MICROBIAL SURVIVAL, MATURITY AND HEALTH HAZARDS IN DIFFERENT COMPOSTING SYSTEMS

BY

KUTSANEDZIE FELIX

B.Sc. (Hons.) Agriculture (KNUST)

KNUST

A Thesis submitted to the Department of Agricultural Engineering,

Kwame Nkrumah University of Science and Technology, Kumasi

in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

BIOENGINEERING

DEPARTMENT OF AGRICULTURAL ENGINEERING

FACULTY OF MECHANICAL AND AGRICULTURAL ENGINEERING

COLLEGE OF ENGINEERING

JUNE, 2009

DECLARATION

I hereby declare that, with the exception of the pieces of information sourced from books duly referenced, all other information contained in this dissertation emanated from my research towards the fulfillment of the award of an MSc. Degree.

Kutsanedzie Felix **Signature**.......................... **Date**11th November, 2009

(Candidate: PG 9508606)

KNUST

CERTIFICATION BY

Dr. E.D. Aklaku **Signature**.......................... **Date**9th Nov. 2009

(Supervisor)

Ing. Ato Bart-Plange **Signature**.......................... **Date**11—11—09

(Supervisor)

NYANSAPƆ WↃSANE NO BADWENMA

CERTIFIED BY

Ing. Ato Bart-Plange **Signature**.......................... **Date**11—11—09

(Head of Department)

LIBRARY
KWAME N RUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

ABSTRACT

Temperature, moisture and pH are known parameters that affect compost microbial community, maturity and quality, but vary in different systems. In assessing the efficiency of composting systems, the need to produce quality compost devoid of pathogenic microorganisms that pose health hazards in the food chain is very important. Temperature, moisture and pH development in four composting systems were studied for thirteen weeks to assess their effects on total viable count, total coliform count, total fungi count, helminth eggs count and germination index. ANOVA preformed on temperature values recorded in the different systems were significantly different as p-value yielded 4.75×10^{-7}. However the moisture and pH values recorded were not significantly different as these gave p-values of 0.9842 and 0.0632 respectively. P-values for total viable count, total coliform count, total fungi count and germination indices in the different systems yielded 0.027, 0.9994, 0.9681 and 0.0904 respectively. No helminth eggs were seen in all the systems. Generally, the total viable count reduced while total fungi increased at the end of week 12 in all systems. However, total coliform reduced to 0 during week 4.The pH values of compost masses ranged between 7 and 9 whereas moisture reduced from the range of 61% - 72 % to 40% - 60% from week 4 to week 12 in all systems. Temperatures recorded in DAT and HV systems were higher than those recorded in FA and T-W systems during the process. The lowest temperatures were recorded in FA allowing more microorganisms to survive in it. Bacteria such as *Staphylococcus spp.*, *Streptococcus spp.*, *Bacillus spp.*, *Corynebacterium spp.*, *Yersinia spp.*, *Listeria spp.*, *Campylobacter spp.*, *Enterobacter spp.* and *Clostridium spp.* were identified in the systems. *Penicillium spp.*, *Aspergillus spp.*, *Mucor spp.* and *Rhizopus spp.* were the fungi identified. *Bacillus spp.* and *Corynebacterium spp.*, present in a starter added to the compost masses in each system to facilitate the decomposition, survived in all the systems. Other microorganisms such as *Listeria spp.*, *Staphylococcus spp.*, *Penicillium spp.* and *Mucor spp.* survived the process in some of the systems. *Listeria spp.*, *Penicillium spp.* and *Staphylococcus spp.* are known to cause diseases in humans. The final germination indices recorded were 189.54%, 198.11%, 202.83% and 222.64% in DAT, HV, FA and T-W systems respectively indicating the measure of compost maturity in the systems.

KNUST

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

TABLE OF CONTENTS

Title Page i
Declaration ii
Abstract iii
Table of Content iv
List of Tables x
List of Figures xii
Abbreviations/Symbols xv
Acknowledgement xvi
Dedication xvii
CHAPTER ONE: INTRODUCTION 1
1.1 Background 1
1.2 Study Area and Systems 3
1.3 Research Questions 4
1.4 Problem Statement 4
1.5 Objectives 5
1.6 Specific Objectives 5
1.7 Justification 6
CHAPTER TWO: LITERATURE REVIEW 10
2.1 Definition of Composting and Compost 10
2.2 Composting Systems 10
2.3 Compost Microorganisms Sources 11
2.4 Microbe Types and Requirements 12
2.4.1 Fungi. 12
2.4.2 Bacteria 13
2.4.3 Actinomycetes 13
2.5 Composting Process 14
2.5.1 Mesophilic Phase 15
2.5.2 Thermophilic phase 15

KNUST

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

2.5.3 Cooling/Maturation stage 16
2.6 Temperature 17
2.7 Moisture 17
2.8 Hydrogen ion concentration (pH) 18
2.9 Variations in Temperature, Moisture and pH during Composting 18
2.10 Compost Maturity 21
2.11 Determination of Compost Maturity 23
2.12 Sampling of compost material 24
2.13 Holding samples 24
2.14 Preparation of samples 24
2.15 Methods used for the Enumeration of Microorganism 25
2.16 Total Viable Counts 25
2.16.1 Plate Count (Pour) 25
2.16.2 Plate Count (Surface) 26
2.16.3 Roll Tube 26
2.16.4 Burri Strip or Slant 27
2.16.5 Membrane Filters 27
2.17 Tube Dilution 27
2.17.1 Most Probable Numbers (MPN) 28
2.18 Identification of Microorganisms 28
2.19 Helminths Eggs Identification Counting Techniques 29
2.19.1 Sedimentation technique 29
2.19.2 Flotation technique 29
2.19.3 Concentration technique 29
2.19.4 The Baerman technique 30
2.19.5 McMaster egg-counting technique 30
CHAPTER THREE: MATERIALS AND METHODS 32
3.1 Composting Materials 32
3.2 Description of the Composting Systems Under study 33

KNUST

3.2.1 Dome Aerated Technology 33

3.2.2 Horizontal-Vertical Aeration Technology 35

3.2.3 Forced Aeration Technology 35

3.2.4 Turned Windrows 36

3.3 Temperature Determination 37

3.4 Sampling of Compost Mass for Physicochemical and Microbiological Analysis in the laboratory 37

3.5 Physicochemical Analysis 38

3.5.1 Compost Masses pH Determination 38

3.5.2 Moisture Determination 38

3.6 Microbiological Analysis 38

3.6.1 Sterilization Procedures 38

3.6.1.1 Glass wares 39

3.7 Media Preparation 39

3.7.1 Standard Plate Count Agar 39

3.7.2 Violet-Red Bile Agar 39

3.7.3 Blood Agar 40

3.7.4 MacConkey Agar 40

3.7.5 Brilliant Green Agar 40

3.7.6 Eosin Methylene Blue Agar 40

3.7.7 Sabouraud Agar 41

3.8 Preparation of Gram Stains 41

3.9 Standard Operating Procedures 41

3.9.1 Serial Dilution for Total Viable and Coliform Count 42

3.10 Fungi Count 43

3.11 Culturing of Microorganism 43

3.12 Sub-culturing of Microorganisms 44

3.13 Identification of Microorganisms 44

3.14 Preparation of Smears 45

KNUST
NKANSAPO W'OSANE NO BADWENMA

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

3.15 Gram Staining of Bacteria 45

3.16 Examination of Slides 46

3.17 Counting of Helminths Eggs 46

3.18 Germination Index Determination 47

3.19 Result Analysis 48

CHAPTER FOUR: RESULT AND DISCUSSION 49

4.1 Hydrogen ion Concentration (pH) 49

4.2 Moisture Content 51

4.3 Temperature 53

4.4 Total Viable Count 55

4.5 Total Coliform Count 57

4.6 Total Fungi Count 58

4.7 Germination Index 60

4.8 Helminth Egg Counts 61

4.9 Microbial Community 62

4.10 Effects of pH and Moisture on Composting Systems 63

4.11 Effects of Temperature on Factors Determined in the Dome Aerated Technology 64

4.11.1 Total Viable Count 64

4.11.2 Total Coliform Count 65

4.11.3 Total Fungi Count 66

4.11.4 Germination Index 67

4.12 Effects of Temperature on Factors determined in the Horizontal-Vertical Technology 69

4.12.1 Total Viable Count 69

4.12.2 Total Coliform Count 70

4.12.3 Total Fungi Count 71

4.12.4 Germination Index 71

KNUST

4.13 Effects of Temperature on Factors Determined in the Forced Aeration System 73
4.13.1 Total Viable Count 73
4.13.2 Total Coliform Count 75
4.13.3 Total Fungi Count 75
4.13.4 Germination index 76
4.14 Effects of Temperature on the Factors Determined in the Turned-Windrow System 78
4.14.1 Total Viable Count 78
4.14.2 Total Coliform Count 79
4.14.3 Total Fungi Count 79
4.14.4 Germination Index 80
CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS 82
5.1 Conclusion 82
5.2 Hydrogen ion Concentration 82
5.3 Moisture Content 82
5.4 Temperature 83
5.4 Total Viable Count 84
5.5 Total Coliform Count 84
5.6 Total Fungi Count 85
5.7 Germination Index 85
5.8 Microbial Community 86
5.9 Health hazards 86
5.10 Recommendations 87
REFERENCES 89
APPENDICES 99
APPENDIX A: Summary of Analysis of Variance for pH, Temperature and Moisture Content for the Different Composting Systems 99
APPENDIX B: Summary of the Coefficient of Correlation Values Between

KNUST

pH, Temperature and Moisture Content Recorded in the Different Composting Systems. 100
APPENDIX C: Summary of Tukey's Test Critical Values for Pair Wise Comparisons of the Means of Temperatures and Total Viable Counts Recorded in the Different Systems 101
APPENDIX D: Table of Critical Values for the Tukey Test at $\alpha = 0.05$ 102
APPENDIX E: Formula and Method Used in Extrapolation of Critical Value from Tukey's Test Table 103
APPENDIX F: Summary of ANOVA of Total Viable Counts, Total Coliform Counts, Total Fungi Counts and Germination Indices in the Different Composting Systems 104
APPENDIX G: Summary of the Cultural Features and Gram stain reactions of bacteria Identified 105
APPENDIX H: Summary of Cultural Features of Fungi Identified 106
APPENDIX I: Weekly Percentage Frequencies of Bacteria Identified in the Different Composting Systems 107
APPENDIX J: Weekly Percentage Frequencies of Fungi Identified in the Different Composting Systems 109
APPENDIX K: Some of the Microorganisms Identified in the Compost Masses 110
APPENDIX L: Tables of pH, Moisture Content, Temperature, Total Viable Count, Total Coliform Count, Total Fungi Count and Germination Index Recorded in the Different Systems. 111

KNUST

LIBRARY
KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
KUMASI-GHANA

LIST OF TABLES

Table1: Period necessary for composting depending on the implemented methods and substrates used 11

Table A.1: ANOVA for pH in the Composting Systems 99

Table A.2: ANOVA for Moisture Content in the Composting Systems 99

Table A.3: ANOVA for Temperature in the composting systems 99

Table B.1: Correlation Between Measured Parameters in DAT Composting System 100

Table B.2: Correlation Between Measured Parameters in HV Composting System 100

Table B.3: Correlation Between Measured Parameters in FA Composting System 100

Table B.4: Correlation Between Measured Parameters in T-W Composting System 100

Table C.1: Tukey's Test Critical Values for the Pair Wise Comparisons of Temperatures Recorded in the Different Composting Systems 101

Table C.2: Tukey's Test Critical Values for the Pair Wise Comparisons of Total Viable Count Recorded in the Different Systems 101

Table D.1: Table of Critical Values for the Tukey's Test at $\alpha = 0.05$ 102

Table F.1: ANOVA of the Total Viable Counts in the Composting Systems 104

Table F.2: ANOVA of Total Coliform Counts in the Composting Systems 104

Table F.3: ANOVA of Total Fungi Counts in the Composting Systems 104

Table F.4: ANOVA of Germination Indices in Composting Systems 104

Table G.1: Summary of the Cultural Features and Identification of the Bacteria present in compost masses 105

Table H.1: Summary of the Cultural Features of the fungi present in the compost masses 106

Table I.1: Weekly Percentage Frequencies of Bacteria Identified in Compost Masses in Systems DAT and HV 107

KNUST

Table I .2: Weekly Percentage Frequencies of Identified Bacteria in Compost Masses in the Systems FA and TW 108

Table J.1: Weekly Percentage Frequencies of Fungi Identified in the Compost Masses in the Different Composting Systems 109

Table L.1: Average weekly pH of compost masses recorded in the composting systems 111

Table L.2: Average weekly moisture content (%) of compost masses recorded in the composting systems 111

Table L.3: Average weekly temperatures (°C) of compost masses recorded in the composting systems 112

Table L.4: Average weekly Total Viable Count (LogCFU/g of Compost) recorded in the Composting Systems 112

Table L.5: Average weekly Total Coliform Count (LogCFU/g of Compost) recorded in the Composting Systems 113

Table L.6: Average weekly Total Fungi Count (LogCFU/g of Compost) recorded in the Composting Systems 113

Table L.7: Average weekly Germination Indices of Compost masses recorded in the Composting systems 114

KNUST
NYANSAPƆ WƆSANE NO BADWENMA

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

LIST OF FIGURES

Fig. 1: Phase changes during the composting process 13

Fig. 2: Containment of the starter added to compost masses 32

Fig. 3: A schematic diagram of the Dome Aerated Technology (DAT) system 33

Fig. 4: (a) Cross sectional area of the DAT system 34

(b) Pile preparation at VREL 34

Fig.5: (a) DAT system covered with Toptex (fleece) sheet 34

(b) Bamboo dome removed after 2 weeks for reshaping of pile 34

Fig. 6: (a) Perforated uPVC pipes 35

(b) Prepared pile 35

Fig.7: (a) Perforated pipe network connected to the blower 36

(b) Mounting the pile 36

(c) Mounted pile for Forced Aerated System 36

(d) Pile covered with fleece 36

Fig. 8: (a) Sandberger ST 300 turning feed stock pile 37

(b) Covered windrow pile 37

Fig.9: (a) Prepared Peptone water 39

(b) Plate Count Agar 39

(c) Violet-Red Bile Agar 39

Fig.10: Compost samples being prepared for serial dilution 41

Fig.11: Colonies viewed under the colony counter 42

Fig.12: Drying of media for culturing of microorganisms 43

Fig.13: Growth observed on MacConkey Agar 44

Fig. 14: Preparation of smears 45

Fig.15: Examination of microorganisms under the microscope 46

Fig.16: Filling of McMaster Egg Counting Chamber with prepared samples 47

Fig.17: Weekly variations in pH of compost masses in the different composting systems 50

KNUST

Fig.18: Weekly variations in moisture content of compost masses in the different composting systems 52

Fig.19: Weekly variations in temperatures of compost masses recorded in the different composting systems 54

Fig.20: Weekly variations in the Total Viable Count of compost masses recorded in the different composting systems 56

Fig.21: Weekly variations in the Total Coliform Count of compost masses recorded in the different composting systems 57

Fig. 22: Weekly variations in the Total Fungi Count of compost masses recorded in the different composting systems 60

Fig.23: Weekly variations in Germination Indices of compost masses recorded in the different composting systems 61

Fig.24: Influence of Temperature on Total Viable, Total Coliform and Total Fungi Counts in DAT system. 64

Fig.25: Variations in Temperature and Germination Index in the DAT system 67

Fig.26: Variations in Germination Index, Total Viable Count and Total Fungi Count in the DAT system 69

Fig. 27: Temperature variations on Total Viable Count, Total Coliform Count and Total Fungi Count in the HV system 69

Fig.28: Variations in Temperature and Germination Index in the HV system 72

Fig.29: Variations in Germination Index, Total Viable Count and Total Fungi Count in the HV system 73

Fig.30: Variations in Temperature, Total Viable Count, Total Coliform Count and Total Fungi Count in the FA system 74

Fig.31: Variations in Temperature and Germination Index in the FA system 76

Fig.32: Variations in Germination Index, Total Viable Count, Total Coliform Count and Total Fungi Count in the FA system 77

Fig.33: Variations in Temperature, Total Viable Count, Total Coliform Count and Total Fungi Count in the T-W system 78

KNUST

Fig.34: Variations in Temperature and Germination Index in the T-W system 80

Fig.35: Variations in Germination Index, Total Viable Count, Total Coliform Count and Total Fungi Counts in the T-W system 81

Fig.36: *Bacillus spp.* viewed under microscope 110

Fig.37: *Corynebacterium spp.* viewed under the microscope 110

Fig.38: *Staphylococcus spp.* viewed under the microscope 110

KNUST

NYANSAPɔ WɔSANE NO BADWENMA

ABBREVIATIONS/SYMBOLS

ANOVA	Analysis of Variance
AOAC	Analytical Office of Applied Chemistry
d.f	Degree of freedom
DAT	Dome Aerated Technology
FA	Forced Aeration System
Fig.	Figure
GI	Germination Index
HV	Horizontal-Vertical System
k	Number of means
LogCFU/g of compost	Logarithm of Colony Forming Unit per gram of compost
MPN	Most Probable Number
n	Number of samples
pH	Hydrogen ion Concentration
q_{cal}	Tukey's Test calculated values for two means
q_{crit}	Critical value from Tukey's Test
r	Correlation Coefficient
rpm	Revolution per minute
s_w^2	Within system variance
TEMP.	Temperature
TCC	Total Coliform Count
TFC	Total Fungi Count
TVC	Total Viable Count
T-W	Turned Windrow System
α	Significance level
$\bar{x}$	Mean of Sample

KNUST
NYANSAPO WOSANE NO BADWENMA

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

ACKNOWLEDGEMENTS

Holding a fond recollection of the litanies of problems and the several near occasions of accidents from which I escaped narrowly, I have all the cause to say God has been my source of refuge and thus far He has brought me. May His name forever be praised, honoured and glorified.

For the criticisms, corrections and suggestions offered by my supervisors Dr. E.D. Aklaku, Ing. Ato Bart-Plange of the Department of Agricultural Engineering, KNUST and the late Dr. A.D. Agyei of CSIR (Animal Research Institute), without whose help the quality of this project would have been substandard; I must say a big thank you and indeed register a deep appreciation for all you have done for me.

Dr. Hope Otysina, Mr. Cornelius Takyi-Arthur, Mr. Stephen Ogbete, Mr. Julius Beyuo, Mr Humprey Amafu-Dey, Mr. Tobias Bonsi and Mr. Abraham Okoe-Teye all of the Microbiology and Parasitology laboratories of the Animal Research Institute of the Council for Scientific and Industrial Research for making me tap from their rich experience and expertise in achieving this success. May God richly bless you.

I am greatly indebted to Mr. and Mrs. Jacob Kutsanedzie, sisters and brother for their relentless efforts in encouraging and financially supporting me throughout my research. May God replenish the seed you have sown abundantly.

My sincere appreciation also goes to Mr. George Rockson for his pieces of advice and support as a project partner; you must definitely share in this success!

I would like to register my utmost appreciation for the Head of CSIR (Animal Research Institute) and Manager of Volta River Estate Limited Farms for allowing access to their facilities in the course of carrying out the research.

Finally, to all loved ones whose prayers and contributions in diverse ways have helped bring about this success, you all stand very tall in my memory.

KNUST

Dedication:

This book is dedicated to the memory of the late Dr. A.D. Agyei of Animal Research Institute (CSIR), the renowned researcher, one time national best agricultural researcher (1999) and two times the National President of the Research Staff Association (2002/2003) of the CSIR for his guidance and contributions to the success of this work.

KNUST

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Organic waste generation emanates from activities of living things. Growth in the world's population has the consequent effect of increasing organic waste generation and accumulation. Sequel to this, scientists the world over continue to gear efforts toward studying appropriate organic waste management and disposal methods, in order to offset the associated problems of environmental pollution and possible health hazards that emanate from waste accumulation and disposal.

Land-filling, incineration, composting and anaerobic digestion are the known waste disposal methods. Composting continues to gain importance as its product serves as a source of amendment to improve soil organic matter level and soil fertility. The benefits of composted organic wastes to soil structure, fertility as well as plant growth have been increasingly emphasized (Chen *et al.*, 1992; Murwira et al., 1995; Esse *et al.*, 2001).

Several reasons make composting an option highly adoptable as a future waste disposal system relative to the others. It is a technology that can be used at any scale from household to regional and also makes spot treatment possible with reduction in waste transport. The compost product can be dried and sold on the soil product market whereas product from anaerobic digestion is normally slurry which is marketable to agriculture. Composting is also flexible as compared to anaerobic digestion and incineration vis-à-vis size and time frame for planning and construction as well as pay-back time for investment (Sundberg, 2005). Pace-Moody (2003) revealed that 10% to 15% of solid waste collected in Accra is composted. Composting in Accra

KNUST

occurs at the Teshie-Nungua Compost Plant, located east of the city centre and was established in 1980 by the Ministry of Local Government and the Accra Metropolitan Assembly. This facility composted more than 60 tonnes of domestic waste each day in 2000. The daily volume of composted waste has now reached between 125 and 188 tons per day. The compost is bought and used in vegetable gardens and farms around the city as a soil conditioner which forms a substitute for imported chemical fertilizers. There are also large private farms in Ghana, notable among these are the Volta River Authority Limited Farms (VREL) and Blue Sky Pineapple Farms. VREL Farms located at Akrade in the Eastern Region, composts organic manure bought from neigbouring animal farms, households and agro-waste generated from their own farms for fertilizer. The organic fertilizer obtained is used in the cultivation of pineapple and banana for export. Blue Sky Pineapple Farms located on the Accra-Nsawam road also uses compost prepared from its farm waste for cultivation of pineapples for export.

KNUST

There are many composting systems used for treatment of different wastes under different conditions. Common systems at large scale include: naturally aerated windrow systems-long rows with triangular cross section; forced aeration static pile systems; tunnel systems-closed rotating cylinders (Sundberg, 2005).

Composting is an exothermic aerobic biological process that stabilizes biodegradable organic matter. It is a microbial process and the overall performance of the composting process is therefore the combined effect of individual organisms (Miller, 1993). Decomposition rates are affected by all factors that commonly affect microbial growth, i.e. carbon, nitrogen, oxygen, moisture, pH, temperature, and nutrient levels (Miller, 1993). The heat generated during composting is reported by Golueke (1977) to help in destruction of pathogens. Factors affecting the rate of decomposition may vary from one composting system to the other, hence affecting compost microbial growth and maturity. Composting is a biotransformation process and for it to be termed as successful, its final product must have pathogens capable of posing health hazards to plants and end users reduced or totally eliminated. More so, it must be matured before application onto the field. This study therefore focuses on the efficiency of different composting systems in the reduction or elimination of pathogenic microorganisms, increasing the rate of maturity, and thus obtaining a more valuable final product via temperature, pH and moisture conditions developed in the systems.

1.2 Study Area and Systems

Volta River Estate Limited Farms is located at Akrade in the Eastern Region of Ghana and owns five farms. They are engaged in banana and pineapple production. One of the five farms uses organic fertilizers for production and the others chemical fertilizers. The organic fertilizer is prepared via composting using waste materials from the farm and environs. Volta River Estate Limited is a private company and proceeds from the farms are exported to the Netherlands. Composting is prepared on site using the windrow system.

For this study, the following systems mounted on the farm's composting site were considered:

1. Mechanically turned windrow pile (T-W)
2. Dome Aerated Technology passive pile (DAT)
3. Horizontal-Vertical passive aerated pile (HV)
4. Forced aerated static pile (FA)

KNUST

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

1.3 Research Questions

During composting, heat is generated and this increases the temperature in the compost matrix. The heat generated helps reduce or destroys pathogens as well as affects the survival of the beneficial microorganisms responsible for degradation. Different temperature, pH and moisture ranges are expected in different composting systems.

KNUST

The research poses the following questions:

1. What temperature, pH and moisture range will be recorded in the different composting systems under study?
2. What will be the initial and final population of microorganisms in the different composting systems?
3. What type of microorganisms will be present initially and at maturity of the compost in the different systems?
4. How will temperature, pH and moisture content affect the rate of compost maturity in the different systems?
5. Which microorganisms constitute the compost microbial community in the different systems?
6. Will the temperature, pH and moisture conditions in the different systems help eliminate pathogens without retarding the decomposition process?

1.4 Problem Statement

Although the use of composting is increasingly gaining grounds as a means of making organic fertilizers, the process is besieged with the following problems:

1. The presence of zoonotic and pathogenic micro organisms in the final product which poses health hazards to both farmers and consumers of compost grown foodstuffs
2. Lack of enough data in literature for inactivation of pathogenic microorganisms during composting in different systems.
3. Lack of information on the selection of systems that provide better control conditions, hence higher process efficiency which results in quality product and decreased number of pathogens.
4. Lack of data on compost quality and rate of maturity in different systems.

To tackle the stated problems, different composting systems were mounted at the composting site of VREL to study the temperature, pH and moisture conditions prevailing in each system in relation to total viable count, total coliform count, total fungi count, microbial identification and germination index determination for their compost matrixes for a period of thirteen weeks.

KNUST

1.5 Objectives

The prime objective of this study was to assess the performance of the different composting systems in terms of the rate of compost maturity and reduction or elimination of pathogenic microorganisms which pose health threats to compost producers, end-users and compost grown product consumers.

1.6 Specific Objectives

In order to achieve the prime objective, the research targets the following specific objectives:

1. To determine the weekly total viable count for the composting period.
2. To determine the weekly total faecal coliform count for the composting period.

3. To determine the weekly helminths eggs for the composting period.
4. To quantify the weekly total fungi population for the composting period.
5. To identify the bacteria and fungi present from the beginning to the end of composting period.
6. To determine the weekly average temperature, pH and moisture content of compost matrixes in each system.
7. To determine the compost maturity in each system using the germination index method.
8. To find the health hazards posed by microorganisms that survived the process to compost producers, end users and compost grown product consumers.

KNUST

1.7 Justification

Composting of food waste and agricultural by-products in different systems is increasingly gaining attention; but selection of a system is dependent on nature of waste to be composted, availability of labour, and economic conditions. However, of more concern to the public health is the need to use lower capital investment and less technical know-how systems capable of effectively reducing pathogens as well as increasing compost maturity through temperature developments during composting to ensure a final safe product.

Temperature has been the most used parameter in the reduction of pathogens during composting. In-vessel systems are reported by Cekmecelioglu *et al.* (2005) as having advantage over windrow composting because of shortened mesophilic (25-45°C) and thermophilic (45-75°C) stages, hence higher process efficiency resulting in a decreased number of pathogenic microorganisms in final product. *Salmonella spp* and *Escherichia coli* were inactivated in 9 days at 60-70°C in a bench scale composting of food waste with leaves (Cekmecelioglu *et al.*, 2005). The United States Environmental Protection Agency (USEPA) requires a process to further reduce pathogens (PFRP) prior to land application of composted biosolids (Hay, 1996). To meet this requirement, a minimum inactivation temperature of 55°C must be achieved for minimum period of 3 days during in-vessel and aerated static pile composting. The minimum conditions for windrow composting are 55°C for 15 days and five turnings during the composting period (Cekmecelioglu *et al.*, 2005). However, there are variations in regulations from one country to another. In Switzerland, regulations require temperature above 55°C for at least 3 weeks or 60°C for at least a week, whereas, in Denmark, the requirement is a temperature above 55°C for at least 2 weeks. The German rules require a temperature above 60°C for a week for in-vessel composting and above 55°C for at least 2 weeks or above 65°C for at least a week (Droffner and Brinton, 1995). Temperature becomes thus the most obvious indicator of compost safety.

KNUST

Change in pH values during composting are due to changes in the chemical composition. Different microorganisms have different sensitivity to organic acids. Bacteria are more sensitive to acids than fungi (Atlas and Bartha, 1998). During composting, pH falls below neutral in the beginning due to the formation of organic acids and later rises above neutral because the acids are consumed and ammonium produced (Beck-Friis *et al.*, 2003). This variation in pH during the process considerably affect microorganisms and the rate of decomposition as organic acids are suppressive to microbial activity and growth at low pH.

