Addis Ababa University

School of Graduate Studies

Addis Ababa Institute of Technology

School of Chemical and Bio Engineering

Environmental Engineering Stream

Optimization of Municipal Solid Waste Vermicomposting Parameters

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By

Teklebrahan Gebrekrstos

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfilment of the Degree of Master of Science in Environmental Engineering

Advisor

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Addis Ababa

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LIST OF ABBREVIATIONS

C/N	Carbon to Nitrogen ratio
MSW	Municipal Solid Waste
CD	Cow Dung
AAEPA	Addis Ababa Environmental Protection Authority
GHGs	Green House Gases
CAPs	Cast-Associated processes
GAPs	Gut-Associated Processes
TKN	Total Kjeldahl Nitrogen
OM	Organic Matter
CEC	Cation Exchange Capacity
CFUs	Colony Forming Unites
MM	Metro-Mix
APPRC	Ambo Plant Protection Research Center
DoE	Design of Experiments
MC	Moister Content
WSD	Worm Stocking Density
RSM	Response Surface Methodology
TOC	Total Organic Carbon
ANOVA	Analysis of Variance
MSWM	Municipal Solid Waste Management

ISWM	Integrated Solid Waste Management
VS	Volatile Solids
EC	Electerical Conductivity
NPK	Nitrogen-Phosphorous-Potassium
3D	Three Dimensional
VC	Vermicompost
BOD	Bilogical Oxygen Demand
TOM	Total Organic Matter
SS	Sum of Squares
Df	Degree of Freedom
MS	Mean Square
Std. Dev.	Standard Deviation
2FI	Two Factor Interaction
C.V	Coefficient of Variation
CI	Confidence Interval
VIF	Variance Inflation Factor

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ABSTRACT

Today, increasing population and rapid development are resulting for generation of large amount of solid wastes in developing countries. Most of these wastes are usually disposed in open dumping site, open spaces, rivers or burned in the streets without any treatment due to lack of proper management techniques, awareness and commitment of the society and the government, which creates severe environmental pollution and health hazards, hence it was thought to attempt use of municipal solid waste for cheap and ecofriendly treatment methods like vermicomposting. It is the process of compost formation by earthworms. Optimization of process parameters for the vermicomposting of municipal solid waste using Esenia fetida was investigated using response surface methodology (RSM). The three parameters and their ranges namely moisture content (60-90%), C/N ratio (20-30), and worm stocking density (0.8-2.0 kg/$\mathbf{m^2}$) were chosen from the previous study of vermicomposting. The experimental data on the quantity of N, P and K were fitted into a quadratic polynomial model using multiple regression analysis. The experimental results and software predicted N, P and K values were comparable. The individual parameters effect as well as effect of interactions between the vermicomposting parameters on N, P and K was analysed using various graphical representations. A three-level three factorial Box-Behnken design technique under RSM using numerical optimization method was used to optimize their interactions, which showed that a moisture content of 74.62%, C/N ratio of 23.21, and worm stocking density of 2 kg/$\mathbf{m^2}$ were the best conditions. Under these conditions, the maximum predicted yield of N, P and K was 2.82%, 1.58% and 1.28%, respectively. The produced vermicompost was found to be better in desired level of composition of macro-nutrients i.e., N, P and K and the efficacy of the prepared vermicompost has been studied on the spinach vegetable plant. A plastic pot set-up with soil was used to determine the effects and efficiency level of vermicompost on the yields of spinach. The study was conducted through effect of increasing concentration of Vermicompost (0% (control), 50% and 100% w/w) in target plant growth. Total leaves wet weight and number of leaves has been studied. Results showed that the number of leaves produced using 100% VC was 2.67 times greater than the number of leaves produced using 0% VC and 1.6 times greater than produced using 50% VC and leaf wet weight of the spinach plant produced using 100% VC was 2.2 times greater than produced using the control (soil) and 1.2 times greater than produced using 50% VC. Thus, vermicomposting of municipal solid waste is an excellent and ecofriendly method of municipal solid waste management.

Key words: *Optimization; vermicomposting; municipal solid waste; Esenia fetida; response surface methodology; spinach*

1 INTRODUCTION

1.1 Background

With the fast development of housing colonies and an increase of population and change in life styles have witnessed increase of municipal solid waste by several folds during the last few years [1]. Currently, world cities generate about 1.3 billion tones of solid waste per year. This volume is expected to increase to 2.2 billion tones by 2025 [2]. The current daily waste generation of Addis Ababa City is estimated to be 5569.62 $\mathbf{m^3}$ [3]. However, currently out of the quantity of solid waste generated and collected from the city of Addis Ababa, only 5% is composted and 5% is recycled [3]. The remaining 90% can be disposed in open dumping site, open spaces, rivers or burned in the streets without any treatment i.e. gas and leachate collection system. Concerns about the environment and problems of solid waste disposal are issues that are increasingly demanding attention globally [4]. Solid waste is defined as the organic and inorganic waste materials produced by different sources and have lost value in the eye of their owner [5]. Its typical composition includes paper, glass, wood, plastic, reusable goods, soil, chemicals, food wastes, plant debris, textiles and rock with organic materials making up 50-70% of all municipal solid waste [6]

Ecological impacts such as land degradation, water and air pollution are related with improper management of municipal solid waste. It is therefore necessary to improve the nutrient status of rural and urban wastes to reduce pollution loads. Hence the importance of efficient solid waste management is increasingly recognized. Before final disposal one can process and treat the waste so as to reduce the "wasteful wasting of waste". Some of the techniques available to achieve this objective are volume reduction, recovery of resources and energy recovery [1]. That is way the world is entering new branch of bioinformational technology. Vermitechnology has been proposed globally as a tool to reduce organic waste, including animal manure, industrial waste, kitchen and agricultural waste as well as municipal organic waste [7].

Vermicomposting is a non-thermophilic process that involves using earth worms to alter physical, chemical and biological reactions in organic wastes such that soil quality is improved. During the vermicomposting process organic materials are converted to stable compounds (humus) and the final product, vermicompost is rich in humus and available plant micro- and macro-nutrients. The rampant use of chemical fertilizer contributes largely to the deterioration of the environment through depletion of fossil fuels, generation of carbon dioxide (CO_2) and contamination of water resources [8]. And now a days, agricultural system has become extremely expensive due to requiring heavy inputs and becoming out of reach for farmers because they are economically very poor.

The effluents of organic matter from rural and urban areas can be used as a vermicompost, which is composed of organic matter without toxicant. The conversion of bio-waste into vermicompost has became safe, proper and appropriate way for the safe hygienic [9]. Vermicoposts have numerous advantages. Through vermicomposting, important plant nutrients such as N, P, K and Ca present in the feed material are converted into forms that are more soluble and available to plants than those in the native compounds [10].

Few studies are available on MSW management through vermicomposting. They are confined only along with cow dung (MSW + CD) using *L. mauritii* and *E. fetida*, MSW + Horticultural waste, market waste + cow dung and MSW+cow dung using P. ceylanesis [6]. Inspite of important works on management of municipal solid waste by vermicomposting little is known about the factors affecting the efficiency of of the process like, the density of earthworms necessary, carbon to nitrogen ratio and moisture content. Hence, the present study deals with, the municipal solid waste vermicomposting, then it used for the optimization of vermicomposting process parameters like, C:N ratio, moisture content and earthworm stocking density using *Eisenia fetida* earth worm species. The vermicomposting process was carried out for a period of 60 days and finally used to evaluate the quantity of N, P, K and other physico-chemical parameters of the MSW before and after vermicomposting.

1.2 Statement of the problem

Increasing population and rapid development in Addis Ababa city are resulting for generation of large amount of solid waste. According to AAEPA out of the total waste generated in Addis Ababa city, 70% by weight and 50% by volume are organic and can be compostable . In different estimates, every day the share of compostable solid waste has been assumed to be 2,784.81 $\mathbf{m^3}$ [3]. However, currently out of the quantity of solid waste generated and collected from the city of Addis Ababa, only 5% is composted and 5% is recycled [3]. The remaining 90% can be disposed in open dumping site, open spaces, rivers or burned in the streets without any treatment i.e. gas and leachate collection system. Even though, high proportion of the waste generated is organic and can be compostable, none of significant formal practical work still implemented either in compost or to generate bio-fuel to alleviate the problems. These disturb the natural environment of soil, surface water, ground water and lead to air pollution and concern for public health. There are a number of possible reduction methods of controlling MSW such as composting, biogas production, reuse and recycling. However, composting has been reported to be efficient mechanisms of waste lessening, removing foul odour and converting into valuable resources in developing countries [11]. Nevertheless, the study carried out so far is inadequate to get sufficient information with respect to MSW alternative composting methods such as vermicomposting and vermicomposting process parameter optimization, to accelerate composting and maximize the fertility parameter of the compost.

In the other hand production of chemical fertilizers in industries is an environmentally damaging process in its entire life-cycle, since harnessing of raw materials from the earth crust, to their processing in factories and their use in agriculture farms [12]. It generates huge amount of toxic and hazardous wastes and pollutants at every stage of production and use. It also uses copious amount of energy in production process and emits huge volumes of greenhouse gases (GHGs). It leads to loss of soil fertility due to imbalanced use of fertilizers that has adversely impacted agricultural productivity and causes soil degradation. It is an economically unproductive process of development. Huge money has to be spent on infrastructure development for production of chemical fertilizers and in installations of equipments for pollution control, transport and then on safe disposal of hazardous waste in engineered landfills. Its application in farms pollutes the soil and water bodies and kills beneficial soil organisms with severe economic and environmental implications.

Therefore, this research was conducted at Kirkose compost demonstration site, so as to demonstrate the optimum vermicomposting process conditions using biodegradable MSW and horse manure as a feedstocks, by using *Eisenia fetida* earthworm species and to evaluate the vermicompost as a fertilizer.

1.3 Objective

1.3.1 General objective

The general objective of this study is to optimize the vermicomposting process parameters to produce high quality compost (vermicompost) from organic municipal solid waste using *Esenia fetida*.

1.3.2 Specific objectives

- Segregation of organic and inorganic fraction of municipal solid waste
- Characterization of the feed materials
- Pre-composting of the feed materials
- Multiplication of earthworms
- Optimization of process parameters
- Characterization of the compost product
- Evaluation of the compost performance

1.4 Significance of the study

The output of this study will;

- Give and add new knowledge to municipal solid waste management and composting research.
- Protect environmental pollution which results from uncontrolled waste management.
- Provide experimental results about the nutrient content, optimum operating conditions and other quality parameters of vermicompost.
- Popularize the use of vermicompost and develop skills of vermicompost preparation.
- Provide baseline information for farmers and stakeholder institutions, environmental agencies and those engaged in organic agricultures.
- Serve as a clue for further research in vermicomposting technology and vermicompost analysis.

2 LITERATURE REVIEW

2.1 Introductions to vermicomposting

Using selected species of earthworms to help compost organic waste, known as vermicomposting, is a process that has been widely adopted throughout the world. Indeed, many countries such as Australia, the USA and several European countries have developed thriving vermicomposting industries [13]. The roots of vermicomposting are thought to stem from the established business of vermiculture, which is the breeding of earthworms mainly for the fishing bait market. In recent years, growing awareness of the ability of earthworms to decompose and stabilize a wide variety of wastes has changed the focus of the industry from producing earthworms to producing compost. However, in practice it is almost impossible to separate the production of earthworms from the processing of waste and many vermicomposting businesses focus on both aspects. However, maximum earthworm production is best achieved using low earthworm densities during vermicomposting coupled with frequent earthworm harvesting. Maximising the waste processing rate depends on maintaining high earthworm densities throughout the vermicomposting process and this is clearly in conflict with producing maximum earthworm biomass from the system.

Many years of experience of vermicomposting has shown that it can be a useful method of composting and one that is suited to a wide variety of wastes but while it has some advantages compared with traditional composting, it also has many disadvantages. Unlike windrow composting, vermicomposting has the potential to produce an additional product in the form of earthworms and many vermicomposting systems have been started or sold on the basis of the profits to be earned from selling these. However, in an attempt to sell commercial vermicomposting systems, exaggerated claims have often been made about the ability of the vermicomposting process to produce large numbers of marketable earthworms and to transform a wide variety of wastes into premium quality vermicompost. While some of these claims are justified, many have not been adequately researched, especially on a large-scale and under sub-optimal processing conditions.

Pollution caused by municipal solid waste has become a serious problem and causes health hazards. Management of solid waste has become one of the biggest problems we are facing today. The rapid increase in the volume of waste is one aspect of the environmental crisis, accompanying recent global development (Rapid urbanization, encroachment of fertile area and booming population is leading to generation of massive amount of waste). The problem has further increased in cities because of shortage of dumping sites and strict environmental legislation, so scientists are seeking for management alternatives, which should be ecofriendly, cheap and fast [14].

Vermitechnology based municipal waste management has become possible and gains attention. Solid waste management is associated with the control of waste generation, storage, collection, transfer and transport, processing and disposal in a manner that is in accordance with the environmental considerations [6]. To reduce the load on disposal system, solid waste must be processed. Municipal solid waste (MSW) is highly organic in nature, so vermicomposting has become an appropriate alternative for the safe, hygienic and cost effective disposal of it. Vermicomposting can be one of such processes.

Vermicomposting is a simple biotechnological process of composting, in which certain species of earthworms are used to enhance process of waste conversion and produce a better end product. Vermicomposting differs from composting in several ways [15]. It is a mesophilic process, utilizing microorganisms and earthworms that are active at 10-32°C (not ambient temperature but temperature within the pile of moist organic materials). The process is faster than composting; because the material passes through the earthworm gut, a significant but not yet fully understood transformation takes place, where by the resulting earthworm castings (worm manure) are rich in microbial activity and plant growth regulators, and fortified with pest repllence attributes as well. In short, earthworms, through a type of biological alchemy are capable of transforming garbage into "gold" [16].

The stabilization process of organic waste materials involves the joint action of between earthworms and microorganisims. Although microbes are responsible for biochemical degradation of organic matter, earthworms are the important drivers of the process, conditioning the substrate and altering the biological activity [9]. As a process for handling organic residuals, it represents an alternative approach in waste management, in as much as the material is neither land filled nor burned but is considered a resource that may be recycled [5]. In this sense, vermicomposting is compatible with sound environmental principles that value conservation of resources and sustainable practices. Vermicompost, specifically earthworm castes are the final product of vermicomposting process. Vermicomposts are rich in microbial populations and diversity, particularly fungi, bacteria and actinomycetes and have large particulate surface areas that provide many micro sites for microbial activity and for strong retention of nutrients [17]. Chemical analysis of vermicastes showed that two times more available magnesium, 15 times more available nitrogen and seven times more available potassium compared to the surrounding soil [18].

2.2 Municipal solid wastes and management

Municipal solid waste includes all wastes collected by municipal authorities including that from household, commercial and industrial premises [19]. It generally implies all wastes generated in a community with the exception of industrial process wastes and agricultural solid wastes. Municipal solid waste also called urban solid waste is a waste type that includes predominantly household waste (domestic waste) with some times the addition of commercial wastes collected by the municipality with in a given area. They are in either solid or semi-solid form and generally exclude industrial hazardous wastes. There are many categories of MSW such as food waste, rubbish, commercial waste, institutional waste, street sweeping waste, industrial waste, construction and demolition waste and sanitation waste. MSW contains recyclables (paper, plastic, glass, metals, etc), toxic substance (paints, pesticides, used batteries, medicine), compostable organic mater (fruits, and vegetable peels, food waste) and soiled waste (blood stained cotton, sanitary napkins and disposable syringes).

MSWM encompasses the functions of collection, transfer, resource recovery, recycling and treatment. The primary target of MSWM is to protect the health of the population, promote environmental quality, develop sustainability and provide support to economic productivity. To meet these goals, sustainable solid waste management systems must be embraced fully by local authorities in collaboration with both the public and private sectors. Although in developing countries the quantity of solid waste generated in urban areas is low compared to industrialized countries, the MSWM still remains in adequate. The best approach to solving a community's solid waste problem is integrated solid waste management- using a combination of techniques and programs to manage the municipal waste stream. An integrated system is designed to address specific set of local solid waste management problems and its operations are based on local resources, economics and environmental impacts. The idea behind integrated solid waste management (ISWM) is that a combination of approaches can be used to handle targeted proportions of waste stream [19]. Local officials should consider a series of activities, each of which is designed to complement the others. For example, a recycling program can have positive impacts on the development of a waste-to-energy facility, source reduction, recycling, combustion and land filling can have positive impacts on the local municipal waste management problem. To reduce waste management problems at the national level most effectively, states, municipalities and the waste management industry should first consider source reduction- reducing the amount and the toxicity of solid waste generated. Recycling of useful waste materials is the next most desirable approach.

Finally vermicomposting, composting, incineration and land filling complete the solid waste hierarchy. Suitable combination of these alternatives is considered an integrated solid waste management program. The implementation of the integrated solid waste management (ISWM) systems depends on several important factors such as the country statutes, environmental requirements, the strategies in environmental management, energy policy, economic and technological feasibility, and the education and environmental awareness of the people.

2.3 Environmental impact of MSW

Environmental impacts of poor solid waste management lead to the deterioration of ground and surface water quality, as well as air and land pollution. The impact depends on the waste composition and disposal practices. The traditional landfill practice produces various land fill gases such as methane, Carbon dioxide, carbon monoxide, nitrogen, hydrogen sulphide and ammonia. The percentage of green house gases (methane and carbon dioxide. 40-60%) is high. Some of these gases have pungent odour (ammonia and hydrogen sulphide) and are poisonous. If proper collection or venting system is not provided these hazardous fumes can create a health risk for the population near the landfill. Some of the most important negative impacts that may result if solid wastes are not managed properly are [19];

- Uncollected wastes often end up in drains, causing blockages which result in flooding and in sanitary conditions.
- Flies bread in some constituents of solid wastes, and flies are very effective vectors that spread diseases.
- Mosquitoes breed in blocked drains and in rain water that is retained in discarded cans, tyres and other objects. Mosquitoes spread disease including malaria and dengue
- Rats find shelter and food in waste dumps. Rats consume and spoil food, spread disease damage electrical cables and other materials and inflict unpleasant bites.
- The open burning of waste causes air pollution; the products of combustion include dioxins which are particularly hazardous.
- Uncollected waste degrades the urban environment, discouraging efforts to keep streets and open spaces in a clean and attractive condition. Solid waste management is a clear indicator of the effectiveness of a municipal administration. If the provision of this service is inadequate large numbers of citizens (voters) are aware of it. Plastic bags are a particular aesthetic nuisance and they cause the death of grazing animals which ate them.
- Waste collection workers face particular occupational hazards, including strains from lifting, injuries from sharp objects and traffic accidents.
- Dumps of waste and abandoned vehicles block streets and other access ways.
- Dangerous items (such as broken glass, razor blades, hypodermic needles and other health care wastes, aerosol cans and potentially explosive containers and chemicals from industries, may pose risks of injury or poisoning, particularly to children and people who sort through the waste.

- Heavy refuse collection trucks can cause significant damage to the surface of road that were not designed for such weights.

- Waste items that are recycled with out being cleaned effectively or sterilized can transmit infection to later users (examples are bottles and medical supplies).

- Polluted water (leachate) following from waste dumps and disposal sites can cause serious pollution of water supplies. Chemical wastes (especially persistent organics) may be fatal or have serious effects if ingested, inhaled or toughed and cause widespread pollution of water supplies.

