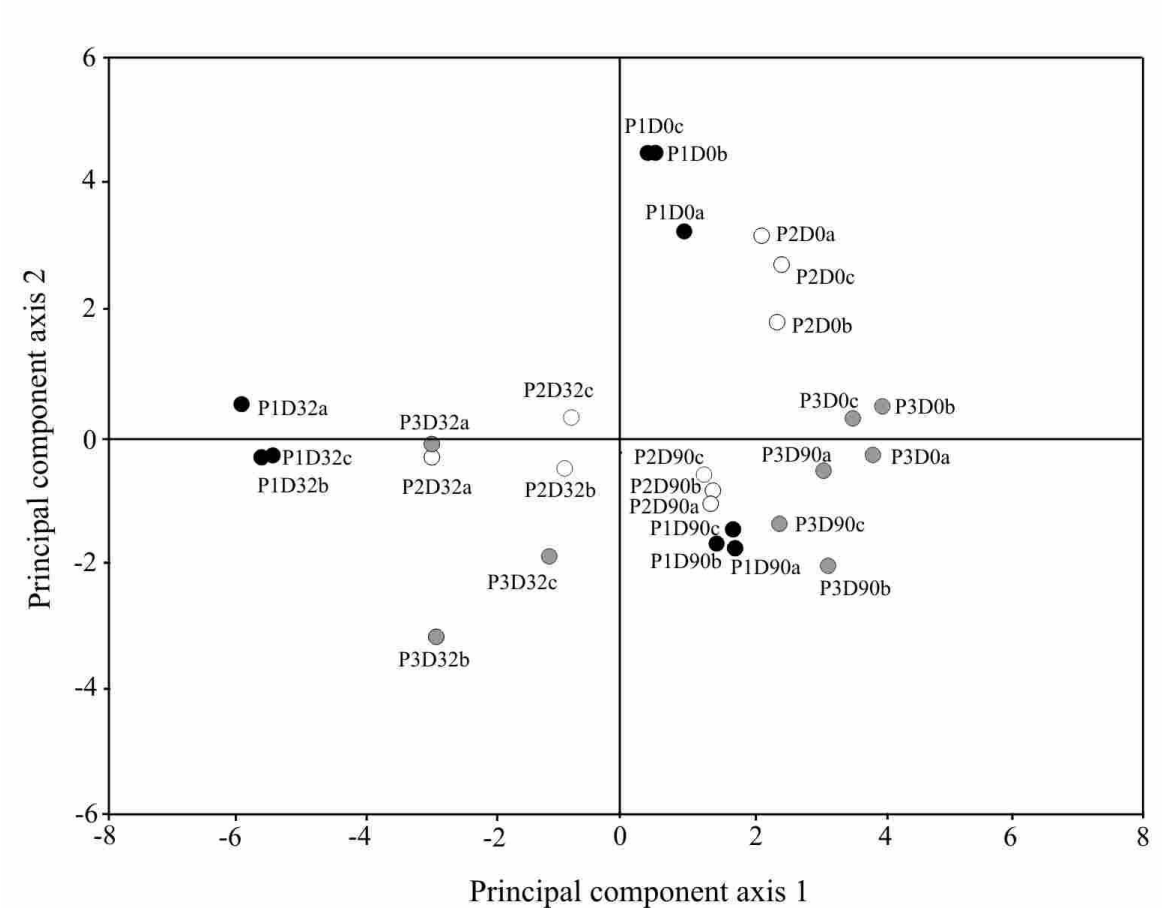


Figure 3.2 Principal Component Analysis (PCA) of compost enzymes on day0 (D0), 32(D32) and 90(D90) from three compost piles. P1:Pile1-coffee husk+cow dung; P2:coffeehusk+fruit/vegetable waste; Pile3:coffee husk.

PCR-DGGE

The bacterial and fungal DGGE profiles were generated from a total of 27 samples including three replicate compost samples taken on d 0, 32 and 90 from Piles 1, 2 and 3(Figure 3.3 and Figure 3. 4). Overall, DGGE profiles of triplicate samples had a degree of similarity higher than 95%.

DGGE patterns showed that the bacterial community profiles clustered into two main groups with 46% similarity (Figure 3.3). Samples from day 0 grouped together and in

distance from the 32 and 90 day samples, which were very similar (clustered with 84% similarity). This indicates a change in the bacterial community composition concurrent with the dynamics of the composting process. The beginning of the process was probably characterised by a bacterial community involved in the degradation of labile organic substrates, while the bacterial DGGE profiles of DNA after 90 day of composting presumably reflects a community involved in the stabilization of organic matter. However, towards the end of the process, the microbial community structure tended to stabilise, as suggested by the high similarity of DGGE profiles. Similar results were found by Takaku *et al*. (2006) and Novinscak *et al*. (2009).

In contrast, the three composts collected on day 0 were found to group separately (Figure 3.3), as the two different co-substrates used introduced different and diverse microbial communities into the composting process. Interestingly, Pile 2 (coffee husks and vegetable and fruit wastes) and Pile 3 (coffee husks only) were found to group together within this cluster (68% similarity). Also, compost samples from Piles 2 and 3 clustered together and distinctly from Pile 1 after 32 d of composting. Nevertheless, all 90day compost samples were found to group together (Figure 3.3). This suggests that the resulting composts were homogenous materials with a well-defined bacterial community.

UPGMA analysis also clustered fungal profiles into two groups, with 63 % similarity (Figure 3.4): one group comprised samples from day 0, while the other comprised composts from days 32 and 90. Within this cluster, these two composts were found to group separately with a degree of similarity of 72% (Figure 3.4). Composting time had a significant effect on fungal communities, as was shown by fungal counts (Table 3.2). Cumulatively, these findings suggest there was a succession of fungal communities

during the composting process related to the different phases involved in the process. Moreover, for bacteria, fungal DGGE profiles of the three composts collected on days 0 and 32 clustered separately (Figure 3.4). These groupings were not observed in 90 d samples (Figure 3.4), indicating that a lower diversity was achieved at the end of the composting period.

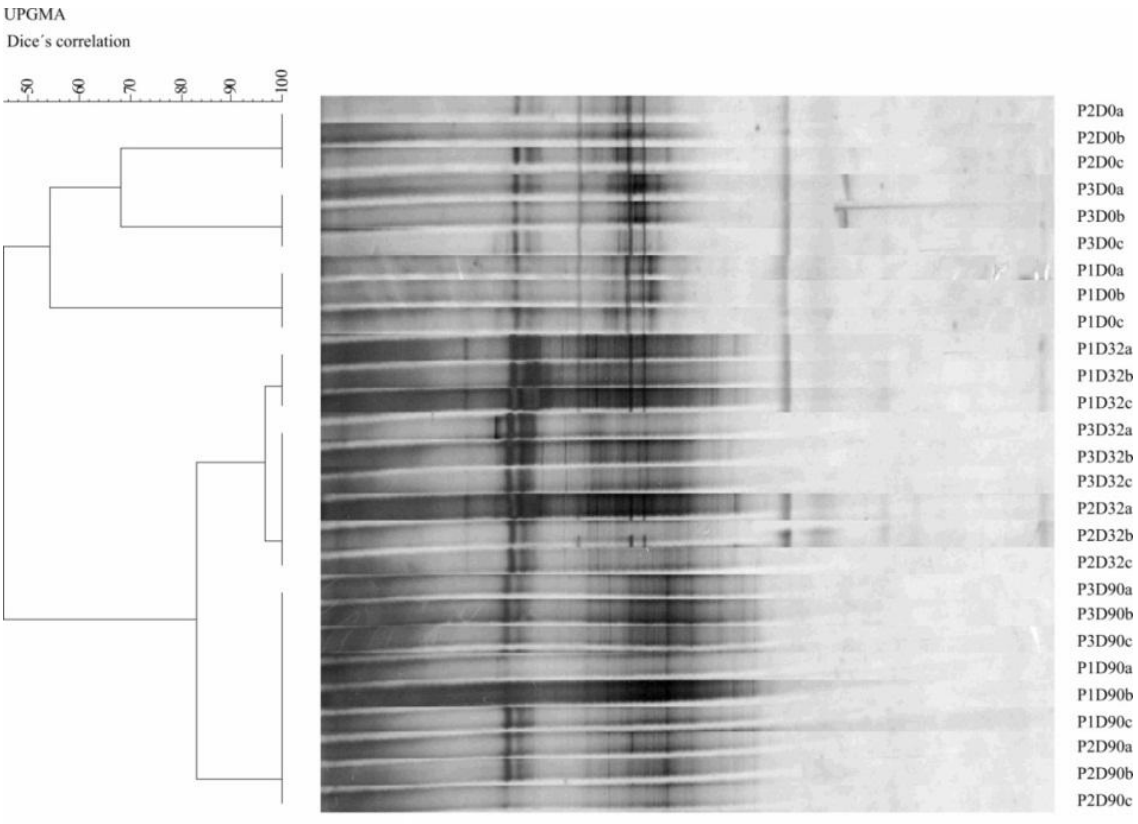


Figure 3.3 Cluster analysis of bacterial DGGE fingerprints based on 16S rDNA of compost samples collected on days0(D0), 32(D32) and 90(D90). Values indicate the percentage of similarity based on the Dice correlation coefficient. P1:Pile1-coffee husk+cow dung; P2:Pile2-coffee husk+fruit/vegetable waste; P3:Pile3-coffee husk

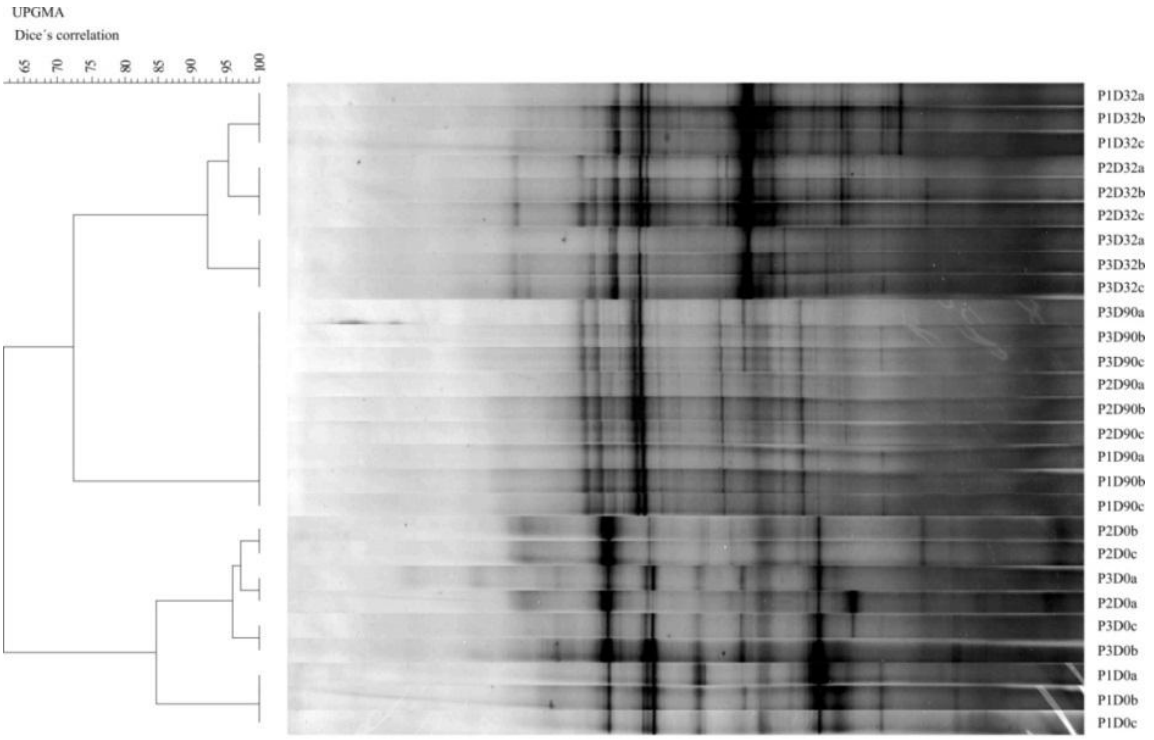


Figure 3.4 Cluster analysis of fungal DGGE fingerprints based on 18SrDNA of compost samples collected on days 0(D0), 32(D32) and 90(D90). Values indicate the percentage similarity based on the Dice correlation coefficient. P1:Pile1-coffee husk+cow dung; P2:Pile2-coffee husk+fruit/vegetable waste;P3:Pile3-coffee husk.

Microarray

The SNRs obtained after hybridization of samples on the COMPOCHIP microarray were determined and are shown in Table 3.5. The changes in bacterial communities in the three different piles, and over the course of composting are clearly shown in this table. Figure 3.5 shows a principal component analysis (PCA) loading plot, whereby the two first axes explain 65.2% of the variance, the first axis representing 44.3% of the variance, the second axis representing 20.9%. The lengths of the arrows indicate the significance for compost sample differentiation, and arrows point in the direction of samples with above average signal. Probes with similar arrow directions have high covariance, meaning they

tend to occur jointly on the microarrays. Certain probes (indicated by the arrows) can be seen to be more influential in discriminating the samples, namely, the probes KO 443 and 444 (*Stenotrophomonas maltophilia*), KO609, KO 610 and KO 614 (*Brevundimonas/Caulobacter*), KO 500 (*Derxia gummosa*) KO 612, 615,616 and 617 (*Flavobacterium/Flexibacter*), KO 541 (*Pseudomonas putida*), KO 252 (*Acinetobacter*) and KO 342 (*Actinomyces* sp.). Canonical analysis clearly shows the different grouping of the compost samples according to the age of the compost. The composts collected on day 0 were found to cluster separately on the positive side of the first principal component axis, while the 90 d composts all clustered quite closely together on the negative side of the first principal component axis. These groupings occurred because of the greater diversity of microorganisms in the composts at the start of the experiment, while at the end of the composting period, the diversity and bacterial numbers had decreased, and the various composts were more similar. The result supports the findings of the DGGE study.

Table 3.5 Microarray results showing SNR values of the different composts.

