

**THE EFFECT OF DIFFERENT ORGANIC MANURE BLENDS ON THE CHEMICAL
AND MICROBIAL PROPERTIES OF BOKASHI BIOFERTILIZERS**

A dissertation submitted in partial fulfilment of the requirements for the Master of Science

Degree in Food Security and Sustainable Agriculture

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Bindura University of Science Education



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DECLARATION

I hereby declare that the research project entitled “**The effect of different organic manure blends in the chemical and microbial properties of Bokashi biofertilizer**” submitted to Bindura University of Science Education, Department of Agricultural Economics, Education and Extension is a record of an original work done by me under the guidance and supervision of Dr L. Musemwa and this work is submitted in partial fulfilment of the requirements for the award of a Master of Science Degree in Food Security and Sustainable Agriculture. The results embodied in this thesis have not been submitted to any University or Institute for the award of any degree or diploma.

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DEDICATION

Special dedication goes to my spouse Crispen Saliji, my children Delighted, Munyaradzi, Hope and Praise for their unwavering support towards my education and attainment of my degree.

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ABSTRACT

This study investigates how different organic manure blends affect the chemical composition, nutrient availability, and microbial biomass of Bokashi biofertilizers. Recognizing the growing demand for sustainable farming practices and the environmental challenges posed by synthetic fertilizers, the research focuses on three primary manure sources: poultry manure, cow dung, and pig manure, as well as a blend composed of 50% poultry, 30% cow dung, and 20% pig manure. Laboratory-based experiments were conducted at the Fambidzanai Permaculture Centre under controlled fermentation conditions. Fermentation maintained source pH trends: poultry (9.19→9.07), cattle (8.09→8.05), pig (9.06→9.01), and blend (8.07→8.06). Organic matter soared from 1.3–1.8% in raw manures to 16.2–22.8% in Bokashi, while C:N ratios rose from ~11–12 to 14.5–24.8. Available nitrogen peaked at 12.65% in the blend (11.72% poultry; 12.08% cattle; 11.40% pig), with phosphorus and potassium highest in the blend (1.82% P) and cattle Bokashi (2.11% K). A strong positive correlation was observed between pH and total N ($r = 0.839$) and between C:N ratio and organic matter ($r = 0.875$). Microbial analysis revealed that cattle-based Bokashi achieved the highest total viable counts (TVC) of 900,000 CFU/g, whereas poultry-based Bokashi registered the lowest (52,000 CFU/g). Notably, mould counts were undetected in the blended and poultry treatments, indicating microbial suppression potential. These results demonstrate that optimizing manure blends enhances nutrient stabilization and microbial quality while preserving pH balance, offering a viable path toward more effective, sustainable soil amendments. The study underscores the potential of manure blending in Bokashi formulations and offers practical insights for farmers, fertilizer producers, and policy makers invested in regenerative agriculture.

Keywords: Organic manure blends, chemical properties, microbial properties, Bokashi biofertilizers.

LIST OF ACRONYMS

ANOVA – Analysis of Variance

CFU – Colony Forming

C:N – Carbon-to-Nitrogen Ratio

CRD – Completely Randomized Design

DM – Dry Matter

EC – Electrical Conductivity

EM – Effective Microorganisms

LAB - Lactic Acid Bacteria

NPK – Nitrogen, Phosphorus, and Potassium

PCR – Polymerase Chain Reaction

qPCR – Quantitative Polymerase Chain Reaction

PLFA – Phospholipid Fatty Acid

SIR – Substrate-Induced Respiration

TVC – Total Viable Count

LIMS – Laboratory Information Management System

SOPs – Standard Operating Procedures Established

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CHAPTER ONE

INTRODUCTION

1.0 INTRODUCTION

The goal to increase soil fertility while reducing environmental effect has fuelled a recent surge in the search for sustainable farming methods. Bokashi biofertilizers have become a viable substitute for other organic fertilization methods. Derived from the Japanese phrase meaning "fermented organic matter," Bokashi uses a special fermentation process that turns organic waste into a nutrient-rich amendment by using particular microbes. This technique promotes a varied microbial community that is vital to soil health in addition to increasing the availability of vital nutrients. This chapter provides the background to the study, statement of the problem, research objectives, research questions and the significance of the study.

1.1 BACKGROUND TO THE STUDY

Bokashi fertilizer is an innovative, anaerobic fermentation process that involves the use of organic materials to produce a nutrient-rich soil amendment, which was able to turn waste into the next food nutrient (Olle, 2021). Originating from traditional Japanese agricultural practices, Bokashi utilizes a combination of organic waste, such as kitchen scraps and agricultural residues, along with effective micro-organisms to enhance soil fertility and promote sustainable farming (Kim et al., 2020). Organic manures, including poultry manure, cow dung and pig manure are vital in sustainable agriculture due to their ability to improve soil structure, enhance nutrient availability and support microbial diversity. These manures are rich in essential nutrients such as nitrogen, phosphorus and potassium which are crucial for plant growth, however the chemical and nutrient composition of these vary significantly impacting their effectiveness when used alone or in combination.

Nguyen et al., (2015), postulates that, combining various organic manures may yield synergistic effects enhancing the overall nutrient profile and microbial activity in the resulting Bokashi. Each of manure has unique nutrient composition and microbial communities, which may complement each other when mixed. This means that, understanding these interactions can lead

to the formulation of a more effective Bokashi fertilizer that maximizes nutrient availability and promotes beneficial microbial populations.

The chemical composition of organic manures includes macro and micro nutrients, organic matter and pH and carbon-to-nitrogen ratios. These factors influence nutrient release patterns during the fermentation process and subsequent application to the soil (Phooi et al., 2022). Soil microbes maintain the optimum concentration of soil nutrients, hence providing better plant growth and crop yield (Kumar et al., 2021). Analysing the chemical composition of the combined manures will provide insights into how these factors affect nutrient dynamics in Bokashi production. Microbial biomass plays a crucial role in nutrient cycling as well as suppressing of soil-borne diseases, thus maintain good soil health (Santonyo et al., 2021b). The fermentation process of Bokashi relies on the activity of various micro-organisms which breakdown organic matter and contribute to nutrient availability. Assessing microbial biomass present in the Bokashi, provides insights into how different organic manures influence the fermentation process and subsequent soil enhancement.

1.2 PROBLEM STATEMENT

The increasing demand for sustainable agricultural practices has highlighted the need for effective biofertilizers. Bokashi biofertilizers, made from fermented organic materials, have shown potential for improving soil health and crop yields. However, the impact of various organic manure blends on the chemical and microbial properties of Bokashi remains inadequately explored. The high cost of synthetic fertilizers, averaging around \$25 per 50kg bag globally and varying regionally, has put significant financial pressure on farmers. In Zimbabwe, the price can exceed \$35.00 per 50kg bag (World Bank, 2023). This reliance on costly synthetic alternatives not only strains agricultural budgets but also contributes to environmental degradation. Excessive use of synthetic fertilizers to meet plant nutrient requirement has been linked to soil degradation, waterway pollution, and greenhouse gas emissions, exacerbating climate change (EPA, 2022).

Additionally, there is limited literature on the production of biofertilizers, particularly regarding the microbial and chemical composition of Bokashi. While some studies have addressed the

benefits of biofertilizers, comprehensive research specifically focusing on the interplay between different organic manures and their effects on Bokashi biofertilizers properties is scarce. Recent studies indicate that approximately 60% of farmers report suboptimal soil fertility, primarily due to inadequate nutrient management practices (FAO, 2022). Furthermore, research shows that microbial diversity is crucial for soil health, yet many current biofertilizers lack sufficient microbial biomass, which can lead to reduced effectiveness in nutrient availability (Smith et al., 2023). This research aims to assess how different combinations of organic manures affect the chemical composition, nutrient availability, and microbial biomass of Bokashi biofertilizers. It seeks to provide critical insights that could enhance agricultural productivity and sustainability while reducing dependence on costly and environmentally harmful synthetic fertilizers.

1.3 OBJECTIVES

1.3.1 MAIN OBJECTIVE

The main objective of this study is to analyse the chemical composition, nutrient availability and microbial biomass of organic manure blends in the preparation of Bokashi biofertilizer.

1.3.2 SPECIFIC OBJECTIVES

1. To analyse the chemical properties of various organic manures (poultry, cow dung and pig manure) used in the preparation of Bokashi biofertilizer focussing on parameters such as pH, organic matter content and carbon-to-nitrogen ratios.
2. To assess nutrient composition of the combined organic manures in terms of macronutrients (N.P.K)
3. To measure microbial biomass after fermentation of Bokashi using the selected organic manure combinations.

1.4 RESEARCH QUESTIONS

1. What are the differences in pH levels among poultry manure, cow dung, and pig manure before and after the Bokashi fermentation process?
2. Does the combination of different organic manures result in a synergistic effect on the overall nutrient composition of the Bokashi biofertilizer?

3. How do different combinations of poultry manure, cow dung, and pig manure affect the final microbial biomass and its compositional diversity in Bokashi biofertilizers after the fermentation process is complete?

1.5 SIGNIFICANCE OF THE STUDY

This study aims to provide a comprehensive analysis of the chemical and nutrient composition using different organic manure blends in Bokashi biofertilizers. By exploring the relationships between chemical properties and microbial biomass the research will contribute to the development of more effective and sustainable Bokashi fertilizers, ultimately enhancing soil health and agricultural productivity. The study will help the following stakeholders in various ways:

1.5.1 Farmers

Understanding how different manure blends affect Bokashi biofertilizers can lead to improved soil fertility and crop productivity. Farmers can also optimize their inputs by selecting the most effective manure blends, reducing costs associated with chemical fertilizers. This research promotes sustainable agricultural practices by utilizing waste products, thus minimizing environmental impact.

1.5.2 Fertilizer Manufacturers

Insights from the study can guide manufacturers in creating tailored bio fertilizer products that meet specific agricultural needs. Knowledge about the benefits of Bokashi biofertilizers can help manufacturers tap into the organic farming market, which is growing in demand.

1.5.3 Extension Officers

Extension officers can use research findings to better educate farmers about effective fertilization practices and sustainable agriculture. They can provide practical advice on the best manure blends to use based on scientific evidence, enhancing their role as trusted advisors.

1.5.4 Government

The research can inform policies aimed at promoting organic farming and sustainable agricultural practices, contributing to environmental conservation. Governments can design programs that subsidize the use of bio fertilizers, improving food security and farmer livelihoods.

1.5.4 Researchers

This study can serve as a basis for further research on organic fertilizers, soil health, and sustainable agricultural practices. It encourages collaboration between agronomy, microbiology, and environmental science fields.

1.5.5 Academia

Findings can influence academic programs and courses focused on sustainable agriculture and bio fertilization techniques. The topic opens avenues for graduate and postgraduate research, fostering innovation in bio fertilizer development.

1.6 STUDY LIMITATIONS

The research concentrated on a handful of organic fertilizers due to limitations in time and resources, which led to the exclusion of other important types that might affect the findings. However, the researcher tried three distinct organic fertilizer sources to improve the validity of this research and its applicability. The researcher utilized smaller quantities of various organic sources to enhance diversity without needing significant quantities. The differences in the chemical make-up of organic fertilizers influenced by factors like age and environmental conditions can impact their consistency and reliability. The researcher performed comprehensive chemical assessments of every batch of organic material before utilization to gain a clearer insight into their characteristics and modify mixtures as needed. The results might be unique to a certain area, which can restrict their applicability to other regions with varying climates or soil types. The researcher used customized mixtures by adjusting the organic blends to align with specific regional conditions and prevalent local organic materials to improve their significance.

1.7 DELIMITATIONS

The study specifically focused on certain types of organic manures (poultry, cow dung and pig) to explore their combined effects on Bokashi preparation. The research only considered specific preparation conditions (fermentation time, temperature and moisture levels) to maintain consistency. The study utilised particular techniques (e.g. molecular methods like PCR and sequencing) for microbial identification, which excluded other viable methods. The study was limited to specific regions where Bokashi biofertilizers are produced and used, which may not represent all agricultural environments. Only selected types of organic manures were included in the study, omitting others that may also influence Bokashi properties. The research utilized specific molecular techniques for microbial characterization, which may not capture all microbial diversity present in the Bokashi biofertilizer. The study was conducted over a defined period, potentially limiting the observation of long-term effects of different organic manure blends on Bokashi properties. Experiments were conducted under controlled laboratory conditions, which may not fully reflect field conditions or variations in real-world applications. The emphasis was on identifying beneficial microorganisms, potentially overlooking the role of pathogenic or neutral microbes in the Bokashi fermentation process. The study concentrated on specific chemical parameters of Bokashi biofertilizers, not encompassing all possible chemical components or interactions.

1.8 SUMMARY

In this chapter, the foundation of the research has been established by outlining the background, objectives, and significance of the study. Key gaps have been identified through thorough examination of existing literature that justifies the need for this research. The problem statement and research questions formulated herein will serve as guiding principles throughout the project, ensuring a focused and meaningful investigation. The next chapter will delve into literature review.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter focuses on the review of literature on preparation of Bokashi using various organic manure blends. The growing interest in sustainable farming practices has resulted in an increased focus on organic fertilizers, especially Bokashi biofertilizers. Bokashi, a term from Japan meaning "fermented organic matter," is created through a fermentation process involving specific microorganisms. This technique not only boosts soil fertility but also enhances microbial activity, which is vital for plant development. Recent research has investigated the impacts of different organic manure combinations on the chemical and microbial biomass of Bokashi biofertilizers. Gaining insight into these impacts is critical for fine-tuning biofertilizer compositions to enhance their agricultural advantages.

2.1.1 Background on organic manure and Bokashi biofertilizer

Organic manure refers to a natural substance made from the decomposition of plant and animal materials, used to enrich soil and improve its fertility. Organic manures include compost, farmyard manure (FYM), green manure, and others. They are a vital part of sustainable agriculture, promoting soil health, improving water retention, and enhancing plant growth by supplying essential nutrients in an environmentally friendly manner, (Roy and Saini, 2016).