2.4 Vermicomposting process

In contrast to composting, the vermicomposting process can be operated in batch, semibatch, and even continuous modes [20]. The time taken to complete the composting of a substrate is of the order of 6-8 week, whereas the 'vermicomposting' is accomplished as quickly as the time it takes for a feed to be ingested by an earthworm, digested, and excreted just a few hours. Vermicomposting involves the following steps:
Step-one: Ingestion of the substrate particles by the earthworm.
Step-two: Physical size-reduction of the ingested particles by the action of the earthworm gizzard, which is located next to the worm mouth.
Step-three: Digestion of the substrate as it passes through the earthworm body and is acted upon by the microorganisms and enzymes present in the earthworm gut.
Step-Four: Exit of the subtracts as 'vermicast' a few hours after the ingestion. The number of hours depends on the nature of the substrate, the worm species, and the length of the worm body. In general, earthworms of shorter body length take lesser time to deliver the vermicast than longer bodied earthworms, and epigeic earthworms process their feed faster than the anecics or the endogeics.

In contrast to composting, vermicomposting does not accompany exothermic reactions; hence, there is no measurable rise in temperature in the vermireactors [20]. Nor do vermireactors need supplementary aeration. In fact, most species of earthworms are not able to thrive at temperatures exceeding 40^oC; hence, if put in an under-composting substrate pile, most worms would perish when the pile temperature rises (as it is must to ensure proper composting) to greater than 55^oC. Also, in contrast to composting, there is no necessity for the periodic turning or mechanical mixing of the substrate. Rather, the natural moving and burrowing activities of the worms accomplish the task of keeping the substrate well-mixed. These activities of worms also keep the substrate adequately aerated and, hence, prevent anaerobic regions from developing in the vermireactors. The effect of earthworms on the decomposition of organic waste during the vermicomposting process is, in the first instance, due to gut-associated processes (GAPs).

These processes include all the modifications that the decaying organic matter and the microorganisms undergo during transit through the earthworms intestines. These modifications include the addition of sugars and other substances, modification of the microbial diversity and activity, modification of the microfaunal populations, homogenization, and the intrinsic processes of digestion, assimilation, and production of mucus and excretory substances such as urea and ammonia, which constitute a readily assimilable pool of nutrients for microorganisms [21]. Decomposition is also enhanced through the action of endosymbiotic microbes that reside in the earthworm gut. These microbes produce extracellular enzymes that can degrade cellulose and phenolic compounds, thereby further enhancing the degradation of ingested material. Other physical modifications of the substrate are caused by the burrowing activities of earthworms, including aeration and homogenization of the substrate, which also favour microbial activity and further decomposition [22].

The proximate activities of earthworms enhance the mineralization of both carbon and nitrogen in the substrate significantly, and such effects are in proportion to the earthworm population densities [23]. Upon completion of GAPs, the resultant earthworm casts undergo cast-associated processes (CAPs), which are more closely associated with the aging processes, the action of the microflora and microfauna presents in the substrate, and the physical modification of the egested materials. During these processes the effects of earth- worms are mainly indirect and derived from the GAPs. It is important to note that in vermicomposting systems, earthworm casts are almost always mixed with material not ingested by the earthworms, and the final vermicompost consists of a mixture of the two different fractions. During this ageing process, vermicompost reaches its optimum in terms of biological properties that promote plant growth and suppress plant diseases.

Currently, there is insufficient information regarding when this optimum is achieved, how we can determine it in each case, and whether this optimum has some kind of expiration date. It is important to note that it is possible that the optimal quality may be achieved only in natural ecosystems built from a correct site-specific balance of soil, plants, microorganisms, macroorganisms including earthworms, and climate. However, it is not possible yet to determine easily when a vermicompost is optimal and thus, this can be known only after its application. All things considered, composting requires labor and machinery to accomplish fragmentation, mixing, and aeration, while in vermicomposting these tasks are by-and-large accomplished by earthworms. The vermicast can be harvested about one to two months after setting up the bin. The compost looks like rich, dark soil and can be separated from the worms by exposing the bins to light and placing fresh bedding next to it. In the presence of light, the worms will move away form the compost and burrows into the fresh bedding.

2.5 Vermicomposting systems

Unlike thermophilic composting, which depends on the rising temperature of the organic mass, vermicomposting can be successfully managed on large or small scales, and it is perhaps most widely practiced in kitchens in home-made bins or crates on a small scale. There are several types and designs of vermicomposting methods; however, most of them fall into two basic groups: the bin method, and the windrow method [24]. Perhaps the greatest challenge to vermicomposting is to assure that the feedstocks do not attain a temperature high enough to begin the thermophilic process of decomposition, as this would kill the worms. Production is greatest when temperature, moisture and aeration are adequate, and feedstock is within acceptable chemical parameters [25].

2.5.1 Bin method

Bins are used for small scales vermicomposting process. Vermicomposting bins are usually constructed from non-aromatic wood or suitable plastic containers. Bottom of the bins include several holes for drainage. Top of the bins are usually covered by porous material, such a burlap sack, to provide aeration and prevent moisture loss. A layer of bedding, lining the bottom is placed in the bin. Worms are placed in the beddings and feed is continuously added on top of the bedding in small amounts, usually less than 10 cm at a time. Vermicomposting based on bin method is usually performed indoors which makes it easier to avoid harsh environmental conditions [24].

2.5.2 Windrow method

Windrows are usually used for large scale vermicomposting process. Windrows are generally built outdoors on a concrete sloped surface to drain water and avoid pests. A typical height of a windrow is three feet or less and distance between each windrow is no more than twenty feet [24]. Windrow vermicomposting can be carried out in several different ways; however, static pile or batch windrow vermicomposting is the most common. In static pile windrow vermicomposting large amount of organic material mixed with bedding is provided to worms. Then, vermicomposting is performed until the entire feed is consumed.

2.6 Biology of Eisenia fetida

There are an estimated 1, 800 species of earthworm worldwide [26]. But the most commonly used is *Eisenia fetida*, commonly known as the compost worm, manure worm, red worm, and red wiggler. The earthworm has a cylindrically shaped, segmented body that tapers off at both ends. Each segment is a separate fluid-filled compartment surrounding a central digestive tract or gut, which runs the length of the worm's body [27].

Earthworms breathe through their skin and must be in an environment that has at least

40 percent moisture (at least as damp as a wrung out sponge). If their skin dries out they cannot breathe and will eventually die. Instead of teeth, *Eisenia* have a gizzard like a chicken. They eat bacteria, fungi, other compost organisms and decaying organic matter small enough to fit into their mouth. In doing so, they can consume close to their body weight in compost material every 24 hours. However, on average, they consume half their body weight every 24 hours. Worm excrement is commonly called castings. While they

Figure 1: *Eisenia fetida* in cow dung

may look and feel like tiny flecks of sticky soil, they are full of beneficial soil microbes.

Scientists have yet to conclude exactly why *Eisenia* castings are good for plants, but they seem to contain nutrients that plants can easily use and disease- suppressing microbes. The mucous covering on the castings also appears to slow down nutrient release. In addition, enzymes in the gut of *Eisenia* may kill many pathogens harmful to plants, horses, or humans that pass through its gut. In any case, castings will not burn plants, even seedlings, and they have a neutral pH. Under ideal conditions, *Eisenia* can double their population every three months. They are hermaphroditic (having both male and female reproductive parts) and become sexually mature when the familiar band (the clitellum) appears around their body, closer to their mouth. Each worm with a clitellum is capable of producing four cocoons per week containing, on average, two baby worms each.

Eisenia tolerate temperatures from 39 to 90 F. Their ideal range is 65 to 75 F. They tolerate moisture levels from 40 to 100 percent but prefer 60 to 80 percent. *Eisenia* tolerate a pH range of 2 to 9, preferring a range of 5.5 to 7. *Eisenia* are sensitive to sunlight or electric light. Their breathing becomes depressed after as little as five minutes of light exposure. At this point *Eisenia* become confused and disoriented, making it difficult to find shelter. More than 30 minutes exposure to sunlight can kill *Eisenia*. They normally do not live in densities greater than 1,000 worms per cubic foot of material. *Eisenia* search out these desirable conditions in a windrow provided there is a food source.

2.7 Factors influencing vermicomposting process

Several studies have been conducted to determine optimal conditions for a successful vermicomposting process. Physical and chemical conditions such as oxygen, moisture content, C/N ratio, temperature, feedstock particle size, species selection and earthworm stocking density and pH are the most common and is need to be considered to perform successful vermicomposting.

2.7.1 Oxygen

Earthworms have no specialized respiratory organs; they obtain oxygen by diffusion through the body wall and lose carbon dioxide by diffusion. However, earthworms are very sensitive to anaerobic conditions, and their respiration rates are depressed in low oxygen concentrations of around 55 to 65%, e.g., at oxygen levels of 0.25 its normal partial pressure [28]; feeding activities might be reduced under these suboptimal conditions. Individuals of *E. fetida* and other species have been reported to migrate in large numbers from a water-saturated substrate in which the oxygen conditions had been depleted or in which carbon dioxide or hydrogen sulphide had accumulated. However, they can live for long periods in aerated water, such as in trickling filters in waste-water treatment plants.

2.7.2 Moisture content

Moisture content plays an important role in the growth of earthworms. Low moisture content can significantly affect earthworm survivability, activity and reproduction [24]. Vermicomposting systems should be maintained at 60 to 80% moisture content [26]. Gunadi et al. [29] reported the maximum weight of *E. fetida* in the pig manure solids at 75% moisture content. Singh et al. [30] observed that a moisture content of 80% is optimum for stabilization of waste in minimum processing time using *Perionyx excavatus* earthworm species. Dominguez and Edwards [31] noted that 85% moisture content is the most favourable for earthworm growth in pig manure. Gurav and Pathade [32] examined vermicomposting of temple waste using *Eudrilus eugeniae* at MC of 50, 60, 70 and 80%. Results indicated that 80% MC is the optimum vermicomposting process.

Palsania et al [33] studied effect of moisture content variation over kinetic reaction rate during vermicomposting process. They have noticed that, the moisture content $75 \pm 5\%$ is the optimal at which the vermicomposting is fastest. Pandit and Maheshwari [34] studied optimization of vermicomposting technique for sugar cane waste management using *Eisenia fetida*. Their result showed that, 80% moisture content were optimum for vermicomposting of sugar cane wastes through this earthworm species and the vermicompost obtained was rich in nitrogen, phosphorus and potassium i.e. 2.3, 2.57 and 1.72% respectively. Parthasarathi [35] observed that, enhanced microbial population and activity and nitrogen, phosphorous, potassium contents were found in fresh vermicomposts from 65-67% moisture than other moisture levels due to the ideal moisture of *Perionyx-excavatus* for better multiplication of microbial population while passing through the worm gut with more activity, thereby enhancing the mineralization process resulting enhancement of nitrogen, phosphorous, potassium contents, whereas these decreased with decline in moisture content, immobilization and inactivation of microorganisms and/or increase in time (aging). Reinecke and Venter [36] reported that the optimum moisture content for E. fetida was above 70% in cow manure.

2.7.3 C/N ratio

Carbon and nitrogen are two primary nutrients required for cell growth; therefore, an optimal carbon to nitrogen ratio is necessary for a successful vermicomposting process. There is not a fixed C/N ratio for vermicomposting. The optimal C/N ratio depends on the type of feed substrate, the species of earthworm, and the desired final product (stabilization of feed or earthworm production). Ndegwa and Thompson [37] found that decreasing C/N ratio increased earthworm biomass production, while increasing C/N ratio produced a more stable end-product. Ndegwa recommended a C/N ratio of 25 for the production of stable vermicompost and a C/N ratio of 10 for the earthworm breeding using biosolids as a feed substrate. Naddafi et al. [38] studied the effect of C/N ratio on vermicomposting of waste activated sludge. They have found that TKN and organic to mineral P of the worm-worked waste activated sludge decreases with increase in C/N ratio. Hailu [39] examined the effects of C/N ration on vermicomposting of Rice husk and cow dung with fresh biosolids. He observed that an increase contents of available P, K organic carbon (C) and also relatively neutral pH at C/N ratio of 25. Kumar et al. [40] observed that a C/N ratio of 30 using a sewage sludge along with cattle manure and saw dust produced the best compost, showed in higher loss of TOC, BOD and higher gain in total nitrogen, and phosphorous, implying that the total amount of biodegradable organic material is stabilized.

2.7.4 Temperature

Earthworms have fairly complex responses to changes in temperature. Neuhauser et al. [41] studied the potential of several species of earthworms to grow in sewage sludge, and they concluded that all these species have a range of preferred temperatures for growth ranging between 15 and 25^{o}C. In their studies, cocoon production was restricted more by temperature than growth, and the species studied produced most of the cocoons at 25^{o}C. Edwards [42] studied the life cycles and optimal conditions for survival and growth of *E.fetida. D. veneta, E. eugeniae.* and *P. excavatus.* Each of these four species differed considerably in terms of response and tolerance to different temperatures. The optimum temperature for *E.fetida* was 25^{o}C, and its temperature tolerance was between 0 and 35^{o}C. *Dendrobaena veneta* had a rather low temperature optimum and rather less tolerance to extreme temperatures. The optimum temperatures for *E. eugeniae* and *P. excavatus* were around 25^{o}C, but they died at temperatures below 9^{o}C and above 30^{o}C. Optimal temperatures for cocoon production were much lower than those most suitable for growth for all these species. Temperatures below 10^{o}C generally resulted in reduced or little feeding activity; below 4^{o}C, cocoon production and development of young earthworms ceased completely [43]. In extreme temperature conditions, earthworms tend to hibernate and migrate to deeper layers of the windrow or soil for protection. The unfavourable effect of high temperatures (above 30^{o}C) on most species of earthworms is not entirely a direct effect because these warm temperatures also promote chemical and microbial activities in the substrate, and the increased microbial activity tends to consume the available oxygen, with negative effects on the survival of earthworms.

2.7.5 Feedstock particle size

Particle size is critical to the size and structure of the vermicomposting because of its effect on pile aeration [44]. The smaller the size of the particles of organic material, the greater is the surface area available for attack by the micro-organisms and earthworms. Very small particles, however, pack tightly together so that the spaces between them will be small and narrow. This prevents the movement of air into the composting heap and the movement of carbon dioxide out of the heap. If the particle size is very large, the surface area for attack is much reduced; the reaction will then proceed slowly or may stop altogether. A compromise on particle size is necessary therefore. Large, brushy yard trash should be reduced in volume for uniformity of size and to obtain a particle size that will promote airflow in the composting pile. The ground material provides a greater surface area for the microorganisms (e.g., fungi, bacteria, and actinomycetes) which will promote decomposition. The pile should be constructed with a variety of particle structures that permits the chimney effect to occur within the pile. The chimney effect allows cool air to be drawn into the bottom of the pile and heated air to be vented through the top, providing the necessary oxygen for microbial activity. In a properly constructed pile, this venting is apparent when the area along the ridge of the pile is giving off visible vapours.

2.7.6 Species selection and worm stocking density

Earthworm density refers to the weight of earthworms used/m^2 of the vermireactor. Earthworm density is very critical because the earthworms function as a bioreactor inside the vermireactor themselves [45]. This is because the bioconversion processes of the organic waste take place inside the earthworm gut whereby the organic waste is the input and the vermicasts are expelled into the vermireactor as a product. The organic waste can stay inside the earthworms' gut for a period of 24 hours after ingestion [45]. During this time the organic waste goes through subsequent physical size reduction in the earthworm's gizzard. The organic waste which is the substrate in this case is acted upon by enzymes and microorganisms in the earthworm gut. Furthermore, the rate of vermicasts produced is depended on the length of the earthworm, the shorter the earthworm, the shorter the residence time inside the earthworm gut.

The best worms are the composting worms. There are several types of vermicomposting worms such as *E. fetida* (Red Wiggler or Tiger worm) and *Lumbricus rubeluus* [46]. These worms have a big appetite, reproduce quickly and tolerate a wide range of temperature. Sinha et al. conducted a comparative study on three species of earthworm on biodegradation of some community wastes, reported that *Eudrilus euginae* is found to have the highest feeding, growth and biodegradation capacity followed by *E. fetida* and *Perionyx excavatus*. Edwards et al. [47] evaluated the suitability of six different species for decomposing agricultural waste and sludge: *E. fetida*, *Dendrobaena veneta*, *D. subrubicunda*, *Lumbricus rubellus*, *Eudrilus eugeniae* and *Perionyx excavatus*. They found that while *D. veneta* showed rapid growth and attained a greater weight at maturity than *E. fetida*, its cocoons produced fewer hatchlings. The choice of species for use in vermicomposting largely depends on temperature.

The amount of worm needed is depend on the size of the worm bin. There is a considerable range in worm stocking density in the literature. Ndegwa et al. [48] studied the effects of various stocking density on the vermicomposting of biosolids. Among the four initial stocking densities of *E. fetida* they found a density of 1.60 kg m^{-2} to be optimal for the production of worm biomass. Garg et al. [49] examined growth and fecundity of an epigeic earthworm (*E. fetida*) during vermicomposting of two different wastes (cow dung and textile mill waste water sludge). The results showed that *E. fetida* growth rate is faster at higher stocking densities; however, biomass gain per worm is faster at lower stocking densities. In this study a worm population of 27-53 worms per kg of feed is found to be the most favourable stocking density. Neuhauser et al. [50], recommended an optimum stocking density of 2.95 kg-worms/m^2 for activated sludge and 0.77 kg-worms/m^2 for horse manure when unlimited feed was supplied to earthworms.

2.7.7 pH

Most species of epigeic earthworms are relatively tolerant to pH, but when given a choice in the pH gradient, they moved toward the more acid material, with a pH preference of 5.0. However, earthworms will avoid acid soils of pH less than 4.5, and prolonged exposure to such soils could have lethal effects [22]. Minor increases in acidity caused by addition of fresh wastes to the vermicomposting bed can be neutralized by the intestinal calcium secretions of earthworms and excreted ammonia. Lime is commonly added to vermicomposts. Singh et al. [30] have reported that earthworms are pH sensitive and generally near neutral initial substrate pH is found to be optimal for stabilization of waste with minimal processing time. The substrates having strong acidic initial pH are found to be less suitable for vermicomposting. Similarly, Gurav and Pathade [32] found that pH 8.0 is optimum pH of vermicomposting of temple waste.

2.8 Chemical and biological characteristics of vermicompost

In vermicomposting, the relatively quick fragmentation of particulate matter is achieved by the grinding muscular gizzard of the worm with the help of enzymatic secretions such as amylase, cellulase, protease, lipase, chitinase and lichenase [25]. Also, the presence of certain actinomycetes aids the formation of clay humic complexes and other cementing agents. The result is a loosely aggregated, granular material whose stability depends on organic matter and moisture concentrations and bacterial and fungal polysaccharides structure. The fertility value of vermicompost produced using different organic waste is given in Table 1.

Table 1: Average chemical composition of vermicompost [52]

Number	Component	Quantity
1	Humic acids(%)	6-18
2	pH	6.5 - 7.2
3	Total nitrogen (%)	0.9 - 3
4	Phosphorous (%)	0.9-2.5
5	Potassium (%)	0.6-2.5
6	Calcium (%)	4.5-8
7	Magnesium (%)	0.3-2.3
8	Iron (%)	0.5-2.5
9	Copper (mgkg^{-1})	3.5-5.1
10	Manganese (mgkg^{-1})	60-80
11	Zinc (mgkg^{-1})	28-35
12	Bacterial flora (Colonies per 1g of biohumes)	up to 20,000 billion
13	Moisture (%)	30-50
13	Dry organic substance (%)	30-70

2.8.1 Nitrogen

Mitchell [53] reported a 17% increase in N concentration in cow manure vermicomposted in a 1.5 m $\times$ 1.5 m $\times$ 0.2 m bed (0.225 $\mathbf{m^3}$ manure), but a 13% decrease in a 1.5 m $\times$ 1.5 m $\times$ 0.3 m bed (0.09 $\mathbf{m^3}$ manure) and a 3% decrease in a 3.5 m $\times$ 1.5 m $\times$ 0.25 m bed (0.19 $\mathbf{m^3}$ manure). Decreased N concentrations were associated with high levels of unconverted material. Additionally, the author concluded that nutrient concentrations were reduced following vermicomposting due to assimilation into worm biomass. Similarly, Ndegwa et al. [37] reported that while N concentration did not change during vermicomposting, total N reductions paralleled decreases in total solids, and they concluded that N was either volatilized or taken up in the worms tissue. Elvira et al. [54], reported 55 to 100% increases in total Kjeldahl N (TKN) concentrations due to mineralization of organic matter. Similarly, Warman and AngLopez [55] observed 42 to 85% increases in total N in three vermicomposted wastes after 45 and 68 days. After 90 days, however, total N concentration had returned to levels only slightly above initial concentrations of 13, 24, and 20 g $\mathbf{kg^{-1}}$.