		P1D0			P2D0			P3D0			P1D90			P2D90			P3D0		
		a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Acinetobacter	KO 252	10.28	6.45	23.63	2.01	1.28	2.31	2.54	2.63	1.40	1.86	2.60	2.32	2.36	3.94	3.74	2.87	2.02	2.86
Acinetobacter calcoaceticus	KO 254	2.54	1.38	1.71	2.68	1.38	3.14	1.85	2.50	1.62	2.07	2.17	1.98	2.33	2.48	1.37	1.75	2.16	1.70
Acinetobacter lwofii	KO 233	5.01	1.06	2.92	1.44	0.95	1.52	1.07	1.08	1.03	1.09	1.04	0.99	1.04	1.09	1.07	1.08	1.07	1.02
Actinomyces sp.	KO 342	3.62	1.18	1.55	4.61	2.08	8.74	5.19	5.01	2.03	27.50	5.57	21.91	10.30	14.23	10.95	26.79	21.54	14.31
Agrobacterium tumefaciens	KO 23	2.28	1.56	2.27	2.01	1.13	1.49	1.71	1.71	1.67	1.43	2.09	1.56	1.89	2.09	2.63	2.98	1.75	3.16
Alcaligenes faecalis	KO 350	8.26	1.68	2.38	2.81	1.17	1.98	1.81	2.78	1.59	1.13	1.27	1.29	1.46	1.19	1.25	1.50	1.19	1.10
Alpha proteobacteria	KO 240	27.22	13.46	9.37	25.36	5.97	19.27	28.25	26.16	10.08	55.44	67.77	43.96	27.25	75.22	78.60	79.85	22.73	33.07
Azotobacter beijerinckii	KO 277	6.21	4.94	5.18	4.70	2.88	5.02	6.46	7.27	8.24	4.37	8.86	4.61	6.82	9.01	6.34	3.73	2.46	3.51
Brevundimonas	KO 614	1.57	1.02	1.19	6.80	19.12	31.84	23.07	2.02	7.84	1.18	1.13	1.58	1.22	1.21	1.88	2.57	1.49	2.54
Brevundimonas	KO 612	7.06	0.98	1.00	14.87	16.17	15.76	1.46	3.00	1.08	0.99	1.03	1.02	1.02	1.14	1.47	1.13	1.16	1.16
Brevundimonas/Caulobacter	KO 610	12.84	1.00	1.48	26.79	15.77	16.22	5.59	12.93	6.41	1.55	1.40	1.41	1.06	2.04	2.44	2.47	1.95	1.46
Brevundimonas/Caulobacter	KO 609	7.34	0.97	1.22	15.78	13.98	14.92	4.50	5.54	6.37	1.31	1.17	1.26	1.01	1.41	1.60	1.29	1.51	1.27
Brevundimonas/Caulobacter	KO 611	1.65	1.02	1.06	7.10	2.88	4.21	1.49	1.82	1.38	1.08	0.99	1.04	1.01	1.03	1.08	1.05	1.09	1.08
Chryseobacterium	KO 495	12.50	2.84	3.80	9.31	4.99	4.30	30.83	34.93	24.69	2.14	1.57	2.32	1.46	2.65	21.53	7.90	6.99	19.41
Chryseobacterium	KO 494	1.94	1.20	1.51	4.69	2.26	3.72	5.30	14.01	10.84	1.49	1.31	1.51	1.47	1.35	14.13	2.44	1.46	3.06
Chryseobacterium	KO 496	1.24	1.19	1.28	2.55	2.08	1.73	2.77	3.51	2.25	1.05	1.01	1.16	1.11	1.29	3.52	1.22	1.17	1.96
Clostridium A	KO 255	1.20	1.29	5.17	1.47	1.16	1.32	1.59	2.14	1.67	1.06	1.07	1.13	1.26	1.35	0.97	1.03	1.05	1.18
Comomonas-Acidovorax	KO 274	1.16	1.08	8.41	1.95	1.23	1.51	1.16	1.31	1.13	1.16	1.26	1.30	1.12	1.44	1.13	1.08	1.08	1.05
DELTA495a	KO 639	14.98	2.24	4.00	7.58	2.26	4.85	3.82	5.69	6.53	12.54	7.96	8.36	11.03	16.80	12.17	13.26	16.33	7.92
Derxia gummosa	KO 500	25.80	3.51	14.70	7.30	5.20	6.28	2.46	5.39	9.72	0.97	1.01	1.05	1.23	1.07	1.13	1.04	1.08	1.12
Derxia gummosa	KO 502	1.59	1.27	1.43	7.20	2.22	3.24	11.55	6.31	4.30	1.04	0.98	1.12	1.31	1.21	6.92	1.72	1.46	2.38
EnterobacteriaceaeD	KO 246	5.64	1.91	5.15	1.23	1.06	1.38	13.60	5.13	7.26	1.77	1.70	1.32	1.22	5.46	1.70	1.58	1.11	1.54
Flavobacteria	KO 615	2.44	1.06	4.00	27.08	5.55	16.59	5.35	9.68	14.32	4.53	5.09	4.30	1.86	4.28	29.58	14.04	11.33	9.97
Flavobacterium/Flexibacter	KO 617	1.12	1.01	1.40	25.40	11.61	28.75	1.34	2.13	1.71	3.99	2.56	2.14	1.58	3.52	5.48	2.65	4.16	3.65
Flavobacterium/Flexibacter	KO 616	0.99	1.00	1.01	8.74	6.98	8.60	1.20	1.23	1.13	1.25	1.35	1.10	1.35	1.23	1.48	3.78	2.71	3.10
Flavobacterium/Flexibacter	KO 618	1.01	1.03	1.00	3.60	1.35	1.22	1.01	0.98	1.01	1.02	1.02	1.04	1.04	1.10	1.15	1.59	1.52	1.37
Lactobacillus panis	KO 505	6.66	5.15	4.72	2.93	1.53	2.25	2.65	3.38	2.64	1.28	1.10	1.23	1.52	1.17	1.29	1.33	1.26	1.14
Low G+C	KO 319	5.63	1.91	11.61	4.41	2.36	10.06	1.86	2.35	1.83	22.66	17.03	14.94	9.84	14.41	29.88	51.30	34.77	49.28
Methylobacterium	KO 513	6.93	5.39	4.67	2.45	1.66	3.33	2.27	2.51	1.84	1.54	1.34	1.28	1.54	1.47	1.36	1.51	1.41	1.18
Methylobacterium	KO 514	5.08	1.08	1.05	1.06	1.06	1.15	1.74	1.13	1.10	1.00	0.96	1.14	1.02	0.96	1.00	1.02	1.03	1.00
Methylobacterium	KO 512	3.47	1.35	1.04	1.03	1.03	1.22	1.60	1.22	1.23	1.04	1.02	0.97	1.03	1.04	1.01	1.04	1.07	1.02
Microbacterium	KO 515	2.31	1.29	1.15	1.59	1.25	1.55	1.41	1.43	1.23	2.05	2.57	1.52	2.27	2.31	3.12	3.26	3.66	3.72
Microbacterium	KO 516	1.72	1.27	1.04	1.32	1.15	1.38	1.32	1.28	1.17	1.86	2.23	1.54	2.13	1.71	2.83	2.44	3.11	2.90
Nitrobacter	KO 293	2.63	2.95	7.25	3.29	2.07	3.22	4.73	5.22	2.19	4.07	9.06	4.28	3.92	5.82	3.91	5.89	3.87	4.86
Nitrosomonas communis	KO 301	1.54	2.13	2.99	2.07	1.75	2.27	2.20	3.22	1.64	1.58	2.14	1.91	2.03	3.35	1.20	1.67	1.86	1.43
Nitrosomonas oligotropha	KO 419	7.65	2.12	3.63	1.57	1.17	1.49	1.11	1.71	2.06	0.94	1.06	1.00	1.10	1.07	1.13	1.07	0.99	1.01
Nitrospira/Nitrosovibrio/Nitrosomonas	KO 293	9.25	3.46	6.00	1.99	2.50	1.35	1.51	3.29	1.52	1.34	1.30	1.41	1.19	1.46	1.25	1.28	1.10	1.32
Pantoea	KO 528	9.35	1.13	4.12	1.06	1.27	1.48	9.96	3.09	13.87	1.14	0.99	1.13	1.30	3.83	1.22	1.22	1.17	1.00
Pantoea	KO 529	1.00	1.07	1.01	1.03	1.03	1.01	2.53	2.85	5.02	0.97	0.94	0.97	0.99	1.46	1.02	1.03	0.99	0.99
Propionibacterium	KO 433	4.35	1.19	2.35	1.44	1.23	1.32	1.12	1.15	1.66	0.97	0.95	1.00	1.06	0.98	1.06	1.06	1.00	1.01
Pseudomonas	KO 536	24.31	13.05	20.07	11.79	8.38	9.23	13.80	16.90	17.85	5.21	2.71	3.61	2.12	3.06	7.43	5.39	6.79	2.69
Pseudomonas	KO 537	8.40	11.32	3.29	0.92	1.33	3.66	1.04	1.09	1.07	1.72	1.46	1.56	1.17	1.53	2.08	1.37	1.51	1.21
Pseudomonas aeruginosa	KO 244	4.78	1.59	2.57	1.60	1.22	1.83	1.19	1.62	1.64	1.55	1.73	1.62	1.57	1.84	1.54	1.43	1.23	1.23
Pseudomonas aeruginosa	KO 12	1.51	1.38	1.58	1.51	1.32	1.77	1.51	2.64	2.42	1.58	2.04	1.61	1.43	2.01	3.18	1.69	1.46	1.96
Pseudomonas aeruginosa	KO 435	2.92	1.93	4.18	1.07	1.13	1.19	2.75	1.63	2.42	1.04	1.04	1.06	1.12	1.03	0.99	1.06	0.98	1.09
Pseudomonas putida	KO 541	31.19	4.63	16.56	4.61	1.89	3.27	9.71	9.77	7.08	1.79	1.56	1.50	1.09	2.39	1.58	1.31	1.75	1.11
Rhodococcus	KO 544	6.25	1.02	1.05	1.03	1.04	1.02	1.00	1.04	1.03	1.01	1.03	0.99	1.06	1.07	1.02	1.05	0.99	1.03
Salmonella	KO 245	16.68	1.34	3.03	2.03	1.14	1.98	4.49	5.05	7.03	2.20	2.74	1.73	1.68	4.58	2.49	2.11	1.65	1.83
Sphingobacterium	KO 548	2.93	0.97	1.08	1.80	1.40	2.51	4.73	22.17	3.39	1.03	1.02	1.00	1.13	1.07	1.06	1.18	1.44	1.24
Sphingobacterium	KO 549	7.23	1.06	1.41	6.03	4.14	4.65	8.00	21.33	4.48	2.22	1.76	1.67	1.16	1.63	2.20	2.90	2.63	1.80
Sphingobacterium	KO 550	1.54	1.10	1.54	2.88	2.37	2.73	3.66	5.37	2.22	1.24	1.41	1.47	1.63	1.31	1.38	1.44	1.31	1.53
Stenotrophomonas maltophilia	KO 443	95.55	3.59	42.55	21.29	8.52	16.10	7.36	7.16	33.36	1.17	1.08	1.09	1.21	1.30	1.97	1.57	1.52	1.38
Stenotrophomonas maltophilia	KO 444	52.33	1.99	15.82	1.74	1.53	2.82	1.32	1.81	3.44	0.97	1.01	1.09	1.14	1.06	1.01	1.03	1.01	1.05
Stenotrophomonas maltophilia	KO 243	32.79	9.09	26.62	9.28	2.54	5.84	8.19	6.37	6.57	4.06	6.23	4.05	3.30	6.35	4.96	8.50	5.51	6.39
Streptomyces	KO 630	1.43	1.02	1.38	1.34	1.32	1.08	1.43	1.43	1.40	3.60	3.32	1.95	1.82	3.59	4.26	6.26	6.41	2.24
Streptomyces	KO 629	1.66	1.02	1.02	1.14	1.00	1.04	1.01	1.17	1.02	2.71	2.26	1.50	1.17	2.01	2.44	2.72	3.99	1.81
Thermoactinomyces	KO 555	3.78	2.22	3.66	2.41	1.48	1.47	2.31	3.10	2.19	1.32	1.40	1.29	1.26	1.54	1.28	1.31	1.13	1.09
Thermus thermophilus	KO 1	1.80	1.60	2.15	2.44	2.01	1.90	2.05	2.30	2.16	1.82	2.26	3.00	2.60	2.14	2.14	2.36	1.85	2.41
Vibrio cholerae	KO 453	1.05	0.99	4.77	1.05	0.97	0.98	1.05	1.00	1.15	0.98	1.03	1.07	1.12	1.06	1.07	1.10	1.03	1.05
Xanthomonas	KO 561	8.11	1.32	2.80	4.44	1.56	4.58	2.55	3.53	2.50	1.06	1.07	1.12	1.06	1.10	1.07	1.08	1.03	1.05
Xanthomonas	KO 455	7.60	2.42	5.39	2.62	1.84	2.73	1.78	2.06	3.77	1.04	1.06	1.04	1.13	0.97	1.06	1.04	0.99	0.98
Xylella-Xantho-Stenotrophomonas	KO 241	22.23	9.86	30.02	9.69	3.39	9.49	28.57	13.59	10.34	17.24	16.95	11.96	8.03	17.09	17.16	17.11	4.27	8.60
Xylella-Xantho-Stenotrophomonas	KO 242	3.36	3.57	13.86	3.95	2.24	2.92	3.90	5.07	3.27	4.61	10.19	4.59	3.62	4.98	2.67	9.04	2.85	6.07
Yersinia	KO 458	4.75	1.52	2.45	1.08	1.32	2.13	1.22	2.71	3.05	0.99	0.95	1.18	1.00	1.02	1.04	0.99	1.00	0.99
Zymomonas mobilis	KO 642	1.26	3.61	1.10	1.04	0.96	1.17	1.00	1.08	1.08	0.95	0.97	1.12	1.02	1.00	0.95	1.04	0.98	1.00

The green represents hybridisaton signals with SNR values above the threshold of 2

The separate grouping of the 3 different composts collected on day 0 is to be expected, with the different co-substrates used to make the piles. Both cow dung and vegetable and fruit wastes offer a different and diverse input of microbial communities into the composting process, which allowed the three compost piles to group distinctly after a canonical analysis of the microbial communities detected by the microarray. Of interest was the finding of *Brevundimonas* and *Caulobacter* in the starting composts, especially in Piles 1 and 2. Members of the genus *Brevundimonas* were also found to be dominant at the start of a compost process treating sewage sludge and yard waste (Danon *et al*., 2008). In their study, *Caulobacter* was found in composts after a curing time of 41 d. Pedro *et al*. (2001) also found *Brevundimonas* present in the mesophilic phase of industrial and agricultural waste composting. These bacteria may play an important role in the composting process.

Chryseobacterium was also found in higher numbers in the starting composts, in particular, in Piles 2 and 3. Lower levels of *Chryseobacterium* were found at the end of the composting process in these same composts. In a study by Franke-Whittle *et al*. (2009), *Chryseobacterium* levels were found to be higher in fresher composts than in more mature composts. *Chryseobacterium*s species are known for their importance in the degradation of complex biopolymers in composting situations (Al Khadi *et al*., 2004).

Sphingobacterium was detected in the compost Piles 2 and 3 at the start of the experiment, but not at the end of the experiment. *Sphingobacterium* is an aerobic bacterium, known to have broad degradation activity, and frequently occur in compost. It has been reported in non-cured composts by Cayuela *et al*. (2009) and Franke-Whittle *et al*. (2009).

SNR values above the detection limit were also found in most compost piles at the start and end of the experiment for the *Flavobacteria/Flexibacter* probes KO 615,616, 617 and 618. *Flavobacteria* are common in environmental samples and include opportunistic pathogens. Its presence in composts has been reported by others (Danon *et al*., 2008).

The genus *Stenotrophomonas* includes species which are plant and human pathogens, and the presence of these bacteria could be of concern in a mature compost product. The *S. maltophilia* probes KO 243, 443 and 444 yielded positive signals in the day 0 compost piles, while only the probe KO 243 yielded a positive signal in the 90 d composts. This indicates that the KO 243 probe works more efficiently than the other *S. maltophilia* probes, and that this organism has survived the composting process.