All living organisms need water, so moisture is essential for the function of the composting process. There is no upper limit for water needed by microorganisms, but excessive moisture reduces the pores in the compost matrix, thus causing oxygen limitation which eventually affects microbial growth and activities, hence the decomposition rate (Miller, 1993). Water is a donor of hydrogen ions and also contributes to the regulation of cellular pH and temperature.

Mesophilic and thermophilic microorganisms are involved in composting and their succession is important in the effective management of composting process (Beffa *et al.*, 1996; Ishii *et al.*, 2000). Since plant and animal waste possibly contains viral, bacterial, and protozoan zoonotic pathogens, the application of untreated livestock wastes could be a hygienic risk for humans (Dai *et al.*, 2005). Several pathogens known to cause diseases and death in humans have been identified in manure and foodwaste. Some of these microorganisms include *Samonella spp.*, *Listeria spp. Clostridium spp. Staphylococcus spp.*, *Escherichia spp.*, *Aspergillus spp.* and *Penicillium spp.* For instance, *Listeria monocytogenes*, *Salmonella spp.* and *Escherichia coli* O157:H7 infections are important to food safety as these classes of pathogens combine to cause approximately 1.5 million illnesses and 60% of all deaths related to food borne illnesses (Mead *et al.*, 1999). Since these microorganisms are found during composting, it becomes important to treat animal wastes and plant materials harbouring these pathogens, to reduce the risks of pathogenic microorganisms entering the food chain via the use of the compost on farms. It is also important to ascertain whether the compost is matured before applying to the field so as to prevent the associated problems of nitrogen immobilization and phytotoxicity.

Proper composting is thus needed to effectively destroy pathogens and weed seeds through the metabolic heat generated by microorganisms.

KNUST

Since temperature, moisture and pH variations in different composting systems affect microbial activities, it becomes necessary to study how the developments of these factors in the different systems help to inactivate the pathogens, increase the rate of maturity as well as help achieve safe finished compost.

KNUST

NYANSAPƆ WƆSANE NO BADWENMA

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition of Composting and Compost

Composting is an exothermic, aerobic and microbial decomposition of organic materials. A typical composting process goes through a series of phases, including a rapid temperature increase, sustained high temperatures and a gradual cooling of the composting mass (Ryckeboer *et al.*, 2003). Different microbial communities dominate during these various composting phases, each adapted to a particular environment (Ryckeboer *et al.*, 2003). During composting, microorganisms use the organic matter as a food source, producing heat, carbon dioxide, water vapour, and humus as a result of their furious growth and activity. Compost is a solid mature product resulting from composting, which is a managed process of bio-oxidation of a solid heterogeneous organic substrate including a thermophilic phase (Canada Composting Council, 2008).

KNUST

2.2 Composting Systems

There are several systems used in composting and the selection of a system is dependent on the nature of waste, available manpower and economic conditions (Cekmecelioglu *et al.*, 2005). The most common methods for composting include passive heaps, static heaps, windrows and in-vessel composting systems. Passive and static heaps are similar: in that, both involve the stacking of composting materials into mounds, referred to as heaps. Passive heaps, however, are given very little attention, while static heaps are maintained through forced aeration or frequent mechanical turning (Sherman, 2005). In windrow composting, composted materials are mixed and formed into long and narrow heaps, which are subjected to regular agitation (Sherman, 2005). In-vessel composting is characterized as a group of composting methods that use a container or enclosed area to perform composting (Cochran and Carney, 2005). Using an in-vessel composting composting system can also include mechanical or forced aeration of compost material.

The table below describes composting period typically experienced based on material and methods implemented.

Table1: Period necessary for composting depending on the implemented methods and substrates used.

KNUST

Method	Materials	Active composting time		Curing time
		Range	Typical	
Passive composting	Leaves Well-bedded manure	2–3 years 6 months to 2 years	2 years 1 year	— —
Windrow—infrequent turning [a]	Leaves Manure + amendments	6 months to 1 year 4–8 months	9 months 6 months	4 months 1–2 months
Windrow—frequent turning [b]	Manure + amendments	1–4 months	2 months	1–2 months
Passively aerated windrow	Manure + bedding Fish wastes + peat moss	10–12 weeks 8–10 weeks	— —	1–2 months 1–2 months
Aerated static pile	Sludge + wood chips	3–5 weeks	4 weeks	1–2 months
Rectangular agitated bed	Sludge + yard waste or Manure + sawdust	2–4 weeks	3 weeks	1–2 months
Rotating drums	Sludge and/or solid wastes	3–8 days	—	2 months [c]
Vertical silos	Sludge and/or solid wastes	1–2 weeks	—	2 months [c]

[a] For example, with bucket loader.
[b] For example, with special windrow turner.
[c] Often involves a second composting stage (for example, windrows or aerated piles).

Source (Rynk, 1992)

2.3 Compost Microorganisms Sources

The microorganisms needed for composting are found throughout the natural environment. They are present in compost feedstock, water, air, soil as well as machinery the feedstock and compost are exposed to during processing. These sources ensure a high diversity of microorganisms, which help to maintain an active microbial population during the dynamic chemical and physical processes of composting such as shifts in pH, temperature, water, organic matter, and nutrient availability. Only on rare occasions will the addition of microorganisms be warranted. (Compost Microbiology and the Soil Food Web, 2008)

2.4 Microbe Types and Requirements

KNUST

The microbiological components of compost consist of bacteria and fungi. The uniqueness of *Actinomycetes* present them as the third microbiological component, though in actuality actinomycetes are a particular kind of bacteria (Compost Microbiology and Soil Food, 2008). Macdonald *et al.* (1981) noted that the composting process was brought about by several organisms such as bacteria, fungi, actinomycetes, protozoa and may also involve invertebrates such as nematodes, potworms, earthworms, mites and other organisms. Singh (1987), however, noted that the sole agents of decomposition of carbonaceous materials are the heterotrophic microorganisms. The majority of microorganisms responsible for the formation of compost are aerobes in that they require or work best in the presence of oxygen.

2.4.1 Fungi.

Fungi form their individual cells into long filaments called hyphae. Fungal hyphae are larger than actinomycetes and may be more easily seen with the naked eye. They penetrate throughout the composting material, decomposing both chemically and mechanically the more recalcitrant organic matter fraction such as lignins and cellulose. Fungal hyphae physically stabilize the compost into small aggregates, providing the compost with improved aeration and drainage. Ecologically, fungi play a vital role in breakdown of dead plant materials (Compost Microbiology and the Soil Food Web, 2008).

2.4.2 Bacteria.

The most numerous biological component of compost is the bacteria. Although they often can exceed 1 billion microorganisms per gram of soil, bacteria (with the exception of actinomycetes) do not contribute as much to the overall microbiological mass as fungi because of their relatively small size. Although bacteria (with the exception of actinomycetes) exist as individuals and do not form filaments, they also contribute to the stabilization of aggregates through the excretion of organic compounds that bind adjacent organic matter and soil particles together. Bacteria are typically associated with the consumption of easily degraded organic matter. They are the dominant population throughout the entire composting process, whereas the actinomycetes and fungi typically proliferate in the later stages (Compost Microbiology and the Soil Food Web, 2008).

KNUST

2.4.3 Actinomycetes.

While actinomycetes are visually similar to fungi in that they have networks of individual cells that form filaments or strands, they are actually a type of bacteria. These filaments allow actinomycetes to spread throughout a compost pile, where they are typically associated with the degradation of the more recalcitrant compounds. Their filaments contribute to the formation of the stable organic aggregates typical of finished compost. Actinomycetes are tolerant of lower moisture conditions than other bacteria and are responsible for the release of geosmin, a chemical associated with the typically musty, earthy smell of compost (Compost Microbiology and the Soil Food Web, 2008).

2.5 Composting Process

Successful composting is usually characterized by the following three phases: mesophilic, thermophilic, and cooling/maturation. The composting phases are characterized by the changes of dominant microbial communities and soluble nutrients utilized during the composting process (Smith, 1992).

Composting proceeds in predictable stages. During different stages, temperatures and nutrient availabilities vary and affect the kinds and numbers of microorganisms that develop. Initially, the pile is at approximately the ambient temperature. The composting material warms through the mesophilic temperature range (10°C - 40°C) as the microorganisms become more active. Soon, microbial activity raises the temperature of the pile to thermophilic temperatures (41°C - 77°C). This is considered the most productive stage of composting. Composting proceeds at a much faster rate under thermophilic conditions.

KNUST

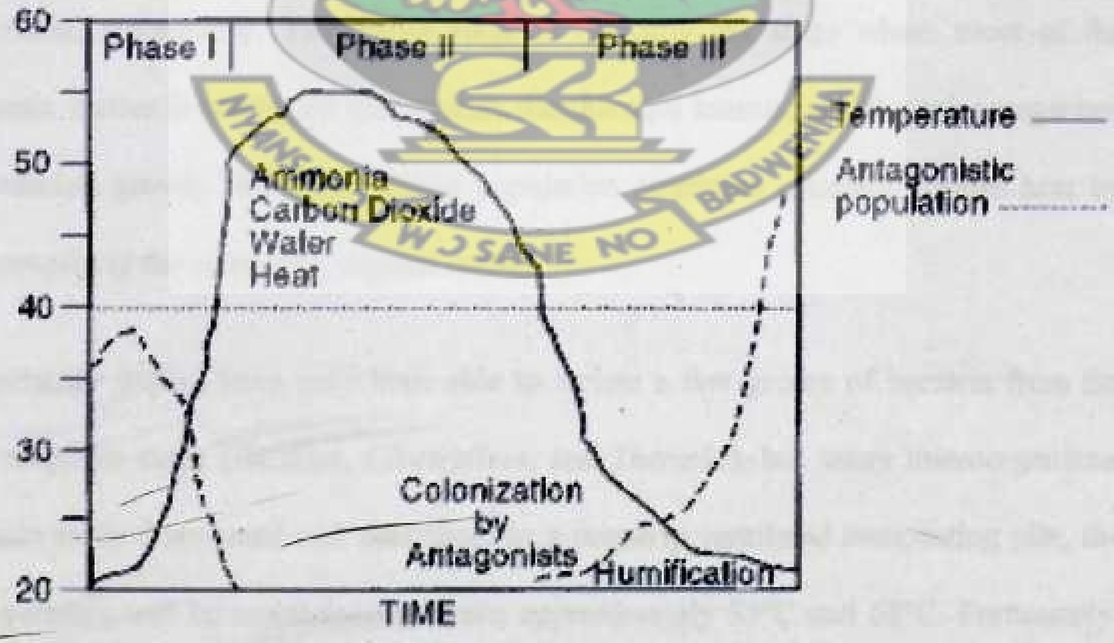


Figure 1: Phase changes during the composting process (Smith, 1992)

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

When readily available substrates within the feedstock are exhausted, temperatures gradually return to the mesophilic range (Compost Microbiology and the Soil Food Web, 2008).

2.5.1 Mesophilic Phase.

The availability of easily usable organic substances at the initial stage enables the proliferation of the fastest-growing microorganisms, the bacteria. Mesophilic bacteria, therefore, dominate initial decomposition. These bacteria release heat from the breakdown of large amounts of easily degraded organic matter. This heat begins to raise the temperature within the pile due to the high insulating capacity of a properly sized compost pile. Within few hours the temperature of the compost pile can rise above the 41°C, the thermophilic threshold (Compost Microbiology and the Soil Food Web, 2008).

KNUST

2.5.2 Thermophilic phase.

As the compost reaches higher temperatures, thermophiles begin to dominate the microbial community. The active stage is typically the stage where most of the organic matter is converted into carbon dioxide and humus, and the microorganism population grows. The thermophilic population continues generating more heat by decomposing the remaining organic matter.

Laboratory studies have only been able to isolate a few genera of bacteria from the thermophilic stage (*Bacillus*, *Clostridium*, and *Thermus*), but many microorganisms remain to be discovered and described. In a properly ventilated composting pile, the temperature will be maintained between approximately 55°C and 68°C. Fortunately, pathogens such as human viruses and infectious bacteria are typically unable to persist in such a hostile environment. The higher temperatures will ensure rapid organic matter processing while simultaneously providing optimal conditions for the destruction of human and plant pathogens as well as weed seeds (Compost Microbiology and the Soil Food Web, 2008).

Since the composting pile is cooler on its outer surface, periodic mixing of the outer layer into the pile is essential for maximum pathogen and seed kill. Mixing or turning the pile also helps to ventilate it by increasing the size and number of pores. This is important because in an unventilated compost pile, the temperatures can exceed 71°C, effectively stopping all microbial activity. The pores also serve as passages for oxygen to enter the pile. Microbes require oxygen to efficiently break down organic matter (Compost Microbiology and the Soil Food Web, 2008).

KNUST

2.5.3 Cooling/Maturation stage

A properly functioning composting pile will eventually deplete itself of a majority of the easily degradable organic substrates leaving some cellulose, lignins and humic materials. Bacteria are generally considered less adept at metabolizing these remaining compounds. Consequently, the bacterial population will decline in numbers as compared to fungi and actinomycetes. Because less heat is generated at this point, the temperature of the composting pile will slowly fall to mesophilic temperatures (Compost Microbiology and the Soil Food Web, 2008).

Finally, the compost is then cured. During the curing stage, the fungi and actinomycete populations predominate, while the bacterial population may decline. Fungi and actinomycetes proliferate on the remaining less degradable organic matter such as chitin, cellulose and lignin. These compounds are more persistent because they are insoluble in water and, due to their size and chemical complexity, cannot pass into the bacterial cell. Thus, degradation of these compounds requires the use of extracellular enzymes. The curing process can vary in duration; a longer curing period provides more assurance that the compost is free of pathogens and phytotoxins (Compost Microbiology and the Soil Food Web, 2008).

2.6 Temperature

Composting, being an aerobic decomposition of waste and a highly exothermic process, generates heat. The heat generated can either remain in the compost mass raising its temperature or leave the mass by either conduction or radiation from the surface or with air passing through it (Sundberg, 2005). Each microorganism can grow within a certain temperature range and most are killed by higher temperatures. Mesophilic micro- organisms have optimum growth temperature at 25ºC - 40ºC and thermophiles with optimum temperature above 45ºC (Madigan *et al.*, 2000). The temperature for maximum degradation rate in composting is nearly 55ºC and much lower at 70ºC (Miller, 1993).

2.7 Moisture

Microorganisms can remain alive in a dried condition but cannot carry out their normal metabolic activities or multiply without water. Water is used to bring more substances into the cells and to dispel waste products (Banwart, 1989). Water is a donor of hydrogen ions, contributes to the regulation of cellular pH and temperature (Beall, 1983). All living organisms need water, so moisture is essential for the function of the composting process. There is no upper limit for water needed by microorganisms as such, but excessive moisture reduces the pores in the compost matrix, thus causing oxygen limitation (Miller, 1993).

KNUST

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

2.8 Hydrogen ion concentration (pH)

Hydrogen ion concentration (pH) is defined as the negative logarithm of the hydrogen ion concentration. It gives an indication of the alkalinity or acidity of the compost mass. Change in pH values during composting are due to changes in the chemical composition. Normally pH falls below neutral in the beginning due to the formation of organic acids and later rises above neutral because the acids are consumed by some microorganisms and ammonium produced (Beck-Friis *et al.*, 2003). Different microorganisms have different sensitivity to organic acids. Bacteria are more sensitive to acids than fungi (Atlas and Bartha, 1998). Changes in pH during composting are also influenced by the carbonic and ammonia systems (Sundberg, 2005). During successful and fully developed composting, the pH often rises to 8-9 (Sundberg, 2005).

KNUST

2.9 Variations in Temperature, Moisture and pH during Composting

Many authors have noted stagnation or decline in microbial activity in the transition from mesophilic to thermophilic conditions in laboratory-scale compost reactors (Schloss and Walker, 2000; Weppen, 2001).The stagnation in microbial activity has in some cases been observed to coincide with low pH in the material (Day *et al.*, 1998). Beck-Friis *et al.* (2001) reported that the change from mesophilic to thermophilic conditions during the initial stage of composting coincided with a change in pH from acidic (pH = 4.5-5.5) to alkaline (pH = 8 - 9).

Different methods have been used to increase the rate of degradation when acidic materials are composted. Nakasaki *et al.* (1998) composted organic household waste at 60°C and observed an increased degradation rate when pH was prevented from decreasing below 7 through liming. Nakasaki *et al.* (1996) demonstrated that the degradation rate at the initial stage of composting can significantly be increased by inoculation with acid-tolerant thermophilic bacteria. Choi and Park (1998) also observed that the growth of thermophilic bacteria in food waste compost at 50°C was stimulated by an addition of thermophilic yeast that breaks down organic acids. Smårs *et al.* (2002) experiments in a composting reactor revealed that the time of the initial acidic phase could be reduced if the process temperature could be kept below 40°C until the pH value in the condensate is above 5.

KNUST

The inhibition of the compost process when low pH is combined with temperature above the mesophilic optimum is a likely explanation for the lag in the transition from mesophilic to thermophilic temperatures (Sundberg and Jönsson, 2003). This has been noticed by many others when composting food wastes or acidic wastes (Haug, 1993; Schloss and Walker, 2000). The transitional phase occurs because the mesophiles are inactivated by the high temperature, and the thermophiles are suppressed by the low pH and organic acids. When the pH rises, the thermophiles are no longer inhibited; their activity increases and the temperature rises further (Sundberg, 2005).

In the fed batch process, Sundberg (2005) noted that addition of a starting culture of active compost caused a quicker start of aerobic degradation and prevented continued conditions of low pH and organic acid concentration. A starting culture supplies microorganisms, which can decompose the acids that are formed and provides a chemical pH-buffer which may reduce the inhibitory effect of the organic acids by reducing the depression of the pH (Sundberg, 2005). Results from experiments with both microbially active and sterilized structural amendments show that microorganisms in a starting culture can accelerate the start-up of the composting process when there is not an adequate microbial community present in the substrate (Nakasaki and Akiyama, 1988; Nakasaki *et al.*, 1993).

Temperature of compost affects the dynamics of the microbial community (Strom, 1985). Several studies conducted on the effect of temperature on the decomposition rate revealed differing conclusions about optimum temperature. Suler and Fintein (1977) found a higher decomposition rate around 50ºC than at higher temperature. Jeris and Regan (1973) showed maximum decomposition rates at 40ºC - 60ºC for different raw materials. Haug (1993) considered around 65ºC to be the optimal.

KNUST

Composting is often done at temperatures other than the microbial optimum because of the difficulty to regulate temperature during the process. Small scale compost is characterised by low temperatures whereas large composting plants often have temperatures higher than 60ºC (Sundberg, 2005).The changes in temperature that occur during the composting process contribute to a succession of different microbial communities over time (McKinley and Vestal, 1985; Klamer and Bååh, 1998). Active regulation of temperature during the initial stage of high decomposition would select for different microbial communities (Sundberg, 2005).

NYANSAPO WO SANE NO BADWENMA

The moisture content at which moisture becomes limiting to microbial activity and oxygen transport varies among materials. The lower limit of moisture content is about 35% - 40% (weight of water x 100/total wet weight) and the upper is about 60% - 70%. If moisture content went below 35% - 40% during the process, measures were promptly taken to increase the moisture content. If moisture was never adjusted, the product could be unstable. If the moisture content of the material went above 60% - 70%, then material would be composted longer to compensate for the reduced oxygen transfer, and thus aerobic microbial activity (VanderGheynst, 2007). Evaporation caused by the heat generated during the composting process reduces the moisture content, which is important for the degradation rate both directly, as it affects microbial activity, and indirectly, as it affects the oxygen supply (Sundberg, 2005). Water evaporates as a result of the elevated temperature and aeration during composting. Food waste compost is susceptible to drying, and it is therefore often necessary to add water to keep the moisture at high level enough to avoid limitations in degradation rate due to drying (Keener *et al*., 1996).

2.10 Compost Maturity

KNUST

The ability to tell the maturity of compost is important to compost makers and end-users. Unstable compost applied in the field maintains a higher microbial activity, leading to increased oxygen consumption. This decreases the supply of oxygen available to plant roots.

In addition, immature compost can contain higher levels of soluble organic matter (i.e., organic acids), which can lead to toxicity problems for certain horticultural applications, such as seed germination (Compost Microbiology and the Soil Food Web, 2008). Zucconi *et al.* (1981) also reported that when unstable or immature compost is used as a soil amendment or plant growth medium, it may reduce oxygen concentration in the soil and immobilize nitrogen, thereby causing serious N-deficiencies in crops.

According to Canada Composting Council (2008), compost is deemed mature if it meets two of the following requirements:

1. C/N ratio is less than or equal to 25.
2. Oxygen uptake rate is less than or equal to 150 mg O_2/kg volatile solids per hour.

3. Germination of cress (*Lepidium sativum*) seeds and of radish (*Raphanus sativus*) seeds in compost must be greater than 90 percent of the germination rate of the control sample, and the growth rate of plants grown in a mixture of compost and soil must not differ more than 50 percent in comparison with the control sample.

4. Compost must be cured for at least 21 days.
5. Compost will not reheat upon standing to 20°C above ambient temperature.

KNUST

Stability is defined as the resistance to further decomposition of compost materials by microorganisms. Stability of composts is often associated with microbial activity (Hue and Liu, 1995). Various parameters that have been used to assess the quality and maturity of composts include the C: N ratio of the finished product, water soluble carbon, cation exchange capacity, humus content, and the carbon dioxide evolution from the finished compost (Garcia *et al.*, 1992; Huang *et al.*, 2001). Germination index, which is a measure of phytotoxicity, has been considered as a reliable indirect quantification of compost maturity (Cunha Queda *et al.*, 2002). It has been suggested that a germination index (a factor of relative seed germination and root elongation) of ≥ 80 indicates the disappearance of phytotoxicity in composts (Zucconi *et al.*, 1981). However, it is difficult to apply these parameters across a wide range of composts prepared from different organic wastes (Roletto *et al.*, 1985).

However, in order to examine the efficiency of the germination index method as a maturity parameter for livestock faeces compost, Ikeda *et al.* (2006) compared the germination index method ~~with~~ other methods, using both immature composts and mature composts sold as products. It was observed that the more the decomposition from livestock faeces came to maturity, the higher the germination index became. The germination index was correlative with BOD and NH_4-N. The composts that showed the germination index of more than 150% indicated low levels of BOD and NH_4-N. In the condition where the immature composts were crushed naturally while being dried, the germination index became higher. When the number of cultivation days was reduced from seven to five days, the germination index was almost the same. It was concluded that the germination index method is a highly accurate maturity parameter, under the conditions of setting more than 150% as a maturity standard, using 5 days for seed cultivation.

2.11 Determination of Compost Maturity

KNUST

In the determination of compost maturity using the germination index method, extract of the compost was prepared by mixing distilled water and compost sample poured into a beaker in the ratio 1:10 with a shaker for 1hour. A Petri-dish was taken, fitted with 5 layers of filter paper pad and then wetted with 5ml of 1:10 compost aqueous extract. The same was done for a second Petri-dish but compost aqueous extracts replaced with distilled water to serve as the control. 25 seeds of *Lepidium Sativum* were placed in each Petri-dish and incubated in the dark at 25°C for 5 days. After 5 days of incubation in the dark, the seed germination percentage and root length of germinated seeds in both the treated and control were determined (Zucconi *et al.*, 1981).

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

The percentages of relative seed germination, relative root elongation and germination index (GI) were calculated as follows:

$$\text{Relative seed germination}(\%) = \frac{\text{No. of seeds germinated in litter extract}}{\text{No. of seeds germinated in control}} \times 100$$

$$\text{Relative root growth}(\%) = \frac{\text{Mean root length in litter extract}}{\text{Mean root length in control}} \times 100$$

$$\text{GI} = \frac{(\%\ \text{Seed germination}) \times (\%\ \text{Root elongation})}{100}$$

Where GI = Germination Index

KNUST

2.12 Sampling of Compost Material

The results of the analysis cannot be more reliable than the sample on which it was based. Microorganisms are not usually distributed homogeneously, so thorough mixing of product prior to sampling is important. When sampling through an entire pile of material, it becomes necessary to avoid introducing a bias by sampling at different locations of the pile and mixing to obtain a representative sample (Banwart, 1989).

W J SANE NO BADWENMA

2.13 Holding Samples

The samples can be analysed immediately for best results. However, when it becomes impossible, it must be kept in a fridge to prevent the growth of any microorganism. If the sample is to be transported from a place distant from the laboratory, it becomes advisable to cover it with ice cubes (Banwart, 1989).

2.14 Preparation of Samples

The main consideration in preparing the sample is to get the microorganisms into a homogeneous suspension so it can be pipetted. The material must be blended with a diluent to obtain a suspension. Alternatively, the sample and diluent can be mixed in a stomacher to obtain a homogeneous suspension of sample and microorganisms (Thomas and McMeekin, 1980). Several diluents have been suggested and used, but Butterfield's buffered phosphate and 0.1 percent peptone water is recommended. Peptone is easy to prepare and also protects the organisms during dilution and plating (Banwart, 1989).

2.15 Methods used for Enumeration of Microorganism

KNUST

There are two basic methods used in estimating the microorganisms in a material. These are total count and total viable count. Total count is the estimation of both the dead and living microorganisms in the material whereas total viable count is the estimation of only the living microorganisms.

2.16 Total Viable Counts

Several methods are used in estimating the total viable microorganisms in a material. Most of these methods are based on the plate count or tube dilution methods. Some of these methods are described below:

2.16.1 Plate Count (Pour)

The standard plate count has been the usual technique used for estimating the living microorganisms in food materials. Appropriate dilutions are plated immediately by transferring a measured aliquot to a sterile Petri plate and adding sterile melted and cooled (42°C - 45°C) agar. The type of agar used for the standard plate count is non-inhibitory and nutritious, unless specific microbial types are to be determined. The medium and inocula are mixed thoroughly to distribute the cells uniformly. After solidification of the agar, the prepared plates are inverted to prevent condensation of moisture on agar surfaces. The temperature and time of incubation varies according to the type of cells being determined. During the incubation period, growth and multiplication of cells occur until visible colonies are formed. The colonies are then counted on plates that contain 30 to 300 colonies. The number of colonies are multiplied by the dilution factor and reported as the number of colony-forming units (CFU) per gramme of material (Banwart, 1989).

2.16.2 Plate Count (Surface)

With this method, the sterile melted and cooled agar is poured in sterile Petri dishes. After solidification, the plates are pre-incubated overnight. The incubation dries the surface of the agar so that, when inoculated, organisms do not coalesce. Aliquots of dilutions are added to the dry surface and uniformly spread over the agar by means of sterile rod, bent into the shape of a hockey stick. The plates are inverted and incubated, and resultant colonies are counted as with the pour plate method (Banwart, 1989).

2.16.3 Roll Tube

The idea of the roll tube is the same as for the pour plate method, except that screw-capped test tubes or bottles are used in place of Petri-plates. Sterilized test tubes are filled with 2 to 4ml of plate count agar. When the melted agar is cooled to 42°C to 45°C, 0.lml of the appropriate sample is added and the tube rolled in cool water horizontal position until the agar is solidified in a thin layer on the inner wall of the tube. The incubated tubes are turned upside down so that any water that condenses collects below the inoculated agar and does not smear the colonies. After incubation, the colonies that develop are counted with the aid of a low magnifier. Multiplying the colony counted by the dilution factor yields the number of organisms per gramme of material (Banwart, 1989).

KNUST

2.16.4 Burri Strip or Slant

This method involves the spreading of a sample over an agar slant with a calibrated loop. Test tubes can be used, but the oval tube gives a larger surface for the growth of colonies. The agar surface must be dry to prevent colonies from coalescing. After incubation (32°C or 37°C for 24hr) in a horizontal position, the surface is examined for microbial growth. Colonies may be counted or comparisons can be made as to the extent of growth that occurs so that high- and low-count products can be distinguished (Banwart, 1989).