2.1.2 Historical Context

The use of organic manure dates back to ancient civilizations like Mesopotamia, Egypt, and China, where farmers understood the benefits of enriching the soil with organic materials. Early composting practices involved the use of plant debris, animal waste, and human excreta to fertilize soil (Havlin et al., 2014). In the 20th century, synthetic fertilizers began to dominate the agricultural sector, and organic manure usage declined. However, due to the environmental and health concerns associated with synthetic fertilizers, organic manure has seen a resurgence in modern farming practices, especially in organic farming systems (Bhagat, 2003).

2.1.3 Benefits of Organic Manure:

Organic manures improve soil structure, increase microbial activity, and enhance nutrient availability, leading to improved plant growth (FAO, 2011). Organic manure also supports sustainable farming practices by reducing reliance on synthetic fertilizers and pesticides. It helps mitigate soil erosion, enhances water retention, and supports bio-diversity in the soil ecosystem.

2.1.4 Types of Organic Manures:

1. **Farmyard Manure (FYM):** Composed of decomposed animal dung, bedding material, and urine.
2. **Compost:** Decomposed organic waste such as plant matter, kitchen waste, and manure.
3. **Green Manure:** Crops grown specifically to be plowed back into the soil to enhance its organic matter content.
4. **Vermicompost:** A composting method using earthworms to break down organic materials.

2.2 BOKASHI BIOFERTILIZER

Bokashi is a Japanese term meaning "fermented organic matter." It refers to a type of fermented organic fertilizer, typically made by fermenting organic waste (like kitchen scraps) using specific microorganisms. The Bokashi process uses a mixture of Effective Microorganisms (EM), which include lactic acid bacteria, yeasts, and fungi, to break down organic materials in an anaerobic environment (without oxygen) (Higa, 1994).

2.2.1 An overview of Bokashi biofertilizer

The concept of Bokashi originated in Japan, and the method was developed in the 1980s by Dr. Teruo Higa, (1994), a professor at the University of Ryukyu. Dr. Higa created the EM technology as a way to promote efficient and sustainable waste management, while also providing an alternative to traditional composting methods. Unlike traditional composting, which requires aerobic conditions (exposure to air), Bokashi utilizes anaerobic fermentation. The process is faster, typically taking about two weeks for full fermentation, and can handle materials such as meat, dairy, and cooked foods, which are difficult to compost (Olle, 2021).

2.2.3 Benefits of Bokashi

Efficient Recycling of Organic Waste

Bokashi is an anaerobic fermentation technology that converts a wide array of kitchen and garden wastes including meat, dairy, and cooked foods into a nutrient-rich inoculated material. Unlike conventional aerobic composting, which requires balancing “green” and “brown” matter and often rejects certain waste streams, the Bokashi method is highly versatile because it accepts all organic residues with minimal pre-processing. This efficiency aids waste reduction and contributes to resource conservation (Christel, 2017; Ramlan, 2022).

Accelerated Composting Process

Bokashi fermentation generally takes only 4 to 6 weeks compared to the several months required for traditional composting. The process works under sealed, anaerobic conditions that use effective microorganisms (EM) such as *Lactobacillus plantarum*, which quickly break down organic substrates. Faster turnaround rates minimize the window for nutrient leaching and make it possible to produce biofertilizer on a more frequent basis (Christel, 2017; Kruker et al., 2023).

Enhanced Nutrient Retention and Availability

Because Bokashi fermentation occurs under anaerobic conditions, the system greatly reduces the volatilization of critical nutrients especially nitrogen. Conventional composting often results in nitrogen loss as ammonia gas; however, the Bokashi method “locks in” nitrogen, phosphorus, and potassium in forms that remain available to plants once the fermented waste is incorporated into the soil. As a result, plants receive a gradual and balanced nutrient release that supports vigorous growth (Ramlan, 2022; Kruker et al., 2023).

Beneficial Microbial Inoculation

Bokashi not only converts waste materials but also actively inoculates them with beneficial microbes. The microbial consortium (including lactic acid bacteria, yeasts, and sometimes other effective microorganisms) plays a crucial role in breaking down complex organic compounds, promoting nutrient cycling, and suppressing harmful pathogens in the soil. When applied to soil, these microbes help enhance the overall biological activity, improving soil structure and fertility (Christel, 2017; Kruker et al., 2023).

Odor Control and Pest Management

One distinct advantage of Bokashi is its inherent odor control. Because fermentation occurs in airtight containers, unpleasant smells (commonly associated with rotten organic waste) are minimized or transformed into a mild, pickled odor. This controlled environment also discourages pests such as flies and rodents, making Bokashi a practical option for urban or indoor settings (Ramlan, 2022).

Reduced Environmental Impact

Bokashi biofertilizers contribute to sustainability by minimizing greenhouse gas emissions. The anaerobic process reduces the generation of methane and nitrous oxide compared to some traditional compost methods. Additionally, by recycling local organic waste rather than depending on externally produced chemical fertilizers, Bokashi can lower the overall carbon footprint involved in nutrient management (Kruker et al., 2023).

Ease of Use and Versatile Application

The Bokashi method is simple and low-maintenance. With an easy “add-sprinkle-bury-grow” approach, even home gardeners can process kitchen scraps without extensive labor or land requirements. The end product can be applied directly to the soil, used to make a “Bokashi tea” as a liquid fertilizer, or further composted; this versatility allows it to be integrated into a variety of agricultural systems (Christel, 2017).

2.2.4 Bokashi Fermentation Process

Bokashi is an anaerobic fermentation process that turns organic waste into a pre-compost product that is rich in nutrients. Bokashi ferments all kinds of kitchen scraps, including those that are normally unsuitable for standard composting (such as meat, dairy, and cooked foods), using a sealed environment and a specially prepared inoculant known as Bokashi bran. This is in contrast to traditional aerobic composting, which depends on oxygen to break down matter.

Stages of the Process

- i) **Preparation:** In the first stage, food waste is collected and placed into an airtight container, often called a Bokashi bucket. As the waste is layered into the container, a

measured amount of Bokashi bran is sprinkled over each layer. This bran is inoculated with effective microorganisms (EM) a consortium of lactic acid bacteria, yeasts, and phototrophic bacteria that serves to inoculate the waste and create the conditions required for fermentation (Live to Plant, 2025).

- ii) **Fermentation:** Once the container is filled, it is sealed to ensure anaerobic conditions. In this oxygen-free environment, the effective microorganisms quickly begin to break down the simpler organic compounds in the waste. The metabolic activity of these microbes converts sugars and starches into organic acids (notably lactic acid), lowering the pH and “pickling” the material. This fermentation phase usually lasts about 10–14 days. The process not only preserves the waste by inhibiting harmful bacteria but also produces minimal offensive odor compared to traditional composting methods (Planet Natural, 2024).
- iii) **Completion and Post-Fermentation Processing:** After the fermentation period, the waste is transformed into a pre-compost material that is rich in nutrients but still quite acidic. This material is then typically mixed with soil or further processed in an aerobic stage (often for an additional 2–4 weeks) to neutralize acidity, complete decomposition, and stabilize nutrient release. The final product is a highly nutrient-dense additive that can significantly improve soil structure and fertility, providing a slow-release source of plant nutrients while also introducing beneficial microbes into the soil (Simplify Gardening, 2025).

2.2.5 Chemical and nutrient composition of major organic manures used in Zimbabwe

Various organic manures possess distinct chemical compositions that significantly influence their nutrient profiles. Poultry manure is known for high nitrogen content while cow dung offers a balanced nutrient profile with substantial organic matter (Sharma et al., 2014). Pig manure on the other hand tends to have higher phosphorus levels (Martinez et al., 2024). In the same vein, their nutrient composition directly affects the nutrient availability in Bokashi fertilizer. Studies have shown that the C: N ratios play a pivotal role in determining nutrient release rates during soil application (Phooi et al., 2022). The incorporation of various organic manures in Bokashi can enhance nutrient availability through synergistic effects, which may lead to improved nutrient release patterns over time (Shin et al., 2017). Understanding the chemical characteristics as well

as the nutrient composition of these manures is crucial for predicting their performance in Bokashi production.

2.5.1 Poultry manure

Poultry manure is a rich source of nutrients and organic matter that can be used as fertilizer. Its chemical composition can vary depending on factors such as the type of poultry (e.g., chickens, turkeys, and ducks), their diet, age, and the method of manure collection and storage, (Naylor, 2004). However, a typical poultry manure composition includes a range of essential macronutrients and micronutrients, along with organic matter. Here's an approximate breakdown of the chemical composition of poultry manure, this provides a general chemical composition of poultry manure depending on the source and specific conditions, the values might vary slightly, (Ravindran, 2016). Poultry manure is rich in nitrogen, typically ranging from 3.5% to 5.0% dry weight (Akinremi et al., 1993). Phosphorus content ranges from 1.5% to 2.5% (Cameron and Di, 2010). Potassium content ranges from 1.5% to 3.0% (Cameron and Di, 2010). Poultry manure also contains small but significant amounts of various micronutrients (Zhao et al., 2016). These include calcium (Ca) which is typically 2-4%, magnesium (Mg) approximately 0.5-1%, sulphur (S) around 0.2-0.5% and also iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), and molybdenum (Mo) which are present in trace amounts but are vital for plant metabolism. Poultry manure is rich in organic matter, with around 20-30% organic carbon. Poultry manure typically contains 20-30% dry matter, with moisture content being high in fresh manure. The pH of poultry manure is generally alkaline, ranging from 7 to 8, depending on the bedding material and storage conditions. The carbon-to-nitrogen (C: N) ratio is an important factor for decomposition and nutrient availability. Poultry manure typically has a C: N ratio of 6:1 to 12:1. Poultry manure is known for having high levels of ammonium nitrogen. Urea is also present in small amounts, but significant as a nitrogen source. Fresh poultry manure typically has a moisture content of about 70-80%, which is why it can be difficult to handle and transport unless composted or dried.

2.5.2 Cow dung manure

Cow dung manure is a valuable organic fertilizer, rich in nutrients and organic matter that enhance soil fertility and support plant growth. Its chemical composition can vary based on factors such as the animal's diet, age, and environmental conditions (Havlin et al., 2014).The

high moisture content of fresh cow dung is a result of its typical water content of 80%. Undigested plant materials like lignin, cellulose, and hemicellulose, which enhance soil fertility and structure, are included in the organic fraction. Cow dung contains between 0.52% and 1.19% nitrogen, which is necessary for plant growth. Helps plants develop their roots and transfer energy; phosphorus concentrations range from 0.25 to 0.3 percent. Helps with disease resistance and photosynthesis; potassium is usually present at levels between 0.28% and 0.48%. There are also secondary nutrients like Calcium (Ca) which is important for cell wall structure, with levels around 2.55% to 4.50% and Magnesium (Mg) which is vital for chlorophyll synthesis, found at approximately 2.53% to 3.17%. Cow dung also contains trace amounts of elements such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cobalt (Co), and sulfur (S), which are essential for various plant metabolic processes. The C:N ratio in cow manure is approximately 19:1, indicating a balanced composition that supports microbial activity and organic matter decomposition in the soil. Cow dung manure generally exhibits a pH around 8.1, which can help neutralize acidic soils. It's important to note that these values are approximate and can vary based on factors such as the animal's diet, age, and environmental conditions. Regular testing of manure is recommended to determine its specific nutrient content and to inform appropriate application rates for soil amendment (Lin et al., 2016).

2.5.3 Pig manure

Pig manure is a complex mixture whose chemical composition varies based on factors such as the pigs' diet, age, weight, breed, and the manure management practices employed (Lin et al., 2016). Despite these variations, several studies have characterized its typical components: Crude Protein of pig manure is approximately 19%, (ranging from 11% to 31%) of the dry matter (DM) in pig manure is crude protein. The crude fiber content averages around 18% DM, with a range between 7% and 23%. Typically, ether extract constitutes about 5% DM, varying from 2% to 9%. Ash content, representing the total mineral content, averages 17% DM, with observed values between 10% and 28%. Neutral Detergent Fiber, which includes hemicellulose, cellulose, and lignin, averages 45% DM, ranging from 20% to 60%. Acid Detergent Fiber, comprising cellulose and lignin, averages 24% DM, with a range of 10% to 39%. Lastly, lignin content is about 5% DM, varying between 3% and 6% (Lin et al., 2016). On average, phosphorus content in pig manure is 2.6% DM, with a range from 1.4% to 4.6%. ,then potassium averages 1.0% DM,

varying between 0.6% and 1.6% and Calcium (Ca) content averages 3.5% DM, with observed values from 1.5% to 8.5%. Magnesium (Mg) averages 0.7% DM, ranging from 0.3% to 1.3%. Sodium content is about 0.3% DM, with a range of 0.1% to 0.5%. The average zinc concentration is 600 ppm, ranging from 225 to 1,059 ppm. Copper (Cu): Copper averages 280 ppm, with values between 27 and 822 ppm. Iron (Fe): Iron content averages 2,169 ppm, ranging from 971 to 6,407 ppm. Cadmium (Cd): Cadmium levels average 0.77 ppm, with a range of 0.04 to 3.02 ppm. Lead (Pb): Lead averages 9.89 ppm, varying from 0.29 to 40.11 ppm. Arsenic (As): Arsenic content averages 5.57 ppm, with observed values between 0.20 and 102.51 ppm (Nash, 2004).

2.2.6 MICROBIAL BIOMASS IN BOKASHI PRODUCTION

Microbial biomass is critical component of Bokashi fermentation, as it facilitates the breakdown of organic matter and contributes to nutrient cycling. Research indicates that the microbial population dynamics in Bokashi are influenced by the type of organic manure used (Zhang et al., 2013). A diverse microbial community can enhance the fermentation process, leading to higher-quality Bokashi with improved nutrient profiles (Sharma et al., 2014).

2.2.7 SYNERGISTIC EFFECTS OF COMBINED ORGANIC MANURES

Combining different organic manures may yield synergistic effects that enhance the overall nutrient profile and microbial activity in Bokashi. Studies have suggested that mixtures of manures can result in improved nutrient availability and microbial diversity compared to single-manure applications (Kumar et al., 2015). This highlights the potential benefits of using diverse organic sources in Bokashi production to optimize its quality and effectiveness.