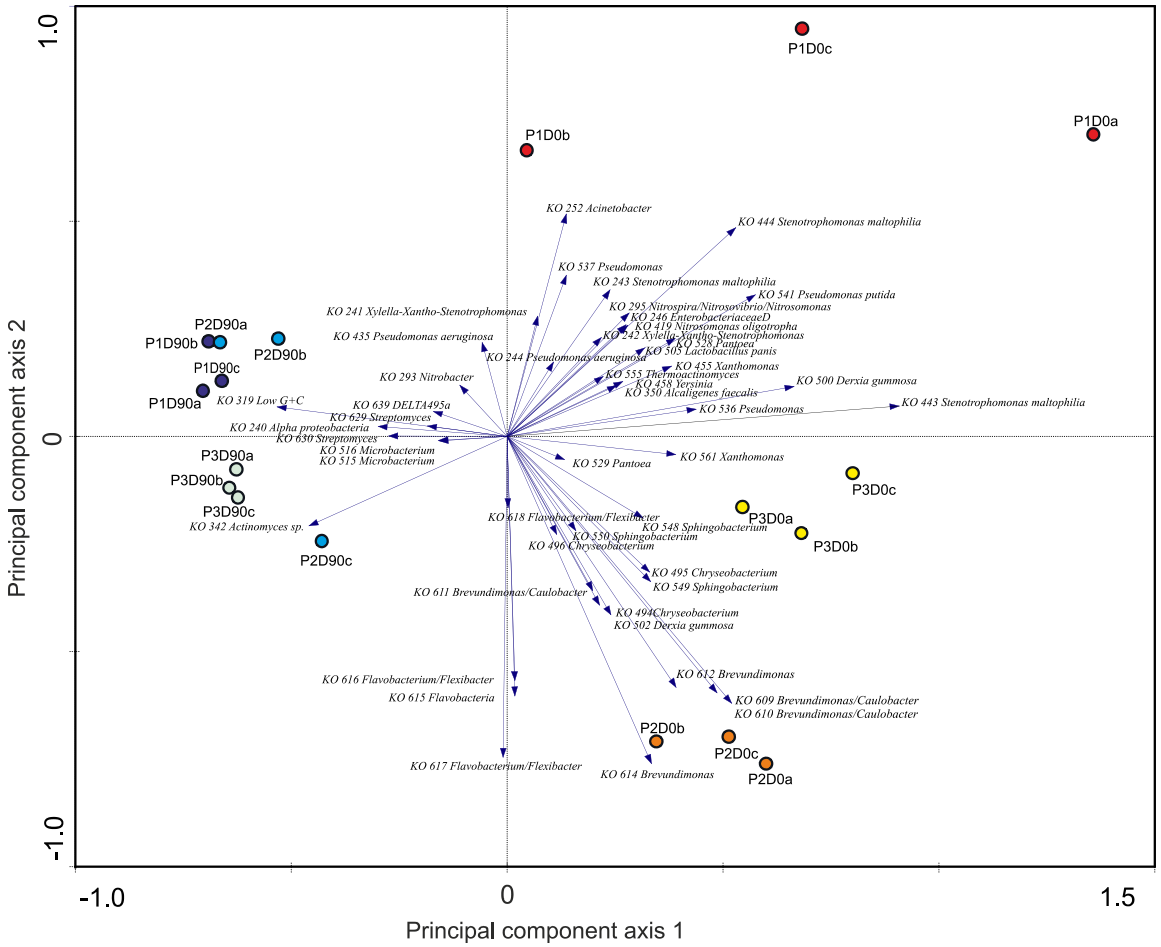


Figure 3.5 Principal Component Analysis (PCA) of compost communities from day 0(D0) and day(D90) composts analyzed by COMPOCHIP microarray. The two axes represent 65.2% of the explained variance. The vectors show the covariance structure of the probe signals. P1:Pile1-coffee husk+cow dung;P2:Pile2-coffee husk+fruit/vegetable waste; P3:Pile3-coffee husk.

In the mature composts, microbial diversity and numbers were lower. The group probes DELTA 495a, Alpha proteobacteria and Low G+C yielded hybridisation signals in all three compost piles. Hybridisation was also seen in all day 90 composts with the KO241 *Xylella/Xanthomonas/Stenotrophomonas*, *Azotobacter beijerinckii*KO277and the KO342 *Actinomyces* probes. The proportion of *Actinomycetes* relative to other bacteria has been suggested as an indicator of compost maturity (Palmisano and Barlaz, 1996). Thus, it is

not surprising that microarray analysis revealed high levels of *Actinomycetes* in the composts from longer curing times.

Low SNR values were also obtained for the *Streptomyces* probes KO 629 and KO 630 as well as the *Microbacterium* probes KO 515 and KO 516 for all 90 d composts. Interestingly, these two organisms were not detected at all in the immature composts. This finding supports the results of Danon *et al*. (2008), whereby *Microbacterium* was also found in composts collected 41 and 131 days after the start of the experiment. Silva *et al*. (2000) also reported the finding of *Microbacterium* on coffee cherries.

Overall, addition of cow dung (CD) and fruit/vegetable wastes (FVW) to coffee husk (CD) as co-substrates contributed in better degradation of the lignocellulosic compounds of CH, resulting in higher carbon losses. The effect of the different microbial activities was reflected in the better maturation of Pile 1 with a germination index (GI) value >80% and C/N ratio of 11 at the end of composting compared to the control. The chemical changes directed with the succession of different microbial groups were accompanied by the release of *hydrolases*, *phosphatases*, *peptidases* and *proteases* in Pile 1 and Pile 2 at the start; and *esterases* at the end of the process. As a result, diverse compost microorganisms could be found to contribute in the complete degradation of coffee husk compost so that the use of DGGE and COMPOCHIP microarray are useful tools to community analysis.

Chapter 4 Maturity and stability determination of flower residue compost in Ethiopia: its nutritional and environmental implication

Fekadu Shemekite*[1] and Fassil Assefa[1]

[1]Addis Ababa University, Department of Microbial, Cellular and Molecular Biology (MCMB)

[*1] Addis Ababa University, Department of Microbial, Cellular and Molecular Biology (MCMB).

e-mail:fshemekite@gmail.com

Abstract

The ever-increasing flower industries in Ethiopia generates large amount of solid organic wastes that poses environmental problems. This necessitates the treatment of those wastes to reduce pollution and produce compost for soil amelioration. To this end, Windrow composting was undertaken using flower residues as major feed stock with cow dung (Pile1: C/N ratio=30), with activated effective microorganisms (EM) and molasses (Pile 2); and flower residue alone (control: Pile 3). The process took 90 days where samples were collected on days 1, 7, 21, 35, 49, 63, 77 and 90 to analyze the chemical and microbiological properties of the composting process. The result showed that significant changes in temperature, moisture and pH starting from the first day to final stage of the process (p=0.001). The effects of the physical states were reflected in significant changes in chemical properties of all compost piles. In effect, NH_4-N was significantly correlated with temperature, water content, pH and organic carbon while NO_3-N showed negative correlation with all parameters except pH. At the end, above 80% GI value, lower NH_4-N (140mg/kg) and NH_4/NO_3 ratio (0.12) together with reduced C/N ratio (14%) in cow dung amended compost gave a clear indication of maturity of this compost. The EM treated pile and the control, however, needed longer time to reach maturity. Besides, the highest bacterial count at the start (Pile 1: 8.99 log ^{MPN} g^{-1} dw) and actinobacteria (Pile 2: 6.67 log ^{MPN} g^{-1} dw) and fungi (Pile 3: 6.64 log ^{MPN} g^{-1} dw) at the end showed microbial

succession. In all treatments, macro (P and K) and micro (Mn, Zn, Fe, Cu) nutrients were found in sufficient amount for horticulture amendment.

Key words/phrases: *NH_4/NO_3 ratio, Effective microorganisms, Flower residues, Germination index (GI), Microbial activity*

Introduction

The flower industry in Ethiopia is currently expanding at a fastest rate for it is one of the priority sectors of the five years plan of the country; and provides cut flowers to local and international markets (Mulugeta, 2009). According to the Ethiopian Horticulture Producer and Export Association (EHPEA) annual report, more than 2000ha of land has been leased to 263 foreign and domestic investors and the sector employed over 70,000 work force; and exported more than 1.7 billion flower stem and earned 160 million USD in ten months in 2012 (EHPEA, 2014).

These flower farms, however, have great concern for they generate huge amount of solid and liquid wastes. Every day about 10 tons of solid wastes are generated as a result of pruning, clearing of unutilized and/or under graded or diseased stems and leaves (Viera and Abarca, 2009). Although few farms tried to manage the solid and liquid wastes through composting and pond systems, most of them dispose the solid waste as heaps in the vicinity and release the wastewater freely to water bodies.

The accumulations of these wastes are believed to be sources of phyto-pathogens, toxic chemicals, and insects (Vieira and Abarca, 2009). On the other hand, the farms have to comply with the country's Environmental pollution control law pursuant to article 20 (Ethiopian Floriculture Code of Practice No.300/2002) in order to protect the society and the environment from any hazard.

This necessitates the search for an environmentally sound waste management system for the solid and liquid wastes for reuse and/or safely release to the environment. One of such methods is the treatment of the solid flower wastes through composting with a dual

purpose of production of organic fertilizer and protection of the environment from these pollutants.

Composting is a microbial degradation of different organic materials under moist, self-heating and aerobic conditions. To this effect, large number of different mesophilic, thermotolerant and thermophilic aerobic microorganisms are involved in the break down organic matter thereby producing relatively stable organic products (Zucconi and de Bertoldi, 1987). These days, attempts are also made to select inoculant microbes into composts through bio-augmentation in order to speed up the process of composting and producing of quality compost. One of these is the application of effective microorganisms (EM) in the compost mixtures. Effective microorganism is a commercial brand made up of mixture of microbial inoculants containing 80 different species (photosynthetic, nitrogen fixing, lactic acid bacteria and yeast) which is presumed to suppress pathogen, enhance decomposition rate of organic matter, increase availability of mineralized nutrients to plants (Ong *et al*, 2001).

However, the production of matured quality compost for agricultural and landscaping purposes within a short period of time depends on several interacting factors. This includes type of feedstock and composting conditions (e.g. facility scale, aeration, temperature, pH, moisture content) (Ishii and Takkii, 2003).

In general, a multitude of physical, chemical and microbiological parameters are used as stability indices to monitor a variety of composting feedstock both as a product quality indicator and a process evaluation index (Cunha-Queda *et al*., 2007; Gómez-Brandón *et al*., 2008; Liu *et al*., 2011). There is a consensus that germination index and N-

mineralization together with other physico-chemical and microbiological parameters can be used as core and surrogate indicators for evaluating compost maturity and quality, respectively (Zucconi *et al.*, 1981; Gómez-Bandón *et al.*, 2008). Generally, composting and compost quality is given so much emphasis that different countries have adopted different national standards for the production of quality compost (Said-Pullicino *et al*., 2007).

The present investigation was, therefore, aimed at evaluating the composting process of flower residues amended with cow dung and EM in a Windrow composting system on the basis of different physico-chemical and microbiological parameters.

Materials and Methods

Arrangement of compost piles and sampling techniques

Composting was conducted at Galica Flower Farm (GFF), located near Holetta town of the Oromia National Regional State (ONRS). The feedstock of shredded flower residues (2-50mm) was composted in three treatments in trapezoidal cross section Windrow composting method (Windrows (1mx1mx2m) for about 90 days (Diaz *et al*., 2002).The first treatment (T1) was a mixture of shredded Flower Residue (FR) with Cow Dung in 3:1 proportion (FRCD, Pile-1), whereas the second treatment (T2) was a mixture of the same Flower Residue with 2 liters activated EM (contained mainly lactic acid bacteria, photosynthetic bacteria and yeasts) and one liter molasses (FREM, Without cow dung) (Pile-2). The third treatment (control) that contained only shredded Flower Residues (FR, Pile3) was included in the experiment.

Moisture content of the different piles was adjusted at approximately 40-60% by sprinkling water regularly (Diaz and Savage, 2007). The composting piles were subjected to manual turning every day during the first week and once in a week thereafter until the end of the experiment. Samples were collected on day 1, 7, 21, 35, 49, 63, 77 and 90 for chemical and microbiological analyses. From each pile around 1kg of composite samples were separately collected from 3 grab subsamples taken along the length of the windrow, screened through 10mm sieves.

Physical and chemical analysis

The temperature was measured as described previously in triplicate before date of turning using a digital thermometer (model H-9283). Moisture content (MC%, w/w) was determined by oven-drying compost samples (10±2g) at 105°C for approximately 24 h and expressed as percentage of total weight loss. pH was measured in aqueous extracts (1:10,w/v) using a pH meter (HD8602,Italy). Total Organic Carbon (TOC) was determined by dry combustion method at 550°C (Allison, 1965).Total nitrogen content (TN) was determined by Kjeldahl digestion analysis (Keenly and Nelson, 1982).

Ammonium nitrogen (NH^{+}_{4}-N) was determined in 10g of freshly sampled compost, which was shaken in 2M KCl extraction solution at 150rpm on orbital shaker for 1h; then filtered through 42-whatman filter paper and steam distillated by 0.2g of ignited and cool MgO and titrate with 0.02 N NaOH, then the result was calculated according the following equation:

$$NH_4^+\text{-N (mg Kg}^{-1}) = \frac{(B-A)*N*14*mcf*100}{S*Z*1000}$$

Where, B: Volume in ml of NaOH solution required for blank titration

A: Volume in ml of NaOH solution required for sample titration

N: Normality of NaOH

S: weight of sample in gram

Z: Aliquot volume

Mcf=correction factor

Nitrate nitrogen (NO^-_3-N) was determined by Devard's distillation method through three steps digestion, distillation and titration, and then calculated the measurement according to the following equation:

$$NO_3^-N = \frac{(B-A)*N*14*mcf*100}{S*Z*1000}$$

Where, B: Volume of NaOH solution required for blank solution

A: Volume of NaOH solution required for sample titration

N: Normality of NaOH

S: weight of the sample in gram

Z: Aliquot of volume

Mcf= Moisture correction factor

Available Phosphorus was analyzed from compost extracts using the molybdovandate phosphoric acid method of Olsen *et al.* (1954). Available potassium was quantified by flame photometry after equilibrium of sample with exchanging cation made of neutral normal NH_4OAc, (Issam and Antoine, 2007). Micronutrients such as Fe, Mn, Cu and Zn were determined by atomic absorption spectrophotometry (AAS) after extracting them with a chelating agent (DTPA) at 248.3 nm, 279.5 nm, 324.7 nm, and 213.9 nm, respectively.

Enumeration of total aerobic bacteria, actinobacteria and fungi

For microbial analysis, compost samples were ground to 0.25 mm, serially diluted in sterile water and inoculated onto the agar using the plate frequency technique (Tiquia *et al*., 2002). Estimation of total aerobic bacteria, actinobacteria and fungi was made by direct plating on appropriate media. For the enumeration of total aerobic bacteria, nutrient agar (NA, Oxoid, England) was used, whereas actinobacteria and fungi were counted on starch casein agar (CSA) and potato dextrose agar (PDA), respectively. Plates were incubated for three days at 30±2°C for the growth of bacteria and fungi, and between five and seven days for actinobacteria. They were estimated using the Most Probable Number (MPN) method (Tiquia *et al*., 2002).