2.8.2 Phosphorus

Most literature indicates increased P concentration during vermicomposting due to loss of dry matter [25]. Ndegwa et al. [37] observed increases between 14 and 39%. Ghosh et al. [56] also reported rapid mineralization of P in worm treatments of five organic wastes.

Total mineral P increased, as well as the amount of unavailable P bound to aluminum (Al), Fe, and calcium (Ca). Most P was fixed as aluminum phosphate following its release from the organic to inorganic form. They attributed lower rates of ferric P fixation to low oxygen; in addition, the iron was present mostly in the Fe^{+2} form, restricting P fixation as ferric phosphate. Kumar and Singh [57] actually inoculated millet (Pennisetum glaucum) and cow manure vermicompost with several strains of nitrogen-fixing and phosphate-solubilizing bacteria. All treatments showed increases of available P and N.

2.8.3 Potassium and micro-nutrients

Increases and decreases of K and micro-nutrients during vermicomposting have been reported in the literature. Elvira et al. [54] reported significant reductions of total K by the end of the vermicomposting process which they attributed to its high water solubility and leaching of the windrows. Casalicchio and Graziano [58] reported highly variable decreases in P, K, Ca, and Mg, but no statistically significant relationships. Similarly, Bansal and Kapoor [59] observed N increases in vermicomposted crop residue and cow manure, but no difference in P, K, or copper (Cu). Gratelly et al. [60] reported a decrease in total Cu concentration, but increases in total K, Ca, Mg, Fe, Mn, and Zn concentrations during the vermicomposting of dairy sludge. Albanell et al. [61] also reported increases in P, Na, and K that they attributed to accelerated mineralization of organic matter. Wong and Griffiths [62] warned of the potential accumulation of toxins in the worm gut and reported Zn levels in vermicompost five times greater than those in the original pig manure feedstock. Edwards [42] noted that low levels of Mg in the final product is common due to low Mg levels in many manures.

2.8.4 Carbon, humification and pH

Vermicomposting leads to a breakdown of carbon and a reduction of volatile solids, resulting in the stability of the finished product. Vincelas-Akpa and Loquet [63] analysed OM transformations in composted and vermicomposted maple leaf yard waste, and found a decrease in the C/N ratio from 62 to 27 over 9 months in the vermicompost compared to a drop from 62 to 30 in the compost waste. While vermicompost and compost treatments both showed losses of organic matter until the seventh month, the initial decrease was greater in vermicompost. In addition, percentage of C actually increased in vermicompost after month 7, whereas it stabilized in the compost. Chowdappa et al. [64] also reported a decrease of 62 to 24 in C/N ratio during vermicomposting of areca (Areca catechu) leaves compared to 62 to 33 for a thermophilic composting process. Casalicchio and Graziano [58] reported no differences in total organic C between compost and vermicompost treatments of sewage sludge, a MSW, and MSW + sludge. After 90 days of vermicomposting the MSW, the ratio of humic to fulvic acids was 18% greater than in compost.

The ratio of humic acid to organic C (humification) was 42% greater in vermicomposted MSW than composted MSW, and 30% greater in the vermicomposted MSW + sludge compared to composting. High humic fractions can result in increased CEC in waste processed with worms. Albanell et al. [61] reported a greater CEC (17%) in vermicomposted sheep manure + cotton waste compared to composted feedstock after 12 weeks. Similarly, humic acid concentration was 31% greater in vermicompost than in compost. Warman and AngLopez [55] also reported increases in CEC from 45 to 80 cmol kg^{-1} after 90 days of vermicomposting kitchen + yard waste. The vermicomposting process generally neutralizes the pH of the substrate.

2.8.5 Microbial characteristics

Due to the mesophilic transformation of organic matter, vermicompost contains greater microbial populations than thermophilic compost, leading to greater potential for odor reduction and nutrient mineralization [25]. Edwards et al. [47] identified large populations of Gram-negative Enterobacteriaceae in vermicompost feedstock. Additionally, a large proportion of the microbial population was composed of protozoa and fungi. The authors suggested that as the worm feeds on organic matter, it digests some of the microbial constituents, and ultimately increases the surface area of the remaining material passing through its gut. This can increase the potential for microbial colonization and subsequent decomposition of the waste material. There are microbial populations fungi, protozoa, and bacterium inside the worm gut itself (mostly in the foregut) that are encouraged by an internal pH between 6.3 and 7.3 [65]. Albanell et al. [61], however, noticed a marked decline in microbial colony forming units (CFUs) after six weeks of vermicomposting sheep manure and cotton waste. Similarly, Hand et al. [66] reported no significant difference in bacterial or fungal populations in cow slurry treated with and without worms. After six weeks, populations decreased more rapidly in vermicompost treatments than in controls. This may have been due to the fact that the control remained in a mesophilic state, rather than achieving a thermophilic state of composting that would have considerably reduced microbial populations.

2.9 Agricultural application of vermicompost

Vermicomposts are excellent sources of biofertilizers and their addition improves the physio-chemical and biological properties of agricultural soil. Vermicomposting amplifies the diversity and population of beneficial microbial communities [67]. Vermicomposts with excellent physio-chemical properties and buffering ability, fortified with all nutrients in plant available forms, antagonistic and plant growth-promoting bacteria are fantabulous organic amendments that act as a panacea for soil reclamation, enhancement of soil fertility, plant growth, and control of pathogens, pests and nematodes for sustainable agriculture.

2.9.1 Effects on soil physical characteristics

The use of vermicompost as soil amendments can have many positive effects on soil physical characteristics following high rates of application. High levels of organic humic matter soil amendment in the form of vermicompost improve soil structure by increasing porosity and reducing the bulk density of an amended soil. Vermicompost with a high concentration of small aggregates may actually increase the bulk density of an amended potting substrate. Atiyeh et al. [68] found that due to smaller particle size the bulk density of pig manure vermicompost was 2.25 times greater than Metro-Mix 360. When vermicompost was added to Metro Mix (MM) at concentrations of 5, 10, 25, 50, and 100% by volume, bulk densities of the potting medium increased proportionally. Total porosity of the 5 to 50% mixtures was reduced 1 to 6%, and percent air space 33 to 53%. The authors concluded that in 5, 10, and 25% MM mixes, vermicompost improved the water holding capacity of the Metro Mix while the Metro Mix improved the percent porosity and airspace of the vermicompost. Masciandaro et al. [69] noted an increase in total surface shrinkage in vermicomposted aerobic and anaerobic municipal sludge. They associated the formation of small cracks (less than 500 μm) in the substrate with an increase in small aggregates, which serve as an index of improved soil structure.

2.9.2 Effects on soil fertility and plant growth

Vermicomposts can significantly influence the growth and productivity of plants due to their micro and macro elements, vitamins, enzymes and hormones [67]. Vermicomposts contain nutrients such as nitrates, exchangeable phosphorus, soluble potassium, calcium, and magnesium in plant available forms and have large particular surface area that provides many micro- sites for microbial activity and for the strong retention of nutrients. Uptake of nitrogen (N), phosphorus (P), potassium (K) and magnesium (Mg) by rice (Oryza sativa) plant was highest when fertilizer was applied in combination with vermicompost [67]. Part from providing mineralogical nutrients, vermicomposts also contribute to the biological fertility by adding beneficial microbes to soil. Mucus, excreted through the earthworm‘s digestive canal, stimulates antagonism and competition between diverse microbial populations resulting in the production of some antibiotics and hormone-like biochemicals, boosting plant growth [43]. Vermicomposts are used as alternative potting media due to their low-cost, excellent nutrient status and physio-chemical characters.

Considerable improvements in plant growth recorded after amending soils with vermicomposts have been attributed to the physico-chemical and biological properties of vermicomposts. Use of vermicomposts as biofertilizers has been increasing recently due to its extraordinary nutrient status, and enhanced microbial and antagonistic activity. The improvement to soil physical structure, soil fertility and soil microbiological characteristics associated with compost application all promote plant growth, as a growth medium for transplants and a soil amendment for field crops. Several studies have evaluated the effect of vermicompost-amended potting media on plant growth greenhouse production. Generally, potting medium with 10 to 20% vermicompost by volume provides adequate fertilization for transplant growth [25]. In one study, germination rates of greenhouse tomatoes increased up to 15% when vermicomposted pig manure was mixed with potting medium at 20, 30 and 40% by volume. The highest marketable yield of fruit was reported in the 20% mixture. Vermicompost application increased plant spread (10.7%), leaf area (23.1%), dry matter (20.7%) and increased total strawberry fruit yield (32.7%) [67].

2.9.3 Soil microbial populations and plant pathogen suppression

In conventional agriculture, incidence of pests and plant diseases is generally curtailed through the use of pesticides and fungicides. While these synthetic inputs are generally effective, pathogens may gain resistance after repeated applications, thus jeopardizing future production and forcing farmers to depend on increasingly intensive control programs or seek alternative forms of bio-control [25]. Similarly, in less industrialized countries, where chemical inputs are often not used due to high cost and limited availability, pathogens must be managed by natural techniques or amendments. Soils with low organic matter and microbial activity are air conducive to plant root diseases and addition of organic amendments can effectively suppress plant disease [67].

Microbial antagonism might be one of the possible reasons for disease suppression as organic amendments enhances the microbial population and diversity. Traditional thermophilic composts promote only selected microbes while non-thermophilic vermicomposts are rich sources of microbial diversity and activity and harbour a wide variety of antagonistic bacteria thus acts as effective biocontrol agents aiding in suppression of diseases caused by soil-borne phytopathogenic fungi.

2.10 Environmental and economic benefits of vermicomposting

A matter of considerable economic and environmental significance is that the cost of food production by vermiculture will be significantly low by more than 60-70% as compared to chemical fertilizers and the food produced will be a safe chemical-free food for the society [70]. It is a win-win situation for both producers (farmers) and the consumers (feeders). The cost of production of vermicompost is simply insignificant as compared to chemical fertilizers. While the former is produced from organic waste, a raw material which is in plenty all over the world, the latter is obtained from petroleum products which is a vanishing resource on earth. Vermicompost can be produced on-farm at low-cost by simple devices, while the chemical fertilizers are high-technology & high-cost products made in factories [70].

Vermicomposting is a natural and efficient way of recycling organic garden and kitchen waste. Given the right environment and appropriate routine attention, our garden and household waste can be converted to valuable compost faster then the traditional composting procedure. Worm composting also prevents stinking smells from the decomposing materials due to the fast action of the worms in eating those garbage. With the right equipments, vermicomposting is quite clean and odorless and can be conducted indoors. For urban dwellers or people with little or no yard space, vermicomposting is the answer for household waste management because composting can be conducted in containers and placed indoors. Wastes and food scraps can be disposed into the vermicomposting containers without burdening the water treatment facility or landfills.

For the gardeners or farmers, vermicompost (with vermicast) is a nutritious fertilizer that can be used for their crops or ornamental plants without incurring cost for the purchase of fertilizers. The granular worm castings, when mixed into garden soils would react as a slow release fertilizer to feed the plants and at the same time acting as a soil conditioner by improving the structure of the soil [71]. Besides that, according to Murphy [71], vermicasts also contains special hormones and enzymes secreted by various types of bacteria living in the worms body. These hormones and enzymes are beneficial in promoting plant growth. Generally, the utilization of vermicompost results in several benefits to farmers, industries, environment and overall national economy [72].

Farmers:

- Less reliance on purchased inputs of nutrients leading to lower cost of production
- Increased soil productivity through improved soil quality
- Better quantity and quality of crops
- For landless people provides additional source of income generation

Industries:

- It is cost-effective pollution abatement technology

Environment:

- Wastes create no pollution, as they become valuable raw materials for enhancing soil fertility

National economy:

- Boost to rural economy
- Savings in purchased inputs
- Less wasteland formation

3 MATERIALS AND METHODS

The study consisted of seven separate but related stages: (i) characterisation of the raw materials used for vermicomposting (ii) pre-composting process involving mixtures of selected organic municipal solid wastes and horse manure, (iii) *Eisenia fetida* breeding, (iv) digestion of the pre-composted mixtures by the earthworms, (v) optimization of the vermicomposting process parameters, (vi) characterisation of some of the chemical and physical properties of the end products (vermicompost) and (vii) Evaluation of the vermicompost as a fertilizer.

3.1 Experimental locations

TOC, %VS, moisture content , *Esenia fetida* breeding and size reduction of both the feed materials and the product (vermicompost) and pH of the wastes were conducted in the laboratory of Chemical and Bio Engineering School of the Institute of Technology, Addis Ababa University. Nitrogen content determination of the feed materials were conducted at AAEPA, soil and water laboratory, the vermicomposting process has been carried out at AAEPA, Kirkos sub city MSW composting site and finally the pH, EC and NPK value of the vermicompost were examined at Wenji Sugar factory, Soil and Water Management Team, Soil Laboratory.

3.2 Equipments and materials

The vermicomposting method used was the bin method. A worm bin which had a capacity of 0.022 m^3 in volume was used. Then the small sized stone and bedding material such as shredded newspaper and cardboard, red worms (*Eisenia fetida*), municipal solid waste, horse manure analytical and analogue balance, oven, conductivity and pH meter, volumetric flasks, beakers, desicator, silica crucible, furnace, semi-micro Kjeldahl distillation unit, spectrophotometer, polythene shaking bottles, funnel, wire mesh and Whatman filter paper were used. In addition sprinkling water can and thermometer was used.

3.3 Earth worm collection and breeding

The recognized earthworm species (*Eisenia fetida*) was brought from Ambo Plant Protection Research Center (APPRC), Ambo, Ethiopia. *Eisenia fetida* were cultured in (0.022 m^3) plastic bins for 2 months. Partially degraded cow dung and horse manure was added for breeding of the earthworms. The reproduction of earthworm depends on several important parameters, which we should always monitor them whenever necessary. The most important of all parameters are temperature, moisture and the pH. The following parameters shown in Table 2 are measured twice a week for a period of eight weeks and the average values are considered.

Table 2: Regulated parameters during the breeding of earthworms

Parameters range	Optimum range	Average measured value
Temperature (^oC)	10-35	18
Moisture (%)	60-90	76
pH	4.5-9	7.8

3.4 Waste collection

MSW was collected from MSW composting site of AAEPA, Kirkos site. Horse manure was obtained from Janmeda horse station, Addis Ababa, Ethiopia. The samples were randomly selected and bagged in plastic container and had been to the stated experimental locations for analysis.

3.5 Preparation and formulation of feedstock

The collected samples were prepared and conditioned for vermicomposting. Sample preparation process includes; separation of organic and inorganic fraction of municipal solid waste, size reduction and sieving and pre-composting of the feeds. MSW of about 20 kg and horse manure of 8 kg was used for the sample preparation. Organic and inorganic fraction separation of MSW was done manually. MSW and horse manure was crushed to reduce the size using Hammer Mill and sieved to give the optimum size of 1-2 mm. Then the feed materials were pre-composted for 2 weeks. Organic fraction of MSW and horse manure was used for preparation of different waste mixtures. Before formulating feedstock, chemical analysis was done on organic municipal solid waste and horse manure. The chemical analysis was done in duplex. The result of the analysis is presented in Table 3 below. C/N ratios and moisture content of the compost mixture were calculated as follows Eq.(1) and (2) [73].

$$\mathbf{G} = \frac{\mathbf{M_1Q_1 + M_2Q_2 + M_3Q_3 + ... + M_nQ_n}}{\mathbf{Q_1 + Q_2 + Q_3 + ... + Q_n}} \tag{1}$$

Where, $\mathbf{Q_n}$ is mass of material n (wet weight basis), G is moisture goal (%) and $\mathbf{M_n}$ is moisture content (%) of material n.

$$\mathbf{R} = \frac{\mathbf{Q_1C_1(100 - M_1) + Q_2C_2(100 - M_2) + ... + Q_nC_n(100 - M_n)}}{\mathbf{Q_1N_1(100 - M_1) + Q_2N_2(100 - M_2) + ... + Q_nN_n(100 - M_n)}} \tag{2}$$

Where, R is C/N ratio of compost mixture, $\mathbf{Q_n}$ is mass of material n (wet weight basis), $\mathbf{C_n}$ is carbon (%) of material n, $\mathbf{N_n}$ is nitrogen (%) of material n and $\mathbf{M_n}$ is moisture content (%) of material n. For any number of independent equations we can usually solve for that same number of unknowns. In this case we have two equations (one for moisture and one for the C/N ratio), and we can solve them for any two unknowns. Normally we use this approach to develop a mix ratio of several different ingredients, knowing the moisture, carbon, and nitrogen contents of each. If we specify the quantities of all but

Table 3: Some physicochemical properties of the feed materials

Parameters studied	Municipal solid waste	Horse manure
VS(%)	75.89	71.21
Ash(%)	24.11	28.79
Total organic carbon (TOC)(%)	42.16	39.56
Total Kjeldahl nitrogen (TKN) (%)	0.56	1.71
C/ N ratio	75	23
Moisture content (%)	53.9	10.66
pH	7.22	8

two ingredients, and the C/N and moisture content we would like to achieve in the mixture, we can solve for those two remaining quantities to get the mix we want. Water will bring up the moisture content without altering the C/N ratio. And since water is cheap and usually readily available, it can be an easy way to develop an appropriate mix.

The solution can be obtained in a number of ways using linear algebra or matrices. With patience, one can use simple algebraic methods to solve the moisture equation for one of the unknown quantities, and then substitute that value in the C/N equation and solve the C/N equation for the other unknown. At that point, back-substitution into the solution of the moisture equation gives both unknowns in terms of known values. The algebraic manipulations required for a mixture of two materials is straightforward.