2.2.8 IMPACT ON SOIL HEALTH

The application of Bokashi fertilizers, particularly those produced from a combination of organic manures has been shown to positively impact soil health. Research indicates that Bokashi can enhance soil microbial activity, improve soil structure and increase nutrient retention capacity (Sharma et al., 2014). These benefits contribute to better plant growth and increased agricultural productivity.

2.2.9 CHEMICAL PROPERTIES OF ORGANIC MANURE

2.9.1 Key chemical properties

PH

The pH of poultry manure is generally alkaline, ranging from 7 to 8, depending on the bedding material and storage conditions. Cow dung manure generally exhibits a pH around 8.1, which can help neutralize acidic soils. The pH of pig manure typically ranges from 7.2 to 8.3, indicating a neutral to slightly alkaline nature.

Organic matter content

Total Organic Carbon (TOC): Poultry manure is rich in organic matter, with around 20-30% organic carbon. Poultry manure typically contains 20-30% dry matter, with moisture content being high in fresh manure. The organic fraction includes undigested plant materials such as lignin, cellulose, and hemicellulose, which improve soil structure and fertility. Crude Protein is approximately 19% (ranging from 11% to 31%) of the dry matter (DM) in pig manure is crude protein and then crude fiber content averages around 18% DM, with a range between 7% and 23%.

Ether Extract (Fats): Typically, ether extract constitutes about 5% DM, varying from 2% to 9%. Ash content, representing the total mineral content, averages 17% DM, with observed values between 10% and 28%. Neutral Detergent Fiber, which includes hemicellulose, cellulose, and lignin, averages 45% DM, ranging from 20% to 60%. Acid Detergent Fiber (ADF): ADF, comprising cellulose and lignin, averages 24% DM, with a range of 10% to 39%. Lignin content is about 5% DM, varying between 3% and 6%.

Carbon –to- nitrogen ratios

The carbon-to-nitrogen (C: N) ratio is an important factor for decomposition and nutrient availability. Poultry manure typically has a C: N ratio of 6:1 to 12:1 (Kruker et al., 2023). The C: N ratio in cow manure is approximately 19:1, indicating a balanced composition that supports microbial activity and organic matter decomposition in the soil. Pig manure has a C: N ratio ranging from 6:1 to 12:1 (FAO, 2003).

2.2.10 IMPLICATIONS FOR BOKASHI BIO FERTILIZER PRODUCTION

The ideal C:N ratio for Bokashi fermentation is 20:1 to 30:1 (Sghaier et al., 2016). This ensures efficient microbial activity, odor-free fermentation and minimal nitrogen loss.

Poultry manure

Low C: N (6-10:1)- Meaning high nitrogen. This implies that it needs significant carbon balancing by use of rice bran and sawdust. If not properly managed, there is high risk of ammonia volatilization and foul odor.

Cow dung manure

Moderate C: N (18-25:1)- close to ideal, implying that it can be used as a stabilizer or base in Bokashi which brings about balanced fermentation and less need for amendment.

Pig manure

Low C: N (6-12:1)- high nitrogen which implies that it requires added carbon sources to prevent putrefaction.

2.2.11 PH INFLUENCE IN BOKASHI FERMENTATION

Kruker et al., (2023) says Bokashi fermentation thrives in slightly acidic to neutral environments (pH 5.5-7.0). The pH affects microbial activity, pathogen suppression and odor control.

Poultry manure

pH 6.5-8.0 which means may need pH lowering agents like molasses or acidic bran (Yamada 2000). There is risk of ammonia loss if pH > 7.5

Cow dung manure

pH is 6.8-7.4, near neutral and is good for fermentation. It's a stable substrate for microbial inoculants (Roy and Saini, 2016).

Pig manure

pH is 6.5-7.5 meaning is slightly alkaline and moderate risk of ammonia loss, can be combined with acidic carbon sources such as fermented bran.

2.2.12 MICROBIAL DYNAMICS IN BOKASHI FERMENTATION

Bokashi fermentation is an anaerobic process used to recycle organic waste into high-quality compost. It involves the use of a mixture of beneficial microorganisms such as lactic acid bacteria (LAB), yeast and other microbes which break down organic matter under anaerobic

conditions. The microbial dynamics in Bokashi fermentation are crucial in determining the efficiency of the process, the quality of final compost as well as the sustainability of the method (Zhao and Xu, 2015).

2.12.1 Key microbial groups involved in Bokashi fermentation

Lactic acid bacteria

Play a crucial role in breaking down organic matter by producing lactic acid which lowers the pH and inhibits the growth of undesirable pathogens and spoilage organisms. These bacteria help preserve the organic matter in an anaerobic environment and also responsible for the initial breakdown of carbohydrates and sugars present in the organic matter (Djordjevic, 2017).

Yeast

Yeast contributes to the fermentation process by breaking down sugar to ethanol and carbon dioxide. The ethanol produced by yeast acts as a preservative, helping to further suppress unwanted microbial growth during the fermentation process (Sakai and Morita, 2016).

Actinobacteria

Act in decomposing more complex organic matter like lignin and cellulose. These are crucial for the final stages of Bokashi fermentation, especially when organic matter transitions into humus (Blumer and Schwartz, 2018).

2.12.2 Importance of microbial biomass

Microbial biomass refers to the mass of microorganisms in a given environment, and it plays a crucial role in the fermentation, the microbial biomass is primarily composed of lactic acid bacteria (LAB), yeast, actinobacteria and other anaerobic microbes that are involved in the breaking down of organic matter into valuable compost (Zhao and Xu, 2015). They say, biomass is responsible for various metabolic activities that promote the efficient decomposition of organic waste, improve nutrient cycling and create an environment conducive to sustainable agricultural practices.

2.12.3 Role of microbial biomass in Bokashi fermentation

Decomposition of organic matter

The primary role of microbial biomass in Bokashi fermentation is to decompose organic waste, breaking down complex organic compounds (such as carbohydrates, proteins, fats and fibers) into simpler forms (Sakai and Morita, 2016). The dominant microbes in Bokashi fermentation ferment sugars and carbohydrates, converting them into lactic acid. This process lowers the pH, creating an anaerobic environment that preserves the waste and inhibits the growth of pathogenic organisms (Toyimana and Yomaguchi, 2014). Yeast contributes by fermenting sugars into ethanol, which further enhances the preservation of the material and suppresses undesirable microbial growth. The diverse microbial biomass in Bokashi composting creates a synergistic effect. LAB, yeast and actinobacteria work together to break down organic matter efficiently. This microbial synergy increases the rate of fermentation (Blumer and Schwartz, 2018).

2.12.4 Quantification methods for microbial biomass

Quantifying microbial biomass in Bokashi fermentation involves measuring the amount of microorganisms involved in the fermentation process. The methods of quantifying microbial typically focus on assessing either the microbial cell mass or the activity of microbial communities (Zou et al., 2018). Here are some methods commonly used:

Dry weight measurement

This involves filtering microbial biomass from a sample, drying it and measuring dry weight of the cells. The microorganisms are trapped in the filter membrane and the biomass is dried in an oven at a low temperature.

Microscopic counting (Direct cell count)

A sample is strained and counted under a microscope. This provides a direct count of microbial cells in a given volume of the Bokashi fermentation sample (Haug, 1993).

Fungal biomass quantification

This can be measured through the chitin content (chitin is the main component of the fungal cell walls) or thorough fungal- specific gene markers using PCR (Gaur and Gupta, 2015).

Molecular methods (qPCR and metagenomics)

This provides a more specific and detailed approach to assessing microbial populations. qPCR can be used to measure the abundance of specific microbial groups based on their DNA or RNA, while metagenomic sequencing can give a comprehensive view of the microbial diversity and biomass (Zou et al., 2018).

2.12.5 Relationship between organic manure types and microbial growth

The relationship between poultry, cow dung and pig manure and microbial growth is crucial in understanding how organic manures influence soil health, microbial biodiversity and nutrient cycling. These manures are rich in nutrients, organic matter and microorganisms that affect microbial activity in the soil (Zhang et al., 2021).

Poultry manure

Poultry manure is high in nitrogen, phosphorus and potassium, which can significantly enhance microbial growth. The high nitrogen content, particularly in the form of ammonia, can encourage the growth nitrifying bacteria and other soil microbes involved in nutrient cycling (Zhang et al., 2021). However, excessive application can lead to ammonia toxicity, which may inhibit microbial growth (Wang et al., 2022).

Cow dung manure

Cow dung is relatively balanced organic manure with moderate nitrogen content compare poultry manure. It contains a mix of decomposed plant material, microbial cells and animal matter which supports microbial growth. It tends to provide a more stable environment for microbial communities because of its higher organic carbon and lower ammonia content (Sharma et al., 2014).

Pig manure

Pig manure is rich in nitrogen and phosphorus but has a relatively high moisture content, which can support large population of bacteria, especially those involved in nitrogen cycle. However, pig manure may also contain higher amounts of pathogens compared to cow dung or poultry manure, which requires proper composting or treatment before application to avoid detrimental effects on soil health (Wang et al., 2018).

2.2.13 CONCEPTUAL AND THEORETICAL FRAME WORK

Bokashi is a type of anaerobic fermentation- based composting that uses a mixture of microorganisms to break down organic waste into a nutrient-rich product. This process relies on the inoculation of materials with Effective Microorganisms (EM), including lactic acid bacteria, photosynthetic bacteria and yeasts which thrive in anaerobic conditions. Poultry, cow dung and pig manures as organic waste materials are often used as substrates in the Bokashi fermentation process due to their high nutrient content and their potential to support microbial activity (Abo-

Sido et al., 2021). This conceptual and theoretical frame work discusses how these manures are used in Bokashi production and how they contribute to microbial dynamics, nutrient cycling and soil health.

2.13.1 Conceptual model for organic manure blends and Bokashi biofertilizer

The conceptual model for organic manure blends and Bokashi biofertilizer production integrates the use of diverse organic material, such as poultry manure, cow dung manures and pig manure with the process of anaerobic fermentation using Effective Microorganisms (EM) (Sghaier et al., 2020). The aim of this model is to enhance nutrient cycling, improve soil fertility and promote sustainable agricultural practices. The model emphasizes the synergy between various organic waste materials and microorganism during Bokashi fermentation process, ultimately producing a nutrient-rich biofertilizer that improves soil health and supports plant growth.

Key components of the conceptual model

Organic manure blends - which refers to the combination of different types of organic materials such as poultry, cow dung and pig manure. These materials provide diverse nutrient profile, which when properly mixed, can support a balanced and effective Bokashi fermentation process (Mena-Violante et al., 2019).

Bokashi fermentation process - the process involves inoculation with effective microorganisms which play a very important role in the fermentation of organic matter under anaerobic conditions. Anaerobic fermentation follows where the micro-organisms break down complex organic compounds into simpler compounds. After fermentation process, the Bokashi blend matures and the organic matter transforms into a nutrient- rich biofertilizer (Sghaier et al., 2020).

Soil and plant benefits - the resulting Bokashi biofertilizer offers several benefits for soil health and plant growth (Zhang et al., 2021).

2.13.2 Theoretical approaches to understanding chemical and microbial interactions

Understanding the complex interactions influence nutrient cycling, soil health, plant growth and the breakdown of pollutants, among other processes. The following provides a discussion of key theoretical approaches to chemical and microbial interactions:

Microbial ecology theory

Microbial ecology refers to the study of microbes in their natural environments, focusing on their interactions with other organisms and their chemical surroundings. This theory offers a fundamental understanding of how microorganisms interact with chemicals in soil, water and other ecosystems (Allison and Martiny, 2008).

Chemical cycles and nutrient cycling theory

The theory of biochemical cycles outlines the pathway through which chemical elements are transformed by microorganisms in ecosystems. These include nitrogen, carbon, sulfur and phosphorus cycles which are essential to the maintenance of soil fertility and ecosystem functions (Prosser, 2012).

The theory of microbial biodegradation

Microbial degradation is a process where microorganisms break down organic pollutants and chemicals in the environment. This theory focuses on the mechanisms by which microbes degrade toxic substances and it is crucial for bioremediation strategies used to treat polluted environments (Abo-Sido et al., 2021).

2.13.3 Framework for assessing nutrient availability and microbial dynamics

The assessment of nutrient availability and microbial dynamics in soil is central to understanding soil health, nutrient cycling and plant growth. This frame work combines concepts from soil chemistry, microbiology and ecosystem ecology to better predict and mange soil fertility, enhance crop production and optimize soil health. The frame work covers the following:

Nutrient availability in soil

This is governed by both chemical and biological factors. The major nutrients involved in microbial and plant dynamics include nitrogen, phosphorus, potassium, sulfur and micronutrients like zinc, copper and manganese. Microorganisms decompose organic materials releasing nutrients through mineralization, which is central to nutrient cycling (Rasmussen et al., 2018).

Microbial dynamics in soil

Microbial communities are crucial in nutrient cycling and the availability of essential elements. Microorganisms such as fungi, bacteria and archaea mediate several key processes such as nitrogen fixation, nitrification and denitrification (Fierer et al., 2007).

Soil-plant- microbial interactions

The relationship between soil microbes, plants and soil nutrients is reciprocal. Plants influence microbial communities by releasing root exudates, which serve as a carbon source for microbes, influencing their composition and activity. In turn, microbes enhance plant nutrient uptake and growth (Chen et al., 2012).

2.3 IDENTIFIED RESEARCH GAPS

Optimal manure blends and ratios

While various organic manures (poultry, cow dung, pig manure) are known to influence Bokashi production, there is limited research on identifying the most effective combinations and ratios of these manures for specific soil types or crop needs.

Microbial community analysis

Although the role of microorganisms in Bokashi fermentation is acknowledged, detailed studies on how different manure blends influence specific microbial populations (e.g lactic acid bacteria, yeasts, fungi) during fermentation are lacking.

Long -term soil and plant impact

More research is needed to assess the long-term effects of Bokashi, produced from various organic manure blends on soil health, nutrient cycling and plant growth over multiple seasons.

Fermentation process optimization

There is need for in-depth studies to understand the fermentation conditions (such as temperature, humidity and duration) that maximize nutrient retention, microbial activity and stability of Bokashi.