Viability test and enumeration of Effective Microorganisms (EM)

The viability of microorganisms in the liquid EM WOLJEEJII (name of EM supplying company) was examined after taking samples from dormant and activated packages separately using appropriate media and incubation time as before. The number of viable bacterial cells from the concentrated packages was $2.48X10^8$cfu/ml; and fungal/yeast count was $3.37X10^7$cfu/ml; whereas, the counts of bacteria and yeasts in the diluted

packages was $1.23X10^7$cfu/ml and $5.73X10^6$cfu/ml, respectively. This number of organisms in the diluted form was used in the composting experiment.

Phytotoxicity test

The phytotoxicity study of the compost samples was determined following the method of Zucconi and de Bertoldi (1987). Aqueous extracts were prepared (10:50, w/v), centrifuged at 10,000 rpm (Wagtech International, 3000 system) and filtered through a 0.45 µm membrane filter. Ten seeds of garden cress (*Lepidium sativum*L.) were evenly distributed on a filter paper in a petri dish and watered with 1 mL of the supernatant. A control treatment consisting of ten seeds watered with 1 mL of distilled water was also included. All Petri dishes were incubated at 22° C for 24 h in the dark and then germination was stopped with 1 mL of ethanol. The number of germinated seeds with at least 1 mm was counted and the length of the germination root was also measured. Then germination index was then calculated following the formula given by Zucconi and de Bertoldi (1987).

Data Analysis

Chemical and microbiological data were analyzed by repeated measures analysis of variance (ANOVAR) in which piles represented the subjects. In addition, the composition of parent material was fixed as the between-subject factor, and the composting time was fixed as the within factor. All the variables met sphercity condition (Mauchly's test) whenever this assumption was violated; it was corrected with Geiser-Greenhouse (G-G) procedure (Potvin *et al.*, 1990). Significant differences in the main effects were further analyzed by paired comparison with the Bonferroni test. To understand the effect of different physico-chemical parameters on the evolution of

inorganic N forms (NH_4-N, NO_3-N and NH_4-N:NO_3-N ratio), Pearson correlation coefficient (r^2) was carried out. Data of all experiments conducted in triplicate were analyzed by statistical computing packages (SPSS, version 21).

Results and Discussion

Physico-chemical profiles of Flower Residue compost

Figure1 shows the temperature profiles of compost made from the three piles (FRCD, FREM and FR). In Pile1 (Cow dung amended compost), the first phase of mesophilic temperature (25-42°C) stayed for three days and temperature reached to thermophilic stage (45-60°C) and remained the same for about thirty two days. This temperature started to gradually decline to reach to the second mesophilic stage (25-42°C) within a week and cooled down to 22-23°C from day 63 until the end of the experiment (90 days). The second treatment (Pile 2; activated EM mix) showed the same trend as Pile 1 with short initial mesophilic stage (3 days) but reached the lower thermophilic stage (43-54°C) and lasted for a relatively short period (18 days); and gradually declined to mesophilic temperature (24-39°C) for15 days, and further cooled down to (21-22°C) after day 63 to day 90.

The Third treatment (Pile 3), however, showed a different trend during the first phase except that the thermophilic stage (45-53°C) was maintained for 12 days (from3 to 14), with a gradual decrease in temperature (23-35°C) from day 35 to day 63. Unlike the other two treatments, there was an abrupt increase in temperature from 23°C to 34°C by as much as 11°C by day 77 and kept on increasing until the last sampling day (day90). The rapid increase in temperature of Pile 3 indicated that the mixture still contained easily

degradable substrates that required more days than the other two piles, suggesting that it was not biologically stabilized even after day 90. However, significant differences were evident in the temperature changes among the three piles as function of compost age (Fig 4.1. Compost piles x time ANOVAR $F_{20, 60} = 53.254$, p=0.0001).

The thermophilic temperature range in Pile 1 (32 days) was the longest as compared to the thermophilic temperature range in Pile 2 (18 days) and in Pile 3 (12 days). Besides, temperature greater than 55°C was maintained for more than 3 days, the minimum requirements for proper disinfection of waste materials from animal and plant pathogens as well as for weed seed suppression (Said-Pullicino *et al*., 2007).

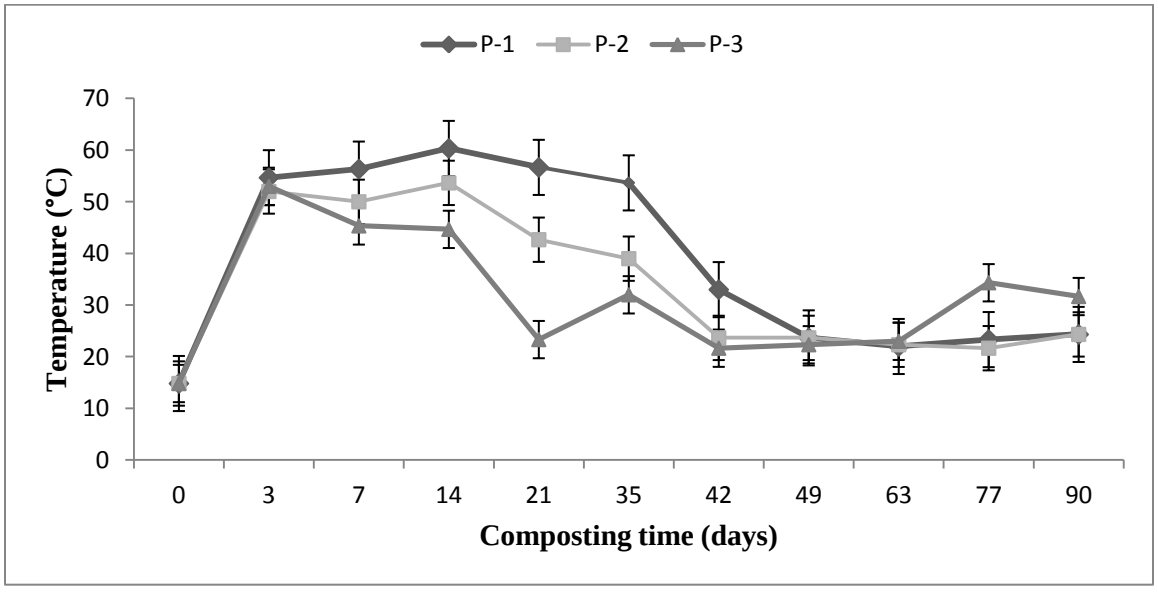


Figure 4.1Temperature changes in the compost piles of FRCD (Pile 1), FREM (Pile 2) and FR (Pile 3)

The compost piles showed a steady decrease in moisture content (40-60%) from the start up to the end of the experiment. Consequently, the moisture content of all compost piles was relatively within the permissible range of 40-70% (Epstein, 1997). Lower moisture loss (18.8%) was observed in Pile 3 compared to 28.2% and 31.8% loss in Pile 2 and Pile

1, respectively as a result of rise in temperature and duration of thermophilic phase that drove the slower vaporization process out of the compost matrix.

This result is similar to Liu *et al*. (2011) who reported 21% loss in pile3, which was a mixture of dairy manure and rice chaff (carbon source) in 85/15 ratio, as compared to Pile 2 (22%) and Pile 1 (24%) that contained 80/20 and 75/25 ratios, respectively. The higher reduction of moisture in Pile1 and Pile 2 can be explained by a better microbial degradation that could generate heat and enhanced desiccation (Rebollido *et al*., 2008). In this study, the moisture content of the matured compost of Pile 1 and Pile 2 was within the range between 30-40%, proportional to the minimum recommended range by Diaz and Savage (2007).

The pH values of the different treatments showed significant differences with compost age (Table 4.1. ANOVAR $F_{7,42}$=108.846, p=0.0001). In all cases, the pH was acidic for one week (6.35-6.38) at the start of the experiment and gradually increased to alkaline pH with the highest pH values measured on day 63 for Pile 1(7.5) and on day 77 for Pile 3 (7.60). At the end, the pH dropped to near neutral (Pile-1:7.15; Pile-2:7.14 and Pile-3: 7.01).

Increase in pH over time can be attributed to a release of ammonia (Table 4.3) associated with protein degradation in cow dung amended composts (Pile 1). Generally, this trend of increasing pH from acidic values at the start of the experiment to alkaline pH during the active stages and neutral at the end is due to microbial nitrification process (Gómez-Brandón *et al*., 2008).

Table 4.1 Physico-chemical characteristics of three compost piles during composting, Pile-1: FRCD; Pile-2:FREM; Pile-3:FR

Parameter	Composting time							
	1	**7**	**21**	**35**	**49**	**63**	**77**	**90**
Pile-1								
MC(%)	71.4	66.4	67.4	68.5	57.2	46.4	43.3	39.6
	(0.66)^{a}	(0.72)^{b}	(0.41)^{b}	(0.57)^{c}	(0.59)^{d}	(0.66)^{e}	(0.37)^{d}	(0.55)^{g}
pH	6.38	6.35	7.38	7.25	7.30	7.51	7.35	7.15
	(0.07)^{a}	(0.05)^{a}	(0.04)^{b}	(0.03)^{c}	(0.06)^{bc}	(0.03)^{d}	(0.07)^{b}	(0.05)^{c}
Pile-2								
MC(%)	61.9 (0.66)^{a}	67.9 (0.72)^{b}	69.7 (0.41)^{c}	67.4 (0.57)^{b}	47.9 (0.59)^{d}	39.2 (0.22)^{e}	30.9 (0.37)^{d}	33.7 (0.55)^{g}
pH	6.82	7.12	7.15	7.09	7.07	7.26	7.25	7.14
	(0.07)^{a}	(0.05)^{b}	(0.04)^{b}	(0.03)^{b}	(0.06)^{b}	(0.03)^{c}	(0.07)^{d}	(0.05)^{b}
Pile-3								
MC(%)	64.5 (0.66)^{a}	65.5 (0.72)^{b}	71.9 (0.41)^{c}	65.7 (0.57)^{b}	44.7 (0.59)^{d}	49.0 (0.66)^{e}	44.0 (0.37)^{f}	45.7 (0.55)^{g}
pH	6.72	7.03	7.13	6.96	7.30	7.38	7.60	7.01
	(0.07)^{a}	(0.05)^{b}	(0.04)^{c}	(0.03)^{b}	(0.06)^{d}	(0.03)^{d}	(0.07)^{e}	(0.05)^{b}

Values are means (n=3) and standard errors of three triplicates. Numbers followed by the same letter in a raw are not significantly different according to Bonferroni test (P=0.05)

Changes in Total organic carbon, Total Nitrogen and C:N ratio

Composting time had a significant effect on the total organic carbon (TOC) content, but this effect varied depending on the starting material (Table 4.2 ANOVAR $F_{7, 42}$=1267.345, p=0.001). The total organic C in all piles varied from 30% to 34% at the start of composting and then decreased as the decomposition progressed, reflecting a notable mineralization of organic matter. At the end of the process, the lowest TOC was obtained in Pile1 (13%) and the highest in Pile 3 (25%). The high TOC mainly came from the concentrations of more recalcitrant compounds present in Pile 3 compared to the other two piles. Pile 3 contained no amendment; hence its decomposition occurred slowly. This study revealed that treatments without or little amendment could contain large quantities of un-mineralized organic matter at the end of the process, similar to Liu *et al*. (2011) that reported a lower proportion of rice chaff in manure compost (Pile 3; 6:1) contained higher concentration of TOC (35%) compared to higher proportion of rice chaff (Pile 1; 3:1) that contained lower concentration of TOC (25%).

At the beginning of the composting, total nitrogen (TN) varied between 0.8% to 1.4% of the dry weight material, with the lowest N in Pile 2 and the highest in Pile 1. Total nitrogen (TN) percentage showed a gradually increasing trend between 12-14% with composting duration (Table 4.2; ANOVAR $F_{7, 42}$=24.009, p=0.0001) which is due to the concentration effect caused by carbon loss associated with mineralization of the organic matter.

Initial C/N ratios of Pile 1, Pile 2 and Pile 3 was 30%, 39% and 36%, respectively (Table 4.2), which was later reduced to C/N ratio of 10%, 16%, and 24% suggesting significant difference among the treatments (Table 4.2. ANOVAR $F_{7 ,42}$=761.654,P=0.0001). This

difference could be explained as C was lost in the form of CO_2 and the mineralization of N through microbial activity (Ryckeboer *et al*., 2003a). The lowest C/N ratio of 10% in Pile 1 was an indication of the matured compost as suggested by Diaz and Savage (2007). This pile showed a similar C/N ratio of 11.4% from 80 days composting of cattle manure (Gómez-Brandón *et al*., 2008). On the contrary, a higher C/N ratio was measured at the end of the composting for the control (Pile 3), indicating a slow kinetic process in this compost. Low initial C/N ratio of Pile 1 caused faster mineralization compared to high initial C/N ratio of Pile 3, similar to Eiland *et al*. (2001) that reported straw containing the lowest initial C/N ratio (i.e. 11) caused a faster degradation of fibers during the first three months of composting (hemicellulose 50-80%, cellulose: 40-60%) than higher C/N ratio (i.e. 54).