KNUST

2.16.5 Membrane Filters

This involves filtering fluid through a membrane filter and aseptically transferring the retained microorganisms onto a nutrient agar or one that is selective, differential or both. After incubation for 6 to 8 hr, the microcolonies can be counted with microscope.There is also the hydrophobic grid membrane filter. The grids are compartments of equal and known size, and the hydrophobic material deters the spreading of colonies. After the organisms are grown on the filter, the number of squares containing colonies is enumerated and converted to most probable number. The results can be determined manually or with an automated counting system (Banwart, 1989).

2.17 Tube Dilution

The tube dilution method is essentially the aseptic inoculation of a series of tubes of sterile nutrient broth with ~~series of~~ dilutions of the material. After incubating the inoculated tubes, the broth is observed for turbidity, which indicates growth of organisms. If no turbidity is evident, it is assumed that no microorganisms were present or were able to multiply. Growth can be detected by streaking on an agar surface the broth that appeared turbid and observing growth after a few hours of inoculation, or by spreading some broth on a slide and looking for microorganisms with the aid of a microscope (Banwart, 1989).

2.17.1 Most Probable Numbers (MPN)

This is a method under the tube dilution method. To use the MPN systems, at least three dilutions are needed. The more tubes that are used in each dilution, the more accurate is the estimate, but for reasons of convenience, three-tube or five-tube series are adopted. After selecting the three series of dilutions, the MPN table is referred to and the most probable number that satisfies the number of positive tubes obtained. This number is then multiplied by the dilution factor to obtain the MPN per gramme of material (Banwart, 1989).

KNUST

2.18 Identification of Microorganisms

Several tests are used to differentiate organisms isolated from selective and differential media. The usual staining reactions and morphological characteristics are used in the identification of microorganisms. Biochemical tests are used depending on the microorganisms to be identified. Various commercial kits and systems for determining biochemical reactions have been evaluated (Cox *et al.*, 1984). Serological reactions can utilize cellular, flagellar, or other antigens. The development of monoclonal antibodies and immunoassays such as radio immunoassay (RIA), enzyme immunoassay (EIA), and enzyme-linked immunosorbent assay (ELISA) are used to detect specific microorganisms as well as toxins and other antigenic agents (Milby and Zare, 1984; Løvborg, 1984). The production of pigment, antimicrobial resistance pattern, phage typing, and bacteriocin typing can be useful for the differentiation of strains of some organisms (Kaneko and Hashimoto, 1982).

2.19 Helminths Eggs Identification and Counting Techniques

2.19.1 Sedimentation Technique

Sedimentation of a sample removes pigment and light debris, and thus provides more concentrated material for microscopical examination. Sedimentation is most often used for helminths where parasite eggs do not float well or are affected by high tonicity solutions. 3g of sample is mixed with 30ml water and mechanically disintegrated. The mixture is passed through 80-mesh sieve and centrifuged in a conical tube for 3 minutes at 1200 rpm. Supernatant is discarded and pellet resuspended in 10 ml conical tube .The sieve and glass are washed at each stage and the washings added to the sample. After a second centrifugation, the supernatant is discarded, and 1 drop of 1% methylene blue added to the pellet and then examined microscopically (Murray *et al.*, 1983).

KNUST

2.19.2 Flotation technique

Differential floatation is based on the fact that helminth eggs float in certain solutions in which a proportion of faecal debris will not. A clear preparation of eggs results as they float to the surface leaving debris in suspension in a lower plane. The higher the specific gravity of the solution, the more eggs of various types will be recovered, although a drawback at higher tonicities is the damaging effect on some eggs. Sodium chloride at slightly less than saturation is most commonly used (Murray *et al.*, 1983).

2.19.3 Concentration Technique

This qualitative technique will recover most helminth eggs, while still removing most of the faecal debris from the preparation. A few glass beads are added to 30 ml of 1% formalin solution in a stout glass or plastic container of 50-100 ml capacity. Samples of 2-3g is added and shaken vigorously to emulsify the samples. The mixture is then

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

sieved through a tea strainer into a plastic beaker and filtrate poured into a conical centrifuge tube. It is centrifuged at 3000 rpm for 5 minutes, supernatant decanted and tube shaken to resuspend deposit. Saturated sodium chloride solution is added to ¾ full and again centrifuged at 1500 at 1500rpm for 2 minutes. Using a 22 x 22 cm square cover slip, a drop from the surface of the tube's content is removed and placed on a glass slide taking care not to introduce bubbles. The slide is examined under a microscope using x10 eyepieces and x10 objective lens (Murray *et al.*, 1983).

2.19.4 The Baerman Technique

KNUST

The Baerman technique is used for the recovery of living larvae from animal material. It is based on the fact that nematode larvae are active, and when material containing them is placed in water, the larvae will sink to the bottom where they may be recovered.The samples are placed in a jar which is filled with water and placed upside down on a Petri dish, the jar is tilted momentarily to allow a little water to cover the bottom of the dish. After 12 to 24 hours, the water around the jar, containing most of the larvae, is pipetted off, or if larvae are likely to be scarce, the whole fluid in the jar may be run off, and the larvae concentrated by sedimentation or centrifugation (Murray *et al.*, 1983).

NYANSAPƆ WɔSANE NO BADWENMA

2.19.5 McMaster egg-Counting Technique

This technique is a refinement of ordinary flotation, and allows an estimation of the number of eggs, oocysts or larvae present in a sample to be made. It is rapid to perform and requires a ~~minimum of~~ special equipment. 2 g of sample is weighed and rubbed through a sieve into a beaker along with 30 ml of saturated sodium chloride. Suspension is mixed and 10 ml poured into a conical centrifuge tube. It is then centrifuged for 2 minutes at 1,200g. Supernatant is discarded and deposit resuspended in 10ml saturated sodium chloride solution. It is mixed thoroughly and McMaster counting chamber filled using Pasteur pipette. It is examined under a microscope using x10 eyepieces (Murray *et al*., 1983).

The usual procedure would be to check the samples for the presence of eggs by one of the techniques and then to quantify the eggs if present by the McMaster technique.

KNUST

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Composting Materials

River reed is the basic feedstock used at VREL; it is harvested in canoes and brought to the site for use. Banana stalks are also obtained from VREL Farms. Other materials in use include: Cocoa seed husk, rice husk, cow manure and poultry manure obtained from farms and households nearby. These form a composition given as follow:

1. River Reed (RR):	75%
2. Clay(C):	10%
3. Banana Stalk/Stem (BS):	5%
4. Cow Manure/Dung (CM):	4%
5. Rice Chaff (RC):	4%
6. Cocoa Seed Husk (CSH):	1%
7. Poultry Manure (PM):	1%

KNUST

Soil-Tech Solutions starter
500 eenheden
SOILTECH™
SOLUTIONS

Fig.2: Containment of the starter added to compost masses

A starter containing genetically modified organisms such as *Bacillus spp.* and *Corynebacterium spp.* was mixed in the proportion 500g in 40L water and added to the feedstocks to facilitate the decomposition process.

3.2 Description of the Composting Systems Under study

3.2.1 Dome Aerated Technology (DAT)

The DAT is a passive aeration system that utilizes thermal convection to drive the aeration process within a windrow of waste. The principle of the DAT method is the creation of large voids in a windrow of waste, using in this case, bamboo structures, called domes and channels. Domes are positioned centrally in the windrow to allow for venting of the hot gases generated by the degradation reactions through the chimneys and channels.

KNUST

The layout of the DAT system is as shown below:

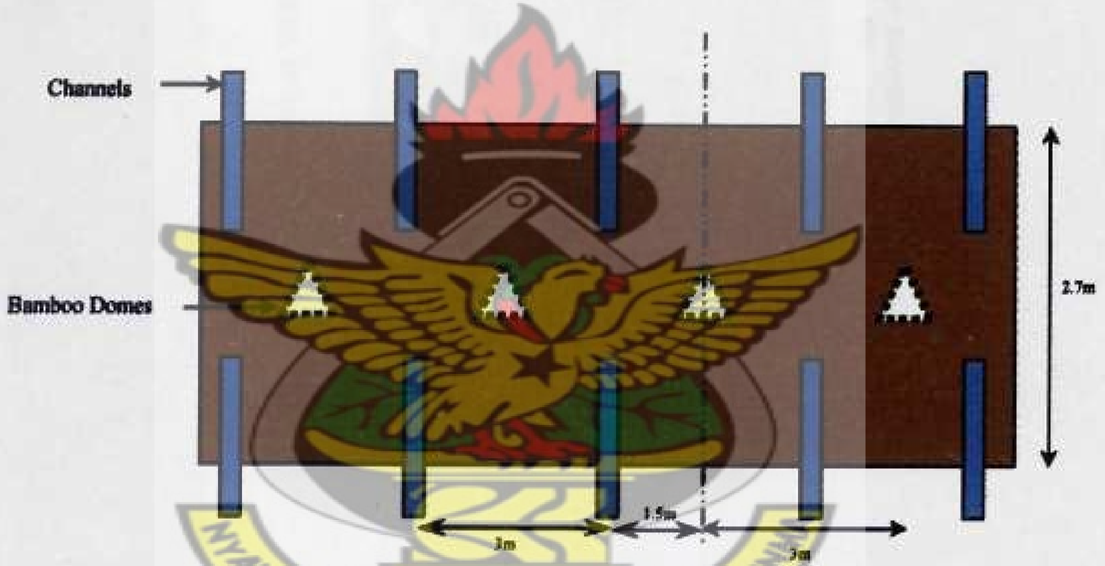
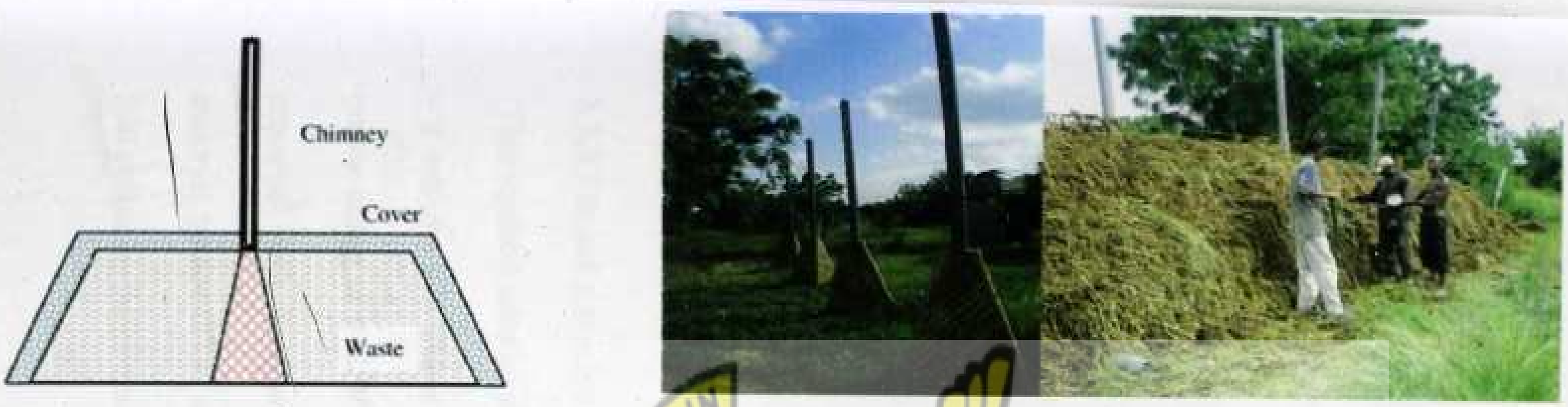


Fig. 3: A schematic diagram of the Dome Aerated Technology (DAT) system

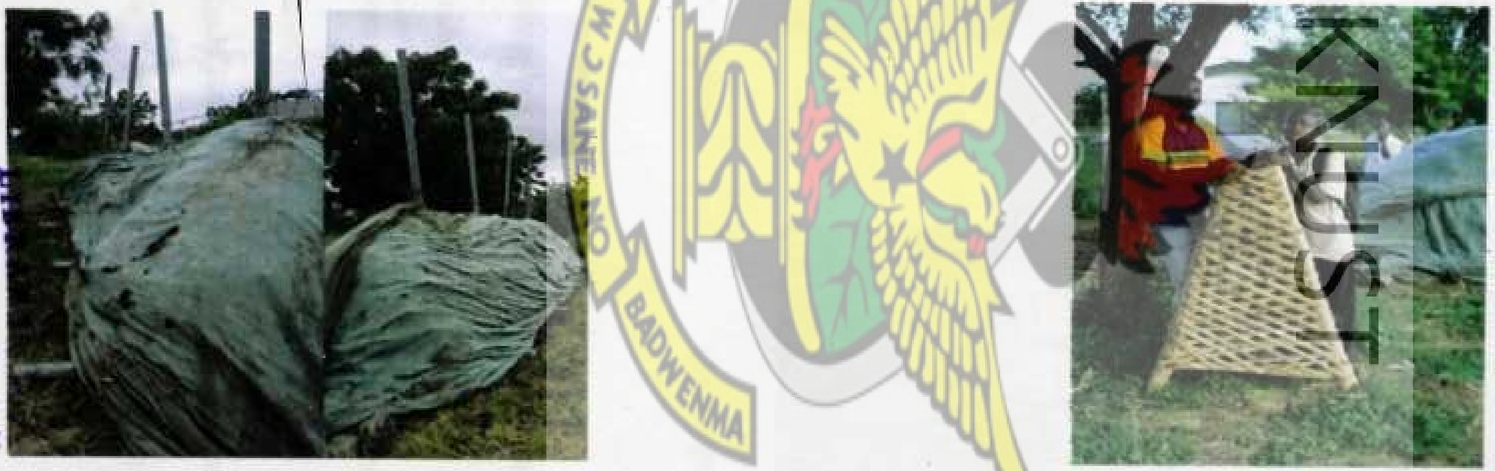
NYANSAPO W J SANE NO ADWENMA

This pile is composed of 4 bamboo domes, 4 chimneys (4 in. dia., and 2.5m high). Additionally, 10 pieces of perforated uPVC (4 in. dia.) which promote the chimneys effect through the compost pile (seen in *fig. 3 and 4*). The chimney pipes were supported by a cable. Design for the construction of the pile is described as follows: 13.7m (L) ×2.7m (B) ×1.8m (H), (as seen in *fig.3, 4 & 5*). The triangular bamboo dome was constructed using the following dimensions: 1.4m high; 0.75m equilateral base.



(a)
Fig. 4: (a) Cross sectional area of the DAT system

(b)
(b) Pile preparation at VREL



(a)
Fig.5: (a) DAT system covered with Toptex (fleece) sheet

(b)
(b) Bamboo dome removed after 2 weeks for reshaping of pile

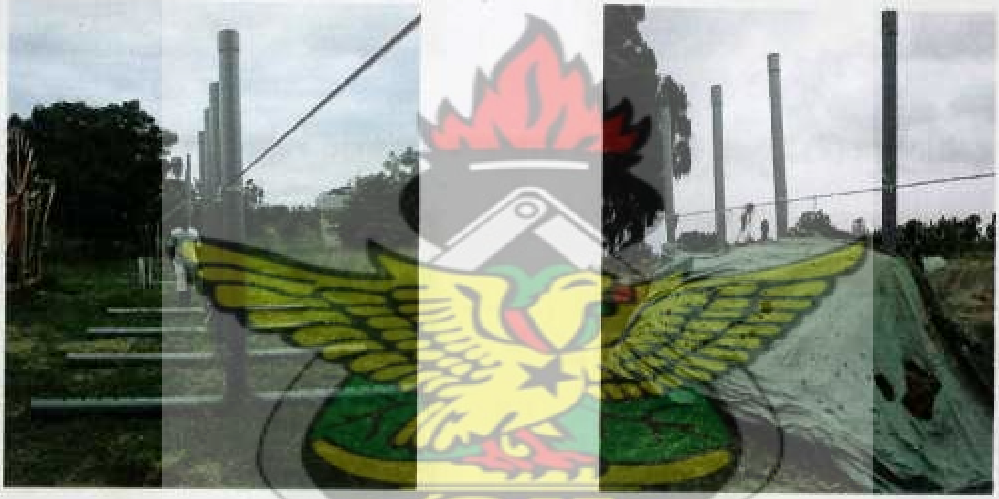
KNUST

KWAME NKRUMAH U… SCIENCE AND TECH… KUMASI-GHANA

3.2.2 Horizontal-Vertical Aeration Technology

The horizontal-vertical aeration technology (also known as the T- aeration) works on the same principle as the DAT. The difference is in the use of 6 in. diameter uPVC pipes with perforated holes to effect the passive aeration. The pipes are inverted T-shaped, they have perforations on the horizontal section allowing ambient air to move into the pile and that of the vertical, allow warm and waste gases to exit compost mass. The dimension of the pile is: 6.8m (L) ×2.6(B) m×1.7m (H); and is covered with the Toptex (fleece) sheet after mounting the piles. The vertical pipe was perforated to about 1.2m high from the bottom (as in *Fig. 6*).

KNUST



(a) (b)

Fig. 6: (a) perforated uPVC pipes; (b) prepared pile

3.2.3 Forced Aeration Technology

This method uses an electrical blower controlled intermittently by timers (Timer ON – 2 minutes and Timer OFF -12 minutes) with a specified flow rate of 18m^3/min and power rating of 0.4kW. Air is blown through perforated pipes 3m long. The pile was mounted with dimensions as follows: 4m (L) ×2m (B) ×1.5m (H), with the same material composition which had been turned four (4) times with the Sandberger ST 300 pulled by 96HP tractor. The pile was mounted a day after turning the mixture.

Wood shavings from a local wood processing facility was used as bulking material to facilitate even air distribution at the bottom of the pile and also prevent feed stock materials from blocking the perforated holes (as seen in *fig.7 (b)*). The blower was covered with a metal can to protect it from the rains, while the fleece was used to cover the pile to prevent excessive drying.

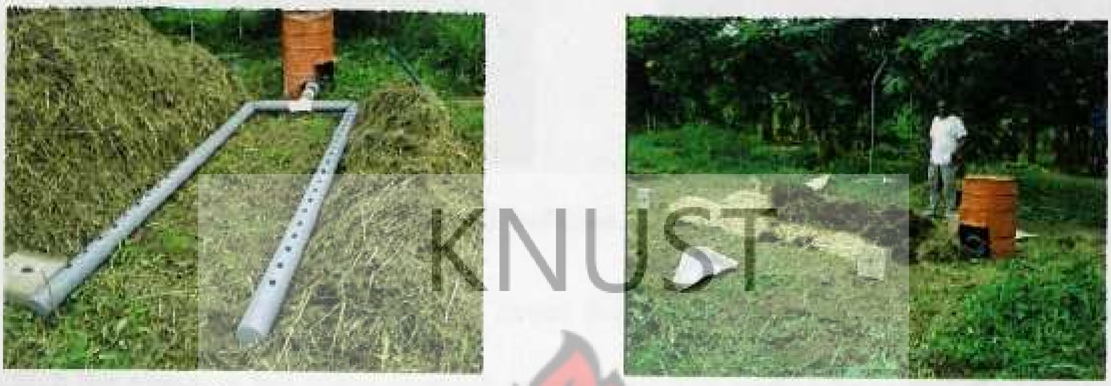

(a) (b)

Fig.7: (a) perforated pipe network connected to the blower; (b) mounting the pile

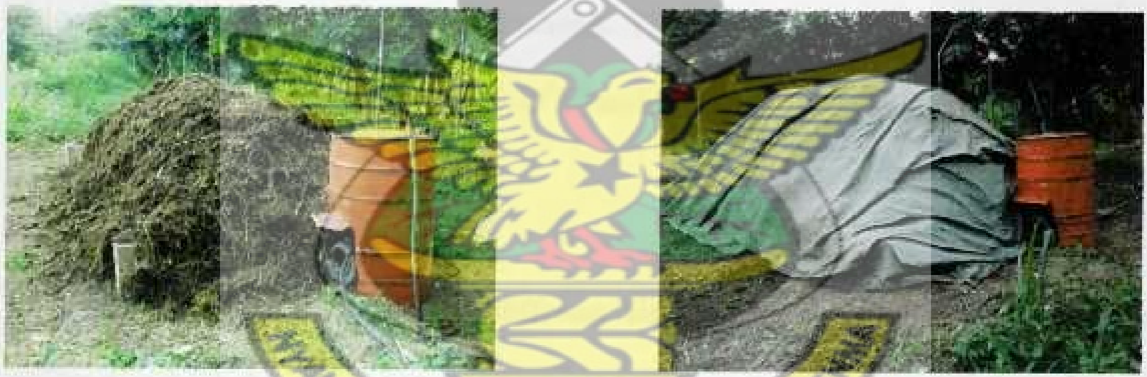

(c) (d)

Fig. 7: (c) mounted pile for forced aerated system; (d) pile covered with fleece

KNUST

3.2.4 Turned Windrows

For the turned windrow system, the feedstock was initially turned four times. The same feedstock was applied to the forced aeration pile. Dimensions for this pile are: 35m (L) ×2m (B) ×0.9m (H), (as shown in *fig. 8*). Turning was conducted on the condition that temperature or CO_2 levels exceed 65^0C or 20% respectively and done with the Sandberger ST 300 pulled by a 90 HP tractor. The front-loader (165 HP) was used to reshape the pile after which a Toptex (fleece) sheet was used to cover the windrow.

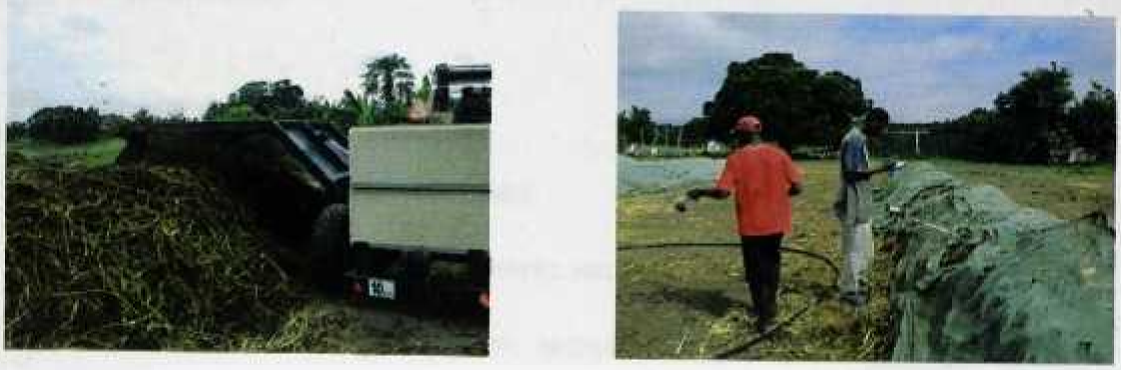


Fig. 8: (a) Sandberger ST 300 turning feed stock pile; (b) Covered windrow pile

KNUST

On the average, windrow is turned about 6-8 times during the first 2 weeks after which the row was left alone as much as possible. The static pile composting methods take between 7 and 8 weeks, before transporting it to the field for use. VREL estimates its maturity stage to be 8 weeks. The main control parameters in use at VREL are the Temperature (max. 60-65^{0}C) and CO_2 evolution (max. 16%).

3.3 Temperature Determination

Temperature readings from three different sides: top, middle and bottom of the compost masses from the different systems under-study were taken daily using the long stem thermometer (Salmoiraghi Co. thermometer model 17506) at the site. The daily ambient temperatures were also taken.

3.4 Sampling of Compost Mass for Physicochemical and Microbiological Analysis in the laboratory

Compost masses were sampled at the top, middle and bottom in the different systems mounted for the research with a forcep every week for laboratory study. The samples taken were bulked to obtain a representative sample. Samples were packed with ice cubes in an ice chest and transported each and every week to the laboratory where they were kept in a freezer at a temperature of 20°C for a day before microbial analysis was performed.

3.5 Physicochemical Analysis

3.5.1 Compost Masses pH Determination

The representative samples from each system were thawed. Three sub-samples of 10g each were taken from the representative sample of each system and poured into labelled beakers for the pH determination. The triplicated sub samples were suspended in distilled water in the ratio (1:10) and shaken on a rotary shaker for 30mins. The supernatant was then poured into a beaker and pH determined using a pH meter (Scientific Instruments Co. (Italy) model 9000/3). The pH of the triplicated samples for each system were averaged to represent pH of compost mass of each system.

KNUST

3.5.2 Moisture Determination

10g each of the representative samples from the different systems were weighed and triplicated for moisture content determination using the oven method. Samples were kept in the oven at 105°C for 24hrs and changes in weight of samples were averaged and used as the measure of moisture content of compost mass of each system.

3.6 Microbiological Analysis

3.6.1 Sterilization Procedures

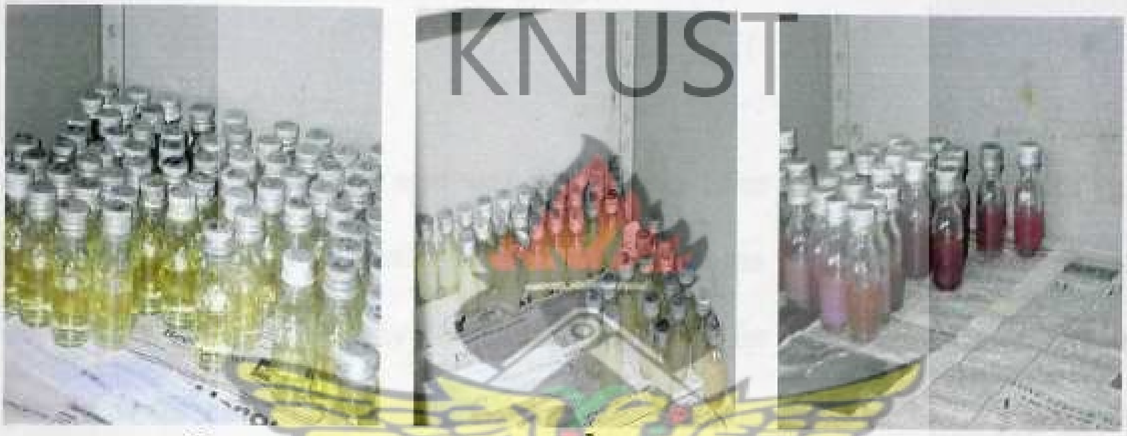
Different methods were employed in sterilizing all equipment, glass wares and media used for the microbiological analysis to prevent contamination by non targeted organisms. Below are the descriptions of the modes of sterilization adopted.

3.6.1.1 Glass Wares

Universal bottles, McCartney bottles and flasks serving as containers of media and distilled water were washed with detergents and rinsed with distilled water. They were then sterilized by autoclaving at 121°C for 15minutes. However, washed, rinsed and dried Petri-dishes were packed into canisters and subsequently sterilized in the hot-air oven at 160°C for 1hour.

3.7 Media Preparation

KNUST



a b c

Fig. 9: (a) Prepared Peptone water; (b) Plate Count Agar; (c) Violet-Red Bile Agar

3.7.1 Standard Plate Count Agar

It was prepared by suspending 23.5g in 1litre of distilled water in a beaker with 1g of agar-agar added per 100ml and boiled to dissolve completely. It is then dispensed 9ml each into McCartney bottles and sterilized by autoclaving at 121°C for 15 minutes.

3.7.2 Violet-Red Bile Agar

38.5g Violet-Red Bile Agar powder was suspended in 1litre of distilled water in a beaker with 1g of agar-agar added per 100ml of distilled water. It was brought to boil to dissolve completely and 9ml each dispensed into McCartney bottles.

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

3.7.3 Blood Agar

The blood agar base was prepared by dissolving 37g of the blood agar base powder into 1litre in a conical flask and adding 1g of agar-agar per 100ml of distilled water. The flask was covered with an aluminium foil. The content was soaked for 10 minutes and swirled to mix. It was then sterilized by autoclaving for 15 minutes at 121°C. 8-9% of sterile defibrinated sheep blood was added after cooling to 47°C. It was well mixed and poured 12-15ml per Petri-dish. After solidifying the Petri- dishes were packed into a canister and stored in a fridge.