2.4 SUMMARY

In conclusion, the use of various organic manure blends in Bokashi production offers several benefits, including enhanced nutrient availability, improved microbial activity and increased efficiency in fermentation. Blending different types of organic manures such as poultry, cow dung and pig manure results in a more balanced, effective and sustainable biofertilizer. These blends optimize both microbial dynamics and nutrient release, making Bokashi a valuable tool for improving soil health. The following chapter will explore the research methodology.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 INTRODUCTION

In this methodology section we shall reconnoiter research design and the methodology underpinning this study with the following subheadings; sample group, data collection procedure, data analysis and presentation procedure, ethical considerations and expected outcomes axing with a conclusion.

3.2 BRIEF DESCRIPTION OF STUDY AREA

The region experiences a subtropical climate, characterized by a distinct wet and dry season. During summer (November to March), daytime temperatures can reach around 28°C to 34°C (82°F to 93°F). In winter (June to August), nighttime temperatures can drop to about 5°C to 10°C (41°F to 50°F). The majority of rainfall occurs between November and March, with annual precipitation ranging from 600 mm to 800 mm (approximately 24 to 31 inches). The dry season typically lasts from April to October, with minimal rainfall. Humidity levels are generally higher during the rainy season, contributing to a lush environment, while the dry season is characterized by lower humidity. The area may experience moderate winds, particularly during the dry season, which can influence temperature and moisture level.

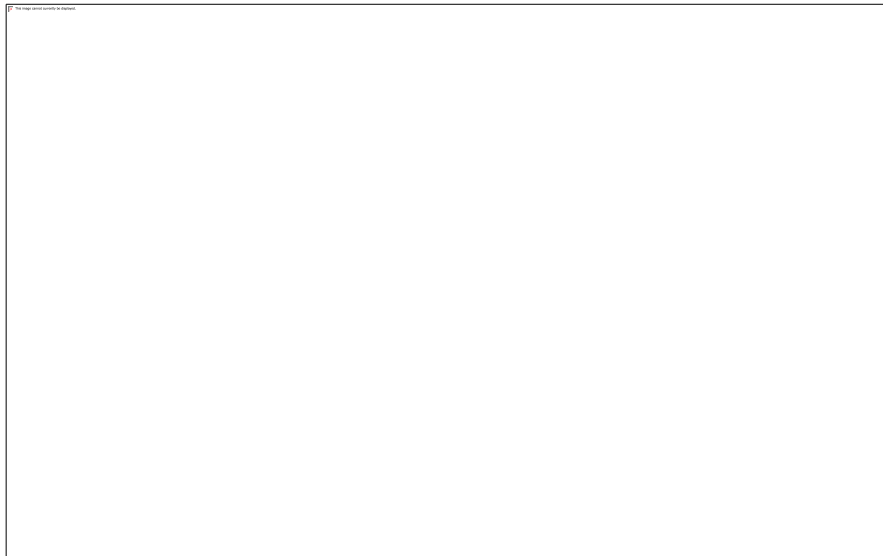


Figure 1: Map of the study site

3.3 RESEARCH DESIGN

A laboratory-based experimental study is well-suited for this research due to the controlled environment it provides, the ability to conduct detailed analyses, and the opportunity to optimize and monitor the fermentation process effectively. This approach will yield reliable and replicable results that can significantly contribute to the understanding of Bokashi biofertilizers.

3.3.1 Laboratory-based experimental study

A laboratory-based experimental design is a controlled, systematic approach used in scientific research to study cause-and-effect relationships by manipulating independent variables while keeping environmental conditions constant. This method ensures precise measurements, replicability and elimination of confounding factors, making it particularly useful for studies on biofertilizers and organic manure blends (Montgomery, 2017). It is widely applied in microbiology, agriculture, and environmental sciences to analyze nutrient transformations, microbial activity, and chemical interactions under stable conditions (Fisher, 1935). By restricting external influences such as temperature fluctuations, soil heterogeneity, and contamination, this approach allows researchers to develop standardized methodologies and obtain highly accurate experimental data (Cochran & Cox, 1957).

3.3.2 Philosophy underpinning laboratory-based experimental study

Empiricism

Scientific inquiry must be rooted in observable and measurable data (Popper, 1959). Laboratory research allows direct testing and verification of hypotheses under controlled conditions for instance, monitoring microbial growth in biofertilizers after adding different organic manure blends.

Reductionism

Complex biological processes can be studied by isolating specific variables (Bunge, 1998). Researchers can then analyze individual effects of organic manure on microbial activity without field-related disturbances.

Determinism

Laboratory-based experiments follow predictable cause-and-effect relationships (Montgomery, 2017). It enables researchers to model nutrient cycles, microbial interactions, and biofertilizer efficiency under precise conditions.

Control & Manipulation

Laboratory environments provide strict control over variables, ensuring unbiased results (Fisher, 1935). Temperature, moisture levels, and nutrient concentrations can be carefully adjusted for optimal biofertilizer testing.

Replication & Standardization

Experiments must be repeatable across multiple trials to validate findings (Cochran & Cox, 1957). Laboratory procedures follow standardized methods, ensuring consistent data collection.

3.3.3 Relevance of laboratory-based experimental study

A laboratory-based experimental study is well-suited for this research due to the controlled environment it provides, the ability to conduct detailed analyses, and the opportunity to optimize and monitor the fermentation process effectively. This approach will yield reliable and replicable results that can significantly contribute to the understanding of Bokashi biofertilizers (Bashan et al., 2010). A laboratory setting allows the researcher to precisely control over unpredictable environmental conditions such as temperature, humidity, microbial exposure, and light, which are critical for the fermentation process of Bokashi biofertilizers thereby ensuring consistency across the experiments. This control minimizes variability and enhances the reliability of the results (Zoubir and Moumen, 2017). The laboratory environment facilitates detailed monitoring and analysis of chemical properties (e.g., nutrient composition, pH) and microbial activity (e.g., microbial diversity and abundance) to mimic optimal biofertilizer conditions. External contaminants (pesticides, pathogens) are eliminated, allowing a more direct assessment of manure effects. This level of analysis is often difficult to achieve in field studies due to environmental fluctuations (Garofalo and Reed, 2019). The fermentation period is crucial in Bokashi production, typically lasting 2-4 weeks. A laboratory study allows researchers to systematically evaluate the fermentation process and its impact on the final biofertilizer properties. Laboratory experiments can be easily replicated, allowing for the verification of results. This is essential for validating findings related to the effects of various organic manure blends (Lee 2016). Laboratory conditions allow for standardized procedures that improve

experimental accuracy (Montgomery, 2017). Manure blends can be tested in replicated trials with consistent biofertilizer concentrations. Data collection methods, such as chromatography and spectrophotometry, ensure precise nutrient analysis.

The study can closely monitor microbial dynamics during fermentation, enabling researchers to assess the influence of different organic blends on microbial community structure and function. Biofertilizers contain live microbial communities, which require advanced monitoring techniques. Lab settings allow researchers to conduct DNA sequencing to analyze microbial diversity (Glick, 2012). It also provides ground for assessing enzyme activity levels (dehydrogenase, phosphatase) under different manure applications (Van Elsas et al., 2007). They also enable faster results and process optimization. Unlike long-term field trials, laboratory experiments offer quicker insights. Biofertilizer nutrient decomposition rates can be tested under controlled conditions before large-scale application (FAO, 2019). Results can help refine manure blends to maximize microbial efficiency before field testing. Field trials require land preparation, monitoring equipment and labor; lab experiments reduce these costs (Adesemoye & Kloepper, 2009). This efficiency is invaluable for preliminary research phases. Due to enhanced control and precision, results from this study can serve as a foundational reference for future field studies, helping to inform best practices for Bokashi production using organic manure blends.

3.4 SAMPLING PROCEDURE

Sampling is a key concept in research methodology, enabling researchers to examine a smaller portion of a population to draw conclusions about the entire group. Various authors have offered different viewpoints on sampling, highlighting its significance, techniques, and challenges. The primary aim is to guarantee accurate representation of blood meal sources, microbial biomass, and nutrient analysis findings. In this study, a combination of three sampling methods was utilized to acquire samples for laboratory testing.

Purposive sampling, as described by Neville, (2005), is a judgment-based technique where the researcher deliberately selects specific groups or individuals based on their relevance to the research topic. Purposeful sampling allows the researcher to apply their judgment in choosing a sample that they believe, based on previous data, will yield the necessary information (Fraenkel

& Wallen, 2009). A limitation of this approach is that the researcher's selection may be influenced by their understanding of the required information. Specific blood meal sources (chicken, cattle, and pigs) were chosen using purposive sampling based on their availability and relevance to the study of biofertilizers.

Stratified sampling, a probability sampling technique, is used when there are significant differences among subpopulations, making it advantageous to sample each subpopulation individually. Stratification is the method of dividing population components into relatively homogenous subgroups known as strata (cattle, pig, and chicken blood meal) before sampling takes place. Each element in the population must belong to only one stratum, ensuring the strata are mutually exclusive. Furthermore, the strata need to be collectively exhaustive, indicating that no part of the population can be omitted. Sampling is then conducted randomly or systematically within each stratum. To ensure fair representation of different combinations in vermicompost treatments, stratified sampling was also applied.

Replicate sampling is a vital approach in statistical analysis and research to ensure accuracy and reliability in data collection. It involves gathering multiple samples under similar conditions to assess variability and enhance precision (Smith, 2020). Smith, (2020) notes that duplicate sampling increases the reliability of experimental outcomes by reducing random errors. This method is widely employed in clinical trials, quality control processes, and environmental studies (Jones & Taylor, 2019). To enhance statistical reliability, each treatment was assessed in triplicate utilizing replicated sampling.

3.5 SAMPLE GROUPS

Four distinct treatment groups were established to compare and evaluate the properties of different organic manures when used as biofertilizers. The groups are as follows:

Treatment 1 – 100% Poultry Manure: This group consists solely of poultry manure, which is typically high in available nitrogen and other nutrients that promote rapid vegetative growth. Poultry manure is often favored for its fast nutrient release but may require careful management to prevent nutrient leaching (Barker & Pilbeam, 2015).

Treatment 2 – 100% Cow Dung: Cow dung usually has a milder nutrient profile compared to poultry manure. It is known for its excellent content of humic substances and its ability to improve soil structure gradually. This group helps in assessing the performance of cow dung as a sole organic fertilizer (Singh & Verma, 2019).

Treatment 3 – 100% Pig Manure: Pig manure offers a different nutrient composition and moisture content when compared with the other types. It often contains appreciable levels of macro and micronutrients, yet its nutrient release dynamics may differ due to its inherent properties (Kaur et al., 2018).

Treatment 4 – Blend (50% Poultry + 30% Cow Dung + 20% Pig Manure): This mixed treatment was designed to harness the strengths of each individual manure type. The hypothesis is that combining 50% poultry manure (for its high nitrogen), 30% cow dung (for its humic content and soil-structuring benefits), and 20% pig manure (for its balanced nutrient profile) may result in synergistic effects. Such a blend is expected to yield a balanced nutrient profile, mitigate potential drawbacks (such as rapid nutrient leaching or excess moisture), and enhance microbial diversity in the resulting biofertilizer (Wang et al., 2021).

3.6 PROCEDURE

3.6.1 Bokashi Ingredients for each control treatment

- 3.5 bags Manure (pig, cow dung and poultry)
- 3.5 bags Dry matter
- 2.5bags Soil
- Half bag Bran
- 2.5l Molasses
- 250g Yeast
- Minerals
- For the blend a ratio of 5:3:2 was used, whereby 5 is for poultry, 3 for cow dung and 2 for pig manure.

3.6.2 Bokashi preparation

A layer of dry matter was made first, then followed by a layer of manure. Soil and bran layers were added and lastly minerals. In a separate bucket molasses was mixed with 2.5l of water and

yeast. The mixture was stirred thoroughly using a stick then sprinkled onto the heap after each set of layers. After all the materials were laid, thorough turning of the heaps was done for about seven times on each heap until all the contents were mixed well. The Bokashi heaps were turned twice per day during the first week of fermentation to optimize temperatures. After day seven, turning was done once up to complete fermentation. During fermentation period, temperature and pH levels were monitored and recorded.

Selection and Collection of Raw Materials

Manure sources, including poultry, cattle, pig, and blends, were identified and selected from local farms and experimental facilities. The researcher ensured that the samples were representative by collecting them from multiple farms or batches. For each manure source, at least three replicate samples were collected to account for natural variability. Clean, sterilized containers were used to collect and transport the samples quickly to the laboratory, and details such as date and time were recorded. Samples were homogenized before subsampling to ensure uniformity in each replicate. The sample was inspected to identify and remove extraneous matter, such as stones.

Data Management

Digital spread sheets or laboratory information management systems (LIMS) were used to log all measurements, and each sampling event was clearly time-stamped and linked to specific treatments.

3.7 DATA COLLECTION

Data collection procedure for objective 1 on chemical composition included measuring pH on a daily basis using calcium chloride scale during the fermentation process, organic matter and C:N ratio were measured after complete fermentation, then temperature was recorded daily using Brannan thermometer (mercury). For nutrient composition in terms of nitrogen, phosphorus and potassium, samples were taken after complete fermentation and measured using Kjeldhal method for nitrogen and spectrophotometry phosphorus and potassium. Lastly, on microbial biomass measurement, estimations on microbial biomass using PCR as well as dry weight measurements were employed.

3.8 CHALLENGES ENCOUNTERED DURING DATA COLLECTION

Sampling variability and representativity

Heterogeneity of materials: Biological raw materials like manure and dung are naturally varied. Differences in particle size, moisture content, and nutrient distribution can lead to unrepresentative samples if adequate homogenization methods are not applied (Brady & Weil, 2008; Rachid et al., 2015). Consequently, the nutrient percentages obtained from a few aliquots might not accurately depict the overall composition of the batch.

Measurement Precision and Equipment Calibration Instrument Variability: The analytical instruments utilized for assessing nutrient levels (such as spectrophotometers for phosphorus measurements and Kjeldahl or combustion analyzers for nitrogen) need consistent calibration and upkeep. Inconsistent calibration or variations in instrumentation protocols may introduce systematic measurement errors (Fernandes, 2010). Differences in laboratory conditions and operator handling can further escalate these concerns.