Table 4.2 Changes of organic carbon, total N and C:N during composting of different piles, Pile-1: FRCD;Pile-2:FREM; Pile-3:FR

Parameter	Days							
	1	7	21	35	49	63	77	90
Pile1								
TOC(%)	34.18 (0.43)^{a}	31.19 (0.13)^{b}	31.17 (0.58)^{b}	26.44 (0.23)^{c}	22.12 (0.16)^{d}	20.85 (0.14)^{e}	16.89 (0.18)^{f}	12.99 (0.46)^{g}
TN(%)	1.14 (0.01)^{d}	1.14 (0.01)^{d}	1.15 (0.01)^{e}	1.16 (0.01)^{c}	1.17 (0.01)^{c}	1.22 (0.02)^{a}	1.23 (0.01)^{b}	1.30 (0.02)^{b}
C:N	29.99 (0.76)^{a}	27.30 (0.47)^{b}	27.10 (0.62)^{b}	22.85 (0.31)^{c}	18.93 (0.25)^{d}	17.09 (0.21)^{e}	13.73 (0.34)^{f}	9.99 (0.36)^{g}
Pile2								
TOC(%)	31.38 (0.43)^{a}	26.33 (0.13)^{b}	25.65 (0.58)^{b}	25.55 (0.23)^{c}	22.44 (0.16)^{d}	18.73 (0.14)^{e}	18.34 (0.18)^{f}	17.49 (0.46)^{g}
TN(%)	0.80 (0.01)^{bc}	0.80 (0.01)^{c}	0.82 (0.01)^{b}	0.83 (0.02)^{d}	0.84 (0.02)^{d}	0.85 (0.02)^{c}	0.87 (0.01)^{b}	0.90 (0.02)^{a}
C:N	39.23 (0.76)^{a}	32.91 (0.47)^{b}	31.28 (0.62)^{b}	30.79 (0.31)^{bc}	27.31 (0.25)^{d}	22.04 (0.21)^{e}	17.08 (0.34)^{f}	16.43 (0.36)^{g}

Cont'd

Parameter	Days							
	1	7	21	35	49	63	77	90
Pile3								
OC(%)	32.54 (0.43)[a]	30.11 (0.13)[b]	30.10 (0.58)[b]	29.69 (0.23)[c]	28.41 (0.16)[d]	25.56 (0.14)[e]	25.54 (0.18)[f]	24.99 (0.46)[g]
TN(%)	0.91 (0.01)[a]	0.92 (0.01)[d]	0.94 (0.01)[d]	0.95 (0.01)[c]	0.98 (0.02)[b]	0.98 (0.02)[b]	1.01 (0.01)[b]	1.02 (0.02)[a]
C:N	35.76 (0.76)[a]	32.73 (0.47)[b]	32.02 (0.62)[b]	31.25 (0.31)[bc]	28.99 (0.25)[d]	26.08 (0.21)[e]	25.24 (0.34)[f]	24.11 (0.36)[f]

Values are means(n=3) and standard error. Values along the raw followed by the same letter are not significantly different according to Bonferroni test (P=0.005)

Evolution of Ammonium-N, Nitrate-N and their ratio

Composting time had significant effect on the transformation of the organic N to inorganic N (NH_4^+–N and NO_3^-–N) (Table 4.2, ANOVAR, $F_{7, 42}$=602.11, p=0.0001). By day 7, the highest concentration of NH_4^+–N (553.7 mg/kg) was found in Pile 1. Likewise, maximum concentration of NH_4^+–N was found by the same day (71.4 mg/kg) in Pile 3, respectively. Sanchez-Monedero *et al*. (2001) reported that highest concentration of ammonium could occur during the first week of the process through mineralization of organic N caused by the intense organic matter degradation. This maximum amount of ammonium-N concentration can arise from the rapid degradation of the organic N provided by the cow dung due to protein degradation (Lerch *et al*., 1992). Generally, the ammonium concentration of all piles decreased gradually after the peak days (days 7-21) towards the end of the composting process.

Comparatively, significant differences have been observed among the three piles along the composting times (Compost pile x time ANOVAR $F_{14,42}$=61.662, p=0.0001) and the lowest NH_4^+–N concentration was observed in Pile 2 throughout the process. This low concentration resulted from the higher C/N of the starting material (Table 4.1), which limited the nitrogen requirements of the microorganisms (Eiland *et al*, 2001). Apart from this, the concentration of NH_4^+–N in Pile 1 fell after day 49 (end of thermophilic phase) to reach final values of less than 0.04% (<400 mg/kg) and stabilized while the concentration of NH_4^+–N in the other two piles was below this limit from the start of composting. This limit was established as one of the best stability indicators for various plant, animal and sludge wastes (Zucconi *et al*., 1987; Bernal *et al*., 1998).

The concentration of nitrate also varied significantly with composting time in all treatments (Table4.2. ANOVAR $F_{7,42}$=147.398, p=0.0001). From the start to end of composting, the decline of NH_4^+–N corresponded with the increase in NO_3^-–N. In general, NO_3^-–N concentration was increased by 284% (863 mg/kg) in Pile 1, by 168% (103.3 mg/kg) in Pile 2, and by 54% (38 mg/kg) in Pile 3 from the start to end of composting. In contrast, the decrease of NH_4^+–N during composting was 66% (272.8 mg/kg) in Pile1, 12.5% (7.5 mg/kg) in Pile 2, and 12.2 % (8.7 mg/kg) in Pile 3.

The higher accumulation of nitrate, particularly in Pile1 at the end of the process can be explained with nitrification, associated with lower temperature (<40°C) (Sánchez-Mondero *et al*., 2001). Furthermore, the good aeration (frequent turning; once in a week), optimum moisture (Table 4.1) and relatively neutral pH (Pile 1: 7.05; Pile 2:7.04 and Pile 3:7.01) were favorable environmental factors for nitrifying bacteria. Most of the nitrification occurred during maturation, leading to a low NH_4-N/NO_3-N ratio in mature compost (Bernal *et al*., 1998).

As a result, the ratio of NH_4^+–N to NO_3^- –N was found to be 0.12, 0.32 and 0.58 for Pile 1, Pile 2 and Pile 3, respectively. This result implied that Pile 1 attained the threshold level of 0.16 by day 63 in which this value was established as a threshold level for stability index for various municipal and agricultural wastes (Bernal *et al*., 1998), whereas Pile 2 and Pile 3 required more days to attain this limit.

The typical trend of N transformation followed that protein converts to organic-N, then to NH_4^+ and finally to NO_3/NO_2^-, with very little denirtification (N_2). This is why a decrease

in NH_4^+-N concentration corresponding with an increase in (NO_3^--N) is common for composting process (Riffalidi *et al*., 1986).

Table 4.3 Changes in inorganic N: NH_4-N and NO_3-N (mg/kg) and NH_4-N to NO_3-N ratio during composting of the three piles

Parameter	Days							
	1	7	21	35	49	63	77	90
Pile-1								
NH_4^+–N	412.8	553.7	435.5	478.3	481.4	174.3	140.0	140.0
	(6.5)[a]	(13.1)[b]	(6.6)[b]	(5.7)[b]	(2.2)[c]	(3.7)[d]	(1.0)[e]	(2.3)[e]
NO_3^- –N	303.7	416.5	425.8	760.7	981.3	1162.1	1167.8	1166.7
	(11.1)[a]	(21.5)[b]	(22.8)[c]	(25.6)[d]	(39.2)[d]	(25.4)[e]	(39.3)[e]	(92.3)[e]
NH_4:NO_3	1.35	1.33	1.03	0.76	0.49	0.15	0.12	0.12
	(0.16)[b]	(0.24)[a]	(0.06)[b]	(0.06)[c]	(0.03)[d]	(0.03)[e]	(0.01)[f]	(0.01)[g]
Pile-2								
NH_4^+–N	60.1	58.9	60.7	60.7	58.5	57.5	55.3	52.6
	(6.5)[a]	(13.1)[b]	(6.6)[b]	(5.7)[b]	(2.2)[c]	(3.7)[d]	(1.0)[e]	(2.3)[f]
NO_3^- –N	61.2	61.2	61.4	63.1	86.3	129.8	131.7	164.4
	(11.1)[a]	(21.5)[a]	(22.8)[a]	(25.6)[b]	(39.2)[c]	(25.4)[b]	(39.3)[b]	(92.3)[d]
NH_4:NO_3	0.99	0.98	0.96	0.96	0.68	0.44	0.42	0.32
	(0.16)[a]	(0.24)[b]	(0.58)[b]	(0.06)[b]	(0.03)[c]	(0.03)[d]	(0.01)[e]	(0.01)[f]

Cont'd

Parameter	Days							
	1	7	21	35	49	63	77	90
Pile-3								
NH_4^+–N	71.3	71.4	69.5	69.1	69.6	67.7	64.4	62.6
	(6.5)^d	(13.1)^d	(6.6)^a	(5.7)^b	(2.2)^c	(3.7)^d	(1.0)^e	(2.3)^f
NO_3^- –N	69.9	70.7	70.9	73.5	75.6	85.7	93.3	107.9
	(11.1)^d	(21.5)^c	(22.8)^b	(25.6)^a	(39.2)^a	(25.4)^a	(39.3)^a	(92.3)^a
NH_4:NO_3	1.02	1.01	0.98	0.94	0.92	0.79	0.69	0.58
	(0.16)^a	(0.24)^a	(0.06)^b	(0.06)^c	(0.03)^c	(0.03)^d	(0.01)^f	(0.01)^g

Values are means±standard error (n=3). Values along the raw followed by the same letter are not significantly different according to Bonferroni test (P=0.001)

Relationship between different N forms and other physico-chemical properties

The evolution of N forms is influenced by the different physico-chemical properties of compost (Table 4.4). In the present study, there were significant correlation between NH_4^+-N and temperature, water content, pH and organic carbon in the piles. In Pile1, NH_4^+-N has significantly correlated with moisture content (r=0.84), temperature (r=0.61), pH (r=0.48) and TOC (r=0.79). Likewise, there were significant correlation between NH_4^+-N and these parameters in Pile 2 and Pile 3, except with pH in Pile 2 (r=-0.43) and with temperature in Pile 3 (r=-0.18). On the contrary, significantly negative correlation was found between NO_3^-N and these parameters except pH which was positively correlated for Pile-1 (r=0.7) and for Pile 2 (r=0.55) (Table 4.3.). Previously, Tiquia (2002) reported that NH_4- N had correlated significantly with these four parameters for spent pig litter sludge and poultry litter piles, indicating that the N transformation in compost were influenced by water content, temperature, pH and carbon during the process. An inverse correlation between nitrate and pH values confirm the idea that the pH values of composting mixtures were directly related to nitrification as previously reported by Sanchez-Monedero *et al*. (2001) for four different biosolid composts. Furthermore, Pile 1 with higher ammonium concentration at the end of thermophilic phase underwent higher degree of nitrification. This suggests that organic N obtained from cow dung was the limiting step in nitrification such mineralization was scant during the last phases of composting, when the supply of ammonium available to the nitrifying bacteria would have been reduced.

Table 4.4 Pearson correlation coefficient (r^2) between different forms of N and physico chemical parameters

	Parameters				
N forms	MC	Temp	pH	TOC	C:N ratio
Pile-1					
Total N	-0.94**	-0.49	0.45	-0.93**	-0.94**
NH_4-N	0.84**	0.61**	0.48	0.79*	-0.88**
NO_3-N	-0.91	-0.48	0.70	-0.96**	-0.96**
Pile-2					
Total N	-0.86**	-0.38	0.55	-0.90**	-0.95**
NH_4-N	0.89**	0.38	-0.43	0.81**	0.87**
NO_3-N	-0.94**	-0.48	0.55	-0.90**	-0.95**
Pile-3					
Total N	-0,86**	0.13	-0.86**	-0.94**	-0.97**
NH_4-N	0.80*	-0.18	0.80*	0.87**	0.90**
NO_3-N	-0.78*	0.17	-0.78*	-0.95**	-0.93**

**** Correlation was significant at 0.01level and * at 0.05 level (2 tailed)**

Availability of macro and micronutrients in compost

The P and K contents of the different treatments from the first up to the last stages of composting are shown on Table 4.5. In both cases the P and K contents of Pile 1 (with manure) was higher than the other two piles, indicating that the manure amendments

increased mineralization and availability of these minerals. Accordingly, Available P contents of Pile 1, Pile 2 and Pile 3 were 7921.3, 6196.9 and 5524.5 mg/kg dw, respectively after the start of composting (Table 4.5).

At the end of composting, the available P contents of Pile 1, Pile 2 and Pile 3 increased by 27%, 13% and 5%, respectively. The treatment also showed same trend with regards to available K showing an increase of 664% in Pile 1, followed by Pile 3 (600%) and Pile 2 (566%). Gómez-Brandón *et al*. (2008) reported that available P in the presence of cattle manure could result in the release more of these compound as early age of composting.

In contrast, Selim *et al*. (2012) showed an increasing trend of extractable P and K as a function of composting time; and the final product on composting of corn stalks mixed with cattle manure. The average increase of available P and available K in the six different heaps of compost were over 200% and 57% in 120 days of composting, respectively.

Table 4.5 Changes in the available P and K during the composting process of three Piles

Days	Pile-1		Pile-2		Pile-3	
	AvP (mg/kg)	AvK (cmol(+)/kg	AvP (mg/kg)	AvK (cmol(+)/kg	AvP (mg/kg)	AvK (cmol(+)/kg)
1	7921.13	31.5	6196.9	16.5	6524.5	22.3
	(21.2)[a]	(0.5)[a]	(21.2)[a]	(0.5)[a]	(21.2)[a]	(0.5)[a]
7	8020.14	47.25	6222.92	31.74	6551.9	37.61
	(11.2)[a]	(0.2)[b]	(11.2)[a]	(0.2)[a]	(11.2)[a]	(0.2)[b]
21	8085.89	78.74	6274.36	61.92	6606.06	68.23
	(13.5)[b]	(0.2)[c]	(13.5)[b]	(0.2)[b]	(13.5)[b]	(0.2)[c]
35	8151.63	110.28	6325.8	91.92	6660.21	98.85
	(9.7)[b]	(0.1)[d]	(9.7)[d]	(0.1)[d]	(9.7)[b]	(0.1)[f]
49	8218.17	141.72	6377.23	92.00	6714.36	129.46
	(11.1)[b]	(0.1)[c]	(11.1)[c]	(0.1)[c]	(11.1)[b]	(0.1)[d]
63	8283.92	173.22	6429.25	52.09	6769.17	130.08
	(88.0)[e]	(0.1)[f]	(28.5)[d]	(0.1)[f]	(88.0)[c]	(0.1)[f]
77	8793.2	204.71	6528.43	82.18	6823.32	134.70
	(22.5)[a]	(0.4)[d]	(22.5)[f]	(0.4)[d]	(22.5)[c]	(0.4)[g]
90	10031.8	233.45	6973.3	110.0	6873.56	154.13
	(5.8)[e]	(0.1)[f]	(5.8)[c]	(0.1)[d]	(5.8)[c]	(0.1)[g]

Values are means ±standard error (n=3). Values within the same column followed by the same letter are not significantly different according to Bonferroni test (P=0.005)

The micronutrient contents (manganese, zinc, iron and copper) of the four composts showed variations (Table 4.6). Although Zn and Cu are not used as index for maturity of compost, they can be related to compost quality in terms of potential contamination of the environment (Tiquia and Tam, 1998). On the other hand, these elements are required in small amount by plants (Wang *et al*., 2004). In the present study, Mn was present in sufficient amount in the final compost for horticultural plants whereas Fe could be sufficiently available only in Pile 1 and Pile 2. According to Wang *et al*. (2004) for the application of dairy manure saw dust compost in cucumber, the recommended sufficiency range for Cu, Fe, Mn and Zn micronutrients were 8-10, 50-300, 50-300 and 25-200 mg/kg, respectively.

Table 4.6 Micronutrients in the three piles (FRCD, FREM and FR) at the end of composting

Micronutrients	Pile-1	Pile-2	Pile-3
Mn (mg/kg)	206.5(6.0)^{b}	230.3(3.9)^{a}	175.6(0.7)^{c}
Zn (mg/kg)	15.3(0.2)^{a}	6.0(0.3)^{c}	7.1(0.3)^{b}
Fe (mg/kg)	67.5(2.9)^{a}	49.5(0.6)^{b}	32.5(0.4)^{c}
Cu (mg/kg)	3.0(0.1)^{a}	2.6(0.02)^{a}	1.49(0.7)^{a}

Values are means $\pm$ standard error of three triplicates. Three piles of the same farm followed by the same letter are not significantly different at p=0.05 significant level according to Tukey's B

Phytotoxicity test

The germination index of samples collected at different times of composting from the three compost piles is shown on Figure 4.2. A GI of 81%, 73% and 66% was recorded at the end of the process for Pile 1, Pile 2 and Pile 3, respectively. Thus, more than 49 days were needed for flower residues amended with cow dung (Pile 1) and activated EM mix (Pile 2) to overcome the threshold limit of 50% stated by Zucconi and Bertoldi (1987) to reduce the toxicity levels.