3.6 Design of experiments (DoE)

The first task before conducting the experiments was selection of potential parameters to be varied. The three main factors selected in this study were moisture content(MC), carbon to nitrogen ratio (C/N) and worm stocking density (WSD). However, as it is mentioned above in the literature review part there are also other factors which affect the vermicomposting process. When varied these factors, it might be exert some effect on the responses but for the present experimentation these factors were held constant at their optimum levels from the literature because of different constraints. The levels of the selected factors is determined from the literature research and is presented in Table 4. The experiment performed as a completely randomized design with three main

Table 4: Level and code of variables used for Box-Behnken design

Factors	Unit	Coded symbol	level 1 (-1)	Level 2 (0)	Level 3 (+1)
Moisture content	(%)	A	60	75	90
C/N ratio	-	B	20	25	30
W. stocking density	kg worm/m^2	C	0.8	1.4	2

factors at three levels and three response variables. The three response variables were the fertility parameters; N, P and K. The experimental design used was a Box-Behnken under response surface methodology (RSM). Box-Behnken design only has three levels (low, medium, and high, coded as -1, 0, and +1) and need fewer experiments; this design is more efficient and easier to arrange and interpret in comparison with others [74]. The geometry of a three-factor Box-Behneken design is shown in Figure 2. The proposed

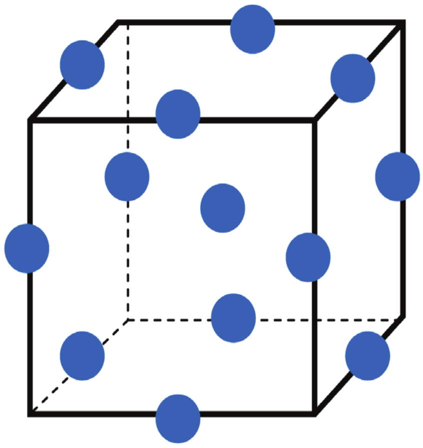

Figure 2: Geometry of a three factor Box-Behnken design [75]

Box-Behnken design required 18 runs for modelling a response surface. Details of the experimental runs with the set of input parameters that were conducted are given in Table 5. Design expert software was used to design the experiment and randomize the runs. Randomization ensures that the conditions in one run neither depend on the conditions of the previous runs nor predict the conditions in the subsequent runs. Randomization is essential for drawing conclusions from the experiment, in correct, unambiguous and defensible manner. Most importantly, parameters corresponding to the central point (0,0,0) are repeated six times to establish that the experimental data is within the normal dispersion and repeatability is ensured.

Table 5: Box-Behnken design and combination of process variables

Run order	A:Moisture content	B:C/N ratio	C:W. stocking density
1	75	20	2.00
2	60	20	1.40
3	90	25	0.80
4	75	25	1.40
5	75	30	2.00
6	60	30	1.40
7	90	30	1.40
8	75	25	1.40
9	75	20	0.80
10	60	25	2.00
11	75	25	1.40
12	75	30	0.80
13	75	25	1.40
14	90	25	2.00
15	75	25	1.40
16	75	25	1.40
17	60	25	0.80
18	90	20	1.40

3.7 Experimental procedure

The organic municipal solid waste was first shredded to reduce the particle size to 1-2 mm, which is the the optimum value [76]. The organic waste was mixed with horse manure to give different combination of C/N ratios and then pre-composted for two weeks monitoring the pH and moisture content in the feed. Pre-composting facilitated in making other organisms that can aid the vermicompost process available, to reduce the temperature of the reactor and also reduction of the organic waste surface area whereby the earthworms will act on. The preferred organic waste feed rate to be used for optimum vermicomposting is 0.75 kg-feed/kg-worm/day [48]. The study was performed in worm bins of 0.022 $\mathbf{m^3}$ capacity. The kind of earthworms used was *Eisenia fetida*. The bins were initially filled to a 2 cm height with 12 mm nominal size chips of stone (aggregates) and shredded newspaper and cardboard as a bedding material, used to ensure proper circulation of air and water. A mixture of municipal solid wastes and horse manure in different C/N ratio and moisture content were used above the bedding material to provide natural habitat to the earthworms. The substrate thickness was restricted to 10 cm to ensure the maintenance of aerobic condition. The vermireactors were kept in dark area at an average temperature of 19^oC.

The daily evaporated water from the bin was determined using the average mass lost every day for 7 days. The measurement was done at 5:00 pm every day and the lost water was determined by subtracting the finial mass from the initial. Measurement (in gram) shows that 1.476 g per day approximately 1.5 g per day was lost. Therefore application of 10.5 g of water or 10.5 ml per each bin per week was done throughout the experiment to maintain the moisture contents. The experiment was carried out for 60 days since the average days for making vermicompost ranges from 56 to 70 [37].

3.8 Parameters analysis

Analysis of pH, EC, N, P and K of the vermicompost was done at Wenji Sugar Factory, Soil and Water management Team, Soil Laboratory. Substrates samples were drawn after 60 days from all the experimental bins to analysis same of the physico-chemical properties of vermicompost. About 0.2 kg of homogenized wet sample (free from earthworms, hatchlings and cocoons) was drawn from each bin and stored at 4 ^oC immediately for analysis. The samples were air dried immediately, ground to pass through 2 mm sieve and stored for physico-chemical analysis. pH, Moisture content (%), Organic Matter (%), Available Phosphorus (%), Total Nitrogen (%), C/N ratio, EC ($\mathbf{dsm^{-1}}$), and Exchangeable Potassium (%) of the samples were measured.

The pH of the samples was measured by pH meter in the supernatant suspension of 1:5 ratios of samples to water mixture. The percentage of moisture content was calculated following equation (3)[44].

$$\mathbf{Moisture}(\%) = \frac{\mathbf{Weight_{Wet}} - \mathbf{Weight_{Dry}}}{\mathbf{Weight_{Wet}}} \times \mathbf{100} \tag{3}$$

Volatile solid/organic matter content was determined by loss ignition method (on dry mass basis) at 550^{o}C for 2 h and is given by equation (4) below.

$$\%\mathbf{VS} = \mathbf{100} - \mathbf{Ash}(\%) \tag{4}$$

The total organic carbon content was calculated from volatile solids using following equation (5) [44].

$$\mathbf{TOC}(\%) = \frac{\%\mathbf{VS}}{\mathbf{1.8}} \tag{5}$$

The percentage of nitrogen content was calculated using Kjeldahl method following equation (6) [77].

$$\mathbf{TKN}(\%) = \frac{(\mathbf{a} - \mathbf{b})}{\mathbf{s}} \times \mathbf{N} \times \mathbf{0.014} \times \mathbf{100} \times \mathbf{mcf} \tag{6}$$

Where;

a= ml of $\mathbf{H_2SO_4}$ required for titration of sample
b= ml of $\mathbf{H_2SO_4}$ required for titration of blank
s= air dry sample weight in grams
N= Normality of $\mathbf{H_2SO_4}$ (0.1 N)
100= ml of the solution
0.014= meq weight of nitrogen in g
mcf= moisture correction factor

The C/N ratio was determined by dividing the total organic carbon content to the total nitrogen content. The amount of phosphorous was calculated using equation (7) [77].

$$\mathbf{P}(\mathbf{ppm}) = (\mathbf{a} - \mathbf{b}) \times \frac{\mathbf{100}}{\mathbf{s}} \times \mathbf{mcf} \tag{7}$$

Where;

a= mg/l of P in sample extract
b= mg/l of P in blank
s= sample weight in gram (5 g)
100= ml of extracting solution
mcf= moisture correction factor

Electrical conductivity (EC) was determined by 1:5 compost:water extract with conductivity meter. Exchangeable K was measured by using Flame-photometer. The detail experimental methods and producers used during this study is given at Appendix 2.

3.9 Statistical analysis

Design-expert 7.0 (Stat-Ease, trial version) was the software used for designing the experiment, statistical analysis (e.g. analysis of variance) and response surface studies. All presented graphs were generated using the software. Box-Behnken under RSM experimental method was used to determine the effect of three operating variables of the vermicomposting process for organic fertilizer production from municipal solid waste. These were moisture content, C/N ratio and worm stocking density. The response variables was total nitrogen, available phosphorous and exchangeable potassium. Significance of the result was set from analysis of variance (ANOVA).

4 RESULTS AND DISCUSSION

4.1 Experimental results

The experimental values of N, P and K obtained under different conditions are presented in Table 6. A total of 18 experimental runs were carried out with a replication of center points for six times which was to estimate the experimental error. These results were input into the Design Expert software version 7.0. for further analysis [78].

Table 6: Experimental observations

Run order	A:Moisture content	B:C/N ratio	C:W.stocking density	N (%)	P (%)	K (%)
1	75	20	2.00	2.63	1.45	1.29
2	60	20	1.40	1.34	0.48	0.28
3	90	25	0.80	1.46	0.73	0.32
4	75	25	1.40	2.56	1.18	1.07
5	75	30	2.00	2.52	1.28	1.02
6	60	30	1.40	0.87	0.21	0.14
7	90	30	1.40	1.83	0.72	0.72
8	75	25	1.40	2.62	1.16	1.04
9	75	20	0.80	1.75	0.64	0.53
10	60	25	2.00	1.76	0.97	0.68
11	75	25	1.40	2.62	1.34	1.07
12	75	30	0.80	1.81	0.78	0.78
13	75	25	1.40	2.56	1.04	1.06
14	90	25	2.00	1.99	1.21	0.63
15	75	25	1.40	2.58	1.15	1.07
16	75	25	1.40	2.63	1.16	1.18
17	60	25	0.80	0.82	0.23	0.11
18	90	20	1.40	1.46	0.43	0.25

4.2 Development of empirical models

In the present study, empirical models for the output responses, N, P and K in terms of the process parameters in actual and coded factors were developed by using the RSM. The sequential model fitting for N, P and K of the samples prepared are given at Appendix 3 (Table 14, Table 15 and Table 16). Three tests were carried out to determine the adequate model. These included Sequential model Sum of Squares, Lack of Fit Tests and Model Summary Statistics. From Table 14, Table 15 and Table 16, it was found that quadratic model was the most suitable model for the the present study, because quadratic model had high R^2, adjusted R^2, predicted R^2 and low PRESS for all responses (N, P and K). The Sequential model Sum of Squares also showed that quadratic model was the highest order polynomial where the additional terms were significant as the PRESS value of cubic model could not be defined in the Model Summary Statistics for all responses. Besides, it can be observed that quadratic model had the highest p-value for Lack of Fit Tests. The regression coefficients of the developed model are determined from the regression analysis. From the three tables it is observed that the quadratic model is the best fit model for all responses in terms of its significance and for this experimental design, the second order (quadratic) model is suggested, as the p-value of this model is also smaller than that of other models. The three developed equations regardless of significant and insignificant model terms for N, P and K respectively are given at Appendix 4. The developed models are further used for optimization of the vermicomposting process.

4.3 Adequacy check for the developed models

Usually, it is essential to confirm first whether the fitted model provides an adequate approximation of the actual values or not. The adequacy of the model was checked by analysis of variance (ANOVA) and some diagnostic plots. Analysis of variance (ANOVA) is employed to test the significance of the developed models. Table 7, Table 9 and Table 10 shows the summary of the analysis of variance (ANOVA) of the three responses N, P and K respectively. The detail ANOVA for the three responses is given at Appendix 4. The F-value is measure of variation of the data about the mean. Generally, the calculated F value should be several times greater than the tabulated value, if the model is a good prediction of their experimental results and the estimated factors effects are real [79]. Also the high F-value and a very low probability indicates that the present models are in a good prediction of the experimental results. The p-value serves as a tool for checking the significance of each of the coefficients. The pattern of interaction between the variables is indicated by these coefficients. The variables with low probability levels contribute to

Table 7: ANOVA results of the quadratic regression model for N

Source	**Sum of squares**	**Df**	**Mean square**	**F-value**	**P-value**	
Model	6.66	9	0.74	463.07	<0.0001	significant
A	0.48	1	0.48	297.65	<0.0001	
B	2.813×10^{-3}	1	2.813×10^{-3}	1.76	0.2211	
C	1.17	1	1.17	732.96	<0.0001	
AB	0.18	1	0.18	110.47	<0.0001	
AC	0.042	1	0.042	26.32	0.0009	
BC	7.225×10^{-3}	1	7.225×10^{-3}	4.52	0.0661	
A^2	3.90	1	3.90	2440.29	<0.0001	
B^2	0.33	1	0.33	206.65	<0.0001	
C^2	0.089	1	0.089	55.49	<0.0001	
Residual	0.013	8	1.597×10^{-3}			
Lack of fit	7.625×10^{-3}	3	1.03×10^{-3}	2.47	0.1771	not significant
Pure error	5.15×10^{-3}	5	1.03×10^{-3}			
Cor Total	6.67	17				

† $\mathbf{R^2}$=0.9981; Pred.$\mathbf{R^2}$=0.9806; Adj.$\mathbf{R^2}$=0.9959; and Adeq.Precision=61.86

the model, whereas the others can be neglected and eliminated from the model. Values of P>F less than 0.0500 indicates model terms are significant. In the present study A, C, AB, AC, A^2, B^2, C^2 for the response N, A, C, AB, A^2, B^2, C^2 for the response P and A,

C, AB, BC, A^2, B^2 for the response K are significant model terms. High F-values and non significant lack of fit relative to the pure error indicated that models were a good fit. The coefficients of determination (coefficient of correlation), $\mathbf{R^2}$ was high for all re-

Table 8: ANOVA results of the quadratic regression model for P

Source	**Sum of squares**	**Df**	**Mean square**	**F-value**	**P-value**	
Model	2.45	9	0.27	37.73	<0.0001	significant
A	0.18	1	0.18	25.00	0.0011	
B	1.25×10^{-5}	1	1.25×10^{-5}	1.736×10^{-3}	0.7230	
C	0.80	1	0.80	111.11	<0.0001	
AB	0.078	1	0.078	10.89	0.0109	
AC	0.017	1	0.017	2.35	0.1641	
BC	0.024	1	0.024	3.34	0.1052	
A^2	1.01	1	1.01	140.83	<0.0001	
B^2	0.23	1	0.23	31.94	0.0005	
C^2	0.040	1	0.040	5.52	0.0468	
Residual	0.058	8	7.201×10^{-3}			
Lack of fit	0.011	3	3.708×10^{-3}	0.40	0.7602	not significant
Pure error	0.046	5	9.297×10^{-3}			
Cor Total	2.50	17				

† $\mathbf{R^2}$=0.9770; Pred.$\mathbf{R^2}$=0.0.9021; Adj.$\mathbf{R^2}$=0.9511; and Adeq.Precision=19.98

sponses, a value > 0.75 indicates the aptness of the model. For a good statistical model , the $\mathbf{R^2}$ value should be close to one [79]. Results ensured a satisfactory adjustment of the quadratic model to the experimental data and indicated that approximately 99.8 %, 97.7% and 98.09% of the variability in the dependent variables of N, P and K respectively could be explained by these models and only 0.2 %, 2.3% and 1.91% of the total variance could not explained by these models for N, P and K respectively. The value of $\mathbf{R^2}$ (correlation coefficient) for all responses is very high and close to one which indicates a good agreement between experimental and predicted values. The predicted $\mathbf{R^2}$ is in a reasonable agreement with the adjusted $\mathbf{R^2}$ for all responses. Also, a ratio of adequate precision greater than 4 is desirable. In the present study the ratio for all responses were greater than 4, which is (61.86 for N, 19.98 for P and 20.44 for K) indicates an adequate signal.

Additionally, the developed response surface models for N, P and K has been checked by using residual analysis. Residuals are usually considered as components of variations, imprecisely fitted to the model and subsequently it is predicted that they behave according to a normal distribution feature. For the evaluation of normality of the residuals, a graphical visualization of the normal probability plot is considered as the proper method. Figure 3, (a), (b) and (c) are the plot of the residuals calculated against the order of experimentation. It is asserted that a tendency to have runs of positive and negative residuals indicate the existence of correlation [80]. The normal plots for N, P and K are shown in Figure 4,((d)-(f)). It can be observed that, the data are spread approximately in a straight line, which show a good correlation between experimental and predicted values for the responses. The plots of observed versus predicted values shows minimal variation be-

Table 9: ANOVA results of the quadratic regression model for K

Source	Sum of squares	Df	Mean square	F-value	P-value	
Model	2.49	9	0.28	45.75	<0.0001	significant
A	0.063	1	0.063	10.41	0.0121	
B	0.012	1	0.012	1.99	0.1965	
C	0.44	1	0.44	73.01	<0.0001	
AB	0.093	1	0.093	15.37	0.0044	
AC	0.017	1	0.017	2.79	0.1332	
BC	0.068	1	0.068	11.17	0.0102	
A^2	1.58	1	1.58	261.42	<0.0001	
B^2	0.076	1	0.076	12.58	0.0075	
C^2	8.673×10^{-3}	1	8.673×10^{-3}	1.43	0.2655	
Residual	0.048	8	6.051×10^{-3}			
Lack of fit	0.036	3	0.012	4.90	0.0598	not significant
Pure error	0.012	5	2.457×10^{-3}			
Cor Total	2.54	17				

† $\mathbf{R^2}$=0.9809; Pred.$\mathbf{R^2}$=0.7655; Adj.$\mathbf{R^2}$=0.9595; and Adeq.Precision=20.438

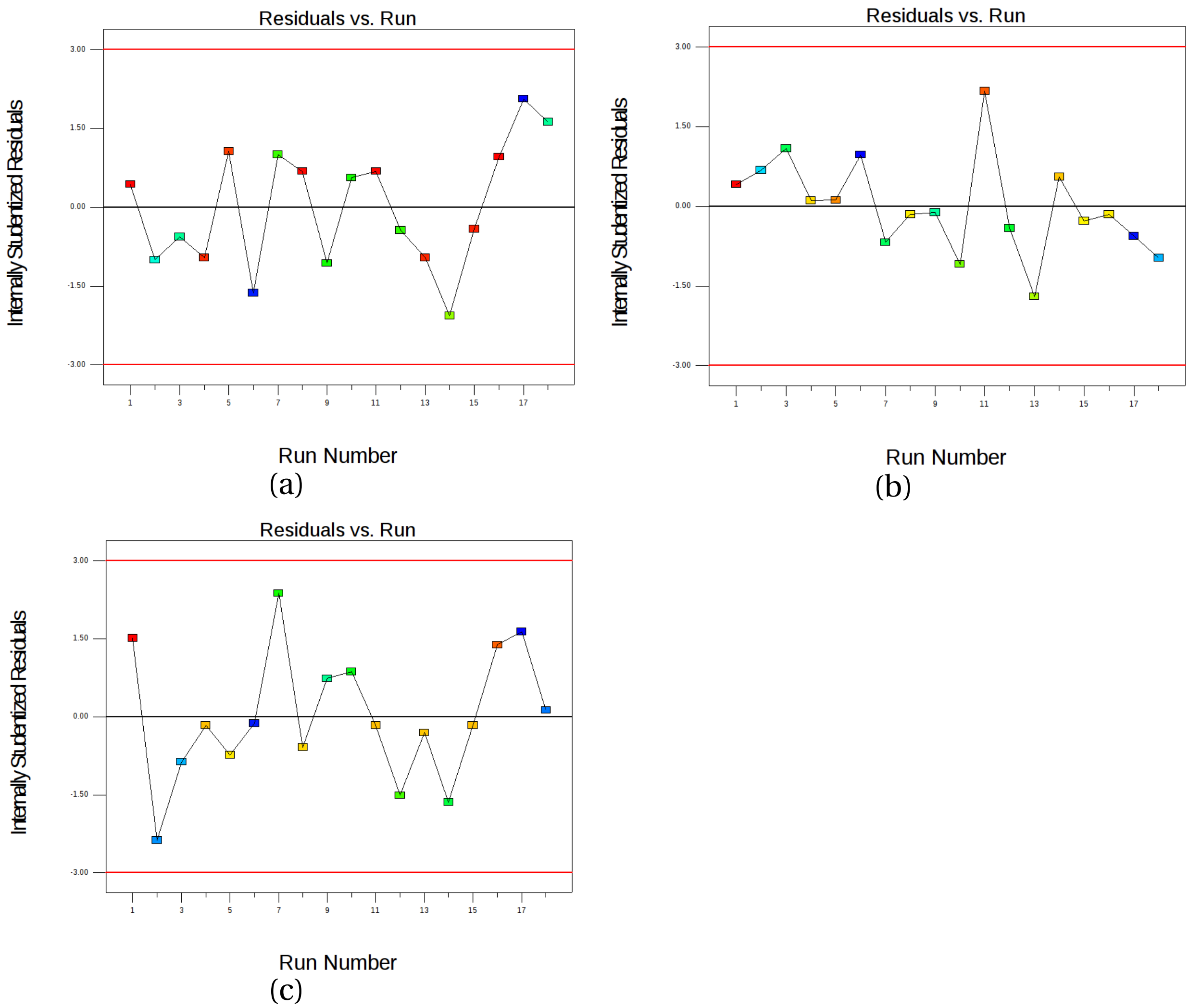

Figure 3: Plot of residuals versus run order of the data; (a) for N, (b) for P and (c) for K

tween the observed and fitted values for all responses (Figure 4, (g)-(i)). From the above ANOVA and analysis of residual plots for the three responses, the model does not reveal inadequacy and this model was used to navigate the design space to find the optimized