KNUST

3.7.4 MacConkey Agar

52g powder of MacConkey agar was dissolved in 1litre of distilled water with 1g of agar-agar per 100ml of distilled water added. It was brought to boil to dissolve completely and sterilized by autoclaving at 121°C for 15minutes.The content was poured at the rate of 12-15ml per Petri-dish after cooling to 45°C.They were then packed into a canister and stored in a fridge.

3.7.5 Brilliant Green Agar

58g of brilliant green agar powder was suspended in 1litre of distilled water in a conical flask and 1g of agar-agar added per 100ml of distilled water. The flask was then covered with aluminium foil. It was heated while shaking and then autoclaved at 120°C for 15 minutes. The medium was allowed to cool to 45-50°C and 12-15ml poured into each Petri-dish. The Petri-dishes were packed into canisters and stored in a fridge after allowing the content to solidify.

3.7.6 Eosin Methylene Blue Agar

34g of Eosin methylene blue agar powder was dissolved in 1litre of distilled water in a conical flask and 1g of agar-agar was added per 100ml of distilled water. The flask was covered with aluminium foil. It was well mixed by swirling and then autoclaved at 121°C for 15minutes.The medium was allowed to cool to 45°C and 12-15ml poured into each Petri-dish. They were packed into canisters and stored in a fridge after allowing medium to solidify.

3.7.7 Sabouraud Agar

30g of Sabouraud powder was dissolved in 1 litre of distilled water in conical flask.1g of agar-agar was added per 100ml of distilled water and flask covered with aluminium foil. The mixture was then autoclaved at 121°C for 15 minutes after which it was poured 12-15ml per Petri-dish and stored in the fridge after solidifying.

KNUST

3.8 Preparation of Gram Stains

It was prepared using 0.5% crystal violet stain (basic stain), Lugols iodine (mordant), 50:50 acetone-alcohol (decolourizer) and 1% aqueous safranin (counter stain).

3.9 Standard Operating Procedures

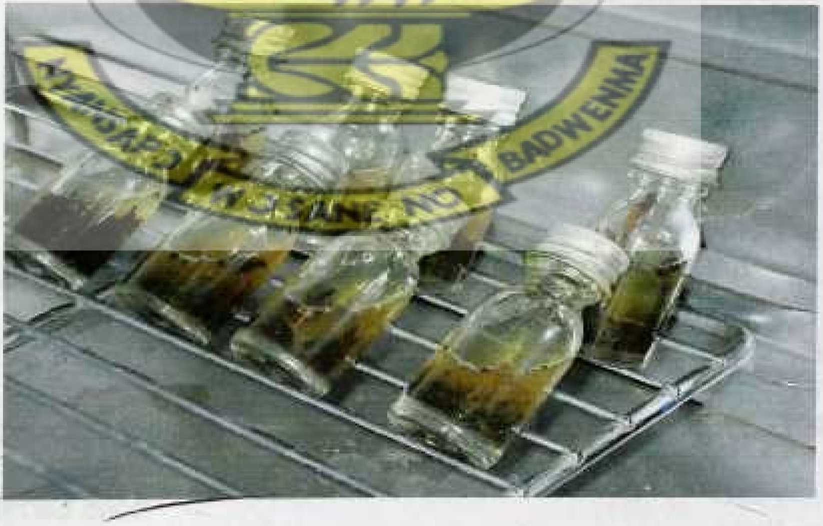


Fig.10: Compost samples being prepared for serial dilution

3.9.1 Serial Dilution for Total Viable and Coliform Count

1g of representative samples taken from each of the systems mounted for study was weighed into 9ml of 0.1% peptone water contained in 4 different McCartney bottles and incubated at 37°C for 15minutes.They were mixed well and 1ml of the supernatant was drawn from each of the bottles and diluted using 10-fold dilution into 4 other McCartney bottles each containing 9ml of sterile 0.1% blank peptone water. Different pipettes were used for each of the dilution. 1ml of the diluents taken from dilution factors: $1{:}10^3$,$1{:}10^4$ and $1{:}10^5$ were transferred into 2 sets of 3 different McCartney bottles one set containing 9ml of molten Plate Count Agar (PCA) and the other set 9ml of Violet Red Bile Agar. Both sets were kept in a water bath at 45°C to prevent solidification (Collins & Lyne, 1983).

KNUST

Fig.11: Colonies viewed under the colony counter

They were mixed by swirling and then poured into sterile Petri-dishes aseptically and allowed to set. The whole set was incubated at 30°C for 24hours. Cultures showing between 30-300 colonies were counted using the colony counter (AOAC, 1983). The process was replicated once.

3.10 Fungi Count

For fungi enumeration, 1ml of the neat representing compost mass taken from each system were transferred onto the Sabouraud agar in labelled Petri-dishes and incubated at 30ºC for 2-7days. The process was replicated once and identified colonies of fungi were counted under the colony counter using cultural characteristics and colour.

3.11 Culturing of Microorganism

KNUST

Fig.12: Drying of media for culturing of microorganisms

This was achieved by streaking the plates of the different agar used. The wire loop was flamed and allowed to cool. The cap of McCartney bottle containing the neat was flamed while held between the thumb and the index finger of the right hand. The left hand was used to open the cap and the mouth of McCartney bottle flamed. The flamed sterile loop was then used to take loopful of culture from the neat and the mouth of bottle quickly flamed and closed. The loopful of culture was streaked evenly over the surface of a prepared agar by sufficiently raising the lid of the Petri- dish. Following this method, cultures from the neat (1:10) from each system were made onto the dried surfaces of blood agar, MacConkey agar and Sabouraud agar. For spore forming microorganisms, the supernatant of the neat from the various systems were pre-incubated at 80ºC for 10 minutes in a water bath. The pre-incubated supernatants were then cultured on blood agar, MacConkey agar and Sabouraud agar. Plates were incubated for 1-2 days at 30ºC for blood agar and MacConkey agar and 2-7days at 30ºC for Sabouraud agar.

3.12 Sub-culturing of Microorganisms

KNUST

Fig.13: Growth observed on MacConkey Agar

For sub-culturing, the streak plate method was used to make pure cultures of colonies identified on the fresh sterile media after growth was observed on mixed cultured plates.

3.13 Identification of Microorganisms

Identification of bacteria was based on the examination of slides of Gram stained microorganisms prepared from pure cultures grown on blood agar, MacConkey agar, Brilliant Green agar and Eosin Methylene Blue agar .The Brilliant Green agar and Eosin Methlyene Blue agar were plated to aid the identification of *Salmonella spp.* and *Escherichia spp.* respectively. Slides were observed using the light microscope at x100 with oil immersion. The colonial and cell morphology of microorganisms and reactions to the Gram stain were used in bacteria identification as shown in appendix

G (Table G.1). Fungi were identified using their colonial morphology and colour reaction on sabouraud agar as shown in appendix H (Table H.1).

Slides kept in 90% alcohol were dried. The wire loop was flamed and two loopfuls of distilled water was placed at the centre of a cleaned slide. The wire loop was lightly used to touch the identified colony on a plated pure culture, and then gently mixed with the distilled water to dilute their concentration and was spread over the centre of the slide to cover approximately 3cm x 1cm.

KNUST

3.14 Preparation of Smears

Fig. 14: Preparation of smears

The process was repeated for all colonies identified on all plated cultures obtained from weekly representative samples of each system. They were then allowed to perfectly dry in air. The smears were flame fixed by passing the slides grasped with forceps horizontally through a Bunsen flame three times.

3.15 Gram Staining of Bacteria

The prepared slide was placed on a staining rack and the smear was flooded with the crystal violet stain for about 30 seconds. The stain was washed off the slide held firmly with forceps in one hand with distilled water and flooded with Lugol's iodine. The Lugol's iodine (mordant) was left for 30 seconds and then washed off with distilled water. Acetone-alcohol was used to decolourize the crystal violet stain. The slide was then flooded with the safrannin (counter stain) and left for a minute. It was washed off with distilled water and allowed to dry finally in air. The process was repeated for all the prepared slides.

3.16 Examination of Slides

Fig.15: Examination of microorganisms under the microscope

KNUST

All prepared slides were examined by light microscope under x100 oil immersion objective lens for bacteria and x10 for fungi. Bacteria were identified based on their reaction to Gram stains, use of cell and colonial morphology, shape, arrangement and colour whereas fungi were identified based on cultural characteristics and microscopy.

3.17 Counting of Helminths Eggs

This was done using the McMaster technique. 3g of sample obtained from each system was weighed into labelled plastic container. The samples were emulsified with 45ml of distilled water and ~~sieved~~ with a wire mesh of 0.15 aperture into a bowl. The sieved solution was poured into Claytone-Lane test tube to 15.5ml mark and centrifuged at 1500 rpm for 3 minutes. The supernatant was poured off and the deposit mixed with saturated NaCl solution. The samples were prepared similarly for each system and resulting solution was loaded into a McMaster egg counting chamber with dropping pipette and examined under the light microscope with x10 eyepiece.

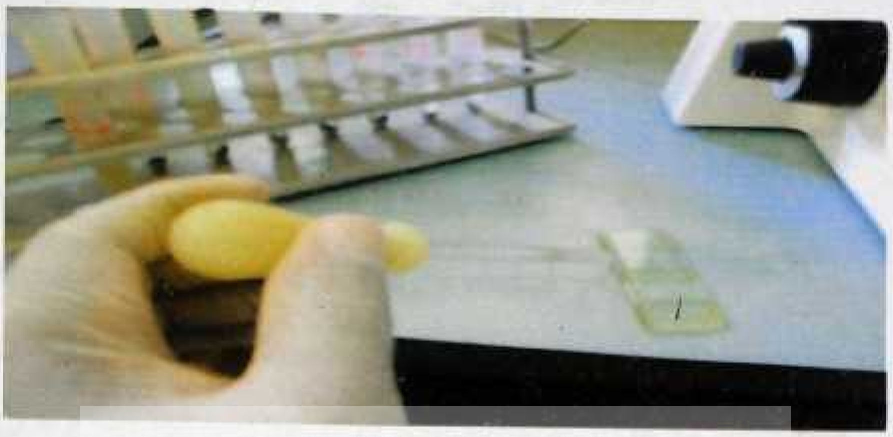


Fig.16: Filling of McMaster Egg Counting Chamber with prepared samples

KNUST
W J SANE NO BADWENA

3.18 Germination Index Determination

Four sterile Petri-dishes were taken and labelled to represent the systems under study. 5 layers of filter paper pad were fitted into each Petri- dish and then wetted with 5ml of 1:10 compost aqueous extract from samples representative of each system. A fifth Petri-dish was taken and fitted with 5 layers of filter paper and distilled water used as the medium of wetting the filter paper pads to serve as control. 30 tomato seeds were placed in each Petri-dish and incubated in the dark at 25°C for 5 days. The germinated seeds were counted and their root lengths measured in each Petri-dish using a rule and a thread in all the set-ups.

The germination index was then computed using the formula below:

$$GI = \frac{Nt}{Ng} \times \frac{AvRLt}{AvRLc} \times 100$$

Where GI = Germination index

Nt =Number of germinated seed in the treated

Ng =Number of germinated seed in the control

AvRLt = Averaged root length of germinated seeds in the treated

AvRLc = Averaged root length of germinated seeds in the controlled

KNUST

3.19 Result Analysis

Results obtained from experiment were summarized and analysed using Excel Software. Analyses of variances for the data obtained in the different systems were performed to determine the significant difference existing between their means. Where data analysed proved that statistical differences existed between systems, Tukey's Test was used in determining the systems in which the differences actually existed. Correlations between parameters measured in the different systems were also determined.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Hydrogen ion Concentration (pH)

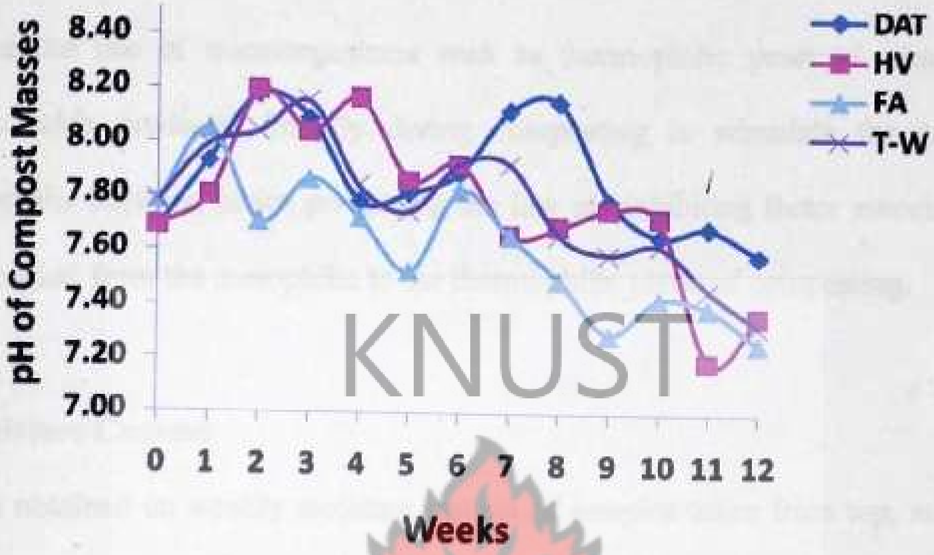
The results of Hydrogen ion concentrations of compost masses in the different composting systems; from the top, middle and bottom locations were averaged and tabulated in Appendix L (Table L.1).

The ANOVA performed on the mean weekly pH values of the compost masses in the different systems for the composting period at 5% level of significance gave a p-value of 0.0632 as shown in appendix A (Table A.1). This indicates that no significant difference existed between the pH values recorded in the different systems. Positive correlation coefficient values between pH and temperature were recorded in all the systems. The correlation coefficient values are: 0.5953, 0.8622, 0.5770 and 0.8029 for DAT, HV, FA and T-W respectively as shown in Appendix B (Table B.1, 2, 3, 4.). Smars *et al.*(2002) reported that the initial acidic phase could be reduced if the process temperature was kept until the pH value of mass is above 5, for the reason that microbial respiration in a well-controlled composting reactor was seriously inhibited when temperature increased above 40°C while the compost mass was still acidic. The pH values recorded in the systems under study were above 5 throughout the composting period so this inhibition was not observed.

The correlation coefficient values between pH and moisture content in systems DAT, HV, FA and T-W shown in Appendix B (Table B.1,2,3,4) are: -0.0816, 0.5102, 0.3048 and 0.4823 respectively. The poor negative correlation coefficient value between pH and moisture for the system DAT may only be due to the poor aeration in that system, since there was no significant difference between moisture contents in all the systems. When aeration is low, oxygen availability in the compost mass is

KNUST

affected, hence the anaerobic conditions created led to the production of acids. Thus this negative correlation coefficient between pH and moisture in DAT may probably be because of acid formation in anaerobic microenvironment as documented by Beck-Friis *et al.* (2003).



KNUST

Fig.17: Weekly variations in pH of compost masses in the different composting systems

Fig.17 shows the trends of the pH values recorded in each system. The pH values for all systems ranged between 7 and 9. Sundberg (2005) reported that during successful and fully developed composting, the pH often rises to 8-9. Nakasaki *et al.* (1993) composted organic household waste at 60°C and observed an increased degradation rate when pH was prevented from decreasing below 7 through liming. Eklind *et al.* (1997) revealed that organic acids such as lactic and acetic acid are frequently produced during the initial microbial degradation of food waste which reduces the pH of compost materials to 4-5.

The peculiar trend taken by the pH values recorded in the four systems may be due to the inclusion of 500g/40L of starter containing genetically modified microorganisms ~~such~~ as *Corynebacterium spp.* and *Bacillus spp.* that might have eliminated the

LIBRARY
KWAME N...
SCIENCE
KUMASI-GHANA

organic acids, thus preventing the characteristic initial low pH of 4-5 reported by Eklind *et al.* (1997). This confirms the result from Nakasaki *et al.* (1996) that the degradation rate at the initial stage of composting can be significantly increased by inoculation of acid-tolerant thermophilic bacteria. Choi and Parks (1998) also reported the use of microorganisms such as thermophilic yeast to eliminate the organic acids produced initially during composting to stimulate the growth of thermophilic bacteria, hence preventing the low pH inhibiting factor associated with the transition from the mesophilic to the thermophilic phase of composting.

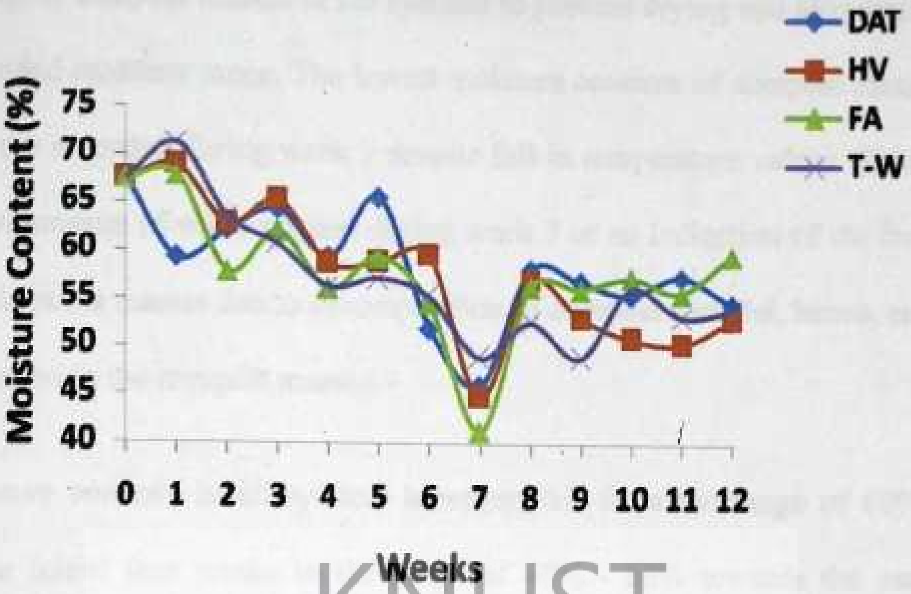
KNUST

4.2 Moisture Content

Results obtained on weekly moisture content of samples taken from top, middle and bottom locations in each composting system using the oven method were averaged and summarised in Appendix L (Table L.2)

Analysis of variance determined for moisture content results for the different systems at 5% level of significance yielded a p-value of 0.9842 as shown in Appendix A (Table A.2). This value indicates that no significant difference existed in the moisture content recorded in the various systems for the composting period.

W J SANE NO BADWEN



KNUST

Fig.18: Weekly variations in moisture content of compost masses in the different composting systems.

Fig.18 shows an initial rise in the moisture content of compost masses in systems T-W, HV, FA and decrease in that of DAT for week 1. The rise and fall moisture patterns illustrated by the curves in Fig.18 for the subsequent weeks, was as a result of the variations in the temperature and aeration in the different systems causing different rates of evaporation of moisture from compost mass in each system. This confirms results by Keener *et al.* (1996), that water evaporates as a result of the elevated temperature and aeration during compost and therefore, the need to often add water when composting food waste to keep the degradation rate high and avoid the susceptibility of compost material to drying.

All the four systems had moisture contents positively correlated with temperatures recorded in them. The correlation coefficients values of moisture contents and temperatures recorded for the systems are: 0.2888, 0.6145, 0.2514 and 0.8587 for DAT, HV, FA and T-W respectively as seen in Appendix B (Tables B. 1, 2, 3, 4). These positive correlation coefficient values were recorded because of the continuous rehydration of compost masses in the systems to prevent drying and also maintain the recommended moisture range. The lowest moisture contents of compost mass in each system were recorded during week 7 despite fall in temperature values. This may be due to low amount of water applied during week 7 or an indication of the creation of more voids in the masses due to decomposition of compost material, hence, enhancing better aeration in the compost masses.

The moisture contents in all systems however, fell from the range of 60% - 72% during the initial four weeks to the range of 40% - 60% towards the end of the process. This agrees with the moisture content recommended for composting process as reported by VanderGhenynst (2007) that, if moisture content went below 35% - 40% during the process, measures should promptly be taken to increase the moisture content. If moisture was never adjusted, the product could be unstable. If the moisture content of the material went above 60% - 70%, the materials would be composted longer to compensate for the reduced oxygen transfer, and thus aerobic microbial activity.

4.3 Temperature

Daily temperatures of compost masses in the different systems under study were taken at three different locations; top, middle and bottom. The daily temperature results were averaged to represent the average weekly temperatures recorded for compost masses in the various systems under study collated for a period of three months. Results obtained are summarized in Appendix L (Table L.3).

Analysis of variance (ANOVA) performed on the temperature results at 5% level of significance gave p-value of 4.75×10^{-7} as shown in Appendix A (Table A.3). It revealed that significant differences existed in the temperatures recorded in the different composting systems. Pair wise comparisons were made using Tukey's test at 5% level of significance to identify the systems between which the significant differences actually existed. Results summarized in Appendix C (Table C.1) indicate that there exist significant differences between the temperatures recorded in systems DAT and FA (q = 8.17), HV and FA (q = 8.16), T-W and FA (q = -4.38), T-W and DAT (q = 3.79) and T-W and HV (q = 3.78). This is because their calculated absolute Tukey's test values (q cal.) were greater than the critical Tukey's test value (q crit) = 3.76 at $\alpha = 0.05$ extrapolated from the Tukey's test table shown in Appendix D. There was no significant difference in temperatures of compost masses in systems DAT and HV (q = 0.015) as shown in Appendix C (Table C.1). The curves representing temperatures recorded in DAT and HV show a similar pattern as seen in Fig. 19. The nature of the curve representing temperatures recorded in FA as seen in Fig.19, illustrates that significant differences actually existed between the temperatures recorded in FA system and all the other composting systems.

KNUST

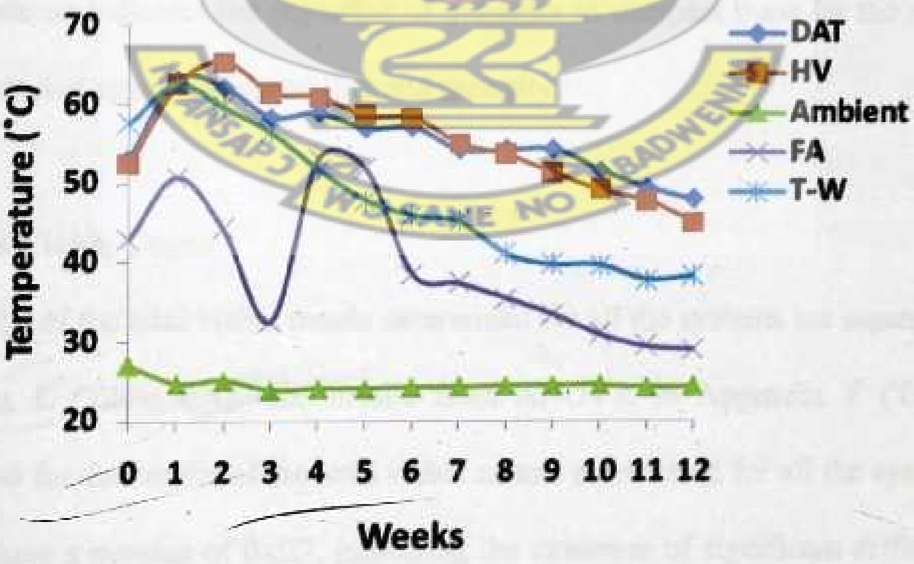


Fig.19: Weekly variations in temperatures of compost masses recorded in the different compost systems

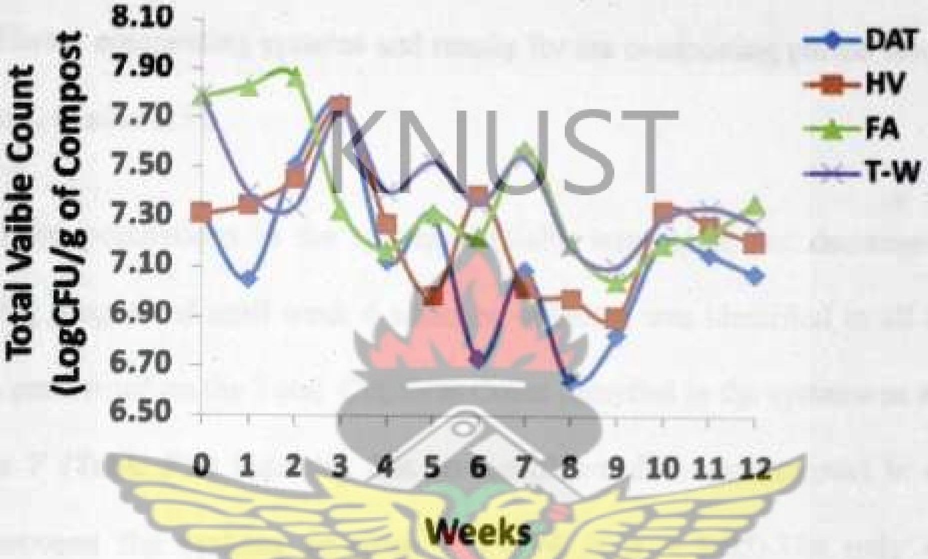
There was an initial temperature rise in systems DAT, T-W and FA from week 0 to week 2 and HV from week 0 to week 3.While temperatures recorded in systems DAT, T-W and HV fell gradually during the subsequent weeks of composting, that in FA experienced a steep fall during week 3 and a rise during week 4. The ambient temperatures ranged between 20°C- 30°C during the process. The gradual fall in temperatures recorded in systems DAT, T-W and HV was due to the natural cooling achieved via passive aeration driven by thermal convection whereas the steep falls in FA may be accounted for by the use of forced aeration to achieve cooling in the system.

KNUST

The earlier falls in the temperature recorded in the systems FA and T-W may result from better aeration, hence better cooling in those systems. The differences in the temperatures recorded in all the systems implied the succession of different microbial communities in all the systems as reported in Miller (1993) and hence the different rates of decomposition as indicated by Sundberg (2005). The final fall in temperatures in all systems indicates the depleting of nutrients in compost mass by the microbes; hence compost approaching stability and maturity.

4.4 Total Viable Count

The results of the total viable counts determined for all the systems are summarized in Appendix L (Table L.4). The results from ANOVA in Appendix F (Table F.1) performed for the results of the total viable counts determined for all the systems at α = 0.05, gave a p-value of 0.027, indicating the existence of significant differences in the total viable counts in the different composting systems. The nature of the curves in Fig.20 illustrates these differences. The compost material used in forming the heaps in systems FA and T-W were turned five times and samples taken to represent both systems. This accounted for the same initial total viable count of 7.79logCFU/g of compost recorded in them. Compost materials in systems DAT and HV were not turned because both are passively aerated systems. Thus, samples were taken from such unturned compost materials to represent both systems resulting in the same initial total viable count of 7.31logCFU/g of compost recorded.



KNUST

Fig.20: Weekly variations in the Total Viable Count of compost masses in the different composting systems

Fig. 20 shows that the microbial populations in all systems during the initial four weeks of composting were highest. During the subsequent weeks of composting, there were fluctuations in microbial populations until week 10 when the populations in each system began stabilising. However the fluctuations in the microbial population in the different systems were significantly different (p-value = 0.027), hence pair wise comparisons were done using the Tukey's test to identify the systems in which the differences in total viable count existed. The results from the Tukey's test summarized in Appendix C (Table C.2) indicates that significant difference existed only in the total viable count recorded in DAT and T-W(q = -3.80 at α = 0.05). The variation in the microbial population at different stages of composting in the different systems denote the succession and inactivation of microbes due to their sensitivity to temperature because moisture contents and pH in all composting systems were not significantly different at $\alpha = 0.05$.