Comparatively, more than 77 days were needed for the control (Pile 3) to reach safe level of 50%. In the present study, around 90 days was needed to pass the GI value of 80% for Pile 1, which was considered as an index of compost maturity and practically free of phytotoxic substances (Zucconi and de Bertoldi, 1987). Pile 2 and Pile 3, in contrast, failed to reach this value and needed more than 90 days to be as safe for soil application. Increases in GI values corresponded with decreases in concentrations of NH_4^+.

In effect, judging from GI values, the level of compost maturity was in the order of FRCD>FREM>FR after 90 days of composting. Manure amended compost could reach to maturity faster than the other feed stock. Other study also showed that manure amended compost could show an increasing trend in GI % (Mathur *et al*., 1993).

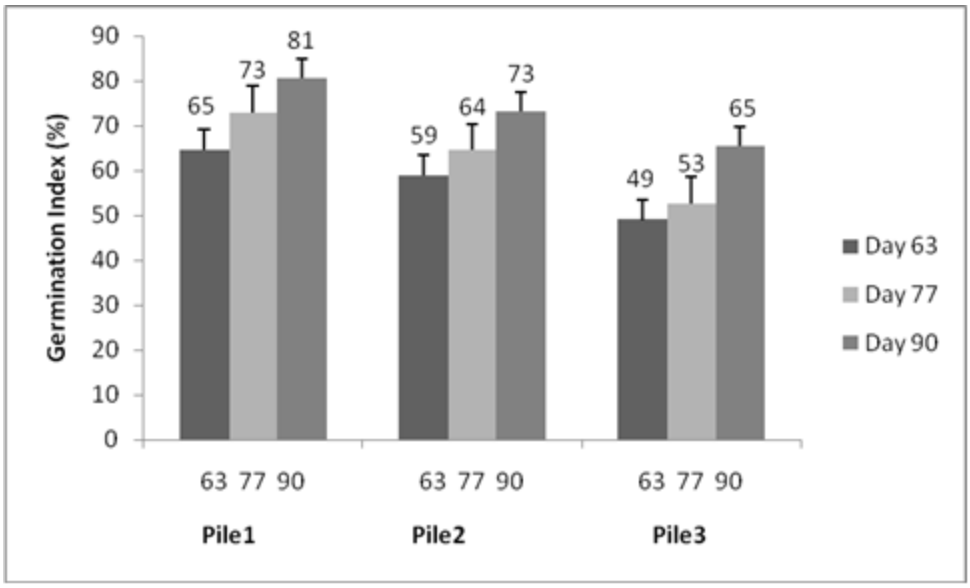


Figure 4.2 Changes in germination index of compost material collected at different times of the process from Flower Residue-Cow Dung (Pile-1), Flower Residue-EM (Pile-2) and Flower Residue only (Pile-3)

Summary of the evaluation of maturity/stability of the different composts

Since the principal requirement of a compost for it to be safely used in soil is high degree of stability and maturity which are most commonly evaluated by the chemical parameters C:N ratio, NH_4-N concentration, NH_4-N:NO_3-N ratio and germination index (GI%) for composts of different sources (Zucconi *et al*., 1985; Zucconi and de Bertoldi, 1987; Mathur *et al*., 1993; Bernal *et al*., 1998). Accordingly, the corresponding values of these parameters are shown in Table 4.7 to evaluate the maturity or stability of the three treatments. Based on this, the C:N ratio, NH_4-N, NH_4-N:NO_3N ratio and GI of Pile 1 was 14,140 mg/kg, 0.12 and 81%, respectively whereas, these values for Pile 2 was 17, 53 mg/kg, 0.32 and 73% and for Pile 3, it was 24, 193 mg/kg,0.38 and 65%, respectively. In all respect, the cow dung amended compost (Pile-1) fulfilled the threshold limit of C:N ratio (<15), NH_4-N (<400 mg/kg), NH_4-N:NO_3N ratio (<0.16) and germination index (<80) to recommend this pile for application to soil. On the contrary, the two piles required more days to attain this threshold limit.

Table 4.7 Maturity of three composts compared with recommended indices

Parameter	Values				Reference
	Pile-1	Pile-2	Pile-3	Recommended	
C:N ratio	13.7	17.1	24.1	5-15	Mathur *et al*.(1993)
NH_4-N (mg/Kg)	140	52.6	193	<400	Zucconi &de Bertoldi (1987)
NH_4-N:NO_3-N	0.12	0.32	0.38	<0.16	Bernal *et al*. (1998)
GI(%)	81	73	65	>80	Zucconi *et al*. (1981)

Enumeration of total aerobic bacteria, actinobacteria and fungi

The microbial profile indicated significant variations among the three different compost piles during the composting process (Table 4.8.). The total aerobic bacteria from pile1 increased significantly at 7days (8.99 $\log_{10}$MPN g^{-1}dwc) and decreased gradually by 1-2 order until day 63, and slightly increased at the end of composting.

Total aerobic bacteria in Pile 2 also peaked at 7 days (8.15 $\log_{10}$MPN g^{-1}dwc), but abruptly declined by approximately 2-3 orders, showing the lowest population at day49 (5.84 $\log_{10}$MPN g^{-1}dwc) and gradually increased to 2 order at the end of composting. In Pile 3, higher number of bacteria was counted in 7 days (7.45 $\log_{10}$MPN g^{-1}dwc) and 90days (7.40 $\log_{10}$ MPN g^{-1}dw). In all piles, significant differences in bacterial number were found along the composting age ($p<0.05$). These findings are expected based on the findings by others (Hassen *et al*., 2001).

The population of actinobacteria in all piles showed an increasing trend, and the largest number was found at 63days in Pile 1, but in the other two piles the same number of actinobacteria was recorded at the end of composting. This shows that easily degradable substrates were exhausted earlier in Pile 1 than the two piles as indicated by the total organic carbon (Table 4.2). In general, minimum actinobacterial counts were detected at various times, i.e. at days 90 in Pile 1, at days 21 in Pile 2 and at days 63 in Pile 3. Indeed these groups are able to utilize big polymers and accounts for a majority of the microorganisms present after cooling temperature as in the case of Pile 1 and Pile 2 (Steger *et al*., 2007).

In this study, the lowest population of fungi was detected at the early stages of composting in all treatments (i.e. Pile 1: 5.59 $\log_{10}$MPN g^{-1}dwc; Pile 2: 5.11 $\log_{10}$MPN g^{-1}dwc; Pile 3: 4.81 $\log_{10}$MPN g^{-1}dwc) whereas the highest population of fungi was found at the end of composting for the two amended piles (i.e. Pile1: 6.64 $\log_{10}$MPN g^{-1}dw; Pile2: 5.66 $\log_{10}$MPN g^{-1}dwc), but for pile3, the highest number of fungi (6.00 $\log_{10}$MPN g^{-1}dwc) was counted immediately after the end of thermophilic phase (day 49).

It is interesting to note that fungi were not eliminated at thermophilic temperatures above 55°C contrary to the findings of Tiquia *et al*. (2002) from cow dung amended compost pile. However, this result was similar to Goyal *et al*. (2005) who reported a mesophilic fungal population (i.e. $144X10^7$ cfu g^{-1}dwc) in sugar trash plus cattle dung compost.

Overall, the total population of aerobic bacteria, actinobacteria and fungi were lower than the report of Tiquia (2002) that the number of heterotrophic bacteria, actinobacteria and fungi varied in the range between 7.09 to 9.80 $\log_{10}$MPN g^{-1}, 6.76-10.51$\log_{10}$MPN g^{-1},

5.08-9.24log_{10}MPN g^{-1} for poultry litter plus yard trimmings compost in 91 days of time. For spent pig litter compost, these numbers also varied from 5.77 to 8.23log_{10}MPN g^{-1} for heterotrophs, from 7.33 to 10.45log_{10}MPN g^{-1} for actinomycetes and from 3.31 to 8.50log_{10}MPN g^{-1} for fungi. The difference in higher number of all microbial groups might be explained with the high N contents from fresh poultry and pig on-litter manures; and the forced aeration composting system used.

.

Table 4.8 Enumeration of microbial populations during composting, Results are the mean of the three replicates; data are expressed as log10MPN g-1 dry weight compost

	Pile-1			Pile-2			Pile-3		
Days	Bacteria	Actinobacteria	Fungi	Bacteria	Actinobacteria	Fungi	Bacteria	Actinobacteria	Fungi
1	8.40	6.71	5.59	7.71	5.59	5.11	6.52	5.71	4.81
	$(0.23)^{e}$	$(0.14)^{b}$	$(0.19)^{a}$	$(0.11)^{f}$	$(0.11)^{ab}$	$(0.07)^{ab}$	$(0.13)^{a}$	$(0.18)^{b}$	$(0.64)^{a}$
7	8.99	7.15	6.00	8.15	5.93	5.20	7.45	6.65	5.15
	$(0.09)^{f}$	$(0.06)^{c}$	$(0.10)^{ab}$	$(0.07)^{g}$	$(0.05)^{c}$	$(0.02)^{ab}$	$(0.42)^{b}$	$(0.11)^{df}$	$(0.26)^{ab}$
21	7.52	7.26	6.40	6.86	5.56	5.36	6.32	5.90	5.48
	$(0.16)^{cd}$	$(0.09)^{c}$	$(0.18)^{b}$	$(0.08)^{e}$	$(0.10)^{a}$	$(0.19)^{ab}$	$(0.06)^{a}$	$(0.13)^{bc}$	$(0.08)^{abc}$
35	7.34	7.15	6.24	6.15	5.90	5.38	6.26	5.97	5.57
	$(0.15)^{c}$	$(0.09)^{c}$	$(0.11)^{ab}$	$(0.03)^{b}$	$(0.01)^{ab}$	$(0.35)^{ab}$	$(0.11)^{a}$	$(0.46)^{bc}$	$(0.06)^{bc}$
49	6.52	7.26	6.04	5.84	6.52	5.08	6.34	5.15	6.00
	$(0.10)^{b}$	$(0.13)^{c}$	$(0.25)^{ab}$	$(0.02)^{a}$	$(0.25)^{d}$	$(0.24)^{sb}$	$(0.08)^{a}$	$(0.14)^{a}$	$(0.25)^{c}$
63	6.15	7.85	6.11	6.40	6.43	5.00	6.53	6.26	5.00
	$(0.20)^{a}$	$(0.23)^{d}$	$(0.27)^{ab}$	$(0.02)^{c}$	$(0.13)^{d}$	$(0.06)^{a}$	$(0.05)^{a}$	$(0.18)^{bcd}$	$(0.09)^{ab}$
77	7.77	6.45	6.20	6.61	6.32	5.08	6.38	6.30	5.36
	$(0.07)^{d}$	$(0.08)^{b}$	$(0.18)^{ab}$	$(0.04)^{d}$	$(0.22)^{d}$	$(0.09)^{ab}$	$(0.12)^{a}$	$(0.22)^{cd}$	$(0.15)^{abc}$
90	6.81	5.75	6.64	7.68	6.67	5.66	7.40	6.92	5.93
	$(0.15)^{b}$	$(0.09)^{a}$	$(0.46)^{b}$	$(0.03)^{f}$	$(0.01)^{d}$	$(0.35)^{b}$	$(0.11)^{b}$	$(0.01)^{d}$	$(0.06)^{bc}$

This study has, therefore, revealed that maturity and stability of flower residue compost was influenced by amendment in which cow dung blended compost (Pile1) accelerated the degradation. The C/N ration, NH_4-N, NH_4-N:NO_3-N ration and GI value of Pile 1 reflected better maturation status compared to EM amended compost (Pile 2) and the control (Pile 3). The end compost in Pile 1 had more nutrients (phosphorus, potassium, nitrogen, manganese and iron) to supplement plant requirements. Besides, the nitrogen transformation of the three piles was influenced by different physico-chemical properties of compost such as moisture, temperature, pH and changes in organic carbon.

Chapter 5 Conclusion and Recommendation

From this study the following conclusion can be drawn:

- Coffee husk supplemented with cow dung and fruit/vegetable wastes together with house hold compost showed faster degradation. Presence of cow dung in the mixture (Pile 1) contributed to earlier elimination of phytotoxic compounds over the 90 days of composting; and germination index can be taken as good indicator of maturity as compared to the control treatment
- The combined physico-chemical parameters such as temperature, total organic carbon, total nitrogen, carbon to nitrogen ratio and pH are important in process monitoring of coffee husk compost. Especially, the C/N ratio of cow dung amended and fruit/vegetable coffee husk was below 15% after 90 days of composting. Therefore, these composts are matured based on their C/N ratio compared to the control that required more days to reach this level.
- The study also showed the importance of API ZYM^{TM} testing for enzyme profile to evaluate compost stability and maturity. Furthermore, the microbes/enzymes relationship at a given time was found to be good indicator of the composting process.
- Apart from this, the use of chemical and biological parameters in flower residue composting were very important indicators of maturity. The inorganic N forms such as NH_4-N, NO_3-N and NH_4-N:NO_3-N ratio are very important indicators of compost maturity of flower residue composts. Besides, germination index greater

than 80% in cow dung amended composts ensures the safety of the end product for the application of soil.

- Finally, the use of classical and molecular techniques was useful in monitoring the composting process and quality control of the product. In effect, more than 65 microbial species were identified with the help of Microarray technology from the process of composting. Certain probes (*Stenotrophomonas maltiphila, Brevundimonas/Caulobacter*, *Derxia gummosa*, *Flavobacterium/Flexibacter*, *Pseudomonas putida, Acinetobacter and Actinomyces* spp) were more influential in discriminating the samples into young and mature compost. The result of DGGE correlated with the result of microarrays in showing the maturity of compost.

Based on the results of the present study, the following can be recommended:

- Since the starting materials and the composting system are crucial factors in standardizing compost maturity and stability, more research on other agricultural and municipal solid matters are necessary.
- Green house and field experiments needs to be conducted to evaluate compost stability and maturity in order to realize the benefits of the product for plant growth.
- Determination of fluvic acid and humic acids need to be included as chemical analysis. The two parameters are important in root growth and development for plants

- Practical use of compost made from coffee husk and flower residue amended with cow dung is highly recommended.

References

Adi, Aj. And Noor,ZM. (2009). Waste recycling of coffee grounds and kitchen waste in vermicomposting. *Bioresour. Technol*. **100**:1027-1030

Al Khadi, SF., Myers, KM., Rasooly, A. and Chizhikov, V. (2004). Genotyping of *Clostridium perfringens* toxins using multiple oligonucleotide microarray hybridization.*Mol. Cell. Probes.* **18:** 359–367.

Albrecht,R. Joffre,R., Gros,R., Le,Petit,T., Terrom, G. and Pėrissol, C. (2008). Efficiency of near-infrared reflectance spectroscopy to assess and predict the stage of transformation of organic matter in the composting process. *Bioresour.Technol*. **99**:445-455

Alfreider,A., Peters,S., Tebbe,C.C., Rangger,A. and Insam, H. (2002). Microbial community dynamics during composting of organic matter as determined by 16S ribosomal DNA analysis. *Comp. Sci.Util*. **10**:303-312.