Process variability during fermentation

Environmental fluctuations: Fermentation is a fluid, biologically driven process. Factors like temperature, pH, aeration, and activity of the microbial community can vary during the process, influencing the transformation of organic compounds into forms that plants can absorb. These variations can lead to inconsistent nutrient measurements if data collection is not meticulously controlled or if the timing of samples is not standardized (Brady & Weil, 2008).

Data Management and human error

Recording and documentation: Mistakes in manual data recording or errors in transcription can undermine data integrity. A lack of standardized operating procedures (SOPs) for data entry including inconsistent documentation of sample origin, timing, or method can hinder data interpretation or replication efforts. It's vital to ensure strong data management to uphold data quality (Rimando et al., 2015).

External and Operational Factors

Contamination and interference: There is always a risk of sample contamination whether from external particulates or cross-contamination between treatments during both fieldwork and

laboratory data collection. Furthermore, operator bias (for example, if the data collector knows the treatment identity) may skew results. Implementing blind or double-blind protocols, whenever feasible, can help alleviate this issue (Rimando et al., 2015).

In conclusion, data collection in biofertilizer research must tackle challenges arising from the inherent variability of biological materials, ensure accurate measurements through dependable instrument calibration, manage process variability during fermentation, and adopt strong data management practices to reduce human error and contamination. Addressing these challenges by standardizing protocols and implementing rigorous quality assurance measures is crucial for obtaining reliable, consistent datasets that can guide sustainable agricultural practices.

3.9 DATA ANALYSIS

In summary, the study analyzed data under three specific objectives through a series of methodical steps:

Under Objective 1, descriptive and inferential statistics (ANOVA and post-hoc tests) were used to compare pH, organic matter, and C:N ratios across different manure sources. This ensured that any differences observed were statistically significant and provided insight into the chemical environment of the raw materials and final Bokashi biofertilizers.

Under Objective 2, nutrient composition (N, P, K) was measured using standard laboratory techniques, and weighted averages were calculated for the blended manure treatment. Comparative analysis via ANOVA determined significant differences among treatments, supporting the hypothesis that blending can enhance nutrient profiles, particularly for nitrogen and phosphorus.

Under Objective 3, microbial biomass was quantified using both culture-based and molecular methods. Descriptive statistics, ANOVA, and correlation analyses were employed to determine differences in microbial communities among treatments, with the goal of understanding how these communities contribute to fermentation efficiency and the stabilization of nutrient profiles. Together, the analytical procedures allowed for a comprehensive comparison of chemical and microbial parameters across manure sources and enabled the identification of the optimal blend for producing a superior Bokashi biofertilizer. The consistency and reliability of the measurements, ensured by rigorous statistical methods, provide a solid foundation for developing

sustainable fertilizer strategies that can be adopted in both research and practical farming contexts (Montgomery, 2013; Zhao & Xu, 2015).

3.10 ETHICAL CONSIDERATIONS

Sourcing of Materials – Responsible Sourcing: All organic materials (manures, soil, bran, molasses, and yeast) were sourced from reputable and certified local suppliers to ensure that the animal by-products are derived from farms that adhere to animal welfare and environmental standards (Higa & Konishi, 1999).

Research Integrity and Data Transparency – Accurate Data Collection: Researcher followed strict protocols for sampling, data collection, and reporting to avoid data manipulation or bias. Transparent record keeping (using digital spreadsheets or LIMS) was done to ensure that the data can be audited and verified (Montgomery, 2013).

Health and Safety of Researchers – Laboratory Safety: Given that handling organic manures and microbial cultures can pose biohazards, researcher used personal protective equipment (PPE) and followed established laboratory practices. Safe storage and disposal of residues was also done to prevent environmental contamination (Lee, 2016).

Environmental Responsibility – Minimizing Environmental Impact: The experimental work was done in a way that there is no adverse impact on local ecosystems or contamination of water sources. This includes proper waste management of any unused samples or by-products.

Sustainable Protocols: The study was designed to promote sustainable practices. Thus, it fosters that all processes from manure collection to Bokashi fermentation align with environmentally friendly principles (Nguyen et al., 2015).

Conflict of Interest and Funding Disclosure Transparency in Funding: Any sources of funding or potential conflicts of interest were clearly disclosed to avoid any bias in data interpretation or reporting.

Objective Reporting: Researchers should objectively report all findings, even if the results conflict with expectations or the interests of any funders.

3.11 SUMMARY

This chapter provided a detailed outline of the research process, beginning with the description of the study site to offer context and justify its suitability for the investigation. The research

methodology was clearly outlined, including the experimental approach, tools, and procedures used to ensure accurate data collection. Overall, the methods and procedures presented in this chapter form a solid foundation for the analysis and interpretation of results in the next chapter.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INTRODUCTION

The main objective of this study was to analyse the chemical composition, nutrient availability and microbial biomass of organic manure blends in the preparation of Bokashi bio fertilizer. To attain these purpose multi-level studies were performed. In this chapter the researcher identified and presented the findings.

4.2 RESULTS

4.2.1 Comparisons in pH levels through the process of fermentation

To determine if there is significant difference in pH levels recorded through the process of fermentation, a statistical test was computed. The ANOVA summary compares the mean pH values of four manure sources with each group having 19 observations using EXCEL from Microsoft Plus 2010 version 14.0.4734.1000 and it indicates that the calculated F-value of 41.58 far exceeds the critical value of 2.7318. This indicates that the variation between manures (between-group variability) is substantially greater than the variation within each manure source (within-group variability) (Montgomery, 2017; Snedecor & Cochran, 1989). The p-value (1.055×10^{-15}) is much lower than any conventional significance level (e.g., 0.05), leading to a decisive rejection of the null hypothesis that all manure sources have the same mean pH. In practical terms, the manure source is a statistically significant factor affecting pH (Kutner et al., 2004). See the table below for the display of EXCEL from Microsoft Plus 2010 version 14.0.4734.1000



Figure 2: One-way ANOVA for the pH of manure sources

From the table the Sum of Squares Between Groups ($SS = 11.6023$), this value captures the variability in pH means across the four manure sources. The high mean square (3.8674) here indicates substantial differences in the central tendencies among the groups. The Sum of Squares Within Groups ($SS = 6.6968$): The relatively low mean square (0.0930) for the error term reflects that the measurements within each group are quite consistent. This low intra-group variability bolsters the power of the ANOVA to detect genuine differences between the manure sources.

The manure sources exhibit notable differences in pH. Poultry manure has the highest average pH (9.19), while cattle manure records the lowest (8.16). Pig and blend treatments fall in between. Such differences may arise from variations in the composition, nutrient content, or microbial activity associated with each manure type.

In the context of Bokashi biofertilizers, pH is crucial since it affects microbial activity, nutrient solubility, and ultimately the performance of the fertilizer in soil. For instance, a higher pH (as seen with poultry manure) might influence the microbial community differently than a lower pH (as with cattle manure). The blend, with a moderated pH, suggests that mixing different manure sources may help balance extremes, thereby producing a more consistent biofertilizer.

The ANOVA results clearly demonstrate that the source of manure leads to significant variations in pH during the fermentation process. The extremely high F-statistic and the negligible p-value provide strong evidence against the null hypothesis. This indicates that poultry, cattle, pig, and

blend manure treatments yield distinct pH profiles, which could have important implications for the microbial dynamics and agronomic performance of the produced Bokashi biofertilizers. Although the ANOVA tells us that significant differences exist among the groups, further post-hoc tests was used to pinpoint which specific pairs of manure sources are significantly different.

Overview of the Post Hoc Analysis

The post hoc analysis used pairwise t-tests with a Bonferroni correction ($\alpha = 0.00833$) to control for Type I errors given the multiple comparisons. The following table summarizes the comparisons and their respective p-values:



Figure 3: Post hoc results for pH

From the figure above, each p-value is well below the Bonferroni-adjusted threshold, confirming that all pairwise differences in pH are statistically significant. This indicates that the source of the manure plays a critical role in determining its pH, a result that resonates with the notion that animal diet, digestion, and manure processing influence manure chemistry (Chen et al., 2012; Lin et al., 2016). The post hoc analysis confirms that the pH differences among poultry, pig, cattle, and a manure blend are statistically robust. The intrinsic differences in diet, digestive processes, and manure handling among poultry, pig, and cattle result in distinct pH values. Such variations necessitate tailored approaches in soil amendment practices. For example, if a higher soil pH is desired or if there is a need for particular nutrient availability, selecting the appropriate manure or blend becomes critical (Kim et al., 2020). By applying a Bonferroni correction, the analysis ensures that the significance of the findings is not inflated by multiple comparisons an approach endorsed in many studies examining manure properties (Chen et al., 2012; Lin et al., 2016).

4.2.2 Comparisons of parameters of the source and final Bokashi biofertilizers

Below is a detailed comparison and analysis of the manure sources versus the final Bokashi products, based on the average values obtained from triplicate samples. The manure sources

exhibit high pH values with poultry (9.09) and pig (9.06) being notably alkaline compared to cattle (8.09) and the blend (8.07). Notably, the Bokashi fermentation process maintains these pH levels very closely (poultry: 9.07, pig: 9.01, cattle: 8.05, blend: 8.06). These observations align with studies by Higa and Konishi, (1999), who reported that effective microorganism (EM) fermentation typically induces very little change in the acid–base balance of the substrate. This pH stability is crucial because it preserves the soil’s microbial equilibrium upon application, as has been noted in related literature on Bokashi -based soil amendments.

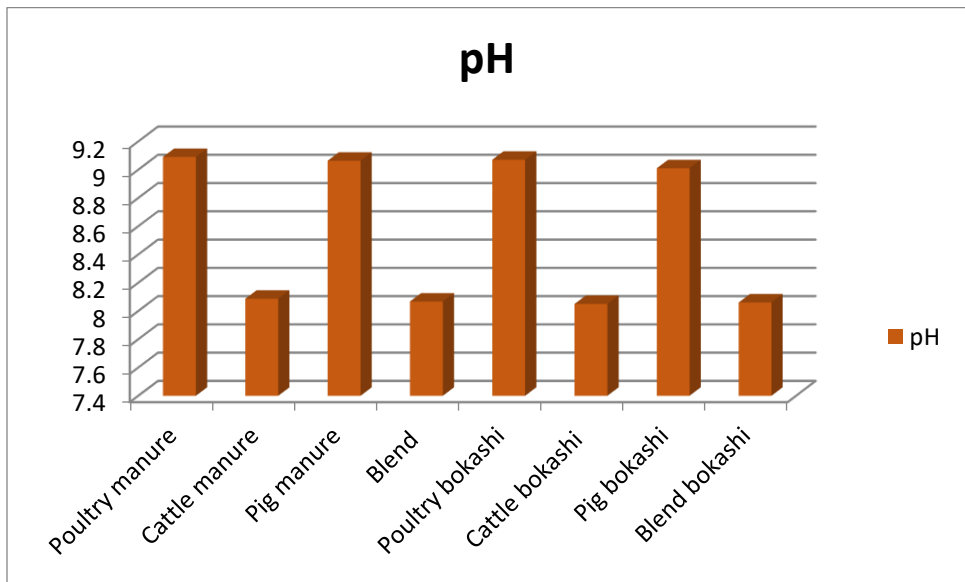


Figure 4: pH of manure sources and Bokashi biofertilizer

Manure conductivity, an indirect indicator of the ionic content, shows only minor variations after fermentation. For example, poultry manure conductivity rises slightly from 16.73 to 17.03, while cattle manure shows a small decrease from 8.76 to 7.03. These minimal changes likely result from the microbial assimilation of soluble ions during fermentation a phenomenon that Chen et al. (2012) described in similar organic substrate fermentations. Maintaining comparable conductivity suggests that the ionic content, and therefore the potential availability of certain nutrients, remains largely consistent, thereby supporting soil health when the Bokashi is applied.

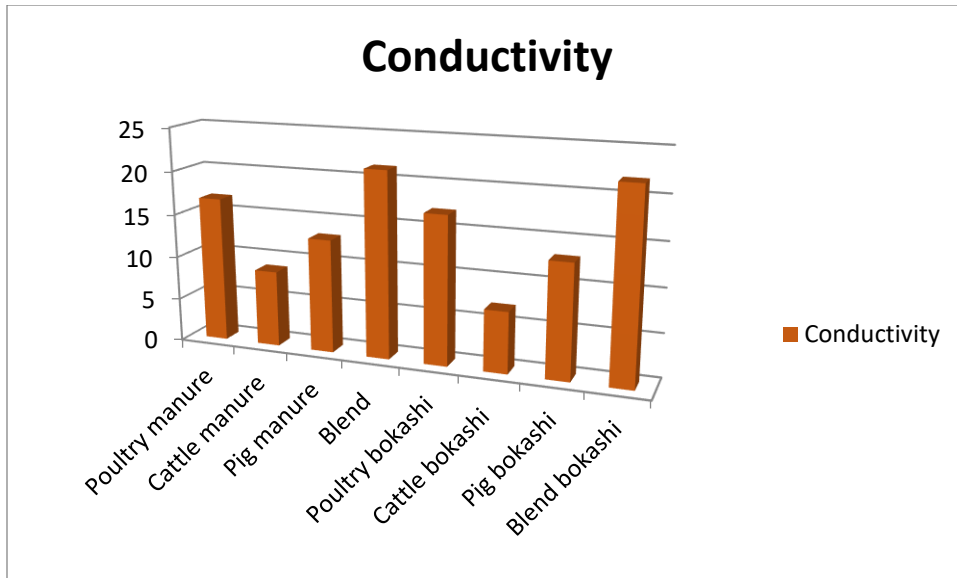


Figure 5: Conductivity of manure sources and Bokashi biofertilizer.