4.5 Total Coliform Count

The Violet –Red Bile Agar was used in the determination of the total coliform count in the different composting systems and results for the composting period tabulated in Appendix L (Table L.5).

KNUST

The coliform populations in the systems initially were high, but decreased as the composting progressed until week 4 when no coliform was identified in all systems. ANOVA performed on the Total Coliform Count recorded in the systems as shown in Appendix F (Table F.2) indicates that no significant difference existed in coliform counts between the systems (p-value = 0.9994 at $\alpha = 0.05$).The only coliform identified in all the systems was *Enterobacter spp.*

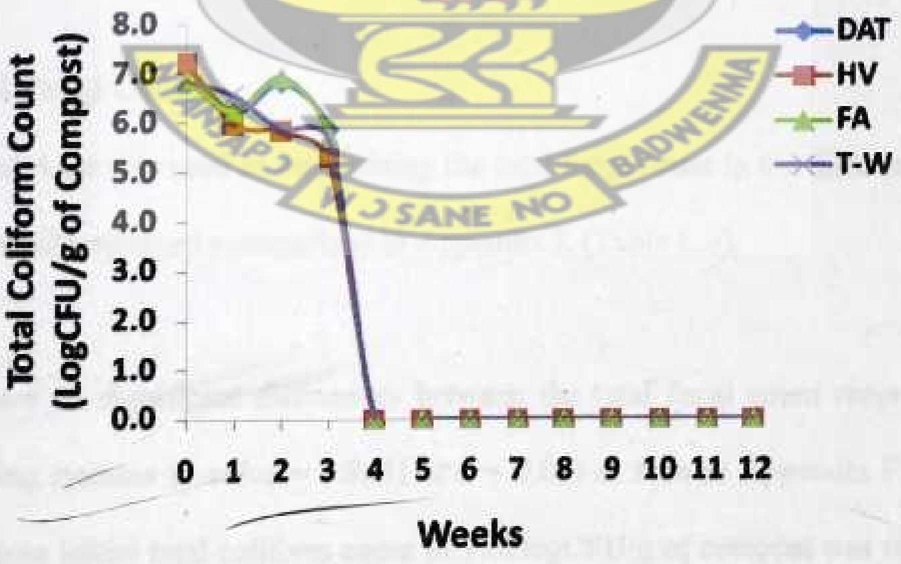


Fig.21: Weekly variations in the Total Coliform Counts of compost masses recorded in the different composting systems

An initial total coliform count of 6.88logCFU/g of compost was recorded in systems FA and T-W while an initial coliform count of 7.20logCFU/g of compost was recorded in systems DAT and HV. Cekmecelioglu *et al.* (2005) reported that to meet PFRP, a minimum inactivation temperature of 55°C must be achieved for a minimum period of 3 days during in-vessel and aerated static pile composting while the minimum conditions for windrow composting are 55°C for 15 days and five turnings during the composting period.

KNUST

The temperatures recorded in DAT, HV and T-W systems were above 55°C for more than 15 days but coliforms persisted till week 4.Though the temperatures recorded were lower than 55°C in the FA system due to the cooling effect of the air forced through the compost mass, coliforms just like in the other systems were not identified during week 4. It was also during week 4 that the highest temperature of 53°C was recorded in the FA system. Coliforms were not destroyed earlier in systems DAT and HV despite the high temperatures recorded in them. This might be because no turning of compost masses was effected in DAT and HV systems during the compost period.

4.6 Total Fungi Count

Sarbourad Agar was used in determining the total fungi count in the different systems and the results obtained summarized in Appendix L (Table L.6).

There were no significant differences between the total fungi count recorded in all composting systems (p-value = 0.9681 at α = 0.05) as seen in Appendix F (Table F. 3).The same initial total coliform count of 1.68logCFU/g of compost was recorded in FA and T-W and 1.11logCFU/g of compost in DAT and HV. There were fluctuations in fungi numbers in all systems during the composting process. However, the fungi numbers recorded in systems DAT, HV, and FA at the beginning were lower than the numbers recorded at the end of week 12 as seen in Appendix L (Table L.6). For T-W, there was a slight fall in the fungi number at the end of week 12. This might be due to early decomposition of cellulose, lignin and chitin as a result of turnings effected. Atlas and Bartha (1998) revealed that fungi are important group during the early phase of composting, because they are more tolerant to acids and less tolerant to temperatures above 35°C-40°C.The initial temperatures recorded in all the systems were above 35°C-40°C. Moreover, no acidic conditions were observed. This might have accounted for the initial low numbers.

KNUST

In Compost Microbiology and Soil Food Web (2008), it was documented that fungi are less tolerant to high moisture conditions and are mainly decomposers of cellulose, chitin and lignin found at the later stages of composting. Therefore, the decreasing moisture contents and the presence of large cellulolytic materials in the systems might have made the number of fungi increase in the latter weeks of composting. There was a rise in the fungi numbers in systems DAT and HV whereas a fall in systems FA and T-W in week 11 and 12 as seen in Fig.22.

NYANSAPO W SANE NO BADWENMA

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

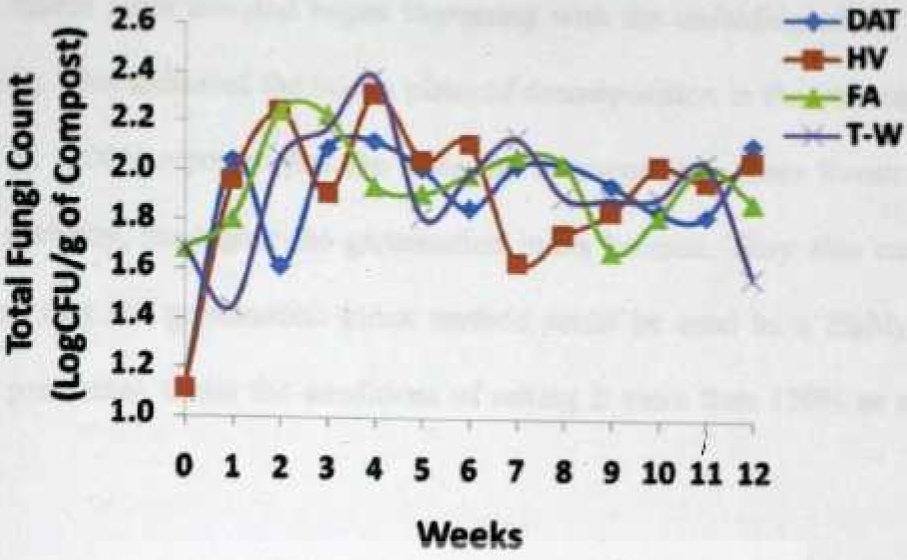


Fig. 22: Weekly variations in the Total Fungi Count of compost masses recorded in the different composting systems

KNUST

The fall in the fungi numbers in systems FA and T-W might be due to faster and earlier decomposition of the cellulolytic materials in these systems leading to the subsequent fall in the fungi numbers during week 12.

4.7 Germination Index

Results obtained from the germination index test performed on samples taken weekly from compost masses in the different systems in order to measure the rate of maturity are summarized in Appendix L (Table L.7).

The same percentage germination index was obtained in systems DAT and HV, and systems FA and T-W for week 0. This is because compost masses in systems FA and T-W were turned five times and samples taken to perform the germination index test representative of both systems. The same was done for systems DAT and HV because compost masses in the two were not turned. There were no significant differences between the germination indices obtained for compost masses in all the different systems (p-value = 0.0904 at $\alpha = 0.05$) as shown in Appendix F (Table F.4). However, initially, values were low and began increasing with the unfolding of the weeks of composting. This indicated the taking place of decomposition in the various systems. Ikeda *et al.* (2006) reported that the more the decomposition from livestock faeces came to maturity, the higher the germination index became. They also came to the conclusion that the germination index method could be used as a highly accurate maturity parameter, under the conditions of setting it more than 150% as a maturity standard.

KNUST

Compost masses in all the systems had germination indices more than 150. Systems T-W and FA had a higher rate of decomposition, hence faster maturity than systems HV and DAT; because the compost masses in the system pairs had final germination indices of 222.64%, 202.83% and 198.11, 189.54 respectively as seen in Fig. 23.

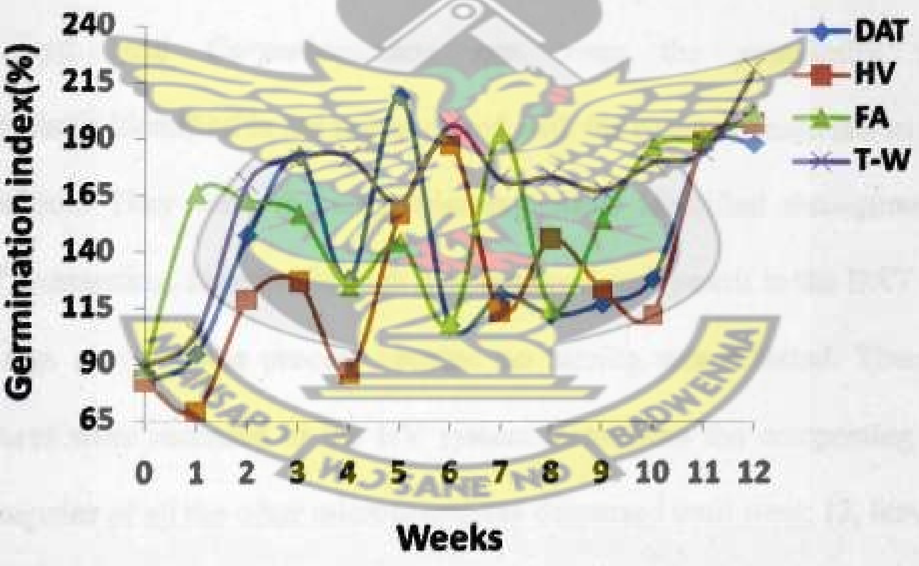


Fig.23: Weekly variations in Germination Indices of compost masses recorded in the different composting systems

4.8 Helminth Egg Counts

The compost masses in all the different systems were analysed for 13 weeks of composting with no helminth egg found.

4.9 Microbial Community

Bacillus spp., Staphylococcus spp., Streptococcus spp., Clostridium spp., Campylobacter spp., Listeria spp., Corynebacterium spp., Yersinia spp., and *Enterobacter spp.* were the bacteria identified during the various weeks of composting in all the systems. The *Enterobacter spp.* was destroyed in all systems during week 4.

In the DAT system, the thermophilic stage lasted for the composting period. The process was dominated during the early weeks by *Bacillus spp., Corynebacterium spp., Listeria spp., Streptococcus spp.* and *Staphylococcus spp.* The frequencies of *Listeria spp., Streptococcus spp.* and *Staphylococcus spp.* decreased towards week 12. However, *Bacillus spp.* (33.33%), *Corynebacterium spp.* (58.33%) and *Listeria spp.* (8.33%) survived the process as seen in Appendix I (Table I.1).

Bacillus spp. and *Corynebacterium spp.* were the genetically modified microorganisms identified in the starter mixed with the compost materials to facilitate decomposition. They were the only microorganisms identified throughout all the weeks of composting. Despite the high temperature development in the DAT systems, *Listeria spp.* survived the process because no turning was effected. Thermophilic temperatures were recorded in the HV system throughout the composting process. The frequencies of all the other microorganisms decreased until week 12, leaving only *Bacillus spp.* (42.86%) and *Corynebacterium spp.* (57.14%) to survive the process as seen in Appendix I (Table I.1).

In T-W system, thermophilic ~~temperatures~~ were recorded from week 0 to week 7. The frequencies of most of the bacteria identified reduced and others destroyed at the end of week 7 as shown in Appendix I (Table I.2). It was only *Bacillus spp.* (28.57%) and *Corynebacterium spp.* (71.43%) which survived the process. Thermophilic temperatures were only recorded in the FA system during week 1, 4 and 5.Though there were decreases and destruction of some microorganisms, *Bacillus spp.* (20%), *Staphylococcus spp.* (6.67%), *Corynebacterium spp.* (66.67%). and *Listeria spp.* (6.67%) survived the process as seen in Appendix I (Table I.2). They survived the process because of the low temperature development in the system and also no turning was effected in compost masses after the compost heap was formed.

Generally, it was noted that as temperature decreases the frequencies of *Bacillus spp.* reduced while that of the *Corynebacterium spp.* increased towards the end of the composting period in all systems, indicating that thermophilic conditions favour the growth and activities of *Bacillus spp.* whereas mesophilic conditions favour the growth and activities of *Corynebacterium spp.*

KNUST

Penicillium spp., Aspergillus spp., Mucor spp. and Rhizopus spp. were the fungi identified in all the systems as seen in Appendix J (Table J.1). Generally, the *Aspergillus spp.* were destroyed during week 4 in all systems. All the other fungi numbers increased toward the end of the composting process until week 12 when *Penicillium spp.* (97.79%) and *Mucor spp.* (2.21%) were identified in DAT system, while only *Penicillium spp.* (100%) were identified in all the other systems.

4.10 Effects of pH and Moisture on Composting Systems.

Moisture content and pH results recorded in all the systems were not significantly different (p-values = 0.0632, 0.9842, at $\alpha = 0.05$) respectively for the two parameters as seen in Appendix A (~~Table A.1,~~ A.2). The moisture and pH developed in the systems were almost similar and had no significant influence on the other factors determined in the different systems.

4.11 Effects of Temperature on Factors Determined in the Dome Aerated Technology.

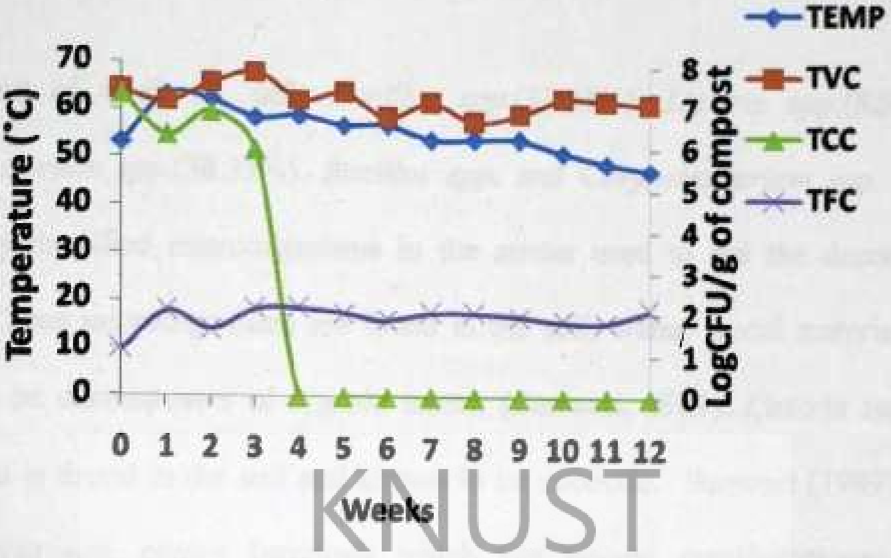


Fig. 24: Influence of Temperature on Total Viable, Total Coliform and Total Fungi Counts in DAT system.

KNUST

4.11.1 Total Viable Count

Fig.24 shows that every temperature change recorded in the DAT system affected the total viable count. Rise in temperature from 53.20°C to 63.24°C (week 0 to week 1), saw a slight decline in total viable count from 7.31logCFU/g of compost to 7.21logCFU/g of compost. Every rise and fall in temperature during the subsequent weeks resulted in the decrease and increase respectively in the total viable count until week 12 when it reduced to 7.08logCFU/g of compost.

The rise and fall in the total viable count shows that temperature inactivated some of the microorganisms and others increased during the process. However, the final total viable count (7.08logCFU/g of compost) was lower than the initial total viable count (7.31logCFU/g of compost) indicating that the total microbial population decreased as the nutrient became depleted. *Bacillus spp., Staphylococcus spp., Streptococcus spp., Clostridium spp., Campylobacter spp., Listeria spp., Corynebacterium spp.,* *Yersinia spp.* and *Enterobacter spp.* were the bacteria identified during the composting process as shown in Appendix I (Table I.1).

At the end of week 12, only *Bacillus spp.*(33.33%), *Listeria spp.*(8.33%) and *Corynebacterium spp.*(58.33%). *Bacillus spp.* and *Corynebacterium spp.* were the genetically modified microorganisms in the starter used to aid the decomposition process. These microorganisms are found in the soil, water, faecal material and are known to be decomposers of organic matter (Banwart, 1989). *Listeria spp.* on the other hand is found in the soil and known to be zoonotic. Banwart (1989) reported that *Listeria spp.* causes listeriosis which has several manifestations including septicaemia (leading to abortion or stillbirth in women), endocarditis, pneumonia, conjunctivitis, pharyngitis, urethritis and meningitis.

4.11.2 Total Coliform Count

Total coliform count decreased from 7.21logCFU/g of compost to 6.26logCFU/g of compost with rise in temperature from week 0 to week 1. Fall in temperature during week 2 resulted in increased total coliform count from 6.26logCFU/g of compost to 6.82logCFU/g of compost. *Enterobacter spp.* was the only coliform that was identified and did not survive the process. The total coliform count was reduced to 0 in week 4 as seen in Fig. 24 when temperature was at 59.08°C, indicating the reduction in faecal contamination. With the high temperatures recorded in the DAT system for the first four weeks, the coliform number could have been reduced earlier had turning been effected.

KNUST
W J SANE NO

4.11.3 Total Fungi Count

As seen in Fig.24, total fungi count rose from 1.11logCFU/g of compost to 2.05logCFU/g of compost in week 0 to week 1, decreased with a fall in temperature in week 2 and rose with further fall in temperature during week 3. There were fluctuations in the total fungi counts during the subsequent weeks until week 11 to week 12 when total fungi count increased with fall in temperature. The total fungi count increased from 1.11logCFU/g of compost in week 0, with slight rises and falls in numbers during the subsequent weeks, until finally reaching 2.13logCFU/g of compost in week 12.

KNUST

The increase in the total fungi counts towards the end confirms the fact that fungi are less tolerant to high temperatures (Atlas and Bartha, 1998); because the temperatures recorded in DAT system reduced gradually towards the end. Also fungi are reported to be decomposers of cellulose, lignin and chitin which bacteria are not able to decompose (Compost Microbiology and Soil Food Web, 2008). This might be the reason for the increase in the total fungi count at the terminal phase of the composting.

The fungi identified were *Aspergillus spp.*, *Penicillium spp.*, *Mucor spp.* and *Rhizopus spp.* as shown in Appendix J (Table J.1). No *Aspergillus spp.* was identified during week 4. The *Penicillium spp.* number kept increasing towards the end of the composting process whereas *Mucor spp.* number decreased in week 12. The final percentage frequencies of *Penicillium spp. Mucor spp. Aspergillus spp.* and *Rhizopus spp.* identified in week 12 were 97.79%, 2.21%, 0% and 0% respectively.

Penicillium spp. is reported to produce mycotoxins such as citrinin, luteoskyrin, ochratoxins and rubratoxin which cause illnesses in humans. Luteoskyrin is associated with high incidence of liver cancer in humans (Banwart, 1989). Dix and Webster (1995) also reported that *Penicillium spp.* and *Aspergillus spp.* may produce mycotoxins, which are harmful to human health; *Aspergillus flavus* produces aflatoxins, which are powerful carcinogens. *Penicillium spp.* and *Aspergillus spp.* may also cause allergy and lung diseases. However, no *Aspergillus spp.* and *Rhizopus spp.* was identified at the end of the composting process. *Mucor spp.* and *Rhizopus spp.* are not known to cause diseases in human (Banwart, 1989).

KNUST

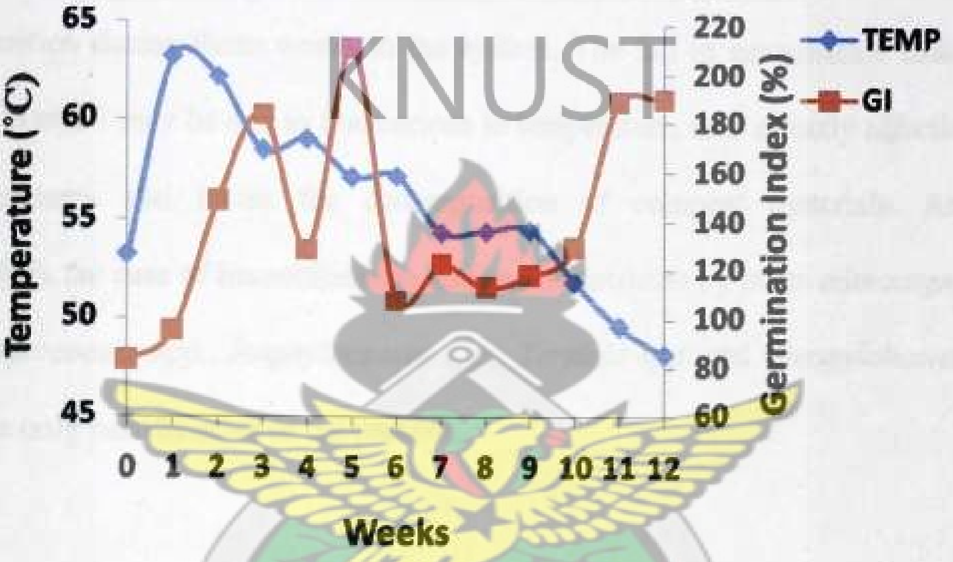


Fig.25: Variations in Temperature and Germination Index in the DAT system

4.11.4 Germination Index

Fig.25 shows that temperatures recorded in DAT system increased initially from 53.20°C in week 0 to 63.24°C in week 2 and gradually decreased during the subsequent weeks till it reached a temperature of 48.14°C in week 12. Germination index also started rising from week 0 to week 4, fell with temperature rise in week 5, rose with decreased temperature in week 6 until week 9 when it started rising again with fall in temperature to the end of the composting process. When the germination index curve is superimposed on the total viable count, total coliform count and fungi count as shown in Fig.26 it is seen that apart from week 0 to week 1 when the total viable count decreased with rise in the germination index, the subsequent weeks saw increase in total viable count with rise in germination index and decrease in total viable count with decline in the germination index until week 10 when the total viable count began falling with rise in germination index and total fungi count from week 11 to 12.

Generally, the germination indices rose from 82.54% during week 0 to 189.54% in week 12. The decrease in germination indices during week 4, 6 and 7, indicated slow decomposition during those weeks in the system. The fall in germination indices in weeks 4, 6 and 7 may be due to fluctuations in temperature, thus directly affecting the microorganisms and hence the decomposition of compost materials. Another possibility is the case of immobilization and use of nutrients by other microorganisms such *Streptococcus spp.*, *Staphylococcus spp.*, *Yersinia spp.* and *Campylobacter spp.* which are only beneficiaries of the process.

KNUST
NYANSAPO WO SANE NO BADWENMA

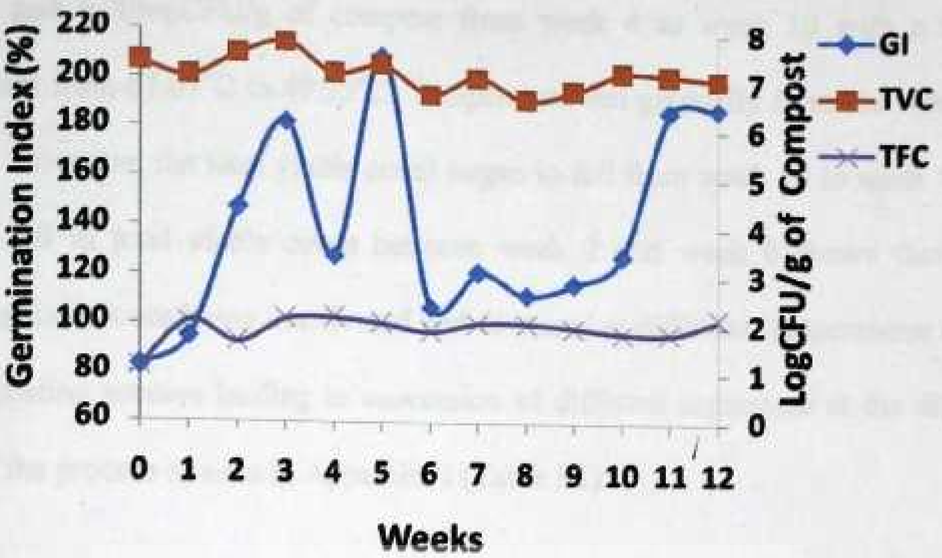


Fig.26: Variations in Germination Index, Total Viable Count and Total Fungi Count in the DAT system

KNUST

4.12 Effects of Temperature on Factors determined in the Horizontal-Vertical Technology

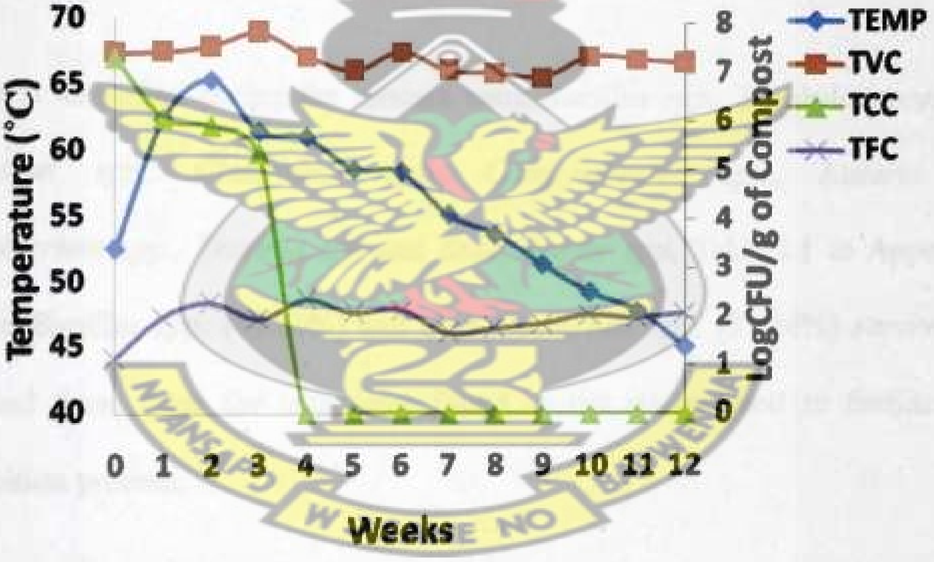


Fig. 27: Temperature variations on Total Viable Count, Total Coliform Count and Total Fungi Count in HV system.

4.12.1 Total Viable Count

Fig. 27 shows that as temperature increased from 52.49°C in week 0 to 65.44°C in week 2, there was a slight rise in the total viable count from 7.31logCFU/g of compost to 7.46logCFU/g of compost. Rise in temperature during week 3 caused a fall in the total viable count. The total viable count ranged between 7.28 logCFU/g of compost and 6.90logCFU/g of compost from week 4 to week 10 with a fall in temperature from 61.07°C to 49.35°C. Temperature fell gradually from the week 3 to week 12. However, the total viable count began to fall from week 10 to week 12.The rise and fall in total viable count between week 2 and week 6 shows that some microorganisms were being inactivated and favoured at different temperatures during the composting process leading to succession of different organisms at the different stages of the process as seen in Appendix I (Table I.1).

The gradual fall in temperature and total viable count towards the end of process indicate the depletion of nutrients and the process approaching stability, hence less heat was generated. The temperature values recorded in the system during the composting period show that only thermophilic conditions were created.

The bacteria identified during the process were *Bacillus spp., Staphylococcus spp., Streptococcus spp., Clostridium spp., Campylobacter spp., Listeria spp., Corynebacterium spp., Yersinia spp* and *Enterobacter spp.* Table I.1 in Appendix I shows that *Bacillus spp.* (42.86%) and *Cornybacterium spp.* (57.14%) survived the process and these were the organisms found in the starter used to facilitate the decomposition process.