Aslam, D.N.,Horwath,W. and Vandercheynst, J.S. (2008). Comparison of several maturity indicators for evaluating phytotoxicity in compost-amended soil. *Was.Manag*. **28**:2070-2076.

Atkinson C.F., Jones D.D. and Gauthier J.J. (1996). Biodegradabilities and microbial activities during composting of municipal solid waste in bench-scale reactors. *Comp. Sci.Util*. **4**: 14-23

Beck-Friis,B.,Smårs,S., Jönsson, H., Eklind, Y. and Kirchmann,H. (2003). Composting of source-separated household organics at different oxygen levels: Gaining an understanding of the emission dynamics. *Comp. Sci.Technol*. **11**:41-50

Beffa, T., Blanc, M., Marilley, L., Lott-Fischer,J.,Lyon,P.F. and Arango,M. (1995). Taxonomic and metabolic microbial diversity during composting. **In***: The Science of Composting.* (de Bertoldi, M., Sequi,P.,Lemmes,B. and Papi,T. eds). **1**:149-161. Blackies Academic and professional, Glasgow, Scotland.

Beguin, P. and Aubert, J.P. (1994). The biological degradation of cellulose. *FEMS Microbiol.Rev.***13**:25-58.

Bernal, M.P., Paredes,C., Mondedero,M.A. and Cegarra,J. (1998). Maturity and stability parameters of composts prepared with a wide range of organic waste. *Bioresour. Technol.***63**: 91-99.

Bernal, M.P., Alburquerque, J.A. and Moral, R. (2009).Composting of animal manures and chemical criteria for compost maturity assessment: A review. *Bioresour.Technol.***100**: 5444–5453.

Blanc, M., Marilley,L., Beffa,T. and Arango,M. (1990). Thermophilic bacterial communities in hot composts as revealed by most probable counts and molecular (16S rDNA) methods. *FEMS Microbiol. Ecol.* **28**:141-149

Bodrossy, L. and Sessitsch. A.(2004). Oligonucleotide microarray in microbial dignostics. Curr. *Opin. Microbiol*. **7**: 246-255.

Boutler-Bitzer, I.J., Trevors, J.T. and Boland,G.J. (2006). A polyphasic approach for assessing maturity and stability in compost intended for suppression of plant pathogen. *Appl. Soil Ecol.* **34**: 65-81.

Brewer, L.J. and Sullivan, D.M (2003). Maturity and stability evaluation of yard trimmings. *Comp. Sci. Util*. **11**(2):96-112.

Brown, A. (1985). Review of lignin in biomass. *J.Appl. Biochem*. **7**:371-387.

Butler,T.A.,Sikora,L.J.,Steinhilber,P.M. and Douglass,L.W. (2001). Compost age sample storage effects on maturity indicators of biosolids compost. *J.Environ.Qual*. **30**(6):2141-2148.

Cabañas-Vargas, D.D., Sàmchez-Mondero, M.A., Urpilainen, S.T., Kamilaki, A. and Stentiford, E.I. (2005).Assessing the stability and maturity of compost at large-scale plants. *Ingenieria*. **9**(2): 25-30.

Caceres, R., Flotats,X. and Marfa,O. (2006). Changes in the chemical and physicochemical properties of the solid fraction of cattle slurry during composting using different aerobic strategies. *Wast. Manage*. **26**(10):1081-1091

Castaldi, P., Garau,G. and Melis,P. (2008). Maturity assessement of compost from municipal solid waste through the study of enzyme activities and water-soluble fractions.*Wast. Manage*. **28**:534-540.

Cayuela, M.L., Mondini, C., Insam, H., Sinicco, T. and Franke-Whittle, I.H.**(**2009). Plant and animal wastes composting: Effects of the N source on process performance. *Bioresour. Technol*. **100**: 3097–3106.

Ceustermans, A., Coosemans, J. and Ryckeboer, J. (2010). Compost microbial activity related to compost stability. **In**: *Microbes at Work. From Wastes to Resources*. (Insam, H., Franke-Whittle, I.H., Goberna, M., eds.). Berlin, Heidelberg; Springer. pp, 135-152

Chikae, M., Ikeda,R., Kerman,K., Morita,Y. and Tamiya, E. (2006). Estimation of maturity of compost from food wastes and agro-residues by multiple regression analysis. *Bioresour.Technol*. **97**:1979-1985.

Choi,H.L.,Richard,T.L. and Ahn, H.K. (2001). Composting high materials: biodrying poultry manure in a sequentially fed reactor. *Comp. Sci. Util*. **9**(4):303-311

Chroni, C., Kyriacou,A., Georgaki,I.,Manios,T.,Katsou,M. and Lasardi,K. (2009). Microbial characterization during composting biowaste. *Wast.Manage*.**29**:1520-1525.

Cooperband, I.R., Stone,A.G., Fryda, M.R. and Ravet,J.L.(2003). Relating compost measures of stability and maturity to plant growth. *Comp. Sci.Util.* **11**:113-124.

Cunha-Queda, A.C., Ribiero, H.M., Ramos, A. and Cabral, F. (2007).Study of biochemical and microbiological parameters during composting of pine and eucalyptus bark. *Bioresour. Technol*. **98**:3213-3220.

CTA (Coffee and Tea Development Authority) (1999). Ethiopia cradle of the wonder bean coffee Arabica (*Abissinica*). Addis Ababa, Ethiopia.

Danon, M., Franke-Whittle, I.H., Insam, H., Chen, Y. and Hadar, Y. (2008).Molecular analysis of bacterial community succession during prolonged compost curing. *FEMS Microb. Ecol*. **65**: 133–144.

de Bertoldi, M., Vallini,G. and Pera, A. (1983). The biology of composting: a review. *Wast. Manage.Res*. **1**:157-176.

de Bertoldi, M., Vallini, G. and Pera, A. (1985). Technological aspects of composting including modeling and microbiology. **In**: *Composting of Agricultural and Other Wastes* (Gasser, J.K.R. ed.), Elsevier Applied Science, London and New York. pp 15-30.

de Bertoldi, M. (1998). Composting in the European Union. *BioCycle* **39**: 74-75

Díaz, M.J., Madejón, E., Ariza, J., López, R. and Cabrera, F. (2002).Co-composting of beetvinasse and grape marc in Windrows and static pile systems. *Comp. Sci. Util*. **10**: 258– 269.

Diaz, L.F., Savage, G.M., Eggerth, L.L. and Chiumenti, A. (2007). **In**: *Compost Science and Technology*, (Golueke, C., Bidlingmaier, W., de Bertoldi, M., Diaz, L., eds), Elsevier.pp 67-87.

Díaz, L.F. and Savage, G, M. (2007). Factors that affect the process. **In**: *Compost Science and Technology*, (Golueke, C., Bidlingmaier, W., de Bertoldi, M., Diaz, L., eds), Elsevier.pp 26-45.

Dick, W.A. and Tabatabi, M.A. (1984). Kinetic parameters of phosphomonoesterase in soils and organic materials. *Sol. Sci*. **137**:7-15

Dziejoski, J.E. and Kazanowska, J. (2002). Heat production during thermophilic decomposition of municipal wastes in the dano-system composting plant. **In**:

Microbiology of composting (Insam, H., Riddech, N. and Klammer, S., eds). Springer-Verlang, Berlin, Geremeny.

EHPEA (Ethiopian Horticulture Producer and Export Association) (2014). Annual report, Addis Ababa, Ethiopia. pp-1-70.

Eiland,F., Klamer,M., Lind,A., Leth,M. and Bååth, E. (2001). Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. *Microbial. Ecol*. **41**:272-280.

Eklind,Y., Beck-Friis,B., Bengtsson,S., Ejlertsson,J., Kirchmann,H., Mathisen,B., Nordkvist,E., Sonesson,U., Svensson,B. and Torstensson, L. (1997). Chemical characterization of source-separated organic household wastes. *Swed. J. Agri. Resear*. **27**:167-178.

Eriksson, K.E.L., Blanchette,R.A. and Ander, P. (1990). Microbial and enzymatic degradation of wood and wood components. Springer-Verlang, Berlin,Germany.

EPA (2010). *Environmnetal management program of the plan for accelerated dudtainable development for eradication poverty 2011-2015*, pp 25. Addis Ababa.

Fan, L., Soccol, A.T., Pandey, A. and Soccol, C.R. (2003). Cultivation of *Pleurotus* mushrooms on Brazilian coffee husk and effects of caffeine and tannic acid. *Micología Aplicada International*.**15:** 15-21.

Fernández-Gómez, M., Nogales, R., Insam, H., Romero, E. and Goberna, M.(2010). Continuous-feeding vermicomposting as a recycling management method to revalue tomato-fruit wastes from greenhouse crops. *Wast. Manage*. **30**: 2461-2468.

Fernández-Gómez, M., Nogales, R., Insam, H., Romero, E. and Goberna, M. (2012). Use of DGGE and COMPOCHIP for investigating bacterial communities of various vermicomposts produced from different wastes under dissimilar conditions. *Sci. Tot. Environ*. **414**: 664-671.

Finstein, M.S. and Morris, M.L.(1975).Microbiology of municipal solid waste composting. *Adv.Appl.Microbiol*.**19**:113-151.

Fogarty, A.M. and Tuovinen, O.H. (1991). Microbial degradation of pesticides in yard waste composting. *Microbiolog. Rev*. **55**: 225–233.

Franke-Whittle, I.H., Klammer, S.H. and Insam, H.(2005). Design and application of anoligonucleotide microarray for the investigation of compost microbial communities. *J. Microbiol. Meth*. **62**: 37-56.

Franke-Whittle, I.H., Knapp, B.A., Fuchs, J., Kaufmann, R. and Insam,H.(2009). Application of COMPOCHIP microarray to investigate the bacterial communities of different composts. *Microb. Ecol*. **57**: 510-521.

Fuchs, J.G. (2002). Practical use of quality compost for plant health and viability improvement. **In**: *Microbiology of composting*. (Insam, H., Riddech, N. and Klammer, S. eds.). Springer Verlang. Heidelberg. pp 435-444

Garcia, C., Hernandez,T., Costa, F., Ceccati, B. and Ciardi, C. (1992). Changes in ATP content, enzyme activity and inorganic nitrogen species during composting of organic wastes. *Can. J. Soil. Sci*.**72**: 243-253.

García-Gómez, A., Bernal, M.P. and Roig, A. (2003). Carbon mineralization and plant growth in soil amended with compost samples at different degrees of maturity. *Wast. Mang. Resour*.**21**: 161-171.

Giglotti, G., Valentini, F., Erriquens, F.G. and Said-Pullicino, D. (2005). Evaluating the efficiency of the composting process: a comparison of different parameters. *Geophys. Res. Abs*. **7**:96-106

Godden, B., Penninckx, M., Pierard, A. and Lannoye, R. (1996). Evolution of enzyme activities and microbial populations during composting of cattle manure. *European J. Appl. Microbiol. Biotechnol*. **17**: 306-310.

Gómez, R., Vazquez-Lima, F. and Sanchez-Ferrer, A. (2006). The use of respiration indices in the composting process: a review. *Was. Manage. Res*. **24**: 37-47.

Gómez-Brandón, M., Lazcano, C. and Domínguez, J. (2008). The evaluation of stability and maturity during the composting of cattle manure.*Chemosph.***70**: 436-444.

Goyal, S.,Dhull, S.K. and Kapoor, K.K. (2005). Chemical and Biological changes during composting of different wastes and assessment of compost maturity. *Bioresour. Technol*. **96**:1584-1591.

Hassen, A., Belguith, K., Jedidi, N., Cherif, A., Cherif, M. and Boudabous, A. (2001). Microbial characterization during composting of municipal solid waste, *Bioresour. Technol*. **80**: 217–225.

Haug, R.T. (1993). *The practical Handbook of Compost Engineering*. Lewis Publishers, Boca Ratan, Florida, pp 718

Herrmann, R.F. and Shann, J.F. (1997). Microbial community changes during the composting of municipal solid waste. *Microb. Ecol*. **33**: 78-85.

Higa, T. (1994). Effective Microorganisms: A New Dimension for Nature Farming. In: Proceedings of the International Conference on Kyusei Nature Farming (Parr, J.F. Hornick, S.B. and Simpson M.E. eds). U.S. Department of Agriculture, Washington, D.C, USA. pp. 20-22.

Hoitink H.A.J. Boehm, M,J. (1999). Biocontrol within the conext of soil microbial communities: a substrate-dependent phenomenon. *Annu.Rev.Phytopath*. **37**:427-446.

Hultman,J., Vasara,T.,Partanen,P.,Kurola,J., Kontro,M.H.,Paulin,L.,Auvinen, P. and Romantschuk,M.(2009). Determination of fungal succession during municipal solid waste composting using cloning-based analysis. *J.Appl. Microbiol*.**108**: 472-487.

Hultman, J., Kurola, J., Raininsalo, A., Kontro, M. and Romantschuk, M. (2010). Utility of molecular tools for optimization of large scale composting, **In**: *Microbes at*

Work. From Wastes to Resources. (Insam, H., Franke-Whittle, I.H., Goberna, M., eds.). Berlin, Heidelberg; Springer. pp, 135-152

Insam, H. and de Bertoldi, M. (2003). Microbiology of the composting process. **In**: *Compost Science and Technology*, (Golueke, C., Bidlingmaier, W., de Bertoldi, M., Diaz, L., eds.), Elsevier. pp. 25–48

Ishii,K., Fukui,M. and Takii, S. (2000). Microbial succession during a composting process as evaluated by denaturing gel electrophoresis analisi. *J.Appl.Microbiol*. **89**:768-777.

Ishii, K. and Takii,S. (2003). Comparison of microbial communities in four different composting processes as evaluated by denaturing gradient gel analysis.*J.Appl.Microbiol*. **95**:109-119.

Jack, A.L.H. and Theis,J.E. (2006). Compost and vermicompost as amendments promoting soil health. **In**: *Biological Approach to Sustainable Systems*. Taylor and Francis Group LLP. p 455

Jakobssen, S.T. (1995). Aerobic decomposition of organic waste 2. Value of compost as a fertilizer. *Resour. Conserv. Recy*. **13**: 57-71.

Joshua, R.S. Macauley, B.J. and Mitchell, H.J. (1998). Characterization of temperature and oxygen profiles in Windrow processing systems. *Comp. Sci. Util*. **6**: 15-28.

Jouraiaphy, A., Amir, S., El Gharous, M., Revel, J.C. and Hafidi, M. (2005). Chemical and spectroscopic analysis of organic matter transformation during composting of sewage sludge and green plant waste. *Int. Biodeterior. Biodegrad*. **56**:101-108.

Lasardi, K.E. and Stentiford, E.I. (1998). A simple respirometric technique for assessing compost stability. *Water Res*. **32**:3717-3723.

Lerch, R.N., Barbarick, K.A., Sommers, L.E. and Westfall, D.G. (1992). Sewage sludge proteins as labile carbon and nitrogen sources. *Soil. Sci.Soc. Am.J*. **56**:1470-1476.

Liu, D., Zhang, R., Wu, H., Xu, D., Tang, Z., Yu, G. and Zu, Z. (2011). Changes in biochemical and microbiological parameters during the period of rapid composting of dairy manure with rice chaff. *Bioresour. Technol*. **102**:9040-9049.