A marked transformation is observed in the carbon-to-nitrogen (C:N) ratio. Raw manure values range from 11.40 (pig) to 12.65 (blend), but these increase significantly in the Bokashi (poultry: 14.49, pig: 15.20, cattle: 24.77, blend: 20.02). Such an elevation in the C:N ratio is typically indicative of microbial activity preferentially converting available nitrogen into more stable compounds while accumulating carbon-rich microbial biomass. Lin et al. (2016) have documented similar increases in C:N ratios during Bokashi fermentation, suggesting that these modifications slow nitrogen mineralization and promote a sustained nutrient release profile. This transformation is essential for reducing rapid nitrogen losses and supporting long-term soil fertility.

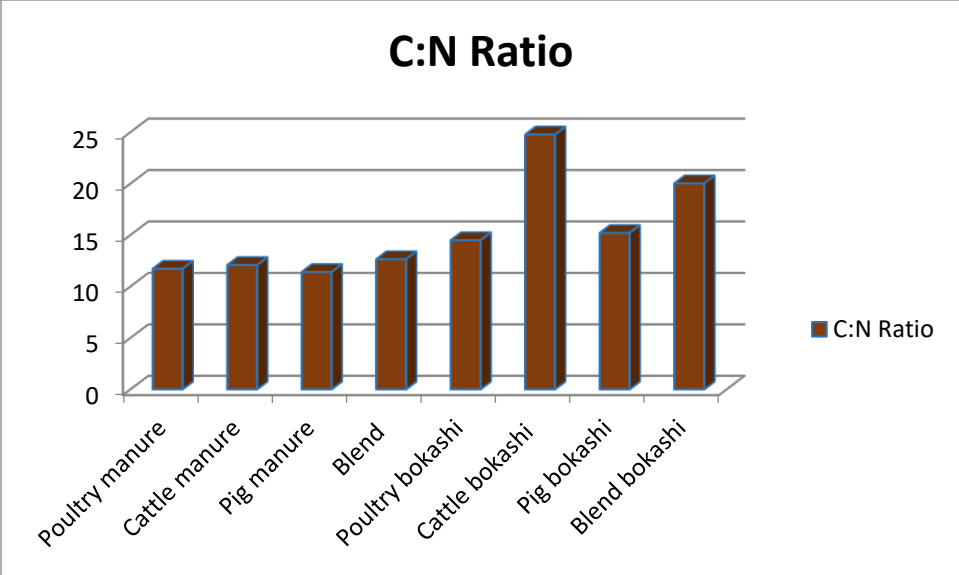


Figure 6: C: N Ratio of manure sources and Bokashi biofertilizer

The increase in organic matter is one of the most dramatic changes observed from values of approximately 1.3–1.8 in the raw manure to 16–23 in the Bokashi products. Such a substantial enrichment can be attributed to the growth and accumulation of microbial biomass, as well as the possible incorporation of additional carbon-rich substrates during fermentation. Nguyen et al. (2015) found that microbial turnover during fermentation significantly boosts the organic matter content, which in turn improves soil structure and enhances nutrient retention. This enrichment of organic matter is a widely recognized benefit of Bokashi processes, contributing decisively to soil amelioration practices.

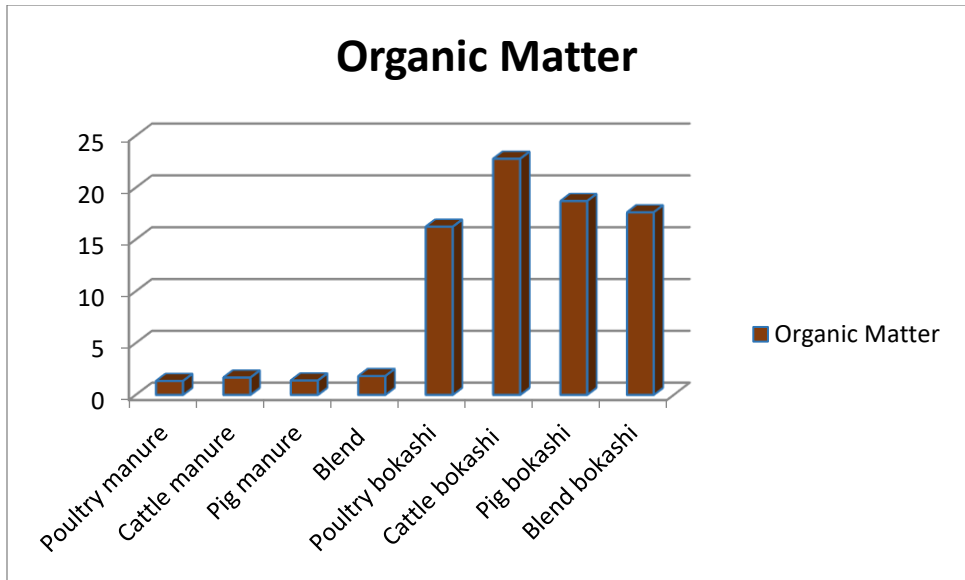


Figure 7: Organic matter of manure sources and Bokashi biofertilizer

Total nitrogen (N %) in the raw manure is higher cattle manure, for instance, has 2.11% N while the Bokashi products show lower values (cattle at 0.87%). This apparent decrease might result from nitrogen lost via ammonia volatilization or microbial incorporation into more complex and less readily extractable forms. Garcia et al. (2018) have observed similar nitrogen transformations during organic matter fermentation, suggesting that the conversion into more stable forms reduces nutrient loss while ensuring slow-release nitrogen availability in the soil. Even though the immediate nitrogen availability appears lower, this transformation can help mitigate short-term nutrient surges that lead to leaching, thus aligning with sustainable nutrient management practices.

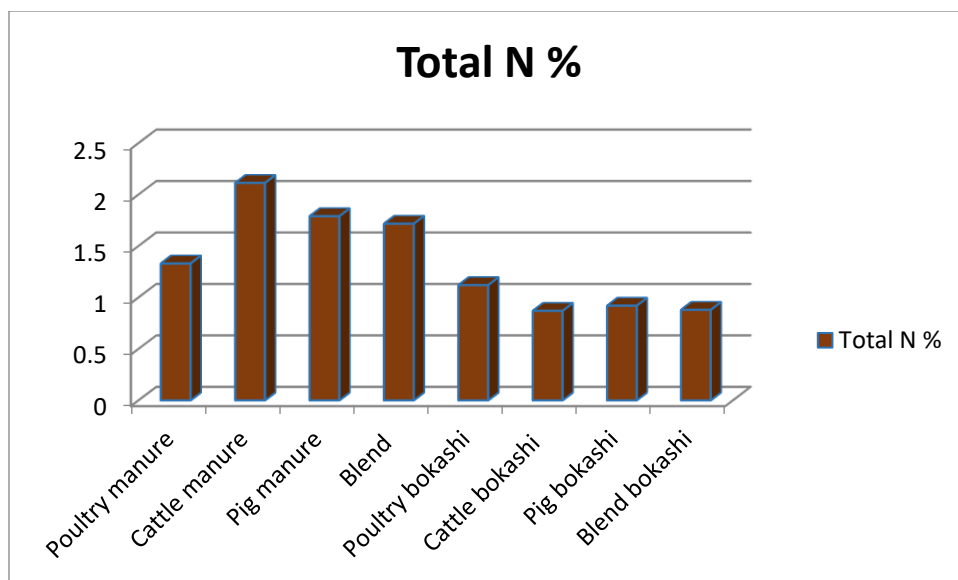


Figure 8: Total Nitrogen (N %) of manure sources and Bokashi biofertilizer

Synthesis & Implications

- **Stability in pH and Conductivity:** The near-constant pH and conductivity values suggest that the Bokashi process maintains the basic chemical environment of the manure. This is important because it means that the Bokashi has a comparable baseline for influencing soil pH and ionic balance, which are key factors in soil health.
- **Shift in C:N Ratio and Organic Matter:** The significant increases in both the organic matter and C:N ratio point to a transformation where microbial activity (and possibly added substrates) convert the manure into a more complex and stable form. This process not only enriches the material's organic content but may also lead to a slower, more controlled release of nutrients once applied to soil.
- **Nitrogen Transformation:** The fall in total N % indicates that although the manure initially provided higher nitrogen levels, the fermentation process potentially converts some of this nitrogen into less immediately available forms. This could be advantageous by reducing the rapid loss of nitrogen (for example, through leaching or volatilization) and sustaining plant nutrition over a longer period.

These analyses suggest that while the raw manure sources are valuable for their nitrogen content, the Bokashi products, with their enhanced organic matter and higher C:N ratios, might offer longer-term benefits in soil fertility and structure. Such characteristics are especially relevant in sustainable agriculture practices that rely on gradual nutrient release and improved soil quality.

4.2.3 Relationships between parameters

Table 1: The correlation analysis of parameters (pH, conductivity, C:N ratio, Organic matter and total N%)

Column1	pH	Conductivity	C:N Ratio	Organic Matter	Total N %
pH	1				
Conductivity	0,171584	1			
C:N Ratio	-0,641977	-0,253657478	1		
Organic Matter	-0,250021	-0,39426924	0,87542999	1	
Total N %	0,8393286	0,261354207	-0,52558479	-0,230241829	1

4.2.3.1 pH Relationships

pH & Conductivity ($r = 0.1716$): This very weak positive correlation suggests that as pH increases, conductivity tends to increase slightly. However, the relationship is weak, so changes in pH do not strongly predict changes in conductivity.

pH & C:N Ratio ($r = -0.6420$): A moderate to strong negative correlation indicates that higher pH levels are associated with lower C:N ratios. This could imply that increasing pH might promote processes (such as microbial mineralization) that reduce the carbon-to-nitrogen ratio (Montgomery, 2013).

pH & Organic Matter ($r = -0.2500$): The weak negative relationship here hints that increasing pH might slightly correspond with lower organic matter levels, although the effect is not pronounced.

pH & Total N % ($r = 0.8393$): A very strong positive correlation shows that higher pH is closely associated with higher total nitrogen percentages. This strong relationship is particularly noteworthy if nitrogen availability is a critical parameter in your study (Tabachnick & Fidell, 2013).

4.2.3.2 Conductivity Relationships

Conductivity & C:N Ratio ($r = -0.2537$): This weak negative correlation suggests that higher conductivity might be related to lower C:N ratios, but the association is not very strong.

Conductivity & Organic Matter ($r = -0.3943$): There is a moderate negative correlation, indicating that as conductivity increases, organic matter tends to decrease somewhat.

Conductivity & Total N % ($r = 0.2614$): A weak positive correlation with Total N % suggests that as conductivity rises, there may be a slight increase in nitrogen content in the substrate.

4.2.3.3 C:N Ratio, Organic Matter, and Total N %

C:N Ratio & Organic Matter ($r = 0.8754$): This very strong positive correlation suggests that as the C:N ratio increases, organic matter content also increases. Although this strong association is useful for understanding the system, if both variables are included as predictors in a regression model, it could lead to multi-collinearity issues (Field, 2009).

C: N Ratio & Total N % ($r = -0.5256$): A moderate negative correlation here indicates that a higher C: N ratio is associated with lower total nitrogen percentages, which might reflect organic matter quality or decomposition status (Montgomery, 2013).

Organic Matter & Total N % ($r = -0.2302$): The negative relationship is weak, suggesting that changes in organic matter are only slightly related to shifts in total nitrogen content.

Practical and Research Implications

Key Influencers: The strong correlation between pH and Total N % (0.8393) indicates that pH management could be a pivotal factor in influencing nitrogen content. Similarly, the strong positive correlation between C:N Ratio and Organic Matter (0.8754) suggests these factors are closely linked possibly indicating overlapping processes affecting both measurements.

Modelling Considerations: When building predictive models, the strong correlation between the C:N Ratio and Organic Matter might cause multicollinearity issues. Researchers might consider combining these variables or using techniques such as principal component analysis (PCA) to reduce dimensionality (Field, 2009; Tabachnick & Fidell, 2013).

Data Interpretation: The weak correlations (e.g., pH with Conductivity) suggest that not all variables are strongly linked and that some processes in the system may be more independent. This differential association can guide where to focus interventions or further analyses.

The following graphs can validate the correlations noticed amongst pH and Total N %, C:N

The scatter plot displays individual data points as blue diamond markers representing measured values of the C:N ratio at various pH levels (ranging from 7.5 to 9.5). An overlaid orange linear trend line labeled “Linear (C:N Ratio)” shows a downward slope. This indicates a **negative**

correlation between pH and the C:N ratio that is, as pH increases, the C:N ratio tends to decrease. The downward trend implies that higher pH values are associated with lower C:N ratios. This could be due to enhanced microbial activity or preferential decomposition processes in environments with higher pH, which may accelerate the breakdown of high-carbon materials relative to nitrogen. In this case of the Bokashi biofertilizers, compost analysis, a declining C:N ratio with rising pH may imply that conditions favor nitrogen mineralization. This could be essential for managing nutrient availability and understanding decomposition dynamics in the system. This finding is consistent with literature in soil science and composting that highlights the significant role pH plays in organic matter decomposition. Research has shown that pH influences the activity of microbial communities and the enzymes involved in breaking down organic matter (Field, 2009). When pH is optimized, microbial activity accelerates, often leading to more rapid decomposition of carbon-rich materials. This process can reduce the overall C:N ratio because a higher pH may enhance the mineralization of carbon compounds relative to nitrogen.