4.12.2 Total Coliform Count

The total coliform count decreased during week 0 to week 2 from 7.21logCFU/g of compost to 5.85logCFU/g of compost with temperature rise from 52.49°C to 65.44 °C. Further fall in temperature from 65.44°C to 61.07°C in week 2 to week 4 led to a fall in the total coliform count from 5.85 logCFU/g of compost to 0, indicating the reduction in faecal contamination. *Enterobacter spp.* was the only coilform identified.

4.12.3 Total Fungi Count

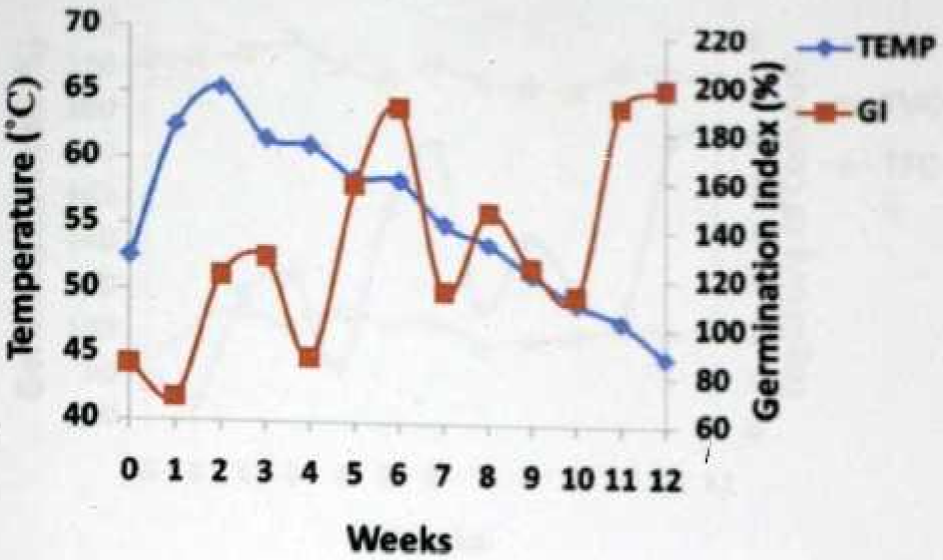
Fig. 27 shows a rise in total fungi count from 1.11logCFU/g of compost to 2.25logCFU/g of compost with rise in temperature from 52.49°C to 65.44°C during week 0 to week 2. The subsequent weeks experienced fluctuations in the total fungi count with a fall in temperature from week 3 to week 7. The total fungi count began increasing during week 8 to week 12 from 1.76logCFU/g of compost to 2.06logCFU/g of compost with a fall in temperature from 53.80°C to 45.19°C. The total fungi count recorded during week 12 was greater than that obtained in week 0. This confirms the fact that fungi are less tolerant to high temperatures and also are responsible for the decomposition of cellulose which are high during the latter stages of composting (Compost Microbiology and Soil Microbiology Web, 2008).

The various fungi identified during the process were *Penicillium spp.*, *Aspergillus spp.*, *Mucor spp.* and *Rhizopus spp.*, with only *Penicillium spp.* surviving the process at the end of week 12 as shown in Appendix J (Table J.1).

4.12.4 Germination Index

Germination index increased from 82.54% in week 0 with falls in week 1, week 4, week 7 and week 10 and rises during the other weeks till week 12 when 198.11% was recorded for the germination index as shown in Fig. 28. The temperature also increased from 52.49°C to 65.44°C from week 0 to week 3 and then fell gradually to 45.19°C in week 12. The rise in germination index from 82.54% to 198.11% denotes the increase in decomposition and maturity of the mass from week 0 to week 12. The gradual fall in temperature toward week 12 denotes the depletion of nutrients, hence less heat generation.

KNUST



KNUST

Fig.28: Variations in Temperature and Germination Index in the HV system.

However, when the germination index curve was compared with the total viable count and total fungi count, it was observed from Fig. 29 that as total fungi count and total viable count rose during week 1, the germination fell. In week 2, rise in total viable count and fungi count gave a rise in germination index. During week 3, the germination index increased with reduction in the total fungi count whereas in week 6, the germination index decreased as the total fungi count increased and total viable count decreased. This indicates that the fungi present were using the nutrient during the early stage of composting hence the low germination index recorded with rises in the fungi count. However, from week 10 to week 12, as the total fungi increased and the total viable count decreased, the germination index increased. This confirm the fact that fungi dominated the process at the end to decompose the cellulose, chitin and lignin coupled with the low temperature that favour their growth as reported in Compost Microbiology and Soil Food Web (2008).

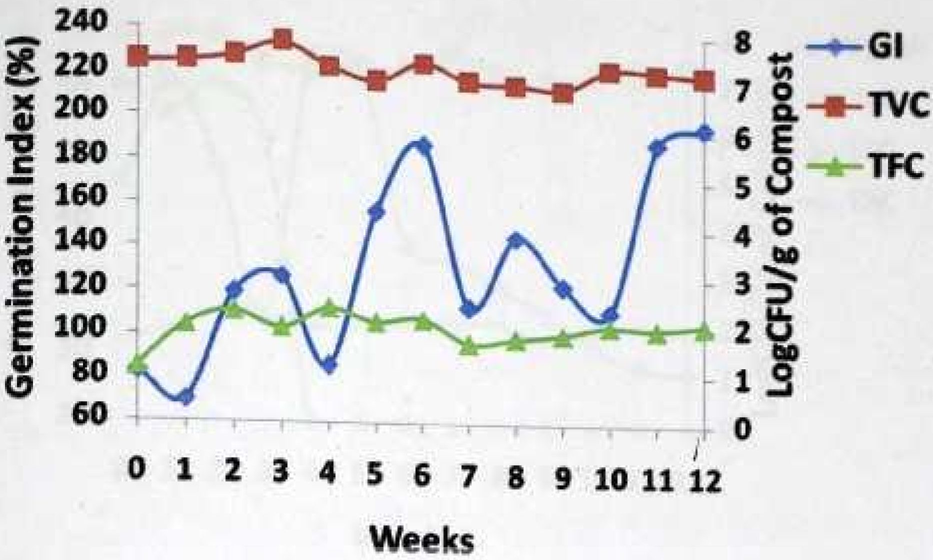


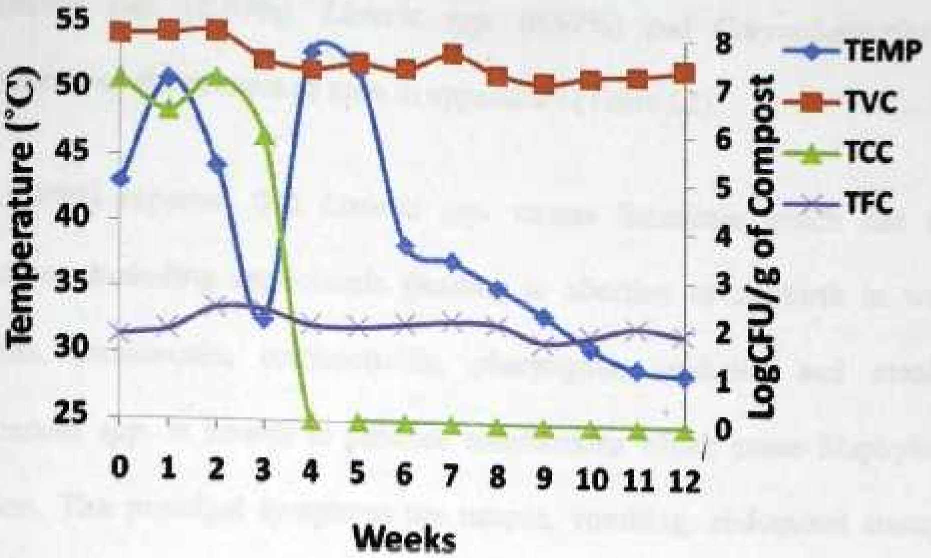
Fig.29: Variations in Germination Index, Total Viable Count and Total Fungi Count in HV system

KNUST

4.13 Effects of Temperature on Factors Determined in the Forced Aeration System.

4.13.1 Total Viable Count

Fig.30 shows that in the forced aeration system, as temperature increased from 42.97°C to 50.83°C during week 0 to week 1, there was a slight rise in the total viable count from 7.79logCFU/g of compost to 7.83logCFU/g of compost. Total viable count further increased to 7.87logCFU/g of compost when temperature dropped to 44.23°C in week 2. This occurred because thermophilic microorganisms such as *Bacillus spp.* and *Cornybacterium spp.* dominated the process during week 0 to week 2 as seen in Appendix I (Table I.2). A further fall in temperature to 32.59 °C reduced the total viable count to 7.33logCFU/g of compost in week 3. This drastic change in temperature from thermophilic to mesophilic condition might have caused the fall in total viable count in week 3.



KNUST

Fig.30: Variations in Temperature, Total Viable Count, Total Coliform Count and Total Fungi Count in the FA system

When temperature rose to the thermophilic zone in week 4 and 5, the total viable count began to increase from 7.17logCFU/g of compost to 7.32logCFU/g of compost. A change again in the temperature from the thermophilic to mesophilic condition reduced the total viable count in week 6.The gradual fall in the temperature from week 6 to week 12 showed variations in the total viable count. There was a rise in the total viable from week 9 to week 12 because of the low temperature development in the system making the mesophilic organisms dominate the process while the thermophilic organisms reduced towards the end. The low temperature development was due to the cooling effect of the forced aeration.

However, the gradual fall in temperature towards the end of the process indicated maturity of compost mass due to less decomposition, hence low heat generation. The bacteria identified during the process were *Bacillus spp., Staphylococcus spp., Streptococcus spp., Clostridium spp., Campylobacter spp., Listeria spp., Corynebacterium spp., Yersinia spp.* and *Enterobacter spp. Bacillus spp.* (20%), *Staphylococcus spp.* (6.67%), *Listeria spp.* (6.67%) and *Corynebacterium spp.* (66.67%) survived the process as seen in appendix I (Table I.2).

Banwart (1989) reported that *Listeria spp.* causes listeriosis which has several manifestations including septicaemia (leading to abortion or stillbirth in women), endocarditis, pneumonia, conjunctivitis, pharyngitis, urethritis and meningitis. *Staphylococcus spp.* is known to produce enterotoxins which cause Staphylococcal intoxication. The principal symptoms are nausea, vomiting, abdominal cramps and diarrhea (Banwart, 1989). *Bacillus spp.* and *Corynebacterium spp.* were the genetically modified microorganisms present in the starter added to aid the decomposition of the compost materials.

KNUST

4.13.2 Total Coliform Count

The total coliform count decreased from 6.88logCFU/g of compost to 6.26logCFU/g of compost when temperature rose from 42.97˚C to 50.83˚C during week 0 to week 1 as seen in Fig.30. The rise in temperature during week 4 from 32.59 ºC to 53.20˚C reduced the total coliform count to 0.The only coliform identified during the process was *Enterobacter spp.* as shown in Appendix I (Table I.2)

4.13.3 Total Fungi Count

There was a slight rise in the total fungi count from 1.68logCFU/g of compost to 1.81logCFU/g of compost during week 0 to week 1 when there was a rise in temperature from 42.97˚C to 50.83˚C.The fall in temperature during week 2 and 3 brought about increases in the total fungi count. The temperature rise in week 4 caused a decrease in the total fungi count. The total fungi count increased from week 5 to week 8, with a fall in week 9, a subsequent rise in week 10, 11 and a fall in week 12 as temperature fell during week 5 to week 12 from 51.94˚C to 29.00˚C.The total fungi count recorded during week 12 (1.89logCFU/g of compost) was higher than the initial total fungi count recorded (1.68logCFU/g of compost). This again supports the fact that fungi dominated the process towards the end because of reduction in temperature and the degradation of cellulose, chitin and lignin as reported in Compost Microbiology and Soil Food Web (2008).The fungi identified during the process were *Penicillium spp., Aspergillus spp. Mucor spp.,* and *Rhizorpus spp. Penicillium spp.* was the only fungi that survived the process in the FA system as indicated in Appendix J (Table J.1).

KNUST

4.13.4 Germination index

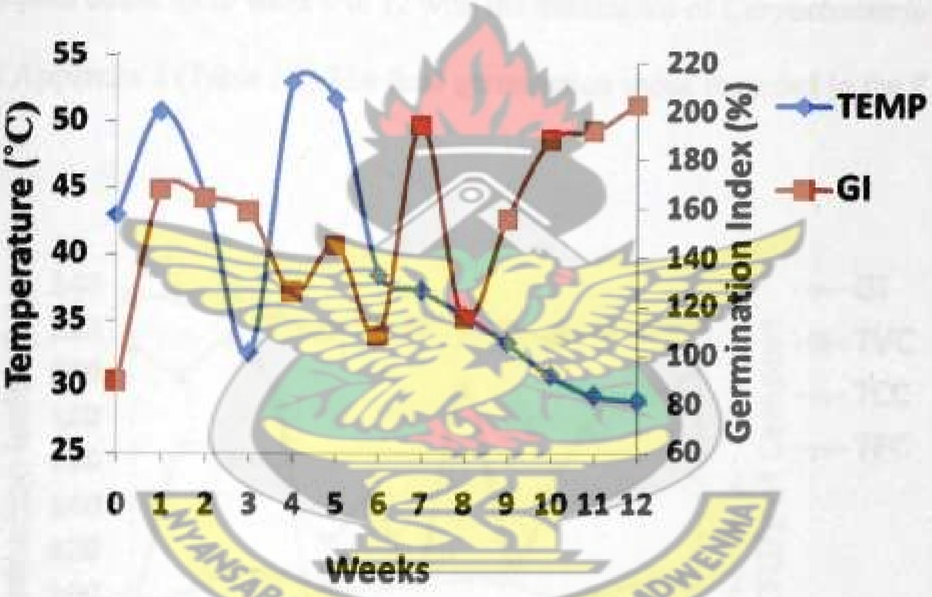


Fig.31: Variations in Temperature and Germination Index in the FA system

Fig. 31 shows that as temperature increased from 42.97 to 50.83 during week 0 to week 1, the germination index rose from 88.58% to 166.17% .When the temperature decreased in week 1 to week 3, the germination index decreased because the temperature changed from thermophilic zone to mesophilic zone affecting the microbial population activities. The change from mesophilic to thermophilic zone during week 3 to week 4 further decreased the germination index as the total viable count declined as seen in Fig.32. However when the thermophilic condition was maintained in week 5, germination index rose with rise in the total viable count. A change to the mesophilic condition, brought about a decline in the total viable count and germination index during week 6 (seen in Fig. 31& 32).The rise in germination index in week 7 with further fall in temperature may be due to the rise in total viable count during that week because of the stable mesophilic condition. The fall in the germination index in week 8 may be accounted for by the fall in the total viable count and also the dominance of *Bacillus spp.* which were inactive in mesophilic conditions as shown in Appendix I (Table I.2). There was a further rise in the germination index and total viable count form week 8 to 12 with the dominance of *Corynebacterium spp.* as seen in Appendix I (Table I.2).The final germination index recorded in the FA was 202.83%.

KNUST

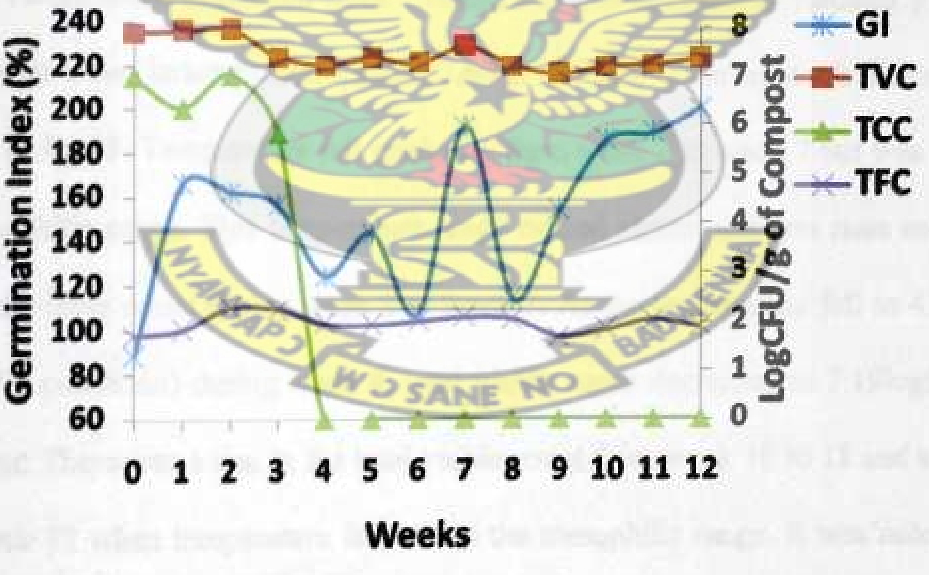
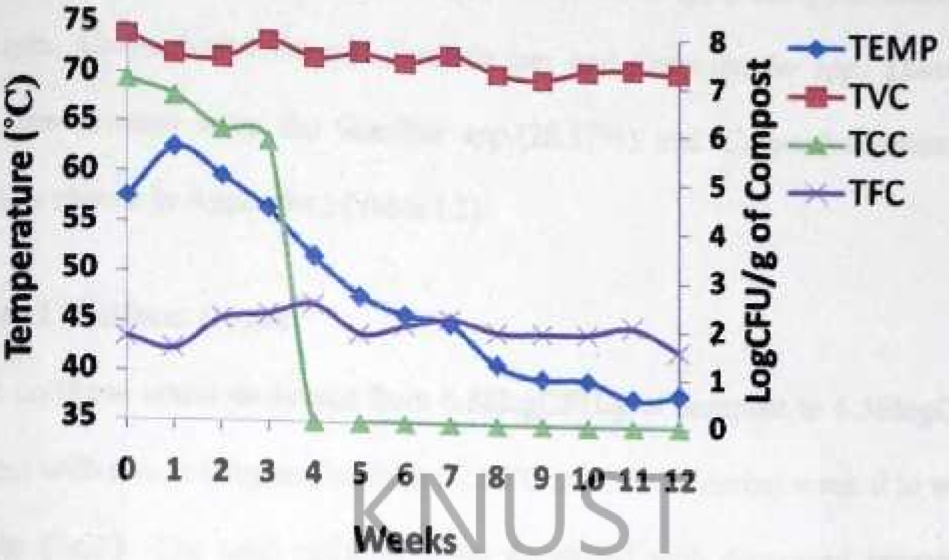


Fig.32: Variations in Germination Index, Total Viable Count, Total Coliform Count and Total Fungi Count in the FA System

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

4.14 Effects of Temperature on the Factors Determined in the Turned-Windrow System.



KNUST

Fig.33: Variations in Temperature, Total Viable Count, Total Coliform Count and Total Fungi Count in the T-W system

4.14.1 Total Viable Count

The total viable count decreased from 7.79logCFU/g of compost to 7.40logCFU/g of compost with a rise in temperature from 57.60°C to 62.56°C during week 0 to week 1 as shown in fig.33. Temperature fell gradually from week 2 to week 7 but was within the thermophilic range. This temperature range caused almost uniform rises and falls in the total viable count during week 2 to week 7. As the temperature fell to 41.17°C (mesophilic condition) during week 8, total viable count decreased to 7.19logCFU/g of compost. There was a rise in the total viable count from week 10 to 11 and a slight fall in week 12 when temperature fell within the mesophilic range. It was noted also that the percentage frequencies of *Corynebacterium spp.* increased from week 9 to week 12 as shown in Appendix I (Table I.2).

The fall in the total viable count during week 12 might be due to nutrient depletion. The gradual fall in temperature toward the end of the process indicated the stability and maturity of the compost. The bacteria identified during the process were *Bacillus spp., Staphylococcus spp., Streptococcus spp., Clostridium spp., Campylobacter spp., Listeria spp., Corynebacterium spp.,Yersinia spp.* and *Enterobacter spp.* Those that survived the process were the *Bacillus spp.*(28.57%) and *Corynebacterium spp.* (71.43%) as shown in Appendix I (Table I.2).

4.14.2 Total Coliform Count

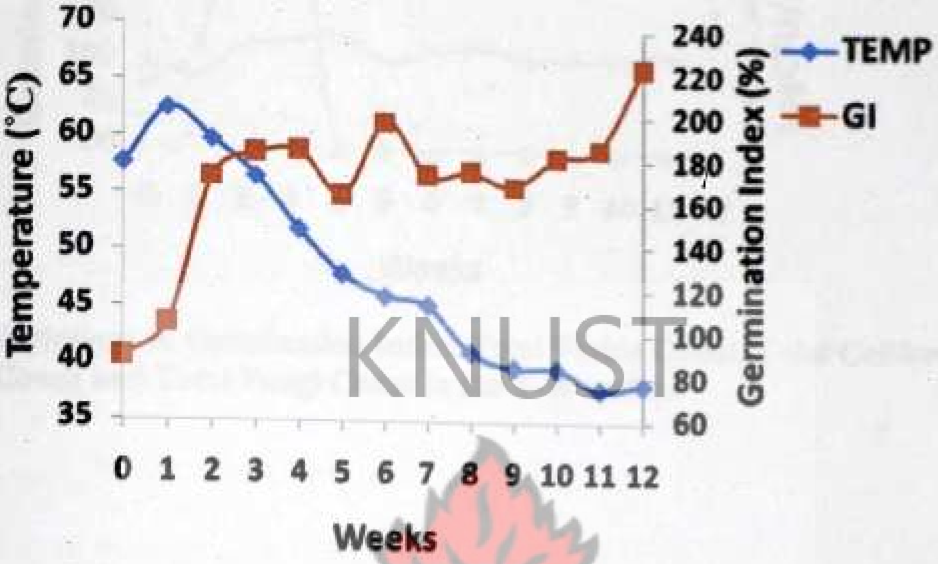
The total coliform count decreased from 6.88logCFU/g of compost to 6.56logCFU/g of compost with rise in temperature from 57.60°C to 62.56°C during week 0 to week 1 as seen in Fig.33. The total coliform count decreased with decreased temperature during the subsequent weeks until week 4 when the total coliform count yielded 0logCFU/g of compost at a temperature of 51.83°C.The total coliform count of 0logCFU/g of compost obtained in week 4 indicated the reduction of faecal contamination. *Enterobacter spp.* was the only coliform identified during the process.

KNUST

4.14.3 Total Fungi Count

Total fungi count decreased from 1.68logCFU/g of compost to 1.45logCFU/g of compost with increased in temperature from 57.60°C to 62.56 °C during week 0 to week 1. The total fungi count increased as temperature fell from week 1 to week 4 and subsequently declined in week 5. The total fungi count further rose to values ranging between 1.90logCFU/g of compost to 2.15logCFU/g of compost during week 6 to week 11. There was a fall in the total fungi count in week 12. This distribution of fungi during 6 to 11 confirms the fact that they tolerate low temperature and largely present at the latter stages of composting to decompose cellulose, chitin and lignin. The fungi identified during the process were *Penicillium spp., Aspergillus spp.,* *Mucor spp.* and *Rhizopus spp.* The only fungus that survived the process was *Penicillium spp.* as shown in Appendix J (Table J.1).

4.14.4 Germination Index



KNUST

Fig.34: Variations in Temperature and Germination Index in the T-W systems

As temperature increased from 57.60°C to 62.56°C during week 0 to week 1, the germination index increased from 88.58% to 103.89% as illustrated in Fig. 34. When temperature decreased the subsequent weeks, germination index increased until a decrease was recorded during week 5. There was a fall in the percentage frequency of *Bacillus spp.* as shown in Appendix I (Table I.2), though a rise in the total viable count was recorded during week 5. The rise in the germination index during week 6 could also be attributed to the rise in the level of *Bacillus spp.* Appendix I (Table I.2), though there was a decrease in the total viable count as seen in Fig.35. The final germination index recorded in the system was 222.64%.

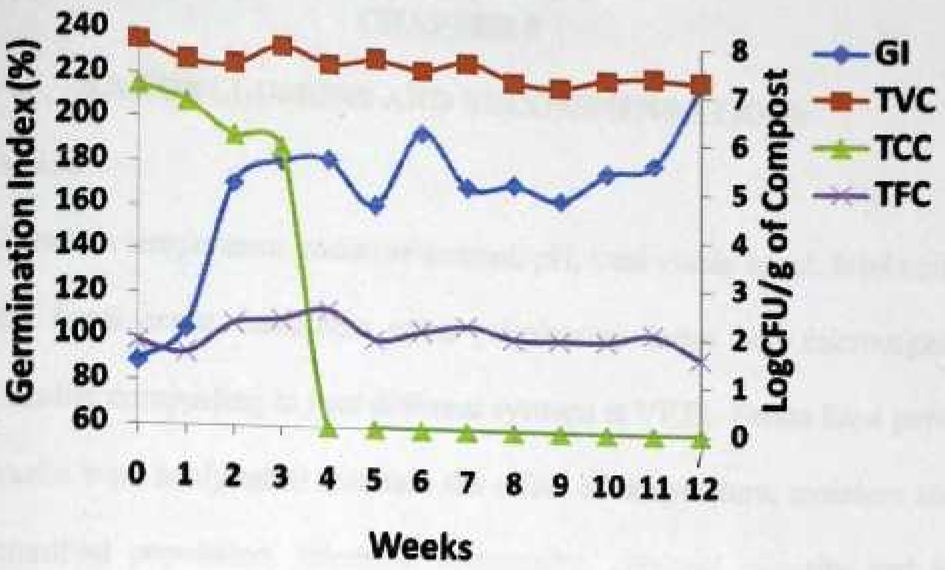


Fig.35: Variations in Germination Index, Total Viable Count, Total Coliform Count and Total Fungi Count in the T-W system.

KNUST

NYANSAPO WO SANE NO BADWENMA

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

CHAPTER 5

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Data collected on temperature, moisture content, pH, total viable count, total coliform count, total fungi count, helminths eggs, germination index and microorganisms identified during composting in four different systems at VREL Farms for a period of thirteen weeks were analysed to ascertain the effect of temperature, moisture and pH on the microbial population, microbial community, compost maturity and health hazards.

KNUST

5.2 Hydrogen ion Concentration

The pH values recorded in the various systems ranged from 7.20 - 8.17, 7.20 - 8.20, 7.27 - 8.05, and 7.33 - 8.16 for DAT, HV, FA and T-W respectively. The pH data recorded in the different systems ranged between 7 and 9 throughout the composting period and analysis of this data showed no significant difference between the systems studied (p-value = 0.06, at $\alpha = 0.05$). No acidic condition was recorded during the process and starter material containing genetically modified microorganisms such as *Bacillus spp.* and *Corynebacterium spp.* was added to facilitate the degradation process. These microorganisms might have fed on the organic acids preventing the characteristic low initial pH recorded during compost.

5.3 Moisture Content

The moisture content of compost masses recorded in DAT, HV, FA and T-W ranged from 46.07% - 67.49%, 44.83% - 68.93%, 41.23% - 67.87% and 49.00% - 71.30% respectively. Moisture content recorded in all the different systems for the span of thirteen weeks were not significantly different (p-value = 0.98, at $\alpha = 0.05$).The moisture content recorded in the four systems for the initial four weeks ranged between 60% - 72% which reduced during the subsequent weeks to the range of 40% - 60%. The positive correlation coefficient values recorded in the different systems between moisture and temperature was due to the regular rehydration of the compost masses in each of the systems.

5.4 Temperature

Temperature values ranged from 48.14°C - 63.24°C, 45.19°C - 65.44°C, 29.00°C - 53.20°C and 37.89°C - 62.56°C in DAT, HV, FA and T-W respectively. There were significant differences in temperatures recorded in the different systems during the composting period (p-value = 4.75×10^{-7}, at $\alpha = 0.05$). Pair wise comparisons were done using Tukey's Test at $\alpha = 0.05$ to ascertain the systems in which the significant differences actually existed. The analysis revealed the existence of significant differences between the temperatures recorded in systems DAT and FA (q = 8.17), HV and FA(q = 8.16), T-W and FA(q = -4.38), T-W and DAT (q = 3.79) and, T-W and HV (q = 3.78) as their calculated absolute Tukey's test values (q cal.) were greater than the critical value (q crit.), at $\alpha = 0.05$ extrapolated from the Tukey's test table and shown in Appendix E (q crit. = 3.76 at $\alpha = 0.05$). There was no significant differences in temperatures of composts in systems DAT and HV (q = 0.015).