Lulu, B. and Insam, H. (2000). Medium-term effects of a single application of mustard residues on soil microbiota and C contents of vertisols. *Biol. Fertil. Soil*.**31**: 108-113.

Mathur, S.P., Owen,G., Dinel, H. and Scnitzer, M. (1993). Determination of compost biomaturity. *Biologic. Agri. Horti.* **10**: 65-85.

Miller, F.C. and Finstein. (1985). Materials balance in the composting of wastewater sludge as affected by process control. *J. Wat. Pollut. Contr. Fed*.**57**:122-127.

Mondini, C., Fornasier, F. and Sinicco, T. (2004). Enzymatic activity as a parameter for the characterization of the composting process. *Soil Biol. Biochem.***36**: 1587–1594.

Murthy, P.S. and Naidu, M.M. (2012). Sustainable management of coffee by-products and value addition-a review. *Resour.Conserv.Recycl*. **66**:45-58.

Muyzer, G., de Waal, E.C. and Utterlinden, A.G. (1993).Profiling of complex microbial populations by denaturing gradient gel electrophoresis (DGGE) analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol*. **59**(3): 595-700

Muyzer, G. and Smalla, K. (1998). Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenh*.**73**: 127-141.

Nakasaki, K., Shoda,M. and Kubota,H. (1985). Effect of temperature on composting of sewage sludge. *Appl.Environ.Microbiol*. **50**:1526-1530.

Nannipieri, P., Giagnoni, P,L., Landi,L. and Renena, G. (2012). Role of phosphatase enzyme in soil. **In**: *Phosphorus in Action. Soil Biology*. (Bünemann, E.K. ed.). Springer-Verlang Berlin, Heidelberg, pp 215-243

Novinscak, A., DeCoste, N.J., Surette, C., Filion, M. (2009). Characterization of bacterial and fungal communities in composted bio-solids over a 2 year period using denaturing gradient gel electrophoresis. *Can. J. Microbiol.* **55**: 375–387.

Palmisano, A.C. and Barlaz, M.A. (1996). *Microbiology of Solid Waste*. CRC Press INC. pp 224.

Pandey, A., Soccol, C.R., Nigan, P., Brand, D., Mohan, F. and Rovossos, S. (2000).

Biotechnological potential of coffee pulp and husk for bio-process. *Biochem. Engineer. J*. **6**: 153-162.

Pedro, M.S., Haruta, S., Hazaka, M., Shimada, R., Yoshida, C., Hiura, K., Ishii, M. and Igarashi Y. (2001). Denaturing gradient gel electrophoresis analyses of microbial community from field-scale composter. *J. Biosci. Bioeng.* **91**(2):159-165.

Potvin, C., Lechowicz, M.J. and Tardif, S. (1990). The statistical analyses of ecological response curves obtained from experiments involving repeated measures. *Ecol*. **71**: 1389–1400.

Raut, M.P., Prince William, S.P.M.,Battacharyya, J.K., Chakarbarti, T. and Devotta, S. (2008). Microbial dynamics and enzyme activities during rapid composting of municipal solid waste: A compost maturity analysis perspective. *Bioresour. Technol*. **99**: 6512-6519.

Rebollido, R., Martinez, J., Aguilera, Y., Melchor, K., Koerner, I. and Stegmann, R. (2008). Microbial populations during composting process of organic fraction of municipal solid waste. *Appl. Ecol. Environ. Res*. **6**(3): 61-67.

Richard, T.L., Hamelers, H.V.M.(Bert)., Veeken, A. and Silva, T. (2002). Moisture relatioships in composting processes. *Comp. Sci.Util.* **10**(4):286-303.

Ryckeboer, J., Mergaert, J., Vaes, K., Klammer, S., De Clercq, D., Coosemans, J., Insam, H. and Swings, J. (2003a). A survey of bacteria and fungi occurring during composting and self-heating processes. *Ann. Microbiol.* **53**: 349–410.

Ryckeboer, J., Mergert, J., Coosemans, J., Deprins, K. and Swings, J. (2003b). Microbiological aspects of bio-waste during composting in monitored compost bin. *J. Appl. Microbiol*. **94**:127-137.

Sathyanarayana, A. and Khan, AB. (2008). An eco-biological approach for resource recycling and pathogen (Rhizoctonia solani Kuhn) suppression. *J. Environ. Protect. Sci.* **2**:36-39

Saha, N., Mukherjee, D., Sen, S., Sarkar, A., Bhattacharya, K.K., Mukhopadya, N. and Patra, P.K. (2012). Application of highly efficient lingo-cellulolytic fungi in composting of paddy straw amended poultry dropping for the production of humus rich compost. *Comp. Sci. Util*. **20**(4): 239-244.

Said-Pullicino, D., Erriquens, F.G. and Gigliotti, G. (2007). Changes in the chemical characteristics of water-extractable organic matter during composting and their influence on compost stability and maturity. *Bioresour. Technol*.**98**:1822-1831.

Sánchez-Monedero, M.A., Roig, A., Paredes, C. and Bernal, M.P. (2001). Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of the composting mixtures. *Bioresour. Technol*. **78**: 301–308.

Schlegelmilch, M., Streese, J., Biedermann. W., Herold, T. and Stegmann, R. (2005). Odor control at biowaste composting facilities. *Was. Manage*. **25**(9): 917-927.

Scholass,P., Hay, A.G.,Wilson, D. B, Gossett, J.M. and Walker, L,P. (2005). Quantifying bacterial population dynamics in compost using 16S rRNA gene probes. *Appl. Microbiol Biotechnol*. **66**:457-463.

Selim, Sh. M., Zayed, M.S. and Atta, H.M. (2012). Evaluation of phytotoxicity of compost during composting process. *Natu.Sci*. **10**(2):69-77.

Sharma, S., Piccolo, A. and Insam, H.(1997). Different carbon source utilization profiles of four tropical soils from Ethiopia. **In**: *Microbial communities-functional versus structural approaches*. (Insam, H. and Rangger, A., eds.). Heidlsberg, Springer. pp 132-139.

Silva, C.F., Schwan, R.F., Dias, E.S. and Wheals, A.E. (2000). Microbial diversity during maturation and natural processing of coffee cherries of *Coffee arabica* in Brazil. *Int.J. Food Microbiol*.**60**: 251-260.

Soumarė, M., Demeyer, A., Tack, F.M.G. and Verloo, M.G. (2002). Chemical characteristics of Malian and Belgian solid waste composts. *Bioresour. Technol*. **81**: 97–101.

Steger, K., Sjögren., Å.M., Jarvis, Å., Jansson, J.K. and Sundh, I. (2007). Development of compost maturity and *Actinobacteria* populations during full-scale composting of organic household waste. *J. Appl. Microbiol*.**103**: 487-498.

Strom, P.F. (1985) Effect of temperature on bacterial species diversity in thermophilic solid-waste composting. *Appl. Environ. Microbiol.* **50**(4):899-905..

Tabatabai M.A. (1994) Soil enzymes. **In**: *Methods of soil analysis. Part 2. Microbiological and biochemical properties*. (Weaver, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A. and Wollum, A. eds.). *Soil Sci. Soci. America*, Madison. pp 775–833.

Takaku, H., Kodaira, S., Kimoto, A., Nashimoto, M. and Takagi, M. (2006). Microbial communities in the garbage composting with rice hull as an amendment revealed by culture-dependent and -independent approaches. *J. Biosci. Bioeng*.**101**: 42–50.

Tenaw Workayehu, Husni, M.H.A., Anuar., A.R. and Zaharah, A.R. (2006). Effect of coffee bean residues and time on soil nitrogen availability. *Malay.J.Soil.Sci.***10**:53-65. ISSN: 1394 7990.

Tenaw Workayehu, and Kelsa, K.(1998). Residual effect of coffee husk/pulp on grain yield of maize. **In**: *Maize Production Technology for the Future: Challenges and Opportunities. Proceedings of the sixth Easteran and Southern Africa Regional Maize Conference*. pp, 271-273. September 1998.CIMMYT Maize program and Ethiopian Agricultural Researh Organization (EARO). Addis Ababa, Ethiopia.

Ter Braak, C.J.F., Smilauer, P. (2002). CANOCO Reference manual and Canodraw for windows user's guide: software for canonical community ordination (version 4.5). Microcomputer power, Ithaca, NY, USA.

Tiquia, S.M., Tam, N.F.Y. and Hodgkiss, I.J. (1996). Microbial activities during composting of spent pig-manure sawdust litter at different moisture contents. *Bioresour. technol*. **55**:201-206.

Tiquia, S.M., and Tam, N.F.Y. (1998). Elimination of phytotoxicity during co-composting of spent pig-manure sawdust litter and pig sludge. *Bioresour. Technol*. **65**: 43-49.

Tiquia, S.M. (2002). Evolution of extracellular enzyme activities during manure composting. *J. Appl. Microbiol*.**92**: 764-775.

Tiquia, S.M., Wan, J.H.C. and Tam, N.F.Y. (2002). Microbial population dynamics and evolution of enzyme activities during composting. *Comp. Sci. Util*. **10**: 150-161.

Tiquia, S.M. (2005). Microbiological parameters as indicators of compost maturity. *J. Appl. Microbiol. Biotechnol.* **99**:79-89.

Tuomela, M., Vikman, M., Hatakka, A. and Itävaara, M. (2000). Biodegradation of lignin in a compost environment: a review. *Bioresour. Technol*. **72**: 169-183

Tuomela, M., Hatakka,A.,Raiskila,S.,Vikman, M. and Itävaara, M.(2002). Biodegradation of radiolabelled synthetic lignin (^{14}C-DHP) and mechanical pulp in a compost environment.*Appl.Microbiol.Biotechnol*.**55**:492-499.

UNEP (2009). *Developing integrated solid waste management plan training manual*. pp 2. International Environmental Technology Center. Osaka/Shiga, Japan.

US-EPA (2006). *Municipal solid waste generation, recycling, disposal in the United States: facts and figures in 2006*. pp 5. United States Environmental Protection Agency, Washington DC 20460.

Vargas-García, M.C., Suarez-Estrella, F., Lopez, M.J. and Moreno, J. (2010). Microbial population dynamics and enzyme activities in composting processes with different starting materials. *Was Manage*. **30**: 771–778.

Vieira, S. And Abarca, L. (2009). Floriculture waste management: best practices to Ethiopia. pp27. University of Se Santa Catarina, Technology Center

Vuorenin, A.H. (2000). Effct of the bulking agenton acid and alkaline phosphophmonesterase and β-D-glucose activities during manure composting. *Bioresour. Technol*. **75**: 133-138.

Waksman,S.A., Cordon,T.C. and Hulpol, N. (1939). Influence of temperature upon the microbiological population and decomposition process in composts of stable manure. *Soil Sci*. **47**:83-98.

Waksman, S.A. and Cordon,T.C. (1939). Thermophilic decomposition of plant residues in composts by pure and mixed cultures of microorganisms. *Soil Sci*. **47**:217-225.

Walter, M.V. (1997). Bioaugmentation. **In**: *Manual of Environmental Microbiology*. American. Soc. Microbiol. (Hurst, C. J., Kundson, G. R., McInerney, M. J., Stetzenbach, L. D. and Walter, M. V. Eds.). pp 753.

Wang, P., Changa, C.M., Watson, M.E., Dick,W.A., Chen,Y. and Hoitink, H.A.J. (2004). Maturity indices for composted dairy and pig manures. *Soil. Biol. Biochem*. **36**: 767-776.

Webley, D.M. (1947). The microbiology of composting I. The behavior of the aerobic mesophilic bacterial flora of composts and its relation to other taking place during composting. *Proceed. Soci. Appl. Bateriol*. **2**:83-89.

Wiersum, K.F., Gole, T.W., Gatzweiler, F., Volkmann, J., Bognetteau, E. and Wirtu, O. (2008). Certification of wild coffee in Ethiopia: experiences and challenges. *Forest Trees.Livelihoods.***18**: 9-21.

Wu, L., Ma. L. Q. and Martinez, G.A.(2000). Composition of methods evaluating stability and maturity of bio-solids compost. *J. Environ. Qual*. **27**:424-429.

Zmora-Nahum, S., Markovitch,O., Tarchitzky, J. and Chen, Y. (2005). Dissolved organic carbon (DOC) as a parameter of compost maturity. *Soil Biol. Biochem*. **37**:2109-2116.

Zucconi, F., Pera, A., Forte, M. and de Bertoldi, M. (1981). Evaluating toxicity of immature compost. *Biocycle*. **22**: 54–57

Zucconi, F., Monacco, A. and Forte, M. (1985). Phytotoxins during the stabilization of organic matter. **In**: *Composting of Agricultural and Other Wastes*. (Gasser, J.K.R. ed.). Elsvier, New York, pp73-86.

Zucconi, F. and de Bertoldi, M. (1987). Compost specification for the production and characterization of compost from municipal solid waste. **In**: *Compost, Production, Quality and Use*. (de Bertoldi, M., Ferranti, M.P., Hermite, P.L. and Zucconi, F., eds.). Elsevier Applied Science Publishers, Barking, pp 30-50.

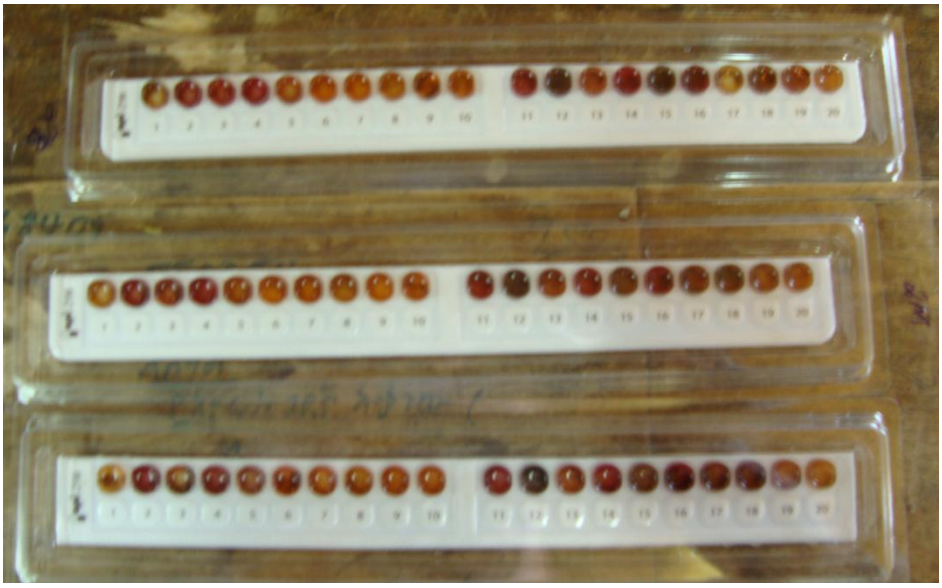
Appendices

Appendix 1 Germination and root growth of garden cress seeds after 24h incubation

Appendix 2 Work in Applied Microbiology Lab

Appendix 3 Coffee husk composting arrangement

Appendex 4 API ZYM™ Kits after testing (extracellular enzymes)



Appendex 5 Microbial enumeration using most probable number