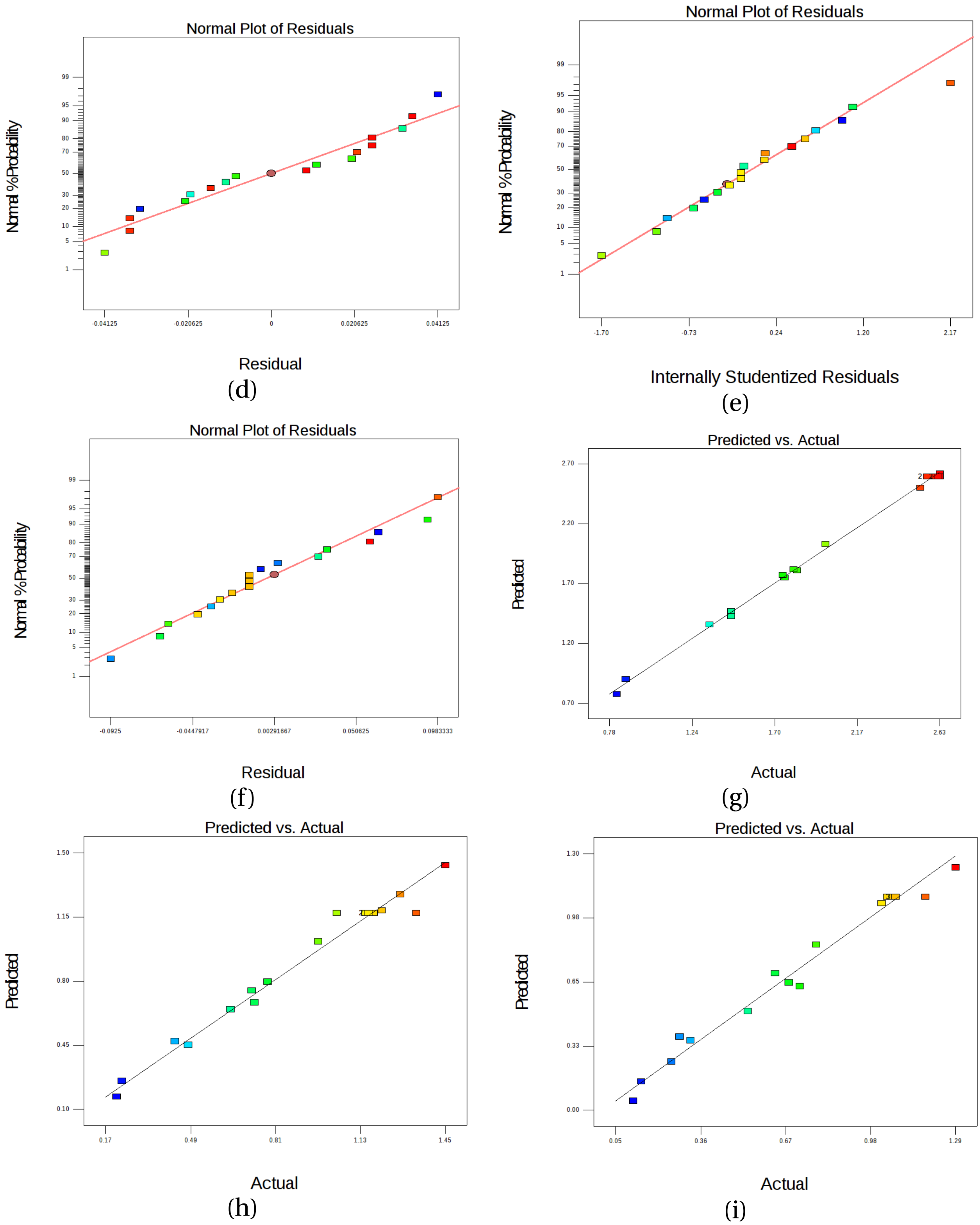

Figure 4: Normal probability and actual versus predicted plots for the three responses; (d) Normal probability plot for N, (e) Normal probability plot for P, (f) Normal probability plot for K, (g) Actual versus predicted for N, (h) Actual versus predicted for P and (i) Actual versus predicted for K

conditions.

4.4 Interpretation of the developed models

In the subsequent headings, whenever direct effect, interaction effect or a comparison between any two input parameters is being discussed and the third parameter would be on its central level. Response surface methodology was used to estimate the effect of three process variables on the quantity of N, P and K. Perturbation, contour and 3D surface plots were drawn by using RSM to investigate the effect of all the factors on the responses. The inferences so obtained are discussed below.

4.4.1 Effect of process parameters on nitrogen (N)

4.4.1.1 Direct effects

It is evident from Figure 5 that, the worm socking density has a positive effect on N. Thus, increasing in worm stocking density resulted an increase in the N content of the vermicompost. This behaviour is due to the fact that, as the density of the earthworms is very high, the material is highly stabilized (there is less unconverted material in the final product) and as a result the N content is high. While, in the case of the moisture content and

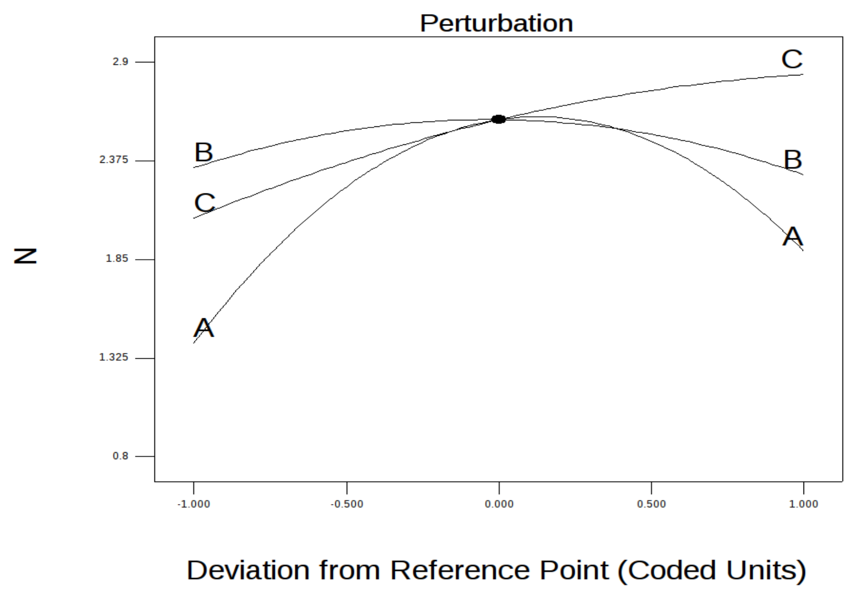

Figure 5: Perturbation plot showing the effect of all factors on N

C/N ratio the result demonstrate that increasing the moisture content and C/N ratio until it reaches its center value would result increasing in the quantity of N, the quantity of N then starts to drop as the two variables tend to increase above the center limit. Such behaviour could be attributed to the following reasons. As the C/N ratio get higher and higher, microbes and worms use all the N for their own metabolic needs and as the C/N ratio get lower and lower, they have surplus of N and it can be lost to the atmosphere as ammonia gas and cause odour problems. Similarly, low moisture content slow the vermicomposting process, by slow down the biochemical reactions and moisture content in excess means pore spaces in the compost pile are filled with water rather than air, leading to anaerobic conditions, so it affects the activity of earthworms and microbes.

Generally, as the results indicate neither too high nor too low moisture and C/N ratio is recommended for the vermicomposting process.

4.4.1.2 Interaction effects

The other benefit of perturbation plot is for selecting axes and constants in contour and 3D plots. From the above perturbation plot it can be noticed that the curve for B (C/N ratio) is almost constant and in this section only the interaction effect of A (moisture content) and C (worms stocking density) is plotted, while the plot of the other interaction effects for all responses is given at Appendix 5. The interaction effects of these variables on the percent nitrogen in vermicomposting process were studied by plotting 3D surface and counter curves against the two independent variables, while keeping constant the C/N ratio at its central value. The three dimensional surface and counter plots for N as a function of moisture content and worms stocking density are shown in Figure 6 and Figure 7 respectively using the design expert statistical software, 7.0.0 trial version. The response plots in Figures are part of a parabolic cylinder, exhibiting a minimum and

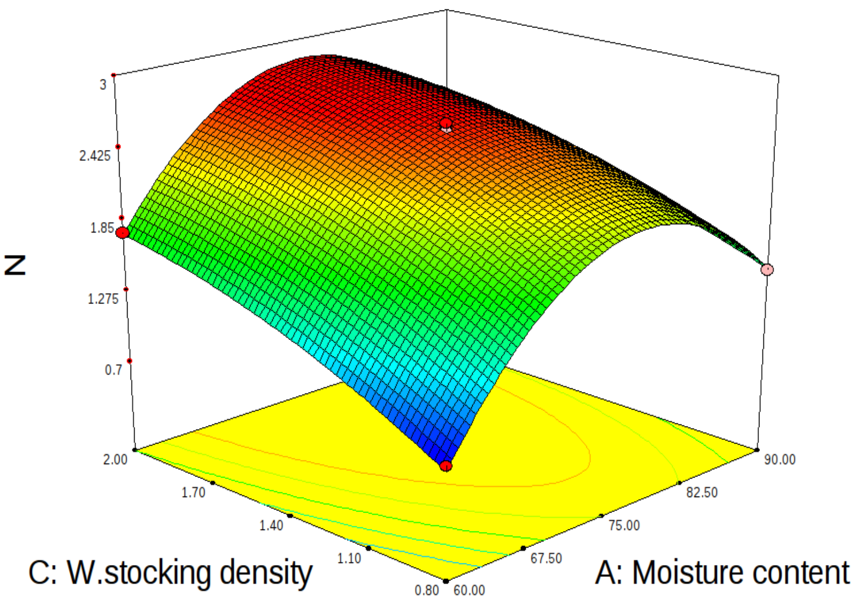

Figure 6: Response surface plot for the effect of moisture content and worm stocking density on N

maximum ridge, respectively in the investigated domain. It can be observed that percent nitrogen increased with increasing worms stocking density. From the figure it can also be noticed that at lower value of moisture content the value for N was also low and N was increased as the moisture content was increased, maximum nitrogen content is observed at moisture content 84.5% and N was decreased as the moisture content was further increased. Optimal level of moisture content is essentially required for vermicomposting (survival of warms and optimal biological activity) as well as for microbial flora present in the process.

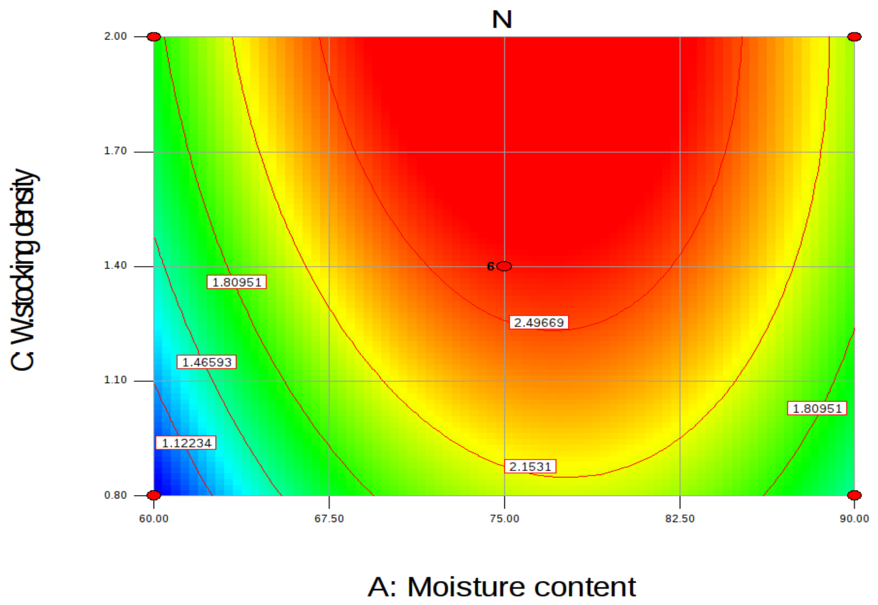

Figure 7: Contour plot showing the effect of moisture content and worm stocking density on N

4.4.2 Effect of process parameters on phosphorous (P)

4.4.2.1 Direct effects

The perturbation diagram for the quantity of P with respect to the three input process factors is shown in Figure 8 where the influence of a process variable around a specific point in the design range is illustrated by this perturbation plot. In this method the re-

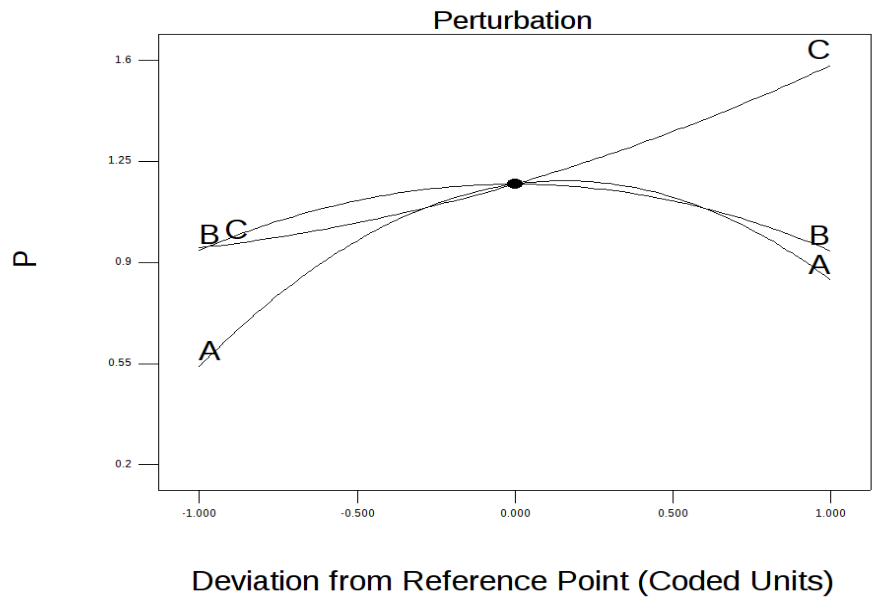

Figure 8: Perturbation plot showing the effect of all factors on P

sponse (the value of P) is plotted with respect to only one variable of the overall process, one at a time over its range considering the additional process variables as remaining constant at their center point. It can be observed from Figure 9, that initially at a lower value of the moisture content the quantity of P is low and as the moisture content is increased also the quantity of P is increased and decreased in P is observed as the moisture content is further increased. In a similar manner C/N ratio has similar effect as the moisture content has on P. In the case of worm stocking density, P is increased sharply as worm stocking density is increased. This phenomena is attributed to the fact that, as the number of earthworms is increased loss of organic matter is became very high and this results release of P minerals which was bound to the organic matter.

4.4.2.2 Interaction effects

Similarly, the curve for B (C/N ratio) is almost constant as for the response N. The 3D surface and counter graphs of P as a function of moisture content and worm stocking density are shown in Figure 9, and Figure 10 respectively, shows that the graphs are curvilinear profile as the empirical model developed is quadratic. It can be seen that increase

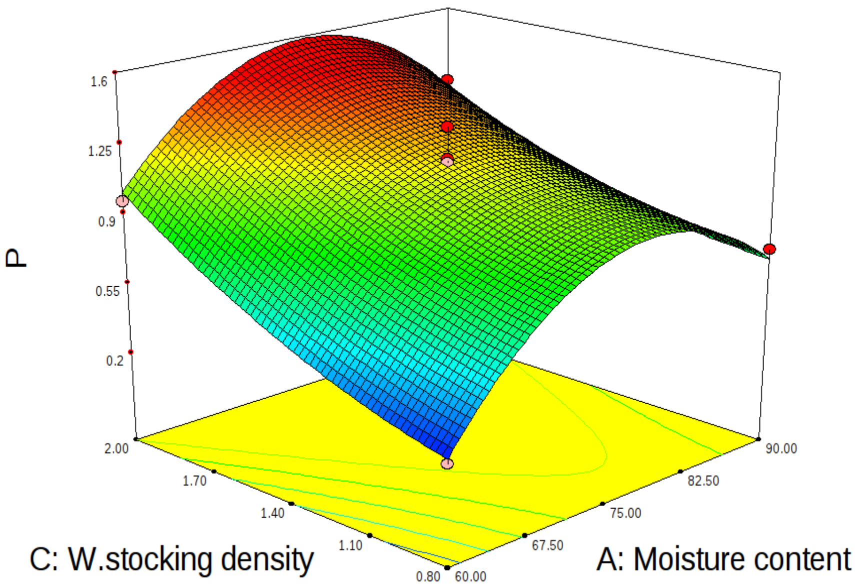

Figure 9: Response surface plot for the effect of moisture content and worm stocking density on P

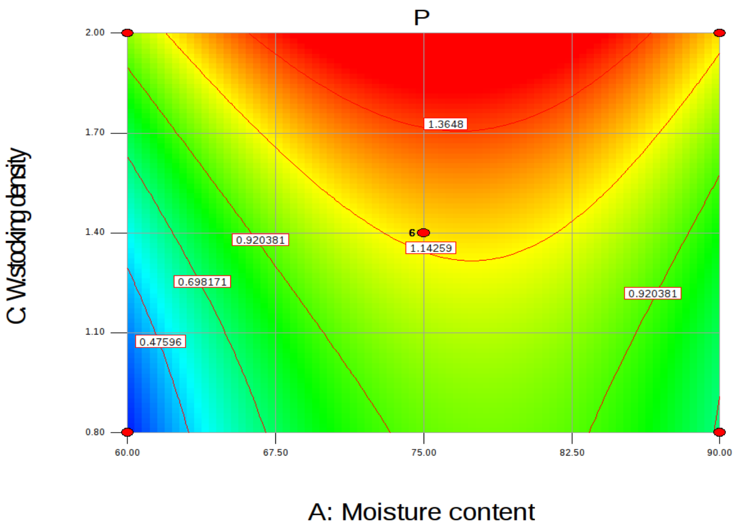

Figure 10: Contour plot showing the effect of moisture content and worm stocking density on P

in moisture content until 85% leads to a sharp increase in P, then P is decreased sharply as the moisture content is further increased. And it also shows that as the worm stocking density is increased, P is increased sharply.

4.4.3 Effect of process parameters on potassium (K)

4.4.3.1 Direct effects

Figure 11 depicts the effect of moisture content, C/N ratio and worms stocking density on the quantity of K during the vermicomposting process. From the perturbation plot it cab be deduced that, the value of of K was increased sharply with increasing worm stocking density. And also it can be deduced from the figure that, increasing the moisture content and C/N ratio until the central value results an increase in K and K decreases as the two process parameters are further increased from the central values.