KNUST

The temperatures recorded in the FA system were the lowest compared to all the other systems. This might be due to the cooling effect of the air forced into the compost mass. The T-W system was the next in which low temperatures were recorded and these can be accounted for by the turning of the compost mass to increase aeration. As a result of the different temperatures recorded in the various systems, different microbes were found in the compost masses in each system at different times.

However, there was a general fall in temperature towards week 12 in all systems indicating the process coming to an end; because of low microbial activities with the consequent low amount of heat generation.

5.4 Total Viable Count

The total viable counts recorded in DAT, HV, FA and T-W ranged from 6.64 - 7.77logCFU/g of compost, 6.90 - 7.75logCFU/g of compost, 7.05 - 7.87logCFU/g of compost and 7.11 –7.79logCFU/g of compost respectively. There was significant differences between the total viable count recorded in the systems (p-value = 0.027, at α = 0.05). Tukey's Test shows that significant difference existed only in the total viable count recorded in DAT and T-W (q = -3.80 at α = 0.05). Generally, the total viable count recorded initially in all the systems reduced at the end of week 12. This was because most of the microorganisms were inactivated by the temperature conditions that developed in the different systems during the composting process. The fall in the total viable counts towards the end of the process also suggested the depletion of nutrients.

KNUST

5.5 Total Coliform Count

The total coliform counts ranged from 5.90 – 7.21logCFU/g of compost, 5.30 – 7.21logCFU/g of compost, 5.78 – 6.95logCFU/g of compost and 5.70 – 6.88logCFU/g of compost in DAT, HV, FA and T-W respectively for the initial four weeks. However, during week 4 to week 12, the total coliform counts reduced to 0.00logCFU/g of compost in the various systems studied.

5.6 Total Fungi Count

The total fungi counts recorded in DAT, HV, FA and T-W ranged from 1.11 – 2.31logCFU/g of compost, 1.11 – 2.32logCFU/g of compost, 1.68 – 2.25logCFU/g of compost and 1.68 - 2.40logCFU/g of compost respectively. The total fungi counts recorded in the different systems during the initial week were lower compared to those recorded during the final week except for the T-W system. The decreased moisture contents, temperatures and the presence of large cellulolytic materials in the systems DAT, HV and FA supported the increase in the total fungi count during the latter week of composting. The low total fungi count recorded in T-W system during the final week might have been due to the turnings effected in the compost mass resulting in the early decomposition of celluloytic materials.

KNUST

5.7 Germination Index

The germination indices recorded were used as a measure of the rate of decomposition and maturity. Germination indices recorded in the different systems were low but considerably increased during the latter weeks of composting because of the release of nutrients by microorganisms. The germination indices ranged from 82.54% - 189.54%, 82.54% - 198.11%, 88.58% - 202.83% and 88.58% - 222.64% for compost masses in DAT, HV, FA and T-W respectively. Compost masses in all the systems had germination indices more than 150% at the end of week 12. Systems T-W and FA had a higher rate of decomposition, hence faster maturity than systems HV and DAT; because the system pairs had final germination indices of 222.64%, 202.83% and 198.11%, 189.54% respectively.

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

5.8 Microbial Community

Different bacteria were identified in the different systems at different stages of the composting and their frequency decreased at different times due to variation in temperatures recorded in the systems. *Bacillus spp., Staphylococcus spp., Streptococcus spp., Clostridium spp., Campylobacter spp., Listeria spp., Corynebacterium spp., Yersinia spp.* and *Enterobacter spp.* were the bacteria identified during the different phases of the composting process in all the systems.

Bacillus spp. (33.33%), *Listeria spp.* (8.33%) and *Corynebacterium spp.* (58.33%) survived the process in the DAT system; *Bacillus spp.* (42.86%) and *Corynebacterium spp.* (57.14%) survived the process in HV system; *Bacillus spp.* (20%), *Staphylococcus spp.* (6.67%), *Listeria spp.* (6.67%) and *Corynebacterium spp.* (66.67%) survived the process in FA system; *Bacillus spp.* (28.57%) and *Corynebacterium spp.* (71.43%) survived in T-W system. More microorganisms survived in the FA system because of the comparatively low temperature development in the system during the composting period.

Aspergillus spp., Penicillium spp., Mucor spp. and *Rhizopus spp.* were the fungi identified during the composting process in the different systems. *Penicillium spp.* (97.79%) and *Mucor spp.* (2.21%) survived the process in DAT system and only *Penicillium spp.* (100%) survived in systems HV, FA and T-W.

5.9 Health hazards

Some of the members of the compost microbial community that survived in the compost process in the different systems are responsible for causing diseases in humans.

Listeria spp. causes listeriosis which has several manifestations including septicaemia (leading to abortion or stillbirth in women), endocarditis, pneumonia, conjunctivitis, pharyngitis, urethritis and meningitis. *Staphylococcus spp.* is known to produce enterotoxins which cause Staphylococcal intoxication. *Penicillium spp.* produces mycotoxins such as citrinin, luteoskyrin, ochratoxins and rubratoxin which cause illnesses in humans. Luteoskyrin is associated with high incidence of liver cancer in humans. However, *Mucor spp.* and *Rhizopus spp.* are not known to cause diseases in humans.

KNUST

5.10 Recommendations

The following recommendations are made to enhance higher rate of maturity in the different systems and to protect compost producers and end-users from the possible health hazards.

1. Higher temperatures affect the microbial activities and the rate of decomposition, hence the need for turning to be effected in systems DAT and HV to improve the rate of compost maturity. In the FA system, aeration must be regulated such that temperatures do not fall so low to prevent the destruction of pathogens in the final product and also affect decomposition by thermophiles.
2. Different temperatures are recorded at different sites of the compost masses, so turning can considerably aid the destruction of pathogens because it allows heat to be distributed evenly in the compost mass.
3. Moisture content and pH ranges of 40% - 60% and 7- 9 respectively can be used for successful composting.
4. Since there were no significant differences at $\alpha = 0.05$ in the germination indices recorded in all the systems, the rates of maturity in all the systems were almost similar; hence in selecting the systems for composting, attention should

be focused on the cost and their ability to help reduce pathogens in the final product.

5. Compost producers and end-users must wear gloves and nose protectives when handling compost to prevent infection by pathogens in the compost.
6. Further research must be conducted to find out if the survived microorganisms would be present in the final produce the composts are applied to.

KNUST

REFERENCES

1. AOAC. (1983). Enumeration of coliforms in selected foods. Hydrophobic grid membrane filter method, official first action. Journal of Association of Official Analytical Chemists, 66:547-548.

2. Atlas, R.M. & Bartha, R. (1998). Microbial Ecology. Fundamentals and applications. Addison Wesley Longman, California, pp.58.

4. Banwart, G.J. (1989). Basic Food Microbiology. 2nd ed. Chapman and Hall, London, pp.105.

5. Beall, P.T. (1983). States of Water in Biological Systems. Cryobiology, 20:324-334.

6. Beck-Friis, B., Smår, S., Jönsson, H. & Kirchmann, H. (2001). Gaseous emissions of carbon dioxide,ammonia and nitrous oxide from organic household waste in a compost reactor under different temperature regimes. Journal of Agricultural Engineering Research 78(4), 432-430.

7. Beck-Friis, B., Smår, S., Jönsson, H., Eklind, Y. & Kirchmann, H. (2003). Composting of source-separated household organics at different oxygen levels: Gaining an understanding of the emission dynamics. Compost Science & Utilization, 11:41-50.

8. Beffa, T., Blanc, M., Marilley, L., Fischer, J.L., Lyon, P.F., & Arangno, M. (1996). Taxonomic and metabolic microbial diversity during composting. In: de Bertoldi, M., Sequi, P., Lemmes, B., Papi, T. (Eds.), The Science of Composting. Chapman and Hall, London, pp.149–161.

9. Bluman, A.G. (2004). Elementary statistics: A step by step approach.2nd ed. McGraw-Hill Companies, New York, pp.595, 742.

KNUST

W J SANE NO BADWE

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

11. Cambardella, C.A., Richard, T.L. & Russell, A. (2003). Compost mineralization in soil as a function of composting process conditions. Eur. J. Soil Biol., 39:117–127.

12. Cekmecelioglu, D., Demirci, A.,Graves, R.E. & Davitt, N.H.(2005). Applicability of Optimised In-vessel Food Waste Composting for Windrow Systems. Journal of Biosystems Engineering. 91(4): 479-487.

13. Cheesbrough M. (1984). Medical Laboratory Manual for Tropical Countries. Butterworth-Heinemann Ltd, London.Vol.2, pp.187-193.

14. Chen, Y., Inbar, Y. & Hadar, Y., (1992). Composted residues reduce pest and pesticide use. Biocycle, 6: 48–51.

15. Choi, M.H. & Park, Y.H., (1998). The influence of yeast on thermophilic composting of food waste. Letters in Applied Microbiology, 26:175-178.

16. Cochran, B. J. & Carney, W. A. (2005). Basic principles of composting. Louisiana State University Agricultural Center. Available at: < http://www. <lsuagcenter.com/NR/rdonlyres/1A247D4F-4E94-4021B09E2DF1043E179E /2908/ pub2622 compost.pdf. >. Accessed on Sept. 14, 2006.

17. Collins, C.H. & Lyne, P.M. (1983). Microbiological Methods. 5th ed. Butterworth and Co, London, pp.5, 89.

18. Composting Council of Canada (2008). A Summary of Compost Standards in Canada. Retrieved on 31st May, 2008 from < http://www. compost.org/ standard.html >

KNUST

19. Compost Microbiology and the Soil Food Web (2008). Retrieved on January 5, 2008, from <http://www.ciwmb.ca.gov/publications/Organics/>.

20. Cox, N. A., Fung, D.Y.C., Goldschmidt, M.C., Bailey, J.S. & Thomson, J.E. (1984). Selecting a miniaturized system for identification of Enterobacteriaceae. Journal Food Protection, 47:74-77.

21. Cunha Queda, A.C., Vallini, G., Agnolucci, M., Coelho, C.A., Campos, L. & de Sousa, R.B. (2002). Microbiological and chemical characterization of composts at different levels of maturity, with evaluation of phytotoxicity and enzymatic activities. In:Insam, H., Riddech, N., Krammer, S. (Eds.), Microbiology of Composting. Springer Verlag, Heidelberg, pp. 345–355.

22. Dai H., Kazutaka K., Yasuyuki, F. & Kiyonori H. (2005). Effect of addition of organic waste on reduction of *Escherichia coli* during cattle faeces composting under high-moisture condition. Journal of Bioresource Technology, 30:30-34

23. Day, M., Krzymien, M., Shaw, K., Zaaremba, L., Wilson, W.R., Botden, C. & Thomas B. (1998). An investigation of the chemical and physical changes occurring during commercial composting. Composting Science and Utilization, 6(2):44-46.

24. Dix, N.J., Webster, J. (1995). Fungal Ecology. Chapman & Hall, London, pp. 337.

25. Droffner, M.L. & Brinton, W.F. (1995). Survival of E. coli and Salmonella population in aerobic thermophilic composts as measured with DNA gene probes. Zentralbl Hygiene, 197:387-397.

26. Eklind, Y., BeckFriis, B., Bengtsson, S., Ejlerttsson, J., Kirchmann, H., Mathisen, B., Nordkvist, E., Sonesson, U., Svensson, B.H. & Torstensson, L. (1997). Chemical characterization of source-separated organic household waste. Swedish Journal of Agricultural Research, 27:167-178.

27. Eriksson, O. (2003). Environmental and economic assessement of Swedish municipal solid waste management in a systems perspective .Doctoral thesis, Royal Institute of Technology, Stockholm, pp.54.

28. Esse, P.C., Buerkert, A., Hiernaux, P. & Assa, A. (2001). Decomposition of and nutrient release from ruminant manure on acid sandy soils in the Sahelian zone of Niger, West Africa. Agri. Ecosyst. Environ., 83:55–63.

29. Fuchs, J.G. (2002). Practical use of quality compost for plant health and vitality improvement. In: Insam, H., Riddech, N., Klammer, S. (Eds.), Microbiology of Composting, Springer Verlag, Heidelberg, pp. 435–444.

30. Garcia, C., Hernandez, T., Costa, F. & Ayuso, M. (1992). Evaluation of the maturity of municipal waste compost using simple chemical parameters. Commun. Soil Sci. Plant Anal, 23:1501–1512.

31. Golueke, C.G. (1977). Biological Reclamation of Organic Wastes. Rodale Press, Emmans-PA, USA, pp.34.

32. Hancock, D.D., Besser, T.E., Kinsel, M.L., Tarr, P.I., Rice, D.H. & Paros, M.G. (1994). The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiol. Infect., 113:199–207.

33. Haug, R.T. (1993). The Practical handbook of compost engineering. Lewis Publishers, Boca Raton, pp.717.

34. Hay, J.C. (1996). Pathogen destruction and biosolids composting. Biocycle, 6, 67-76.

35. Huang, G.F., Fang, M., Wu, Q.T., Zhou, L.X., Liao, X.D., Wong, J.W.C. (2001). Co-composting of pig manure with leaves. Environ. Technol., 22:1203–1212.

KNUST

36. Hue, N.V. & Liu, J. (1995). Predicting compost stability. Compost Science and Utilization, 3:8–15.

37. Ikeda, K. Koyama, F. Takamuku, K. & Fukuda, N. (2006). The Efficiency of Germination Index Method as a Maturity Parameter for Livestock Faeces Composts. Bulletin of the Fukuoka Agricultural Research Center, 25: 135-139.

38. Ishii, K., Fukui, M. & Takii, S. (2000). Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis. J. Appl. Microbiol., 89: 768–777.

39. Jeris, J.S. & Regan, R.W. (1973). Controlling Environmental Parameters for Optimum Composting II: Moisture, free air space and recycle. Compost Science, 3: 8-15.

40. Kaneko, K. & Hashimoto, N. (1982). Five biovars of *Yersinia enterocolitica* delineated by numerical taxonomy. International Journal Systems of Bacteriology, 32:275-287.

41. Keener, H.M., Elwell, D.L., Das, K. & Hansen, R.C. (1996). Remix scheduling during composting based on moisture control.Trans.ASAE:1839-1845.

42. Klamer, M. & Bååh, E. (1998). Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS Microbiology Ecology, 27(1):9-20.

43. Kudva, I.T., Blanch, K. & Hovde, C.J. (1998). Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. Appl. Environ. Microbiol., 64: 3166–3174.

44. Løvborg, U. (1984). Monoclonal antibodies. A short review. America Clinical Production. Review, 3(1):26-28.

KNUST

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

45. Macdonald, D. M. and Griffin, S. E. (1981). Returning wastes to the land. In: food, fuel and fertilizer from organic wastes. Report of ad hoc panel of the advisory committee on technology for international development commission on international relations. National Research council. National Academy Press, Washington, DC. Pp. 10-15.

46. Madigan, M.T., Martinko, J.M. & Parker, J. (2000). Brock-Biology of microorganisms.9th Edition. Prentice-Hall. Upper Saddle River, N.J., pp.991.

47. Mangkoedihardjo, S. (2006). Revaluation of Maturity and Stability Indices for Compost. Journal of Applied Sciences & Environmental Management, 10(3): 83-85.

48. McKinley, V.L. & Vestal, J.R. (1985). Physical and chemical correlates of microbial activity and biomass in composting municipal sewage sludge. Applied and Environmental Microbiology, 47:933-941.

49. Mead, P. S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. & Tauxe, R.V., (1999). Food-related illness and death in the United States. Emerging Infectious Diseases, 5:607 – 625.

50. Milby, K. H. & Zare, R. N. (1984). Antibodies, Lasers, and chromatography. America Clinical Production. Review, 3(1):12-19.

51. Miller, F.C. (1993). Composting as a process based on the control of ecologically selective factors. In: Metting, F.B.J. (Ed.).Soil microbial ecology. Marcel Dekker, New York, pp.515-544.

52. Murray, M., Trail, J.C.M., Turner, D.A. & Wissocq, Y. (1983). Livestock Productivity and Trypanotolerance. Retrieved on 4th July 2008 from < http: //www.ilri.org/ InfoServ/Webpub/Fulldocs/LivProd/Toc.htm#TopOfPage>.

KNUST

53. Murwira, K.H., Swift, M.J. & Frost, P.G.H. (1995). Manure as a key resource in sustainable agriculture. In: Powell, J.M., Fernandez-Rivera, S., Williams, T.O., Renard, C. (Eds.), Livestock and Sustainable Nutrient Cycling in Mixed Farming of Sub-Saharan Africa, International Livestock Centre for Africa (II C.A.) Addis Ababa, Ethiopia, pp. 131–148.

54. Nakasaki, K. & Akiyama, T. (1988). Effects of seedling on thermophilic composting of household waste. Journal of Fermentation Technology 66, 37-42.

55. Nakasaki, K., Yaguchi, H., Sasaki, Y. & Kubota, H. (1993). Effects of pH control on composting of garbage. Waste Management and Research, 11(2): 117-125.

56. Nakasaki, K., Uehara, N., Kataoka, M. & Kubota, H. (1996). The use of *Bacillus licheniformis* HAI to accelerate composting of organic wastes. Compost Science and Utilization, 4(4):47-51.

57. Nakasaki, K., Akakura, N. & Atsumi, K. (1998). Degradation patterns of organic material in batch and fed-batch composting operations. Waste Management & Research, 11:117-125.

58. Pace-Moody, A. (2003). Sanitation and Solid Waste in Accra, Columbia University, USA, pp.1- 4.

59. Roletto, E., Barberis, R., Consignlid, M. & Jodice, R. (1985). Chemical parameters for evaluation of compost maturity. BioCycle, 3: 46–48.

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

61. Rynk, R. (1992). On-farm composting handbook. Northeast Regional Agricultural Engineering Service, Ithaca, NY, pp.22.

KNUST

62. Schneierson, S.S. (1960). Atlas of Diagnostic Microbiology. Abbott Laboratories, North Chicago, Illinois, pp. 44-45.

63. Schloss, P.D. & Walker, L.P. (2000). Measurement of process performance and variability in inoculated composting reactors using ANOVA and power analysis. Process Biochemistry, 35(9):931-942.

64. Sherman, R. (2005). Large-scale organic materials composting. North Carolina Cooperative Extension Service. Available at: < http://www.bae. .ncsu.edu/bae/programs/extension/publicat/vermcompost/ag593.pdf.>. Accessed on October 23, 2005.

65. Singh C.P. (1987). Preparation of High grade Compost by an enrichment on organic matter decomposition. Journal of Biol. Agric. & Hort., 5: 41-49.

66. Smars, S., Gustafsson, L., Beck-Friis, B. & Jönsson, H. (2002).Improvement of the composting time for household waste during an initial low pH phase by mesophilic temperature control. Bioresource Technology, 84:237-241.

67. Smith, R. C. (1992). Composting practices. North Dakota State University. Available online at: <www.ag.ndsu.edu/pubs/plantsci/hortcrop/h885w.htm>. Accessed on March 24, 2007.

68. Sonesson ,U., Björklund, A., Carlsson, M.& Dalemo, M. (2000). Environmental land economic analysis of management systems for biodegradable waste .Resources Conservation and Recycling, 28:29-53.

69. Strom, P.F. (1985). Effect of temperature on bacterial species diversity in thermophilic solid-waste composting. Applied and Environmental Microbiology, 50(4): 899-905.

KNUST
W J SANE NO BADWENMA
NSAP

70. Suler, D.J. & Finstein M.S. (1977). Effect of temperature, aeration and moisture on CO_2 formation in bench-scale, continuously thermophilic composting of solid waste. Applied and Environmental Microbiology, 33(2):345-350.

71. Sundberg, C. & Jönsson, H. (2003). Down-scaling a large composting plant to pilot-scale for systematic research. In: Pullammanapallil, P., McComb, A., Diaz, L., Bidlingmaier, W. (Eds.), Proceedings of the Fourth International Conference of Orbit Association on Biological Processing of Organics. Perth, Murdoch University, pp.388-397.

72. Sundberg, C. (2005). Improving Compost Process Efficiency by Controlling Aeration, Temperature and P^{H}. Doctoral Thesis No.2005:103, Faculty of Natural Resources and Agriculture, Swedish University of Agricultural Sciences, Sweden, pp. 10,11,16-17

73. Tendler, M.D. & Burkholder, P.R. (1961). Studies on some Actinomycetes. Appl. Microbiol., 9:394- 399.

74. Thomas, C. J. & McMeekin, T.A. (1980). A note on scanning electron microscopic assessment of stomacher action on chicken skin. Journal of Applied Bacteriology, 49:339-344.

75. Tiquia, M.S. (2005). Microbiological parameters as indicators of compost maturity. The Society for Applied Microbiology. Journal of Applied Microbiology, 99:816-828.

76. VanderGheynst, J. (2007). How agricultural end-users can assess compost quality. Retrieved on 3rd October 2007from <http://esce.ucr.edu/WASTEMAN /CMJEAN ~1.htm>.

77. Weppen, P. (2001). Process calorimetry on composting of municipal organic wastes. Biomass and Bioenergy, 21(4):289-299.

KNUST

78. Zucconi, F., Forte, M., Monaco, A. & De Bertoldi, M. (1981). Biological evaluation of compost maturity. BioCycle, 22: 27-29.

KNUST

APPENDICES

APPENDIX A: SUMMARY OF ANALYSIS OF VARIANCE FOR PH, TEMPERATURE AND MOISTURE CONTENT FOR THE DIFFERENT COMPOSTING SYSTEMS

Table A. 1. ANOVA for pH in the Composting Systems

Source of Variation	SS	Df	MS	F	P-value	F crit
Between systems	0.4568	3	0.1523	2.5955	0.0632	2.7981
Within systems	2.8159	48	0.0587			
Total	3.2727	51				

KNUST

Table A. 2. ANOVA for Moisture Content in the Composting Systems

Source of Variation	SS	Df	MS	F	P-value	F crit
Between systems	0.0674	3	0.0225	0.0519	0.9842	2.7981
Within systems	20.7896	48	0.4331			
Total	20.8571	51				

Table A.3. ANOVA for Temperature in the Composting Systems

Source of Variation	SS	df	MS	F	P-value	F crit
Between systems	2379.1400	3	793.0460	15.0908	4.8×10^{-7}	2.7981
Within systems	2522.4700	48	52.5515			
Total	4901.6100	51				

NYANSAPO WO SANE NO BADWENMA

APPENDIX B: SUMMARY OF THE COEFFICIENT OF CORRELATION VALUES BETWEEN PH, TEMPERATURE AND MOISTURE CONTENT RECORDED IN THE DIFFERENT COMPOSTING SYSTEMS.

Table B.1 Correlation Between Measured Parameters in DAT Composting System

	PH	Temperature	Moisture Content
PH	1		
Temperature	0.5953	1	
Moisture Content	-0.0816	0.2888	1

Table B.2 Correlation Between Measured Parameters in HV Composting System

KNUST

	PH	Temperature	Moisture Content
PH	1		
Temperature	0.8622	1	
Moisture Content	0.5102	0.6145	1

Table B.3 Correlation Between Measured Parameters in FA Composting System

	PH	Temperature	Moisture Content
PH	1		
Temperature	0.5770	1	
Moisture Content	0.3048	0.2514	1

Table B.4 Correlation Between Measured Parameters in T-W Composting System

	PH	Temperature	Moisture Content
PH	1		
Temperature	0.8029	1	
Moisture Content	0.4823	0.8587	1

APPENDIX C: SUMMARY OF TUKEY'S TEST CRITICAL VALUES FOR PAIR WISE COMPARISONS OF THE MEANS OF TEMPERATURES AND TOTAL VIABLE COUNTS RECORDED IN THE DIFFERENT SYSTEMS.

Table C.1: Tukey's Test Critical Values for the Pair Wise Comparisons of Temperatures Recorded in the Different Composting Systems

	DAT	HV	FA	T-W
DAT	-			
HV	0.02(N)	-		
FA	8.17(S)	8.16(S)	-	
T-W	3.79(S)	3.78(S)	- 4.38(S)	-

N = not significantly different S = significantly different

Critical Tukey Test value (q_{crit}) at 5% = 3.76

KNUST

Note: Only absolute Tukey Test values are to be used in testing

Table C.2: Tukey's Test Critical Values for the Pair Wise Comparisons of Total Viable Count Recorded in the Different Systems.