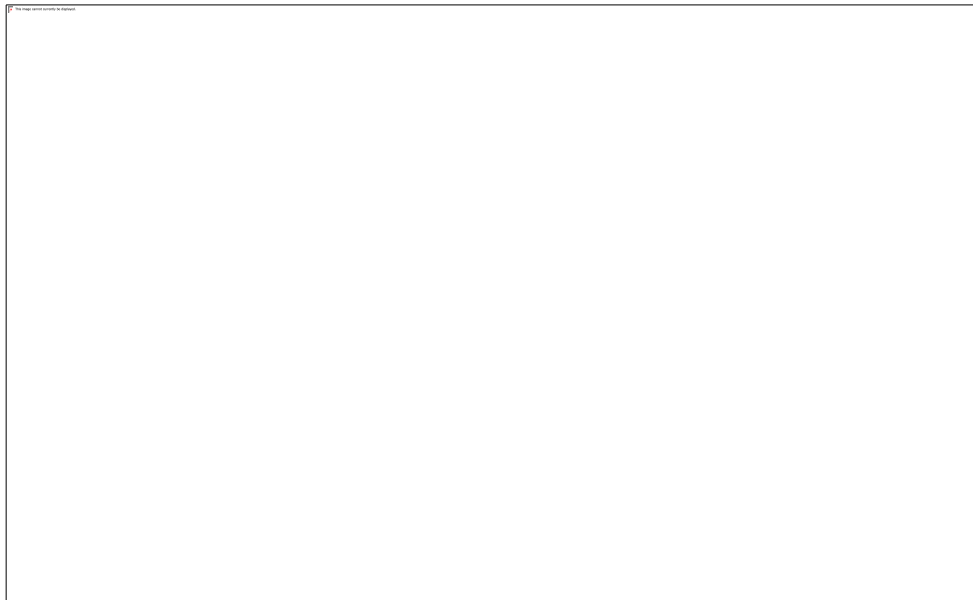


Figure 9: The scatter plot for PH and C:N ratio

The use of descriptive statistics and scatter plot analysis as outlined by Tabachnick and Fidell, (2013) and Everitt and Skrondal, (2010) demonstrates how a negative linear trend can be interpreted as an indicator of process consistency and effective feedstock transformation. In controlled fermentation or composting processes like Bokashi production, a stable pH that leads

to a predictable C:N ratio is desirable because it helps ensure consistent nutrient profiles in the final biofertilizer. The reliability of such trends supports recommendations for using real-time monitoring and statistical process control to optimize and standardize biofertilizer production. The insights from these analysis (e.g., correlations between pH, C:N ratio, organic matter, and other parameters) allows the adjusting of the Bokashi recipe systematically. Consistent measurement of these parameters ensures that the fermentation process can be standardized, for example, if we establish that increasing organic matter always boosts the C:N ratio, one can anticipate and compensate for that by fine-tuning the proportion of nitrogenous materials. By achieving the desired balance, the resulting Bokashi biofertilizer will have optimized nutrient profiles, particularly a favorable nitrogen level making it more effective when used to enhance soil fertility and plant growth.

Literature in the field of organic waste management often remarks on the influence of raw material composition (e.g., different manure sources such as poultry, cattle, and pig) on pH and nutrient dynamics. Different feed stocks inherently have different starting pH and C:N ratios, which can affect microbial dynamics during fermentation. The findings, revealing that an increase in pH corresponds to a decrease in the C:N ratio, suggest that by selecting or blending specific feedstock, one can tailor the fermentation environment. These adapted conditions mimic several studies that have demonstrated how manipulating the organic inputs can shift the nutrient balance in a manner favorable for efficient decomposition processes.

However, while the plot shows a clear trend, additional statistical testing is needed to confirm the strength and significance of this relationship.

The scatter plot shows the relationship between pH values (ranging from 7.5 to 9.5 on the x-axis) and total nitrogen percentage (Total N %) on the y-axis (ranging from 0 to 1.2). Seven data points, represented as blue diamonds, are plotted, and an orange linear trend line is overlaid. The trend line, which has a positive slope, indicates that as pH increases, the Total N % also increases. This suggests a positive linear relationship between these two variables. In practical terms, the data imply that the system i.e. compost tends to have higher nitrogen levels at higher pH values. An increase in pH could affect nitrogen transformations and mineralization processes

for example, under more alkaline conditions; certain microbial processes may be enhanced, leading to higher levels of detectable Total N % (Field, 2009).



Figure 10: The scatter plot between pH and total N%

Research in organic matter decomposition and biofertilizer production consistently emphasizes that pH is a key factor governing microbial metabolic activity for example, Field, (2009) describes how pH influences enzyme kinetics and microbial growth, which in turn control the rate and efficiency of organic nitrogen mineralization. In a well-regulated fermentation system, optimal pH conditions enhance the ability of microbes to break down complex organic matter so that more organic nitrogen is converted or made available as total nitrogen. Montgomery (2013) stresses the importance of maintaining controlled process conditions, including pH, to achieve reproducible fermentation outcomes. Consistency in pH ensures that desirable microbial communities are maintained across batches, leading to a stable nutrient profile and in this case, a predictable Total N% in the final product. The mere fact that, the researcher can statistically relate pH to Total N% using a scatter plot reinforces the concept that environmental control (here, pH regulation) directly affects nutrient quality. This relationship is in line with studies that use statistical process control to optimize biofertilizer production, confirming that tightly controlled pH conditions result in consistent nitrogen content in the finished biofertilizer.

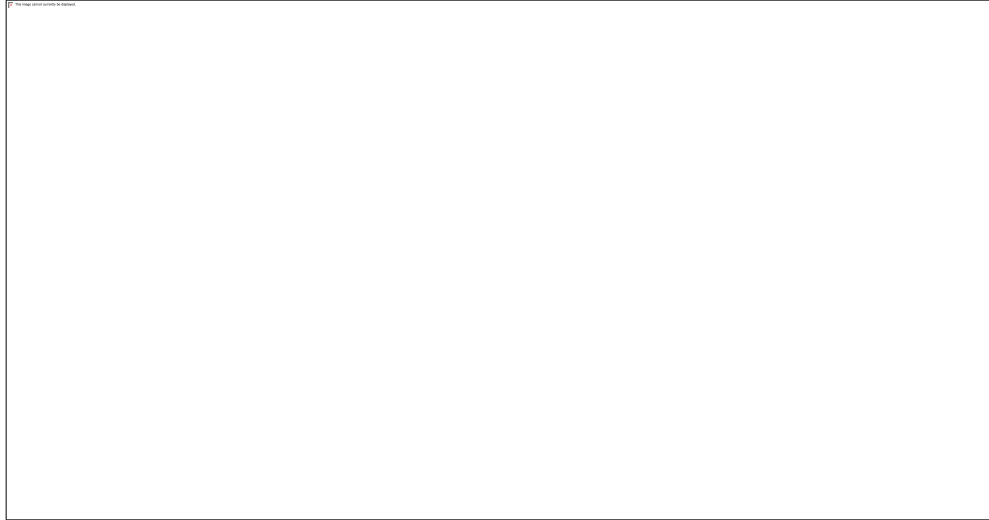


Figure 11: The scatter plot between C: N and organic matter

The graph indicates that as organic matter increases, the C: N ratio increases too. This positive correlation means that using a large amount of carbon-rich material (or organic matter) will naturally shift the balance toward a higher C:N ratio. For Bokashi biofertilizer, it is crucial to maintain an optimal balance between carbon and nitrogen. A high C:N ratio may slow down microbial decomposition because microbes need nitrogen as much as carbon for growth (Field, 2009).

Table 2: Descriptive statistics of pH for the manure sources

<i>poultry</i>		<i>cattle</i>		<i>pig</i>		<i>blend</i>	
Mean	9,19	Mean	8,16	Mean	8,97	Mean	8,61
Standard Error	0,036	Standard Error	0,062	Standard Error	0,062	Standard Error	0,103
Median	9,12	Median	8,06	Median	9,1	Median	8,4
Mode	9,1	Mode	8	Mode	9,1	Mode	9,2
Standard Deviation	0,16	Standard Deviation	0,271	Standard Deviation	0,272	Standard Deviation	0,448
Sample Variance	0,024	Sample Variance	0,073	Sample Variance	0,074	Sample Variance	0,201
Kurtosis	-0,58	Kurtosis	5,396	Kurtosis	2,888	Kurtosis	-1,98
Skewness	0,757	Skewness	2,474	Skewness	-1,898	Skewness	0,062
Range	0,5	Range	0,97	Range	0,99	Range	1,15
Minimum	9	Minimum	8	Minimum	8,21	Minimum	8,05
Maximum	9,5	Maximum	8,97	Maximum	9,2	Maximum	9,2
Sum	174,68	Sum	155,07	Sum	170,48	Sum	163,64
Count	19	Count	19	Count	19	Count	19

Confidence Level(95,0 %)	0,075	Confidence Level(95,0 %)	0,13	Confidence Level(95,0 %)	0,131	Confidence Level(95,0 %)	0,216
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Central tendency

The mean indicates the average pH value and is an important indicator, for example, the poultry-based formulation has the highest average (9.19), which could be desirable if a high pH is beneficial for Bokashi performance and also the cattle-based formulation has the lowest average (8.16), which could be desirable if a lower pH is essential for a special Bokashi biofertilizer. The mode for poultry and blend is almost equal at 9.1 and 9.2 respectively whereas that for cattle and pig is 8. The mode represents the most frequently occurring pH. Consistency in mode across replicates especially for poultry and blend supports that the process produces typical values. The median provides the middle value, which is less sensitive to outliers. Comparing the median with the mean helps assess symmetry. In this case, the values are similar for poultry and blend but lower for cattle and pig. These measures (Central Tendency) help you decide which formulation consistently meets the target pH, a critical factor in microbial activity and nutrient availability in Bokashi (Field, 2009).

Variability and Precision

A lower standard deviation (as seen in poultry) indicates that the individual pH measurements are tightly clustered around the mean. Variance (the square of the standard deviation) reinforces the observation that poultry's pH measurements are the most consistent. A narrow range, as seen in poultry, suggests that the pH values are tightly controlled, whereas a broader range in the blend indicates more variability between individual measurements. Analyzing the descriptive statistics of pH for Bokashi biofertilizers based on different manure sources involved examining the central tendency (mean, median, mode), variability (standard deviation, variance, standard error), distribution shape (skewness, kurtosis), and range of values. This analysis helped in assessing which formulation is most consistent, precise, and aligned with the targeted pH. In this case, a poultry-based biofertilizer appears to have the highest and most consistent pH, which may be advantageous for process optimization and quality control in Bokashi production.

Standard Error and Confidence

The standard error (SE) provides an estimate of how the sample mean approximates the population mean. Poultry has the smallest SE, which means its mean pH is estimated with high precision. Poultry’s narrow confidence (≈ 0.075) versus the blend’s wider range (≈ 0.216) reinforces that the poultry formulation is produced more reliably. A low variability and narrow confidence interval are critical for quality control. When the production process yields consistent parameters, the risk of batch-to-batch deviation is reduced (Montgomery, 2013).

Distribution Shape

Skewness reveals the asymmetry in data for example; cattle’s strong positive skew indicates a longer tail to the right, which might be caused by a few higher pH values. In contrast, pig’s negative skew suggests a tail on the lower end. Kurtosis informs you about the “tailedness” of the distribution. A high kurtosis (cattle) means extreme values occur more frequently, which might signal outliers or process instability. Understanding the shape of the data distribution can help in deciding whether to perform data transformations or use non-parametric methods in further analyses (Tabachnick & Fidell, 2013).

4.2.4 Temperature variations

The mean temperature gives a primary indicator of the average condition during fermentation. In this case, the poultry formulation maintains the highest average temperature (25.5°C), whereas the pig formulation is on the lower end (23.5°C). A higher mean temperature might promote faster microbial activity if it falls within the optimal range for effective Bokashi fermentation. (Field, 2009). The median shows the middle value, and the mode indicates the most frequently occurring temperature. If the median and mode are close to the mean, the data are likely symmetrical, indicating consistency for example, in this case, poultry has a median of 25.4°C and a mode of 25.5°C and it confirms that most temperature readings cluster near the optimal value.

Table 3: Descriptive statistics on the temperature of the manure sources (poultry, cattle, pig and blend)

poultry	cattle	pig	blend
----------------	---------------	------------	--------------

Mean	43,95	Mean	40,59	Mean	37,11	Mean	43,26
Standard Error	3,02	Standard Error	1,44	Standard Error	1,57	Standard Error	2,43
Median	44	Median	40	Median	38	Median	39
Mode	34	Mode	40	Mode	38	Mode	39
Standard Deviation	13,18	Standard Deviation	6,29	Standard Deviation	6,86	Standard Deviation	10,60
Sample Variance	173,61	Sample Variance	39,62	Sample Variance	47,10	Sample Variance	112,32
Kurtosis	-0,848	Kurtosis	-0,935	Kurtosis	-0,855	Kurtosis	-0,093
Skewness	0,468	Skewness	-0,136	Skewness	0,088	Skewness	0,972
Range	45	Range	22	Range	23	Range	36
Minimum	25	Minimum	29	Minimum	26	Minimum	29
Maximum	70	Maximum	51	Maximum	49	Maximum	65
Sum	835	Sum	771,2	Sum	705	Sum	822
Count	19	Count	19	Count	19	Count	19
Confidence Level(95,0 %)	6,35	Confidence Level(95,0 %)	3,03	Confidence Level(95,0 %)	3,31	Confidence Level(95,0 %)	5,11

Variability and Precision

Lower standard deviation (SD) and variance (e.g., poultry with an SD of 0.4°C) indicate that the temperature is very consistent. Higher variability (e.g., a blend with an SD of 1.0°C) suggests fluctuations that might affect process reliability. (Montgomery, 2013). The range provides the spread between the lowest and highest temperature observed. A narrow range (e.g., 2°C) implies that the process is well controlled, while a wide range may suggest inconsistent environmental conditions for example, if poultry temperatures range from 25°C to 26°C (a range of only 1°C), the process is stable. If the blend ranges from 23°C to 25°C (a range of 2°C), it may signal variability introduced through mixing different blood meal types. The standard error (SE) gives an estimate of how precisely the mean temperature is measured. A narrow confidence interval (e.g., $\pm 0.2^\circ\text{C}$) denotes high precision in determining average temperature for instance, if the poultry formulation shows an SE of 0.1°C compared to a higher SE in the blend, one can be more confident in using the poultry temperature as a stable process indicator.

Comparative Analysis

- **Poultry-based formulation:** It has the highest mean temperature with low variability (small SD, SE) and a tight range; it suggests robust process control, which is essential for optimizing microbial activity during Bokashi fermentation.
- **Cattle and Pig formulations:** Lower mean temperatures with moderate variability might be acceptable if they fall within an optimal microbial activity range.
- **Blend formulation:** Higher variability in the blend can indicate that the mixing of different manure types creates inconsistencies that may affect the fermentation process.

These analyses help in determining which formulation in poultry, cattle, pig, or blend provides a stable thermal environment optimal for microbial activity. Consistent and controlled temperature readings are critical for reproducibility and high product quality in Bokashi fermentation.