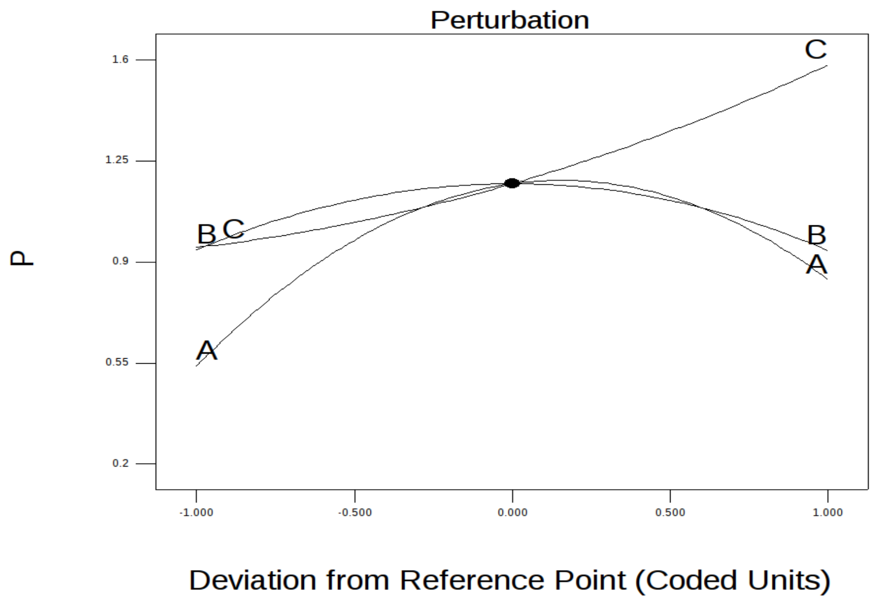

Figure 11: Perturbation plot showing the effect of all factors on K

4.4.3.2 Interaction effects

3D and Contour plot graphs showing predicted response of exchangeable K as a function of moisture content and worm stocking density is shown in Figure 12 and Figure 13 respectively. As moisture content increases until 84.5% at lower level of worm stocking density and as worm stocking density increases at low level of moisture content gives a positive effect on the quantity of exchangeable K. This phenomena may attributed due to the fact that as the moisture content is very low, biochemical reaction during the composting process and earthworm activity is very low and as the moisture content is very high, then aeration is very low then the bioreactor becomes anaerobic. Consequently, this affects the vermicomposting process and more unconverted materials are present in the vermicompost. On the other hand, as the density of the worms is very high, less unconverted material is present in the final product and also the product is rich in the major and minor nutrients.

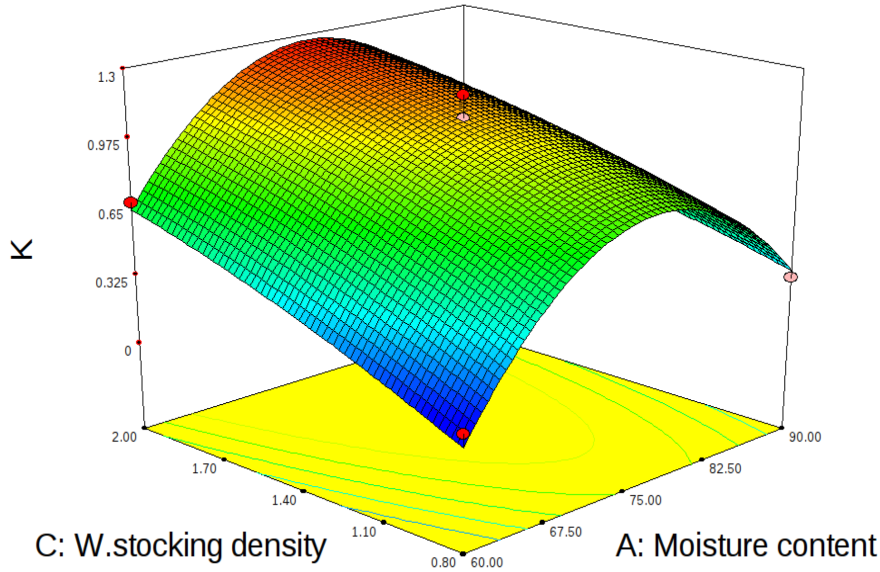

Figure 12: Response surface plot for the effect of moisture content and worm stocking density on K

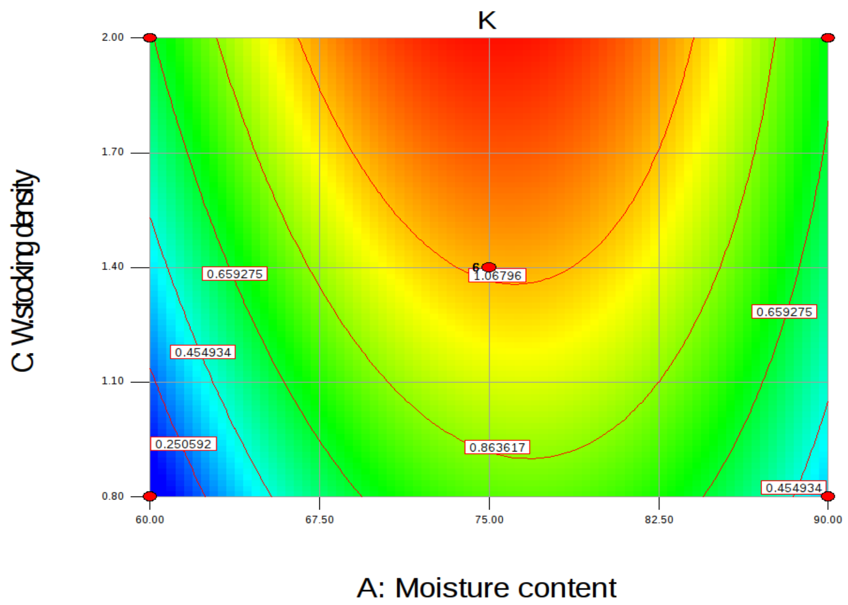

Figure 13: Contour plot showing the effect of moisture content and worm stocking density on K

4.5 Optimization

One of the primary objectives of the present study was to find the optimum process parameters for maximizing the quantity of N, P and K. The process variables such as moisture content, C/N ratio and worm stocking density have been optimized using Box-Behnken experimental design and their output values are executed using design-expert software 7.0. In optimizing the vermicomposting process, the moisture content, C/N ratio and worm stocking density are a set of process parameters held to be "in range" while the %N, %P and %K are set of responses that need to be "maximized". Table 10 shows the summary of factors/responses and goals and the corresponding set of specific objectives that will optimize the process condition. Table 10 exhibits the desired combinations of process parameters that would provide the highest responses. Numerical optimization

Table 10: Constraints applied for optimization

Factors/ Responses	**Goal**	**Lower Limit**	**Upper Limit**	**Lower Weight**	**Upper Weight**	**Importance**
Moisture content	In range	60	90	1	1	3
C/N ratio	In range	20	30	1	1	3
W. stocking density	In range	0.8	2	1	1	3
N	Maximize	0.82	2.63	1	1	3
P	Maximize	0.21	1.45	1	1	3
K	Maximize	0.11	1.29	1	1	3

was used to optimize any combination of one or more goals. The goals may be apply either factors or responses. The model capable of predicting the maximum N, P and K value showed that the optimum values of the process variables were a moisture content of 74.62%, a C/N ratio of 23.21 and a W. stocking density of 2 kg worm/m^2. Under these conditions, the predicted N, P and K were 2.82%, 1.58% and 1.28% respectively. Desirability function was used to identify the optimum levels of factors and to get maximum desirable responses. The optimized batch was selected with maximum combined desirability value i.e. 0.999 using Design Expert® version 7.0.0. The desirability histogram of the optimized solution for the vermicomposting condition is shown in Figure 14. The

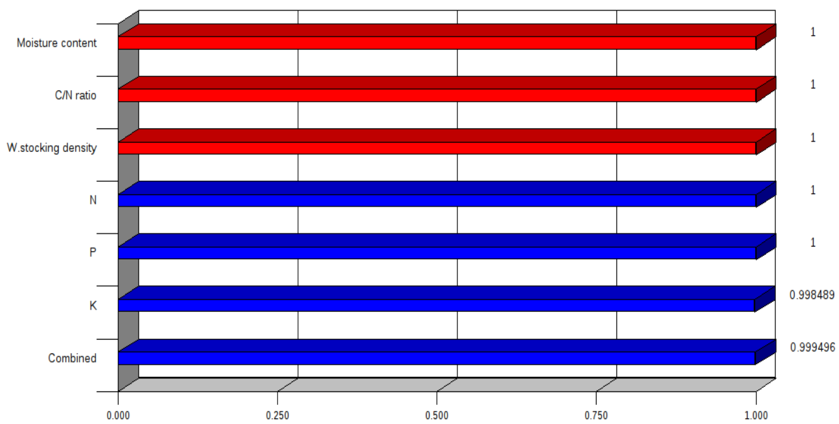

Figure 14: Desirability plot of optimization solution for the responses

histogram shows how well each variables/responses satisfied the criteria and values near one are good. The ramp plot of the optimized solution for the vermicomposting condition is shown in Figure 15. The ramp display combines the individual graphs for easier

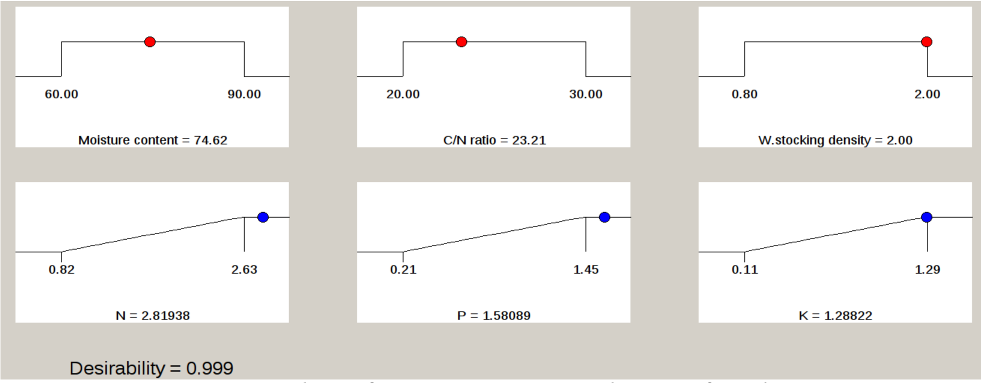

Figure 15: Ramp plot of optimization solution for the responses

interpretation. The dot on each ramp reflects the factor setting or response prediction for that solution.

The optimum value for C/N ratio obtained during this study was less than the optimum value obtained by the previous works, but this difference was not significant (it was consistent with the finding of these authors). The optimum value for moisture content found in the present study was consistent with Gunadi et al. [29], higher than the optimum value obtained by Palsania et al. [33] and Parthasarathi [35], but lower than the optimum value found by the other previous works. Similarly, the optimum worm stocking density found in this study was higher than found by Ndegwa et al. [48] and lower than that of found by Neuhauser et al. [50]. Some possible reasons for the differences may as follows:

- The type of substrate used, different organic wastes has different nutrient status and characteristics. The optimal C/N ratio, moisture content and worm stocking density depends on the type of the feed substrate and desired final product (stabilization of feed or earthworm production).
- The type of earthworm species used, each earthworm has it's own moisture content, C/N ratio, stocking density and other parameter preferences.
- Most of the optimization study studied previously are on the growth, survivability, activity and reproduction of the earthworm species not on the product stability, although the stability is indirectly affected by these factors. Literatures suggest low stocking density is good for worm reproduction and high stocking density is good for product stability.

4.6 Validation of the developed models

In order to validate the developed models, confirmation experiment was carried out with vermicomposting condition chosen from the optimization results, at 74.62% for moisture content, 23.21 for C/N ratio and 2 kg-worms/m^2 for worm stocking density. Table 11 summarizes the actual experimental values, the predicted values and the percentages of error. The validation results demonstrated that the models developed are quite accurate as the percentages of error in prediction were in a good agreement.

Table 11: Validation test results

Moisture content	**C/N ratio**	**W.stocking density**		**N**	**P**	**K**
74.62	23.21	2	Actual	2.71	1.53	1.22
74.62	23.21	2	Predicted	2.82	1.58	1.28
			Error%	4.06	3.27	4.92

4.7 Characterization of the vermicompost

Some physico-chemical characteristics of the vermicompost obtained from the optimum vermicomposting operating conditions is given in Table 12 below. Dark black colour

Table 12: Physicochemical characteristics of the vermicompost

Number	**Component**	**Quantity**
1	Colour	Dark Black
2	Odour	No foul odour
3	pH	8.34
4	EC(ds/m)	10.69
5	Moisture content(%)	24
6	VS (%)	56.03
7	Total organic carbon (%)	31.13
8	Total nitrogen (%)	2.71
9	Available Phosphorous (%)	1.53
10	C/N ratio	11.48
11	C/P ratio	20.34
12	Exchangeable Potassium (%)	1.22

of vermicompost indicated that the decomposition of municipal solid waste successfully. Absence of foul odour indicated that all parameters required for vermicomposting process were present in optimum condition. The high value for EC is due to loss of organic matter and release of different mineral salts in available forms such as phosphate, ammonium, potassium ions etc [82]. A C: N ratio of less than 20 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes [83].

The vermicasts obtained from the organic municipal solid waste contained N (2.71%), P (1.53%), and K (1.22%) as the major trace elements. The vermicasts obtained were richer in N compared to the typical nutrient analysis found in vermicasts i.e. N (1.5-2.5%), P (1.25-2.25%) and K (1-2%). The vermicompost is very rich in nitrogen, only vermicompost from municipal sewage sludge may contain more of it - up to 3.2% [84]. Additionally, the results showed that the amounts of major nutrients in the vermicompost are much higher than results obtained from previous studies. Sudhir et al [85] had analysed vermicompost made from solid waste and they found the nitrogen, phosphorus and potassium to be 1.02%, 0.13% and 0.27% respectively. Some possible reasons for the extremely high values of NPK found in the vermicompost from this study are as follows:

- The characteristics of the municipal solid waste. The initial characteristics of a municipal solid waste is vary with place to place as well as with economic status of the society. Waste obtained from low economic society is richer in nutrients than that of high economic society.

- The NPK value obtained during this study was obtained at the optimum vermicomposting process parameters.

4.8 Evaluation of the vermicompost as fertilizer

Vermicompost contains plant growth promoters with other nutrients and improves physical, chemical and biological properties of soil on repeated application [86]. Many important factors, such as the presence of beneficial microorganisms or biologically active plant growth influencing substances such as phytohormone are released by beneficial microorganisms present in the vermicompost rich soil. Root initiation, increased root biomass, enhanced plant growth and development and sometimes, alterations in plant morphology are among the most frequently claimed effects of vermicompost treatment [87]. A plastic pot set-up with soil was used to determine the effect and efficiency level of vermicompost on the yields of spinach. The study was conducted through effect of increasing concentration of vermicompost (0% (control), 50% and 100% w/w) in target plant growth. It is evident from Table 13 that, the spinach plant used in the stud-

Table 13: Number of leaves and leaf wet weight of spinach produced with different potting media

Treatments	**Number of leaves (No./plant)**	**Leaf wet weight (g/plant)**
T1	3	107.70
T2	5	197.77
T3	8	235.47

† T1=0% VC + 100% soil, T2=50% VC+50% soil and T3=100% VC + 0% soil

ies showed good enhancement of growth in terms of number of leaves as well as leaf wet weight as compared to the control. The number of leaves produced in 8 weeks of experiment for the spinach was 3, 5 and 8 No. of leaves/plant for T1, T2 and T3, respectively. From this it can be observed that, the number of leaves produced using 100% VC was 2.67 times greater than the number of leaves produced using 0% VC and 1.6 times greater than produced using 50% VC. This indicated that vermicompost prepared from municipal solid waste had enhanced the plant growth. The average total leaf wet weight of T1, T2 and T3 were 107.70, 197.77 and 235.47 g/plant respectively. This indicated that the leaf wet weight of the spinach produced using 100% VC was 2.2 times greater than that of produced using the control (soil) and 1.2 times greater than produced using 50% VC. Generally, the study showed that the yield of the plant had positively affected by the application of municipal solid waste based vermicompost and increasing the dose of VC increases the yield of the spinach vegetable plant, which indicates that the performance of the vermicompost was good.

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The results of the present study indicated that municipal solid waste admixed with horse manure and after partial decomposition of the waste materials, it works as an excellent palatable raw material for vermicomposting using *Eisenia fetida* earth worm. Optimization of vermicomposting process parameters has been carried out in order to attain maximum amount of N, P and K. The process parameters moisture content, C/N ratio and worm stocking density were selected and optimized to produce high quality vermicompost. Design Expert software was used to develop design of experiment and analyse the results. Box Behnken design in Response surface method was used to optimize the process condition. In summary, the following conclusions can be drawn:

- For this particular study the quadratic model was the best fit model for all responses as the p-value of this model was smaller than the other models and had the highest p-value for Lack of Fit Tests.
- Among the three variables studied, ANOVA showed that, earthworm stocking density is found to be the most important parameter influencing the vermicomposting process of municipal solid waste followed by moisture content, while C/N ratio has the least effect relative to the two factors.
- The N, P and K values of the vermicompost increased with increase of worm stocking density and moisture content and C/N ratio until the central value.
- The N, P and K value of the vermicompost decreased with an increase of moisture content and C/N ratio further from the central value.
- The predicted value of the responses matches the experimental values reasonably well, with high value of coefficient of determination ($\mathbf{R^2}$) and thus ensuring the satisfactory fit for the regression model.
- Maximum values for N, P and K content of the vermicompost 2.82%, 1.58% and 1.28% respectively were achieved under the optimum conditions; moisture content of 74.62%, C/N ratio of 23.21 and worm stocking density of 2 $\mathbf{kgworm/m^2}$ with high value of combined desirability, i.e. 0.999.
- The variation in percentage error for the responses is 4.06%, 3.27% and 4.92%, which shows that the models developed were accurate.
- The produced vermicompost was found to be better in desired level of composition of macro-nutrients i.e., N, P and K.
- The yield of spinach plant had positively affected by the application of municipal solid waste based vermicompost and increasing the dose of VC increases the yield

of the spinach vegetable plant, which indicates that the performance of the vermicompost was good.

5.2 Recommendation

The present study has enabled to confirm that MSW can be used as a resource to obtain bio-fertilizer, which could contribute to reduce the highly rising quantity of wastes and reduces the associated environmental pollutions. However, during the present study, there had been some constraints or limitations. So it is strongly advice the following recommended tasks should be considered to strengthen the paper. These are;

- The statistical analysis based only on limited macro-nutrients of vermicompost therefore, additional nutrients and same physical properties should be investigated.
- Further optimization study has to be done by incorporating other vermicomposting process parameters like temperature, particle size and pH.

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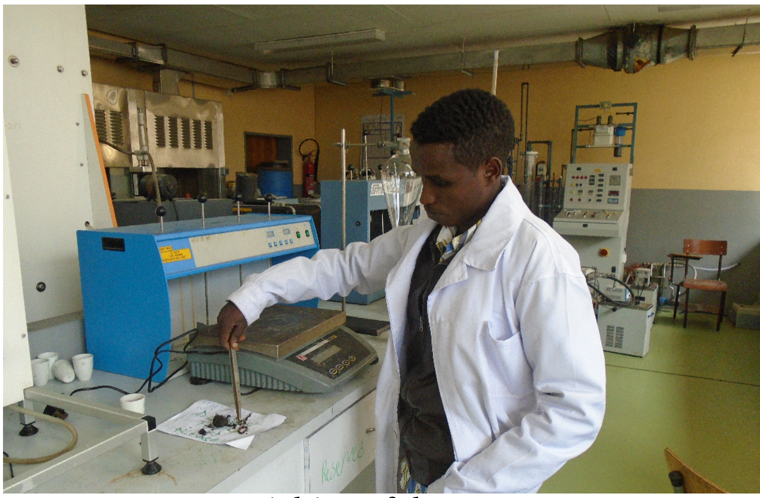
APPENDICES

Appendix 1: Vital pictures during the research work

E. fetida breeding

An adult *E. fetida*



Weighing of the worm

Vermireactors used in the study

Kirkos composting site, AAEPA

Sorted organic MSW

Pot study using MSW based Vermicompost

Appendix 2: Laboratory procedures

- **Estimation of pH**

  1. Make 25 g of compost into a suspension in 125 ml of distilled water and shake on a rotary shaker for 2 hours.
  2. Filter through Whatman No. 1 or equivalent filter paper under vacuum using a Buchner funnel.
  3. Determine pH of the filtrate by pH meter.