	DAT	HV	FA	T-W
DAT	-			
HV	-1.55(N)	-		
FA	-3.66(N)	-2.11(N)	-	
T-W	-3.80(S)	-2.25(N)	-0.14(N)	-

N = not significantly different S = significantly different

Critical Tukey Test value (q_{crit}) at 5% = 3.76

Note: Only absolute Tukey Test values are to be used in testing

APPENDIX D: TABLE OF CRITICAL VALUES FOR THE TUKEY TEST AT α = 0.05

α = 0.05

v \ k	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	17.97	26.98	32.82	37.08	40.41	43.12	45.40	47.36	49.07	50.59	51.96	53.20	54.33	55.36	56.32	57.22	58.04	58.83	59.56
2	6.08	8.33	9.80	10.88	11.74	12.44	13.03	13.54	13.99	14.39	14.75	15.08	15.38	15.65	15.91	16.14	16.37	16.57	16.77
3	4.50	5.91	6.82	7.50	8.04	8.48	8.85	9.18	9.46	9.72	9.95	10.15	10.35	10.53	10.69	10.84	10.98	11.11	11.24
4	3.93	5.04	5.76	6.29	6.71	7.05	7.35	7.60	7.83	8.03	8.21	8.37	8.52	8.66	8.79	8.91	9.03	9.13	9.23
5	3.64	4.60	5.22	5.67	6.03	6.33	6.58	6.80	6.99	7.17	7.32	7.47	7.60	7.72	7.83	7.93	8.03	8.12	8.21
6	3.46	4.34	4.90	5.30	5.63	5.90	6.12	6.32	6.49	6.65	6.79	6.92	7.03	7.14	7.24	7.34	7.43	7.51	7.59
7	3.34	4.16	4.68	5.06	5.36	5.61	5.82	6.00	6.16	6.30	6.43	6.55	6.66	6.76	6.85	6.94	7.02	7.10	7.17
8	3.26	4.04	4.53	4.89	5.17	5.40	5.60	5.77	5.92	6.05	6.18	6.29	6.39	6.48	6.57	6.65	6.73	6.80	6.87
9	3.20	3.95	4.41	4.76	5.02	5.24	5.43	5.59	5.74	5.87	5.98	6.09	6.19	6.28	6.36	6.44	6.51	6.58	6.64
10	3.15	3.88	4.33	4.65	4.91	5.12	5.30	5.46	5.60	5.72	5.83	5.93	6.03	6.11	6.19	6.27	6.34	6.40	6.47
11	3.11	3.82	4.26	4.57	4.82	5.03	5.20	5.35	5.49	5.61	5.71	5.81	5.90	5.98	6.06	6.13	6.20	6.27	6.33
12	3.08	3.77	4.20	4.51	4.75	4.95	5.12	5.27	5.39	5.51	5.61	5.71	5.80	5.88	5.95	6.02	6.09	6.15	6.21
13	3.06	3.73	4.15	4.45	4.69	4.88	5.05	5.19	5.32	5.43	5.53	5.63	5.71	5.79	5.86	5.93	5.99	6.05	6.11
14	3.03	3.70	4.11	4.41	4.64	4.83	4.99	5.13	5.25	5.36	5.46	5.55	5.64	5.71	5.79	5.85	5.91	5.97	6.03
15	3.01	3.67	4.08	4.37	4.59	4.78	4.94	5.08	5.20	5.31	5.40	5.49	5.57	5.65	5.72	5.78	5.85	5.90	5.96
16	3.00	3.65	4.05	4.33	4.56	4.74	4.90	5.03	5.15	5.26	5.35	5.44	5.52	5.59	5.66	5.73	5.79	5.84	5.90
17	2.98	3.63	4.02	4.30	4.52	4.70	4.86	4.99	5.11	5.21	5.31	5.39	5.47	5.54	5.61	5.67	5.73	5.79	5.84
18	2.97	3.61	4.00	4.28	4.49	4.67	4.82	4.96	5.07	5.17	5.27	5.35	5.43	5.50	5.57	5.63	5.69	5.74	5.79
19	2.96	3.59	3.98	4.25	4.47	4.65	4.79	4.92	5.04	5.14	5.23	5.31	5.39	5.46	5.53	5.59	5.65	5.70	5.75
20	2.95	3.58	3.96	4.23	4.45	4.62	4.77	4.90	5.01	5.11	5.20	5.28	5.36	5.43	5.49	5.55	5.61	5.66	5.71
24	2.92	3.53	3.90	4.17	4.37	4.54	4.68	4.81	4.92	5.01	5.10	5.18	5.25	5.32	5.38	5.44	5.49	5.55	5.59
30	2.89	3.49	3.85	4.10	4.30	4.46	4.60	4.72	4.82	4.92	5.00	5.08	5.15	5.21	5.27	5.33	5.38	5.43	5.47
40	2.86	3.44	3.79	4.04	4.23	4.39	4.52	4.63	4.73	4.82	4.90	4.98	5.04	5.11	5.16	5.22	5.27	5.31	5.36
60	2.83	3.40	3.74	3.98	4.16	4.31	4.44	4.55	4.65	4.73	4.81	4.88	4.94	5.00	5.06	5.11	5.15	5.20	5.24
120	2.80	3.36	3.68	3.92	4.10	4.24	4.36	4.47	4.56	4.64	4.71	4.78	4.84	4.90	4.95	5.00	5.04	5.09	5.13
∞	2.77	3.31	3.63	3.86	4.03	4.17	4.29	4.39	4.47	4.55	4.62	4.68	4.74	4.80	4.85	4.89	4.93	4.97	5.01

APPENDIX E: FORMULA AND METHOD USED IN EXTRAPOLATION OF CRITICAL VALUE FROM TUKEY'S TEST TABLE

Formula for Calculating Tukey's Test Values

$$q = \frac{(\overline{x_1} - \overline{x_2})}{\sqrt{s_w^2/n}}$$

q_{cal} = Tukey's Test calculated values for two means

$\overline{x_1}$ = Mean of sample one

$\overline{x_2}$ = Mean of sample two

s_w^2 = Within system variance

n = Number of samples

q_{crit}= Critical value from Tukey's Test

KNUST

Extrapolation of Critical Value of Tukey's Test

The number of means (k = 4) and the degree of freedom (d.f) for S^2_w (52-4) = 48,denoted by v on Table D.1 in appendix D. v = 48 against k = 4 critical value was extrapolated between critical values of v = 40 and v = 60, with k = 4, using the formula below:

$$\frac{40-60}{40-48} = \frac{3.74-3.79}{3.74-x}$$

$$\frac{-20}{-8} = \frac{-0.05}{3.74-x}$$

$$-20(3.74-x) = 0.4$$

$$20x - 74.8 = 0.4$$

$$20x = 0.4 + 74.8$$

$$x = \frac{75.2}{20} = 3.76$$

Where x = critical value corresponding to the Tukey's Test critical value for v = 48 against k = 4

LIBRARY
KWAME NKRUMAH UNIVERSITY OF
SCIENCE AND TECHNOLOGY
KUMASI-GHANA

APPENDIX F: SUMMARY OF ANOVA OF TOTAL VIABLE COUNTS, TOTAL COLIFORM COUNTS, TOTAL FUNGI COUNTS AND GERMINATION INDICES IN THE DIFFERENT COMPOSTING SYSTEMS.

Table F.1: ANOVA of the Total Viable Counts in the Composting Systems

Source of Variation	SS	Df	MS	F	P-value	F crit
Between systems	0.6592	3	0.2198	3.3437	0.0267	2.7981
Within systems	3.1546	48	0.0657			
Total	3.8139	51				

KNUST

Table F.2 : ANOVA of Total Coliform Counts in the Composting Systems

Source of Variation	SS	Df	MS	F	P-value	F crit
Between systems	0.1578	3	0.0526	0.0056	0.9994	2.7981
Within systems	450.5700	48	9.3869			
Total	450.7280	51				

Table F.3: ANOVA of Total Fungi Counts in the Composting Systems

Source of Variation	SS	Df	MS	F	P-value	F crit
Between systems	0.0172	3	0.0057	0.0847	0.9681	2.7981
Within Systems	3.2420	48	0.0675			
Total	3.2591	51				

Table F.4: ANOVA of Germination Indices in Composting Systems

Source of Variation	SS	Df	MS	F	P-value	F crit
Between systems	10276.0163	3	3425.3388	2.2886	0.0904	2.7981
Within systems	71842.4445	48	1496.7176			
Total	82118.4608	51				

APPENDIX G: SUMMARY OF THE CULTURAL FEATURES AND GRAM STAIN REACTIONS OF BACTERIA IDENTIFIED

Table G.1 Summary of the Cultural Features and Identification of the bacteria present in compost masses

Organisms	Cultural Features	Identification
Bacillus spp.	Blood agar: Large 2-5mm grey colonies with wavy edges. Most strains are non-haemolytic	Large Gram positive bacilli occurring singly and in chains
Clostridium spp.	Blood agar: When isolated (rarely), a film of growth is produced. Strict anaerobe. Haemolysis may occur MacConkey agar: No growth	Gram positive, long, thin rods with round spore (unstained) at one end.
Escherichia spp.	Blood agar: 1-4mm colonies, may appear mucoid and some strains are haemolytic. MacConkey agar: Large 2-4mm lactose fermenting colonies.	Gram negative usually motile rods.
Campylobacter spp.	-	Gram negative spiral curved rods
Klebsiella spp.	Blood agar: Large mucoid colonies. MacConkey: Most strains form lactose fermenting mucoid colonies.	Gram negative non-motile capsulated rods.
Listeria spp.	Blood agar: Small droplet-like, beta-haemolytic colonies after 24 - 48h aerobic incubation.	Small Gram positive rods or coccobacilli which are easily decolourized.
Staphylococcus spp.	Blood agar: Smooth,1-2mm cream coloured(occasionally white) colonies. Some strains are haemolytic. MacConkey agar: Small, usually non-lactose fermenting colonies.	Gram positive cocci of uniform size, occurring singly and in groups.
Streptococcus spp.	Blood agar: Alpha-haemolytic raised colonies, growing best in CO_2.Colonies become ringed after 24-48h incubation or after culture on lyzed blood agar. MacConkey agar: No growth	Gram positive capsulated diplococcic or streptococci in short chains.
Yersinia spp.	Blood agar: Small, shiny colonies formed after 24-48h,growing best at20-28°C MacConkey agar: Non-lactose fermenting colonies, growing best at 20-28°C	Gram negative non-motile, capsulated coccobacilli, showing bipolar staining.

Source: Cheesbrough (1984)

KNUST

APPENDIX H: SUMMARY OF CULTURAL FEATURES OF FUNGI IDENTIFIED

Table H.1: Summary of the Cultural Features of the fungi present in the compost masses

Fungi	**Cultural Characteristics**
Aspergillus spp.	Aspergilli cultured on Sabouraud, colonies iniatially appear white. Filamentous surface growths, soon becoming green to dark green as spores are produced.
Penicillium spp.	Penicillia produce rapidly growing colonies on sabouraud which are initially white but becomes bluish green and powdery due to the production of many spores from the aerial mycelium,
Mucor spp.	Colonies appear as individual spherical whitish mass of cell on sabouraud and growth is filamentous in aerobic conditions.
Rhizopus spp.	Colonies appear as a white mass of fluffy cells with stoloniferous growth on sabouraud

Source: Schneierson (1960)

KNUST
NYANSAPO WOSANE NO BADWENMA

APPENDIX I: WEEKLY PERCENTAGE FREQUENCIES OF BACTERIA IDENTIFIED IN THE DIFFERENT COMPOSTING SYSTEMS

Table I .1: Weekly Percentage Frequencies of Bacteria Identified in Compost Masses in Systems DAT and HV

	DAT									HV								
WEEKS	BACILLUS SPP.	STAPHYLOCOCCUS SPP.	STREPTOCOCCUS SPP.	CLOSTRIDIUM SPP.	CAMPYLOBACTER SPP.	LISTERIA SPP.	CORYNEBACTERIUM SPP.	YERSINIA SPP.	ENTEROBACTER SPP.	BACILLUS SPP.	STAPHYLOCOCCUS SPP.	STREPTOCOCCUS SPP.	CLOSTRIDIUM SPP.	CAMPYLOBACTER SPP.	LISTERIA SPP.	CORYNEBACTERIUM SPP.	YERSINIA SPP.	ENTEROBACTER SPP.
0	30.77	23.08	0.00	0.00	7.69	7.69	23.08	0.00	7.69	30.77	23.08	0.00	0.00	7.69	7.69	23.08	0.00	7.69
1	25.00	12.50	12.50	0.00	0.00	0.00	37.50	0.00	12.50	33.33	0.00	0.00	0.00	0.00	33.33	16.67	0.00	16.67
2	12.50	0.00	37.50	12.50	0.00	0.00	0.00	25.00	12.50	16.67	0.00	16.67	8.33	16.67	0.00	16.67	16.67	8.33
3	56.25	0.00	6.25	0.00	0.00	6.25	25.00	0.00	6.25	30.77	15.38	0.00	0.00	0.00	0.00	46.15	0.00	7.69
4	5.56	0.00	33.33	5.56	0.00	33.33	22.22	0.00	0.00	12.50	12.50	37.50	0.00	0.00	0.00	37.50	0.00	0.00
5	36.36	9.09	0.00	0.00	18.18	9.09	27.27	0.00	0.00	44.44	11.11	0.00	0.00	0.00	0.00	44.44	0.00	0.00
6	38.46	23.08	15.38	0.00	0.00	7.69	15.38	0.00	0.00	35.71	14.29	7.14	0.00	0.00	0.00	42.86	0.00	0.00
7	16.67	0.00	33.33	0.00	0.00	16.67	33.33	0.00	0.00	14.29	42.86	0.00	0.00	0.00	0.00	42.86	0.00	0.00
8	26.67	20.00	20.00	0.00	0.00	0.00	33.33	0.00	0.00	46.67	13.33	6.67	0.00	0.00	0.00	33.33	0.00	0.00
9	33.33	8.33	8.33	0.00	0.00	0.00	50.00	0.00	0.00	50.00	0.00	0.00	7.14	0.00	7.14	35.71	0.00	0.00
10	50.00	0.00	7.14	0.00	0.00	7.14	35.71	0.00	0.00	25.00	0.00	6.25	0.00	0.00	25.00	43.75	0.00	0.00
11	25.00	8.33	0.00	0.00	0.00	8.33	58.33	0.00	0.00	40.00	0.00	6.67	0.00	0.00	0.00	53.33	0.00	0.00
12	33.33	0.00	0.00	0.00	0.00	8.33	58.33	0.00	0.00	42.86	0.00	0.00	0.00	0.00	0.00	57.14	0.00	0.00

KNUST

Table I.2: Weekly Percentage Frequencies of Identified Bacteria in Compost Masses in the Systems FA and TW

	FA									TW								
WEEK	BACILLUS SPP.	STAPHYLOCOCCUS SPP.	STREPTOCOCCUS SPP.	CLOSTRIDIUM SPP.	CAMPYLOBACTER SPP.	LISTERIA SPP.	CORYNEBACTERIUM SPP.	YERSINIA SPP.	ENTEROBACTER SPP.	BACILLUS SPP.	STAPHYLOCOCCUS SPP.	STREPTOCOCCUS SPP.	CLOSTRIDIUM SPP.	CAMPYLOBACTER SPP.	LISTERIA SPP.	CORYNEBACTERIUM SPP.	YERSINIA SPP.	ENTEROBACTER SPP.
0	42.86	14.29	0.00	7.14	0.00	0.00	14.29	0.00	21.43	42.86	14.29	0.00	7.14	0.00	0.00	14.29	0.00	21.43
1	37.50	12.50	0.00	0.00	0.00	0.00	37.50	0.00	12.50	27.27	0.00	9.09	0.00	0.00	36.36	9.09	9.09	9.09
2	7.14	14.29	21.43	0.00	14.29	21.43	7.14	7.14	7.14	10.00	0.00	10.00	0.00	0.00	20.00	50.00	0.00	10.00
3	60.00	0.00	0.00	0.00	0.00	0.00	33.33	0.00	6.67	28.57	0.00	0.00	7.14	0.00	7.14	50.00	0.00	7.14
4	5.88	0.00	41.18	11.76	0.00	11.76	29.41	0.00	0.00	30.77	7.69	15.38	0.00	0.00	30.77	15.38	0.00	0.00
5	33.33	25.00	0.00	0.00	0.00	0.00	41.67	0.00	0.00	30.77	7.69	15.38	0.00	0.00	30.77	15.38	0.00	0.00
6	66.67	20.00	6.67	0.00	0.00	0.00	6.67	0.00	0.00	62.50	0.00	6.25	0.00	6.25	6.25	18.75	0.00	0.00
7	14.29	28.57	14.29	0.00	0.00	28.57	14.29	0.00	0.00	36.36	9.09	9.09	9.09	0.00	9.09	27.27	0.00	0.00
8	42.86	28.57	21.43	0.00	0.00	0.00	7.14	0.00	0.00	70.59	5.88	5.88	0.00	0.00	5.88	11.76	0.00	0.00
9	31.25	0.00	0.00	0.00	0.00	0.00	68.75	0.00	0.00	26.67	6.67	13.33	0.00	0.00	0.00	53.33	0.00	0.00
10	42.86	0.00	7.14	0.00	0.00	7.14	42.86	0.00	0.00	46.67	0.00	0.00	0.00	0.00	6.67	46.67	0.00	0.00
11	7.14	7.14	0.00	0.00	0.00	14.29	71.43	0.00	0.00	46.67	6.67	0.00	0.00	0.00	0.00	46.67	0.00	0.00
12	20.00	6.67	0.00	0.00	0.00	6.67	66.67	0.00	0.00	28.57	0.00	0.00	0.00	0.00	0.00	71.43	0.00	0.00

KNUST

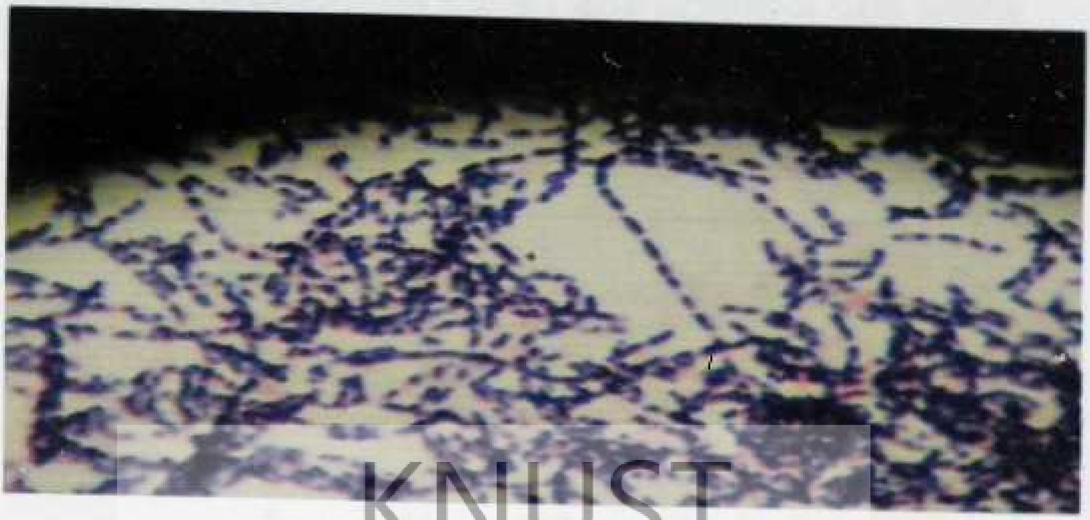
APPENDIX J: WEEKLY PERCENTAGE FREQUENCIES OF FUNGI IDENTIFIED IN THE DIFFERENT COMPOSTING SYSTEMS

Table J. 1: Weekly Percentage Frequencies of Fungi Identified in the Compost Masses in the Different Composting Systems

	DAT				HV				FA				T-W			
WEEKS	PENICILLIUM SPP.	ASPERGILLUS SPP.	MUCOR SPP.	RHIZOPUS SPP.	PENICILLIUM SPP.	ASPERGILLUS SPP.	MUCOR SPP.	RHIZRPUS SPP.	PENICILLIUM SPP.	ASPERGILLUS SPP.	MUCOR SPP.	RHIZOPUS SPP.	PENICILLIUM SPP.	ASPERGILLUS SPP.	MUCOR SPP.	RHIZOPUS SPP.
0	21.43	71.43	0.00	7.14	21.43	71.43	0.00	7.14	6.00	90.00	0.00	4.00	6.00	90.00	0.00	4.00
1	52.25	18.92	28.83	0.00	82.80	5.38	10.75	1.08	95.38	3.08	0.00	1.54	0.00	0.00	96.55	3.45
2	76.19	2.38	19.05	2.38	88.70	1.69	9.04	0.56	88.27	1.68	9.50	0.56	87.07	0.00	12.07	0.86
3	86.61	2.36	10.24	0.79	86.59	6.10	7.32	0.00	95.93	1.16	2.33	0.58	89.47	0.66	9.21	0.66
4	90.44	0.00	8.82	0.74	97.16	0.00	1.90	0.95	96.63	0.00	2.25	1.12	85.54	0.00	14.46	0.40
5	79.44	0.00	18.69	1.87	95.69	0.00	2.59	1.72	93.98	0.00	6.02	0.00	89.39	0.00	10.61	0.00
6	84.72	0.00	15.28	0.00	97.06	0.00	1.47	1.47	94.00	0.00	6.00	0.00	91.92	0.00	8.08	0.00
7	82.14	0.00	15.18	2.68	84.78	0.00	10.87	4.35	98.33	0.00	1.67	0.00	95.07	0.00	4.93	0.00
8	90.09	0.00	9.91	0.00	96.49	0.00	3.51	0.00	98.20	0.00	1.80	0.00	82.72	0.00	17.28	0.00
9	89.13	0.00	10.87	0.00	100.00	0.00	0.00	0.00	97.96	0.00	2.04	0.00	95.00	0.00	5.00	0.00
10	70.42	0.00	29.58	0.00	83.49	0.00	16.51	0.00	97.06	0.00	2.94	0.00	95.06	0.00	4.94	0.00
11	47.83	0.00	52.17	0.00	72.63	0.00	27.37	0.00	72.07	0.00	27.93	0.00	59.29	0.00	40.71	0.00
12	97.79	0.00	2.21	0.00	100.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00

KNUST

APPENDIX K: SOME OF THE MICROORGANISMS IDENTIFIED IN THE COMPOST MASSES



KNUST

Fig.36: *Bacillus spp.* viewed under microscope

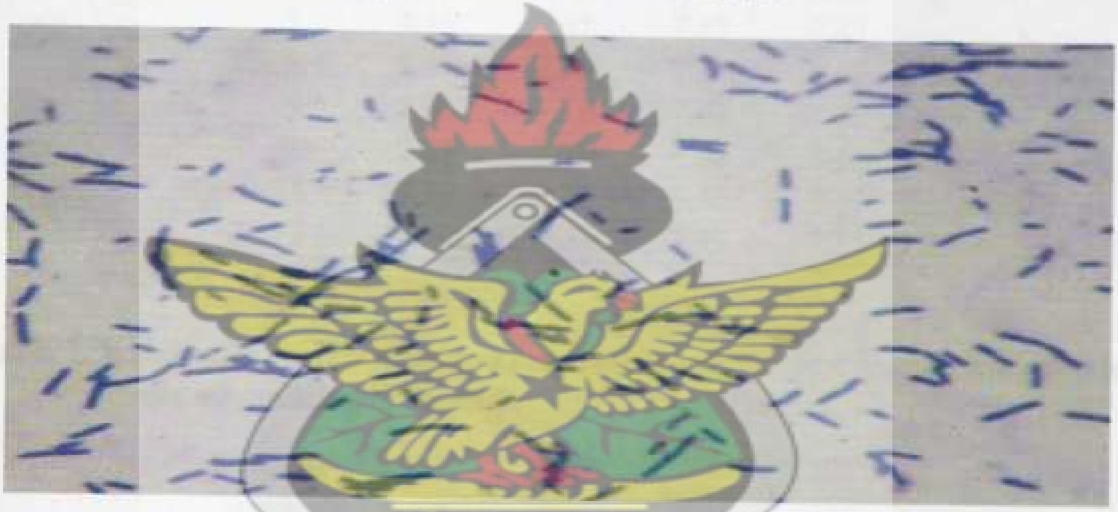


Fig.37: *Corynebacterium spp.* viewed under the microscope

NYANSAPO WO SANE NO BADWENMA

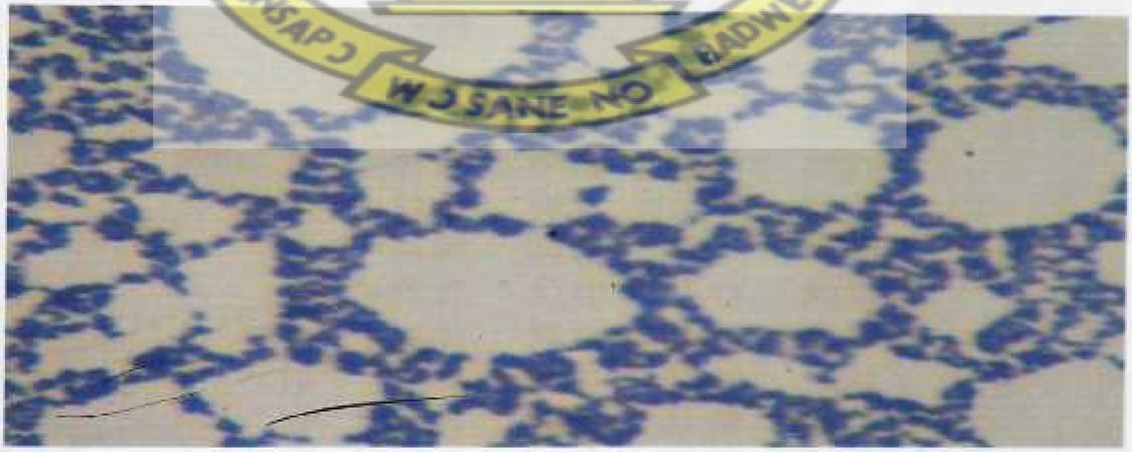


Fig.38: *Staphylococcus spp.* viewed under the microscope

APPENDIX L: TABLES OF PH, MOISTURE CONTENT, TEMPERATURE, TOTAL VIABLE COUNT, TOTAL COLIFORM COUNT, TOTAL FUNGI COUNT AND GERMINATION INDEX RECORDED IN THE DIFFERENT SYSTEMS.

Table L.1: Average weekly pH of compost masses recorded in the composting systems

WEEKS	DAT	HV	FA	T-W
0	7.69	7.69	7.79	7.79
1	7.93	7.80	8.05	7.99
2	8.18	8.20	7.71	8.04
3	8.10	8.05	7.87	8.16
4	7.79	8.17	7.73	7.85
5	7.82	7.87	7.53	7.75
6	7.90	7.93	7.83	7.93
7	8.13	7.67	7.67	7.93
8	8.17	7.70	7.50	7.67
9	7.80	7.77	7.30	7.60
10	7.67	7.73	7.43	7.63
11	7.70	7.20	7.40	7.47
12	7.60	7.37	7.27	7.33

KNUST

Table L.2: Average weekly moisture content (%) of compost masses recorded in the composting systems

WEEKS	DAT	HV	FA	T-W
0	67.49	67.49	67.49	67.49
1	59.27	68.93	67.87	71.30
2	62.17	62.87	57.87	63.53
3	64.43	65.40	62.13	60.77
4	59.40	58.87	56.17	56.40
5	65.73	59.00	59.53	57.27
6	52.03	59.73	54.67	55.40
7	46.07	44.83	41.23	49.00
8	58.27	57.10	56.43	52.73
9	57.03	53.23	56.23	49.00
10	55.67	51.07	57.47	56.33
11	57.57	50.53	55.90	53.40
12	54.73	53.00	59.80	54.07

Table L.3: Average weekly temperatures (°C) of compost masses recorded in the composting systems

WEEKS	Ambient	DAT	HV	FA	T-W
0	27.00	53.20	52.49	42.97	57.60
1	24.75	63.24	62.48	50.83	62.56
2	25.08	62.24	65.44	44.23	59.78
3	23.92	58.51	61.52	32.59	56.56
4	24.08	59.08	61.07	53.20	51.83
5	24.08	57.14	58.70	51.94	47.89
6	24.25	57.26	58.52	38.57	46.06
7	24.33	54.39	55.24	37.43	45.28
8	24.42	54.46	53.80	35.50	41.17
9	24.42	54.53	51.46	33.43	39.83
10	24.50	51.85	49.35	30.94	39.72
11	24.25	49.58	47.94	29.41	37.89
12	24.33	48.14	45.19	29.00	38.33

KNUST

Table L.4: Average weekly Total Viable Count (LogCFU/g of Compost) of compost masses recorded in the different composting systems

WEEKS	DAT	HV	FA	T-W
0	7.31	7.31	7.79	7.79
1	7.05	7.35	7.83	7.40
2	7.51	7.46	7.87	7.34
3	7.77	7.75	7.33	7.74
4	7.13	7.28	7.17	7.41
5	7.31	6.99	7.32	7.53
6	6.73	7.39	7.23	7.36
7	7.09	7.02	7.59	7.55
8	6.64	6.98	7.20	7.19
9	6.83	6.90	7.05	7.11
10	7.21	7.33	7.20	7.30
11	7.16	7.28	7.25	7.35
12	7.08	7.21	7.37	7.29

Table L.5: Average weekly Total Coliform Count (LogCFU/g of Compost) recorded in the composting systems

WEEKS	DAT	HV	FA	T-W
0	7.21	7.21	6.88	6.88
1	6.26	6.00	6.26	6.56
2	6.82	5.85	6.95	5.90
3	5.90	5.30	5.78	5.70
4	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00

KNUST

Table L.6: Average weekly Total Fungi Count (LogCFU/g of Compost) recorded in the composting systems

WEEKS	DAT	HV	FA	T-W
0	1.11	1.11	1.68	1.68
1	2.05	1.96	1.81	1.45
2	1.61	2.25	2.25	2.06
3	2.10	1.91	2.23	2.18
4	2.13	2.32	1.94	2.40
5	2.02	2.06	1.92	1.82
6	1.86	2.13	2.00	2.00
7	2.04	1.64	2.08	2.15
8	2.05	1.76	2.05	1.91
9	1.96	1.85	1.69	1.90
10	1.85	2.04	1.83	1.91
11	1.84	1.98	2.05	2.05
12	2.13	2.06	1.89	1.58

Table L.7: Average weekly Germination Indices (%) of compost masses recorded in the composting systems

WEEKS	DAT	HV	FA	T-W
0	82.54	82.54	88.58	88.58
1	94.61	69.76	166.17	103.89
2	147.41	119.12	162.95	170.92
3	182.63	126.69	157.20	181.78
4	128.13	85.94	125.00	182.81
5	209.66	157.00	144.44	162.80
6	107.45	188.30	107.98	196.81
7	121.66	113.55	193.49	172.53
8	112.45	146.47	114.94	174.27
9	117.51	124.02	155.22	167.92
10	128.77	112.67	187.87	180.90
11	188.24	190.76	191.60	185.71
12	189.54	198.11	202.83	222.64

KNUST