- **Estimation of conductivity**
  Apparatus

  - Analytical balance
  - Oven
  - Conductivity meter
  - 1000 and 500 ml volumetric flasks
  - 250 ml beakers
  - Desiccator
  - Thermometer

  Method

  - Pass fresh sample of organic fertilizer through a 1-2mm sieve
  - Take 20 gm of the sample and add 100 ml of distilled water to it to give a ratio of 1:5
  - Stir for about an hour at regular intervals.
  - Calibrate the conductivity meter by using 0.01 M potassium chloride solution
  - Measure the conductivity of the unfiltered organic fertilizer suspension

  Calculation
  Express the results as millimho's or ds/m at 25^oC specifying the dilution of the organic fertilizer suspension viz, 1:5 organic fertilizer suspensions.

- **Estimation of moisture content**
  Procedure

  1. Weigh to the nearest mg about 5 gm of the prepared sample in a weighed clean, dry crucible.

2. Heat in an oven for about 24 hours at 105^oC to constant weight.
3. Cool in a desiccator and weigh.
4. Report percentage loss in weight as moisture content.

Calculation:

$$\mathbf{MC}(\%) = \frac{\mathbf{100 \times (B - C)}}{\mathbf{B - A}} \tag{8}$$

Where;

A = Weight of the crucible
B = Weight of the crucible plus material before drying
C = Weight of the crucible plus material after drying

- **Estimation of organic carbon**
  Apparatus

i. Silica/Platinum crucible 25 g cap.

ii. Furnace

Procedure

1. Accurately weigh 10 gm of sample dried in oven at 105^oC for 24 hrs, in a pre weighed crucible and ignite the material in a furnace at 650 -700^oC for 6-8 hrs
2. Cool to room temperature and keep in Desiccator for 12 hrs
3. Weigh the contents with crucible

Calculation:
Calculate the total organic carbon by the following formula;

$$\mathbf{TOM}(\%) = \frac{\mathbf{Initial_{wt} - final_{wt}}}{\mathbf{sample_{wt}}} \times \mathbf{100} \tag{9}$$

$$\mathbf{TOC}(\%) = \frac{\mathbf{TOM}}{\mathbf{1.8}} \tag{10}$$

- **Estimation of total nitrogen**
  Apparatus

  – Digestion block

- Semi-micro Kjeldahl distillation unit
- Burettes
- Pipettes
- Erlenmeyer flasks-250 ml
- Magnetic stirrer
- Kjeldahl flasks-300 ml

Reagents

- Concentrated sulphuric acid-96% H_2SO_4, N-free
- Catalyst mixture: Mix by grinding in a mortar 100 parts Na_2SO_4 or K_2SO_4 with 10 parts of copper sulphate ($CuSO_4.5H_2O$) and 1 part selenium powder; mixed thoroughly.
- Sodium hydroxide, 40%: Dissolve 400 g NaOH in about 800 ml of distilled water in a 1 l volumetric flask.
- Boric acid solution, 2%: Dissolve 20 g of boric acid in 600 ml of distilled water in a 1 l volumetric flask and make to volume with distilled water.
- Mixed indicator: Dissolve 0.5 g bromocresol green + 0.1 g methyl red in 100 ml of 95% ethanol.
- Sulphuric acid solution 0.1 N: Pipette 2.82 ml of concentration H_2SO_4 (96%) in a 1 l volumetric flask and make to volume with distilled water.
- Oxalic acid, 0.05 M: Dissolve 6.3035 g $HOOCOOH.2H_2O$ in distilled water in a 1 l volumetric flask and make to volume with distilled water.
- Phenolphthalein indicator, 0.1%: Dissolve 100 mg phenolphthalein in 100 ml 95% ethanol.
- Sodium hydroxide, 0.1 N solution: Dissolve 4 g of NaOH in distilled water.

Procedure

1. Accurately weigh 0.5 g compost sample ($\leq$ 0.5 mm sieve) and transfer into a digestion tube.
2. Add 2 g (1/2 spoon) of catalyst mixture and few carborundum boiling stones, mix well and rinse with a little water just enough to moisten the mixture.
3. add 7 ml of concentration H_2SO_4 and mix by swirling.
4. Place the digestion tube stand with the samples beside the block digester and fit the exhaust manifold on top of it.

5. Place the tubes with rack and exhaust manifold on the digestion block, pre-heated in the fume-hood.
6. Digest for 3 hours or until the digest is white or pale yellow on a block digester pre-heated to 300 ^{o}C.
7. Allow to cool, and cautiously add 50 ml of distilled water, and then cool again.
8. Transfer the acid digest quantitatively to the macro-kjeldahl flasks and rinse using distilled water.
9. Measure 20 ml boric acid solution from a dispenser into a receiver Erlenmeyer flask corresponding to the number of samples. Add to it 2 drops of indicator solution and place under the condenser. Take care that the end of the condenser is immersed in the boric acid solution to prevent any loss of ammonia.

Calculation

$$\mathbf{TKN}(\%) = \frac{\mathbf{(a-b)}}{\mathbf{s}} \times \mathbf{N} \times \mathbf{0.014} \times \mathbf{100} \times \mathbf{mcf} \tag{11}$$

Where;

a= ml of $\mathbf{H_2SO_4}$ required for titration of sample
b= ml of $\mathbf{H_2SO_4}$ required for titration of blank
s= air dry sample weight in grams
N= Normality of $\mathbf{H_2SO_4}$ (0.1 N)
100= ml of the solution
0.014= meq weight of nitrogen in g
mcf= moisture correction factor

- **Estimation of C/N Ratio**
  Method
  Calculate the C/N ratio by dividing the organic carbon value with the total nitrogen value.

- **Estimation of available phosphorous**
  Apparatus

  – Spectrophotometer suitable for measurement at 880 nm
  – Polythene shaking bottles 250 ml
  – Reciprocating shaking machine
  – Analytical balance
  – Funnel racks

- Funnel
- Whatman No. 42 filter paper (or equivalent)
- Volumetric flasks and pipettes as required for preparation of reagents, standard solutions anf color development.

Reagents

- Sodium bicarbonate solution, 0.5 M, pH 8.5 (extracting solution): Dissolve 42 g $NaHCO_3$ in water and make to 1 l. Adjust the pH to 8.5 by adding NaOH 1 M (4 g/100 ml). In case of overshooting of pH above 8.5, add some $NaHCO_3$ 0.5 M.
- Sulphuric acid, 4 M: Slowly add 56 ml concentrated H_2SO_4 (96%) to about 150 ml distilled water in a graduated beaker under constant stirring. After cooling make to 250 ml with distilled water.
- Ammonium molybdate solution, 4%: Dissolve 4 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ in water and make to 100 ml. Store in polythene or pyrex bottle.
- Potassium antimony tartrate solution, 0.275%: Dissolve 0.275 g $KSbOC_4H_4O_6$ in water and make to 100 ml.
- Ascorbic acid solution, 1.75%: Dissolve 1.75% ascorbic acid in water and make to 100 ml.
- Mixed reagent: Successively add with a measuring cylinder to a 500 ml polythene or pyrex bottle and homogenize after addition of each of the following:
    - 50 ml of 4 M H_2SO_4
    - 15 ml of NH_4-molybdate solution
    - 30 ml of ascorbic acid solution
    - 200 ml of water
    - 5 ml potassium antimony tartrate solution

Procedure

1. Weigh 5 g of $\leq$ mm compost into a 250 ml polythene shaking be include two blanks and a reference sample.
2. Shake for 30 minutes on a mechanical shaker
3. Filter through a Whatman No. 42 filter paper
4. If filtrate is not clear, add 1 spoon P-free charcoal, shake again and filter
5. Pipette in (short) test tubes 3 ml of the standard series, the blanks and the sample extract

6. Slowly add 3 ml of the mixed reagents by pipette and swirl (CO_2 evolution)
7. Allow the solutions to stand for at least 1 hour for the blue color to develop to maximum
8. Measure absorbence on spectrophotometer at 882 or 720 nm

Calculation

$$\mathbf{P(ppm) = (a - b) \times \frac{100}{s} \times mcf} \tag{12}$$

Where;

a= mg/l of P in sample extract
b= mg/l of P in blank
s= sample weight in gram (5 g)
100= ml of extracting solution
mcf= moisture correction factor

- **Estimation of C/P Ratio**
  Method
  Calculate the C/P ratio by dividing the organic carbon value with the available phosphorous value.

- **Exchangeable potassium**
  Flame-photometer method
  Total Potassium are usually determined by dry ashing at 650-700 Degree Centigrade and dissolving in concentrated hydrochloric acid.
  Reagent and Standard curve

  – Potassium chloride standard solution: Make a stock solution of 1000 ppm K by dissolving 1.909 g. of AR grade potassium chloride (dried at 60^oC for 1 h) in distilled water 1 ; and diluting up to 1 litre. Prepare 100 ppm standard by diluting 100 ml of 1000 ppm stock solution to 1 litre with extracting solution.
  – Standard curve: Pipette 0,5, 10,15 and 20 ml of 100 ppm solution into 100 ml volumetric flasks and make up the volume upto the mark. The solution contain 0,5, 15 & 20 ppm K respectively.

  Procedure

  1. Take 5g sample in a porcelain crucible and ignite the material to ash at 650-700 ^oC in a furnace.

2. Cool it and dissolve in 5 ml concentrated hydrochloric acid, transfer in a 250 ml beaker with several washing of distilled water and heat it.
3. Again transfer it to a 100 ml volumetric flask and make up the volume.
4. Filter the solution and dilute the filtrate with distilled water so that the concentration of K in the working solution remains in the range of 0 to 20 ppm, if required.
5. Determine K by flame photometer using the K- filter after necessary setting and calibration of the instrument.
6. Read similarly the different concentration of K of the standard solution in flame-photometer and prepare the standard curve by plotting the reading against the different concentration of the K.

Calculation

$$\mathbf{K}(\%) = \mathbf{R} \times \mathbf{20} \times \mathbf{dilute} \tag{13}$$

Where;

R= ppm of K in the sample solution (obtained by extra plotting from standard curve).

Appendix 3: Model fit summary for the three responses (N, P and K)

- **Response 1:** N
  Transform: None
  ⋆⋆⋆ WARNING: The Cubic Model is Aliased!⋆⋆⋆

Table 14: Model fitting summary for N

Sequential model SS					
Source	**SS**	**Df**	**MS**	**F-value**	**P-value**
Mean	71.4	1	71.24	-	-
Linear	1.65	3	0.55	1.53	0.2498
2FI	0.23	370.075	0.17	0.9127	
Quadratic	4.78	3	1.59	997.97	< 0.0001
Cubic	7.625×10^{-3}	3	2.542×10^{-3}	2.47	0.1771
Residual	5.15×10^{-3}	5	1.03×10^{-3}	-	-
Total	77.91	18	4.33	-	-
Lack of fit tests					
Linear	5.01	9	0.56	540.9	< 0.0001
2FI	4.79	6	0.8	774.84	< 0.0001
Quadratic	7.625×10^{-3}	3	2.542×10^{-3}	2.47	0.1771
Cubic	0.000	0	-	-	-
Pure error	5.15×10^{-3}	5	1.03×10^{-3}	-	-
Model summary statistics					
Source	**Std.Dev.**	R^2	**Adj.** R^2	**Pred.** R^2	**Press**
Linear	0.60	0.2472	0.0859	-0.2459	8.31
2FI	0.66	0.2811	-0.1111	-1.3359	15.58
Quadratic	0.04	0.9981	0.9959	0.9806	0.13
Cubic	0.032	0.9992	0.9974	-	-

A model with highest order polynomial but not aliased, insignificant lack of fit, low press and high Adj. $\mathbf{R^2}$ and Pred. $\mathbf{R^2}$ is a good model.

- **Response 1:** P

  Transform: None

  ⋆⋆⋆ WARNING: The Cubic Model is Aliased!⋆⋆⋆

Table 15: Model fitting summary for P

Sequential model SS					
Source	**SS**	**Df**	**MS**	**F-value**	**P-value**
Mean	14.51	1	14.51	-	-
Linear	0.98	3	0.33	3.00	0.0661
2FI	0.12	3	0.040	0.31	0.8165
Quadratic	1.35	3	0.45	62.29	< 0.0001
Cubic	0.011	3	3.708×10^{-3}	0.40	0.7602
Residual	0.046	5	9.297×10^{-3}	-	-
Total	17.01	18	0.95	-	-
Lack of fit tests					
Linear	1.48	9	0.16	17.64	0.0028
2FI	1.36	6	0.23	24.32	0.0015
Quadratic	0.011	3	3.708×10^{-3}	0.40	0.7602
Cubic	0.000	0	-	-	-
Pure error	0.046	5	9.297×10^{-3}	-	-
Model summary statistics					
Source	**Std.Dev.**	R^2	**Adj.** R^2	**Pred.** R^2	**Press**
Linear	0.33	0.3916	0.2613	-0.0725	2.68
2FI	0.36	0.4393	0.1335	-0.0565	5.15
Quadratic	0.085	0.9770	0.9511	0.9021	0.24
Cubic	0.096	0.9814	0.9369	-	-

A model with highest order polynomial but not aliased, insignificant lack of fit, low press and high Adj. $\mathbf{R^2}$ and Pred. $\mathbf{R^2}$ is a good model.

- **Response 1:** K

  Transform: None

  ⋆ ⋆ ⋆ WARNING: The Cubic Model is Aliased!⋆ ⋆ ⋆

Table 16: Model fitting summary for K

Sequential model SS					
Source	**SS**	**Df**	**MS**	**F-value**	**P-value**
Mean	9.74	1	9.74	-	-
Linear	0.52	3	0.17	1.19	0.3486
2FI	0.18	3	0.059	0.35	0.7882
Quadratic	1.80	3	0.60	99.01	$<$0.0001
Cubic	0.036	3	0.012	4.90	0.0598
Residual	0.012	5	2.457×10^{-3}	-	-
Total	12.28	18	0.68	-	-
Lack of fit tests					
Linear	2.01	9	0.22	90.95	$<$0.0001
2FI	1.83	6	0.31	124.38	<0.0001
Quadratic	0.036	3	0.012	4.90	0.0598
Cubic	0.000	0	-	-	-
Pure error	0.012	5	2.457×10^{-3}	-	-
Model summary statistics					
Source	**Std.Dev.**	R^2	**Adj.** R^2	**Pred.** R^2	**Press**
Linear	0.38	0.2035	0.0328	-0.3775	3.50
2FI	0.41	0.2734	-0.1230	-1.5443	6.46
Quadratic	0.078	0.9809	0.9595	0.7655	0.60
Cubic	0.050	0.9952	0.9836	-	-

A model with highest order polynomial but not aliased, insignificant lack of fit, low press and high Adj. $\mathbf{R^2}$ and Pred. $\mathbf{R^2}$ is a good model.

Appendix 4: ANOVA for the three responses (N, P and K)

- **Response 1 :** N
  ANOVA for Response Surface Quadratic Model
  Analysis of variance table [Partial sum of squares - Type III]

Source	**Sum of squares**	**Df**	**Mean square**	**F-value**	**P-value**
Model	6.66	9	0.74	463.07	$<$0.0001
A	0.48	1	0.48	297.65	$<$0.0001
B	2.813×10^{-3}	1	2.813×10^{-3}	1.76	0.2211
C	1.17	1	1.17	732.96	$<$0.0001
AB	0.18	1	0.18	110.47	$<$0.0001
AC	0.042	1	0.042	26.32	0.0009
BC	7.225×10^{-3}	1	7.225×10^{-3}	4.52	0.0661
A^2	3.90	1	3.90	2440.29	$<$0.0001
B^2	0.33	1	0.33	206.65	$<$0.0001
C^2	0.089	1	0.089	55.49	$<$0.0001
Residual	0.013	8	1.597×10^{-3}		
Lack of fit	7.625×10^{-3}	3	1.03×10^{-3}	2.47	0.1771
Pure error	5.15×10^{-3}	5	1.03×10^{-3}		
Cor Total	6.67	17			

The Model F-value of 463.07 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, AB, AC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Lack of Fit F-value" of 2.47 implies the Lack of Fit is not significant relative to the pure error. There is a 17.71% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good – we want the model to fit.

Std.Dev.	0.04	R-Squared	0.9981
Mean	1.99	Adj R-Squared	0.9959
C.V%	2.01	Pred R-Squared	0.9806
PRESS	0.13	Adeq Precisi	61.860

The "Pred R-Squared" of 0.9806 is in reasonable agreement with the "Adj R-Squared" of 0.9959. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 61.860 indicates an adequate signal. This model can be used to navigate the design space.

Factor	Coefficient Estimate	df	Standard Error	95%CI Low	95%CI High	VIF
Intercept	2.6	1	0.016	2.56	2.63	
A-Moisture content	0.24	1	0.014	0.21	0.28	1
B-C/N ratio	-0.019	1	0.014	-0.051	0.014	1
C-W.stocking density	0.38	1	0.014	0.35	0.42	1
AB	0.21	1	0.020	0.16	0.26	1
AC	-0.10	1	0.020	-0.15	-0.056	1
BC	-0.042	1	0.020	-0.089	3.57×10^{-3}	1
A^2	-0.95	1	0.019	-0.99	-0.90	1.02
B^2	-0.27	1	0.019	-0.32	-0.23	1.02
C^2	-0.14	1	0.019	-0.19	-0.098	1.02

Final Equation in Terms of Coded Factors:

```
N  =
+2.60
+0.24  * A
-0.019  * B
+0.38  * C
+0.21  * A * B
-0.10  * A * C
-0.042  * B * C
-0.95  * A^2
-0.27  * B^2
-0.14  * C^2
```

Final Equation in Terms of Actual Factors:

```
N  =
-27.14000
+0.59219  * Moisture content
+0.35608  * C/N ratio
+2.95417  * W.stocking density
+2.80000E-003  * Moisture content * C/N ratio
-0.011389  * Moisture content * W.stocking density
-0.014167  * C/N ratio * W.stocking density
-4.20000E-003  * Moisture content^2
-0.011000  * C/N ratio^2
-0.39583  * W.stocking density^2
```

- **Response 2: P**
  ANOVA for Response Surface Quadratic Model
  Analysis of variance table [Partial sum of squares - Type III]

Source	**Sum of squares**	**Df**	**Mean square**	**F-value**	**P-value**
Model	2.45	9	0.27	37.73	$<$0.0001
A	0.18	1	0.18	25.00	0.0011
B	1.25×10^{-5}	1	1.25×10^{-5}	1.736×10^{-3}	0.7230
C	0.80	1	0.80	111.11	$<$0.0001
AB	0.078	1	0.078	10.89	0.0109
AC	0.017	1	0.017	2.35	0.1641
BC	0.024	1	0.024	3.34	0.1052
A^2	1.01	1	1.01	140.83	$<$0.0001
B^2	0.23	1	0.23	31.94	0.0005
C^2	0.040	1	0.040	5.52	0.0468
Residual	0.058	8	7.201×10^{-3}		
Lack of fit	0.011	3	3.708×10^{-3}	0.40	0.7602
Pure error	0.046	5	9.297×10^{-3}		
Cor Total	2.50	17			

The Model F-value of 37.73 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, AB, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Lack of Fit F-value" of 0.40 implies the Lack of Fit is not significant relative to the pure error. There is a 76.02% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good – we want the model to fit.

Std.Dev.	0.085	R-Squared	0.9770
Mean	0.90	Adj R-Squared	0.9511
C.V%	9.45	Pred R-Squared	0.9021
PRESS	0.24	Adeq Precisi	19.980

The "Pred R-Squared" of 0.9021 is in reasonable agreement with the "Adj R-Squared" of 0.9511. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 19.980 indicates an adequate signal. This model can be used to navigate the design space.

Factor	Coefficient Estimate	df	Standard Error	95%CI Low	95%CI High	VIF
Intercept	1.17	1	0.035	1.09	1.25	
A-Moisture content	0.15	1	0.081	0.22	0.26	1
B-C/N ratio	-1.25×10^{-3}	1	0.030	-0.070	0.068	1
C-W.stocking density	0.32	1	0.030	0.25	0.39	1
AB	0.14	1	0.042	0.042	0.24	1
AC	-0.065	1	0.042	-0.16	0.033	1
BC	-0.077	1	0.042	-0.18	0.020	1
A^2	-0.48	1	0.041	-0.58	-0.39	1.02
B^2	-0.23	1	0.041	-0.32	-0.14	1.02
C^2	0.095	1	0.041	1.74×10^{-3}	0.19	1.02

Final Equation in Terms of Coded Factors:

```
P  =
+1.17
+0.15  * A
-1.250E-003  * B
+0.32  * C
+0.14  * A * B
-0.065  * A * C
-0.077  * B * C
-0.48  * A^2
-0.23  * B^2
+0.095  * C^2
```

Final Equation in Terms of Actual Factors:

```
P  =
-15.74468
+0.29483  * Moisture content
+0.35508  * C/N ratio
+0.97245  * W.stocking density
+1.86667E-003  * Moisture content * C/N ratio
-7.22222E-003  * Moisture content * W.stocking density
-0.025833  * C/N ratio * W.stocking density
-2.14259E-003  * Moisture content^2
-9.18333E-003  * C/N ratio^2
+0.26505  * W.stocking density^2
```

- **Response 3: K**
  ANOVA for Response Surface Quadratic Model
  Analysis of variance table [Partial sum of squares - Type III]

Source	**Sum of squares**	**Df**	**Mean square**	**F-value**	**P-value**
Model	2.49	9	0.28	45.75	<0.0001
A	0.063	1	0.063	10.41	0.0121
B	0.012	1	0.012	1.99	0.1965
C	0.44	1	0.44	73.01	<0.0001
AB	0.093	1	0.093	15.37	0.0044
AC	0.017	1	0.017	2.79	0.1332
BC	0.068	1	0.068	11.17	0.0102
A^2	1.58	1	1.58	261.42	<0.0001
B^2	0.076	1	0.076	12.58	0.0075
C^2	8.673×10^{-3}	1	8.673×10^{-3}	1.43	0.2655
Residual	0.048	8	6.051×10^{-3}		
Lack of fit	0.036	3	0.012	4.90	0.0598
Pure error	0.012	5	2.457×10^{-3}		
Cor Total	2.54	17			

The Model F-value of 45.75 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, AB, BC, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Lack of Fit F-value" of 4.90 implies the Lack of Fit is not significant relative to the pure error. The "Lack of Fit F-value" of 4.90 implies there is a 5.98% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good – we want the model to fit.

Std.Dev.	0.078	R-Squared	0.9809
Mean	0.74	Adj R-Squared	0.9595
C.V%	10.58	Pred R-Squared	0.7655
PRESS	0.60	Adeq Precisi	20.438

The "Pred R-Squared" of 0.7655 is in reasonable agreement with the "Adj R-Squared" of 0.9595. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 20.438 indicates an adequate signal. This model can be used to navigate the design space.

Factor	Coefficient Estimate	df	Standard Error	95%CI Low	95%CI High	VIF
Intercept	1.08	1	0.032	1.01	1.15	
A-Moisture content	0.089	1	0.028	0.025	0.15	1
B-C/N ratio	0.039	1	0.028	-0.025	0.10	1
C-W.stocking density	0.24	1	0.028	0.17	0.30	1
AB	0.15	1	0.039	0.063	0.24	1
AC	-0.065	1	0.039	-0.15	0.025	1
BC	-0.13	1	0.039	-0.22	-0.040	1
A^2	-0.60	1	0.037	-0.69	-0.52	1.02
B^2	-0.13	1	0.037	-0.22	-0.046	1.02
C^2	-0.045	1	0.037	-0.13	0.041	1.02

Final Equation in Terms of Coded Factors:

```
K  =
+1.08
+0.089  * A
+0.039  * B
+0.24  * C
+0.15  * A * B
-0.065  * A * C
-0.13  * B * C
-0.60  * A^2
-0.13  * B^2
-0.045  * C^2
```

Final Equation in Terms of Actual Factors:

```
K  =
-17.16356
+0.36658  * Moisture content
+0.18008  * C/N ratio
+2.36343  * W.stocking density
+2.03333E-003  * Moisture content * C/N ratio
-7.22222E-003  * Moisture content * W.stocking density
-0.043333  * C/N ratio * W.stocking density
-2.67593E-003  * Moisture content^2
-5.28333E-003  * C/N ratio^2
-0.12384  * W.stocking density^2
```

Appendix 5: 3D and contour plots for the three responses (N, P and K)

1. **3D and contour plots for the response N**

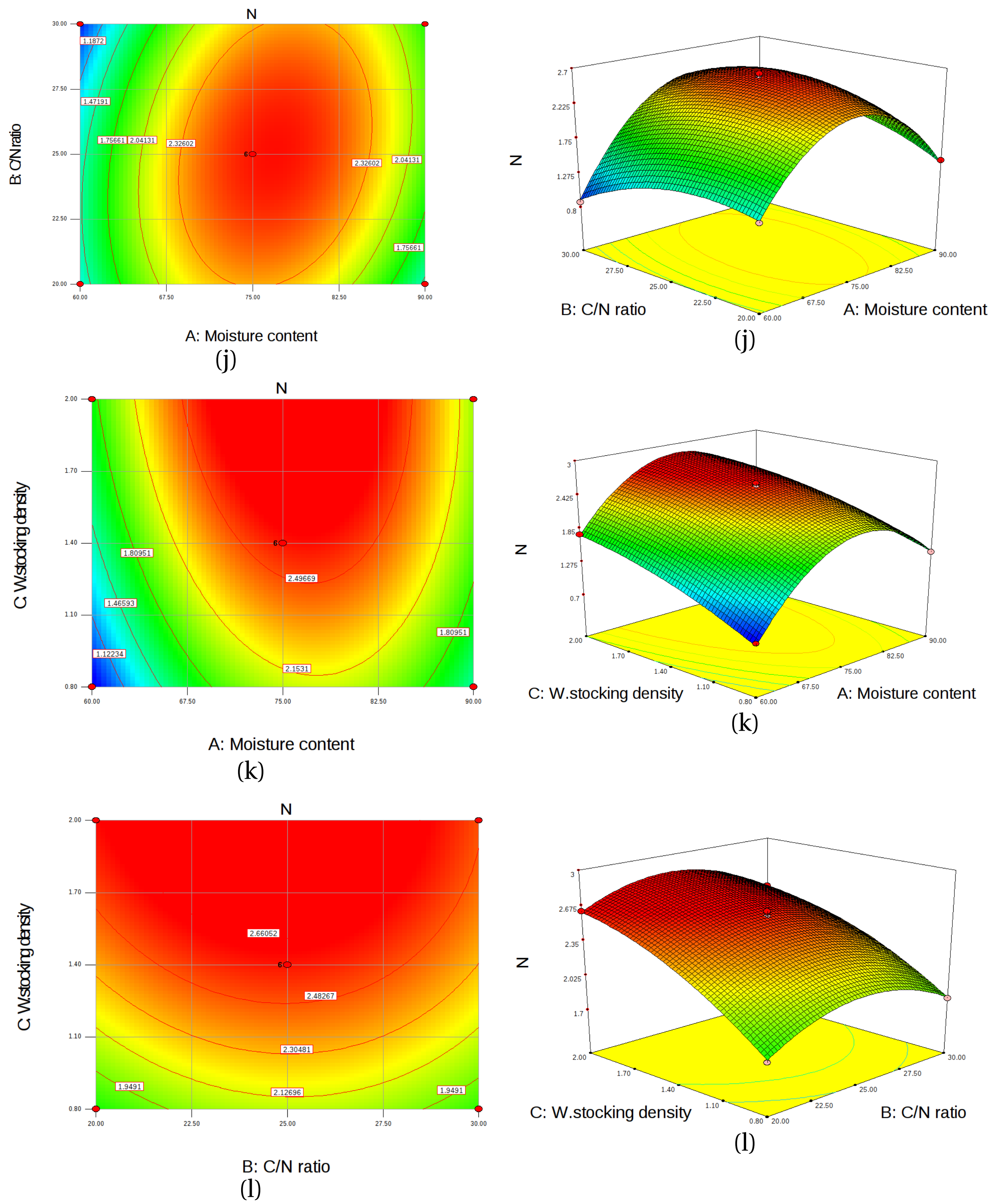
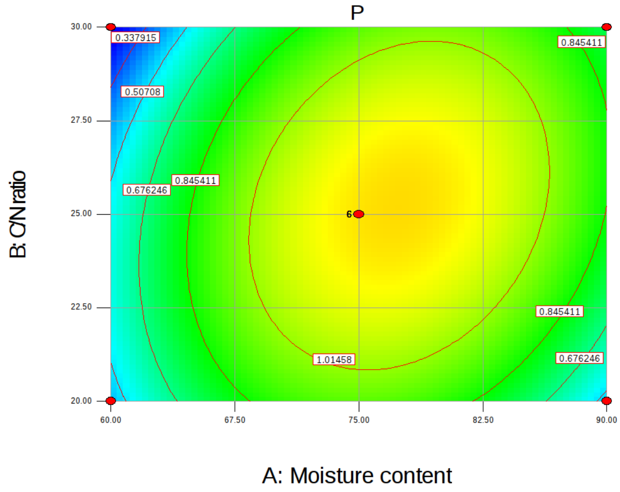
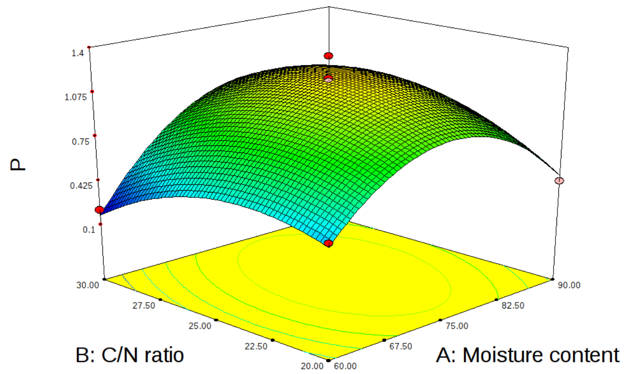


Figure 16: Contour and response surface plots of N of the samples studied as a function of (j) moisture content and C/N ratio, W.density fixed at 1.40 kg worm/m^2 (k) moisture content and W.density, C/N ratio fixed at 25, (l) C/N ratio and W.density, moisture content fixed at 75%

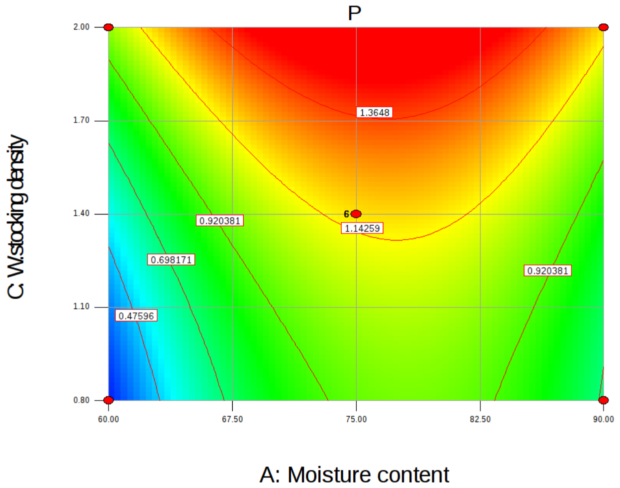
2. **3D and contour plots for the response P**

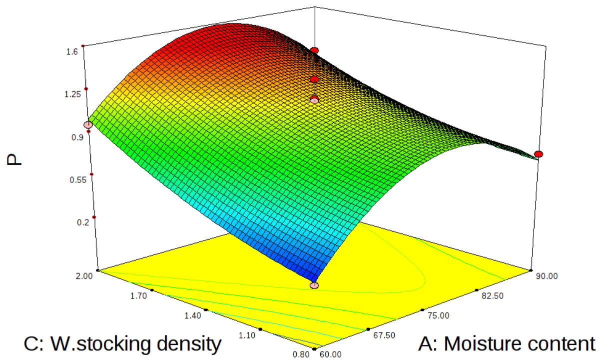




(m)

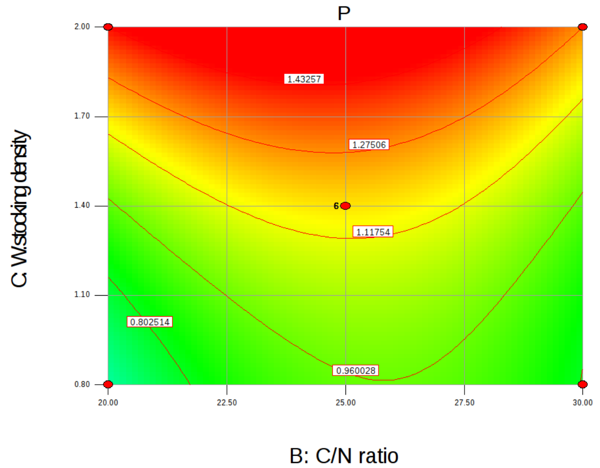
(m)

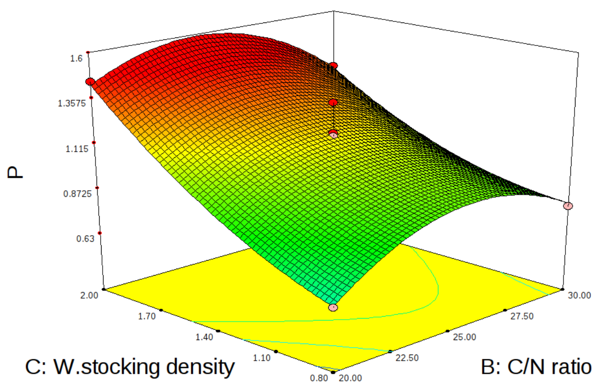




(n)

(n)





(o)

(o)

Figure 17: Contour and response surface plots of P of the samples studied as a function of (m) moisture content and C/N ratio, W.density fixed at 1.40 kg worm/m^2 (n) moisture content and W.density, C/N ratio fixed at 25, (o) C/N ratio and W.density, moisture content fixed at 75%

3. 3D and contour plots for the response K

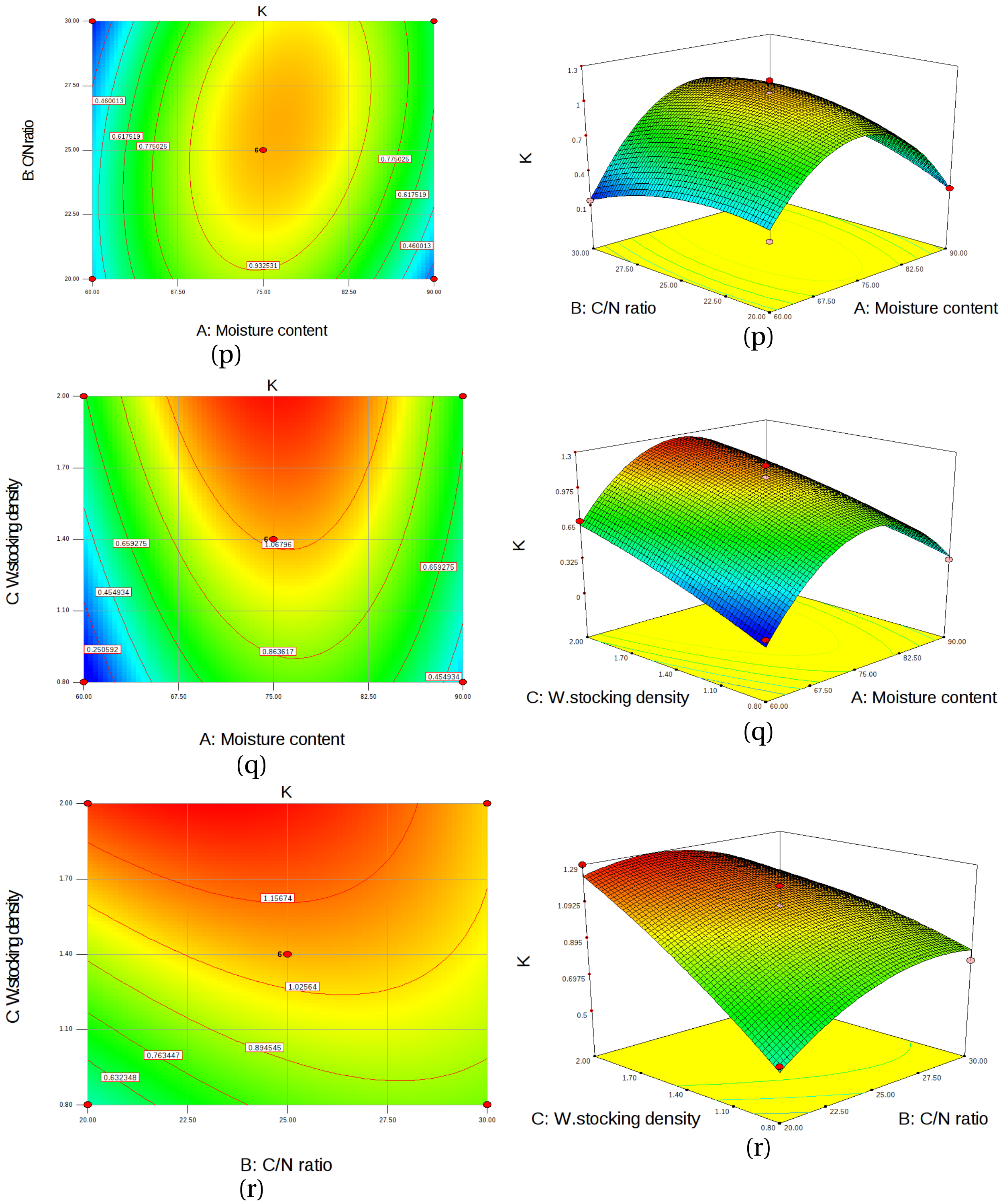


Figure 18: Contour and response surface plots of K of the samples studied as a function of (p) moisture content and C/N ratio, W.density fixed at 1.40 kg worm/m^2 (q) moisture content and W.density, C/N ratio fixed at 25, (r) C/N ratio and W.density, moisture content fixed at 75%

Declaration

I, **the undersigned**, declare that this thesis entitled "**Optimization of Municipal Solid Waste Vermicomposting Parameters**" has not been submitted in any form for another degree, diploma or an award at any university or other institution of the tertiary education. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature and discussions. Information taken from published and unpublished work of others has been acknowledged in the text and a list of references is given. The work was under the guidance of **Teshome Worku (Asst. Prof.)** instructor in Addis Ababa university, School of Chemical and Bio Engineering.

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Name

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Signature

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Place of Submission

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Date of Submission

This thesis has been submitted to the university with my approval as the university advisor.

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Advisor

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Signature

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Date