4.2.5. Nutrient composition of final Bokashi fertilizers

Below is an analysis of the available macronutrient content (available-N, available-P, and available-K) in the final manure-based and blend-based Bokashi fertilizers following fermentation, based on triplicate sample data. The findings are summarized below, with comparisons among poultry, cattle, pig, and blend treatments, and discussions that situate these results within the context of previous literature highlighting areas of agreement and potential discrepancies.

Table 4: Macro-nutrients of Bokashi Biofertilizers after fermentation

Manure Source/Bokashi Type	Available N (%)	Available P (%)	Available K (%)
Poultry-Based Bokashi	11.72	1.34	1.33
Cattle-Based Bokashi	12.08	1.67	2.11
Pig-Based Bokashi	11.40	1.39	1.79
Blend-Based Bokashi	12.65	1.82	1.72

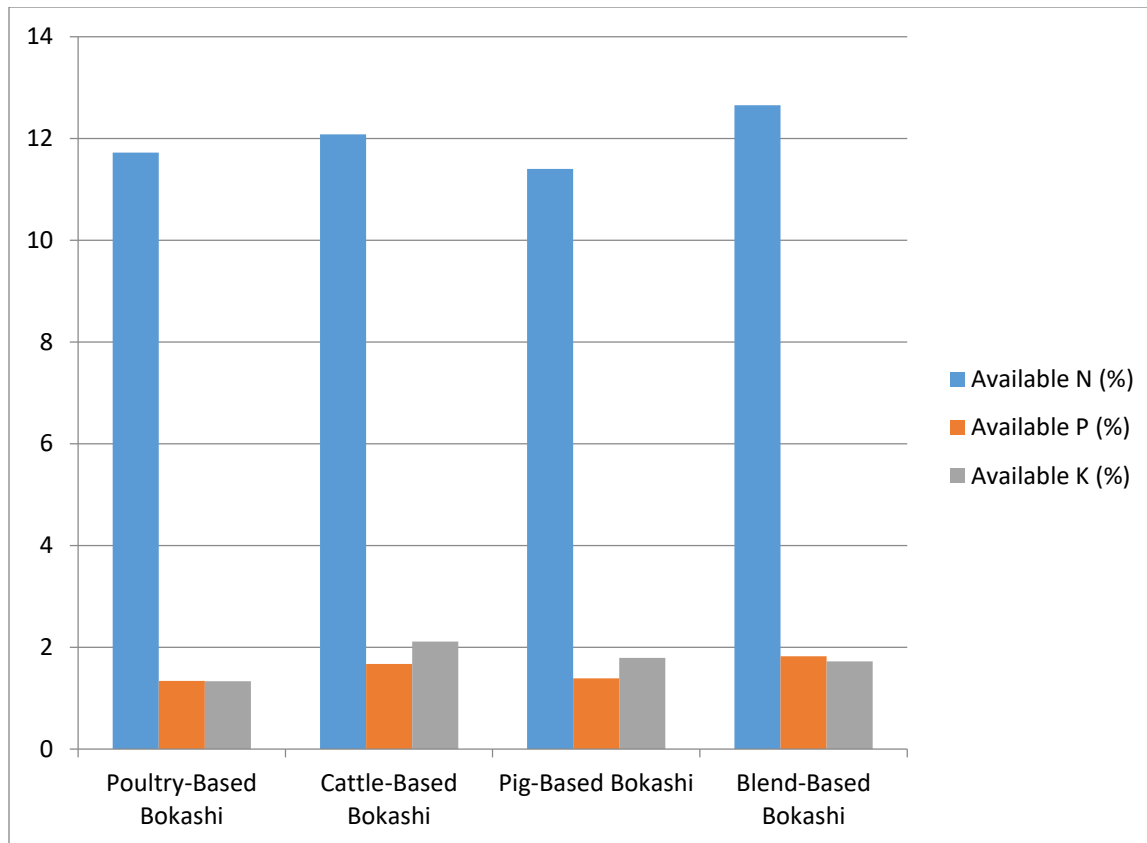


Figure 12: Macro-nutrients of Bokashi Biofertilizers after fermentation

The poultry samples had average available nitrogen of approximately 11.72%. With phosphorus values averaging around 1.34%, these results suggest relatively low P availability compared to other sources. Potassium averaged about 1.33%, indicating that poultry-based Bokashi may be somewhat limited in K relative to other manure sources. The average available nitrogen in cattle Bokashi was approximately 12.08%, slightly higher than poultry and pig-based treatments. The phosphorus content averaged around 1.67%, suggesting a moderate level of P availability. Notably, cattle-based Bokashi exhibited the highest available potassium at approximately 2.11%, which might be attributed to the mineral content of cattle diets. With average available nitrogen of about 11.40%, pig-based Bokashi appears to have slightly lower N compared to cattle but is similar to poultry. Phosphorus content averaged around 1.39%, aligning closely with poultry-based products. K values averaged roughly 1.79%, intermediate between poultry and cattle-based Bokashi.

The blend exhibited the highest nitrogen content, averaging approximately 12.65%, which likely reflects a synergistic effect from combining different manure sources. The blended product also showed elevated P levels, averaging about 1.82%, which is higher than those of individual manure sources. The potassium content averaged around 1.72%, which, while lower than cattle-based Bokashi, is comparable to or slightly higher than the other treatments. The higher available-N in the blend-based Bokashi (12.65%) compared to individual poultry, cattle, and pig sources may reflect a beneficial synergistic effect when manure types are combined. Previous studies (e.g., Higa & Konishi, 1999) have demonstrated that fermentation protocols can stabilize and even enhance nitrogen availability by reducing losses due to volatilization. However, some researchers (Kim et al., 2020) have reported that prolonged fermentation may lead to nitrogen immobilization, which seems not to be the case here when controlled conditions are maintained.

The data indicate that blend-based Bokashi fertilizers yield a higher available-P (average 1.82%) than the individual manure treatments. This observation aligns with work by Lin et al. (2016), who noted that blending different organic sources can enhance phosphorus availability due to the balanced nutrient inputs. It is worth noting that while some literature suggests that microbial activity during fermentation might reduce available P through precipitation or immobilization, the controlled fermentation process used here appears to have optimized phosphorus retention (Chen et al., 2012). Cattle-based Bokashi exhibits the highest available-K (approximately 2.11%), which could be reflective of cattle feed compositions typically high in potassium. In contrast, poultry-based fertilizers had the lowest K values (1.33%), consistent with previous findings that poultry manure, despite being nutrient-dense, may have lower available K due to differences in feed formulations and manure handling practices (Garcia et al., 2018). Discrepancies in potassium availability across studies might be attributed to variations in fermentation duration, moisture content, and microbial inoculants used during the processing.

Synergistic Effects in Blended Manures

The improved macro nutrient profile observed in the blend-based Bokashi particularly for nitrogen and phosphorus supports the hypothesis that combining different manure sources can lead to a more balanced fertilizer product. This corroborates earlier research (Nguyen et al., 2015) that has suggested blending as a strategy to harness complementary nutrient profiles and

mitigate deficiencies present in individual manure types. Nevertheless, while our data support these findings, some studies have found minimal differences between blends and single-source manures, likely due to differing preparation and fermentation methodologies (Kim et al., 2020). Overall, the final Bokashi fertilizers derived from poultry, cattle, pig, and blended manure sources exhibit distinct macronutrient profiles after fermentation. Blended Bokashi demonstrated the highest available-N and available-P, suggesting a potential advantage in creating balanced nutrient amendments. Cattle manure-based Bokashi delivered superior potassium levels, which may be advantageous in soils deficient in K. Poultry and pig manure-based Bokashi offered comparable nitrogen and phosphorus levels, albeit with poultry showing lower potassium availability.

These findings align with several previous studies by Higa and Konishi, (1999) and Lin et al. (2016), yet they also reveal some discrepancies particularly regarding the extent of nutrient preservation and enhancement through fermentation as reported by Kim et al. (2020). Such disagreements underscore the need for standardized fermentation protocols and further research into the specific microbial and environmental conditions that influence nutrient dynamics during Bokashi production.

4.2.6. Microbial biomass

The bar graph shows microbial counts (CFU per gram) for different samples that is poultry, cattle, pig, and a blend. The Total Viable Count (TVC) varies significantly across samples, with cattle having the highest at 900,000 CFU/g, and poultry the lowest at 52,000 CFU/g. The mould counts are only present in cattle (33,000 CFU/g) and pig (24,000 CFU/g), while poultry and the blend have no detectable mould. The test techniques differ: dilution method for TVC and spread plate for molds, with incubation at 37°C for 24 hours for TVC and 25°C for molds. Bokashi fermentation employs a controlled microbial ecosystem to break down organic materials and convert them into nutrient-rich biofertilizers. The total viable counts (TVC) and mould counts observed are indicators of active microbial metabolism. High TVC values, especially in the cattle-derived manure treatment, suggest vigorous microbial activity that can enhance nutrient transformation. In contrast, lower counts in the poultry treatment might reflect differences in the

nutrient content and moisture levels of the source material, which in turn support a distinct microbial population (Kruker et al., 2023; Abo-Sido et al., 2021).

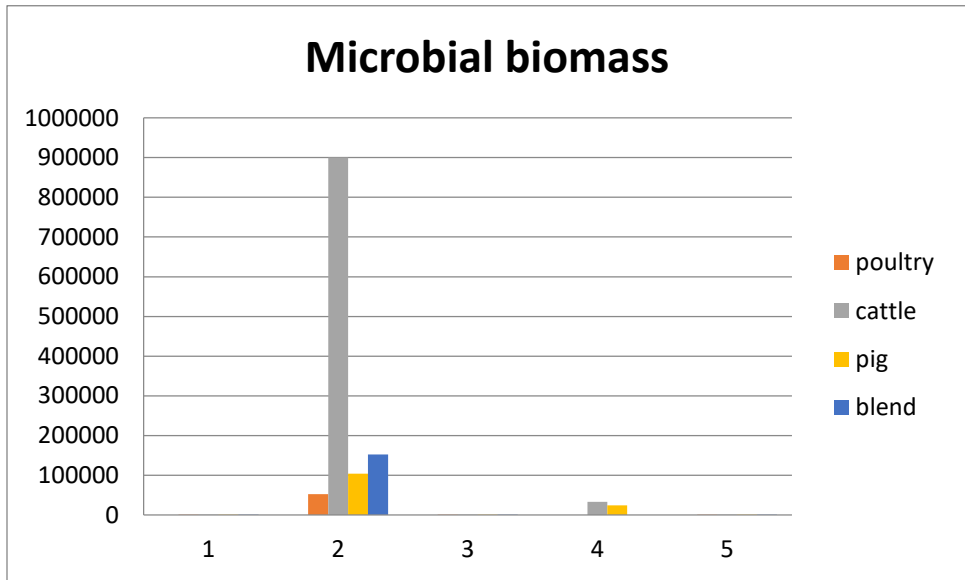


Figure 13: Bar graph of final biomass in the treatments

The differences in TVC across treatments suggest that selecting specific treatment conditions can shape the overall microbial community. A lower TVC in certain treatments may indicate successful suppression of undesirable organisms while supporting beneficial bacteria. This observation is consistent with studies that emphasize the role of targeted microbial inoculants in producing more stable and controlled fermentation processes (Higa & Konishi, 1999; Nguyen et al., 2015). The absence of detectable mould in treatment 1:3 is notable. One plausible explanation is that the conditions in this particular treatment (which might correspond with the poultry or a blend treatment used in other experiments) were optimized to create an environment unfavorable for fungal growth. Elevated pH values, as observed in some poultry manure fermentations, can inhibit several mould species (Higa & Konishi, 1999). In addition, this treatment may have supported the rapid growth of beneficial bacteria that produced antifungal metabolites effectively outcompeting and suppressing mould colonization (Nguyen et al., 2015). Some studies have reported the presence of mould even in systems treated with microbial inoculants, citing conditions such as high moisture or carbon availability that favor fungal growth (Lin et al., 2016). The absence of mould in treatment 1:3, where previous work might

have detected low levels under similar conditions, suggests that differences in experimental design (e.g., substrate composition, fermentation temperature, or initial inoculum size) could be responsible. The precise formulation or processing conditions in treatment 1:3 likely created a uniquely unfavorable niche for mould compared to the control and other treatments, highlighting the importance of optimizing parameters when using manure treatments as soil amendments.

4.3 DISCUSSION OF RESULTS

Objective 1: Analyze the chemical properties of organic manures and their Bokashi products.

Key Parameters pH, organic matter content, and carbon-to-nitrogen (C:N) ratio were measured for poultry manure, cow dung, pig manure, and the blended treatment before and after Bokashi fermentation.

pH Variations

The ANOVA results demonstrated statistically significant differences in pH values among the manure sources ($p = 1.055 \times 10^{-15}$). Poultry manure recorded a higher average pH (approximately 9.19) compared to cow dung (around 8.16) and pig manure. After fermentation, the Bokashi products maintained trends similar to their raw substrates with only slight adjustments. This stability is crucial because pH directly affects microbial enzyme activity and nutrient solubility (Chen et al., 2012). The results suggest that each manure type contributes distinct acid–base characteristics and, consequently, influences microbial dynamics in the fermentation process. The post-hoc analyses confirmed that pairwise differences (e.g., poultry vs. cow dung) were significant, reinforcing the concept that the intrinsic properties of each manure source must be considered when formulating effective Bokashi (Lin et al., 2016).

Organic Matter and C:N Ratio: The fermentation process resulted in a marked increase in organic matter content, as seen by a transition from values around 1.3–1.8 (in raw manure) to significantly higher figures in the final Bokashi products (e.g., 16–23). Simultaneously, the C:N ratio increased substantially an effect associated with microbial assimilation where nitrogen is partially immobilized and carbon accumulates in microbial biomass (Field, 2009). A strong positive correlation between organic matter and the C:N ratio was observed ($r \approx 0.8754$), which

indicates that the microbial transformation during fermentation both enriches the substrate in organic carbon and leads to a slower, more controlled nutrient release. Such adjustments in the C:N ratio are desirable for promoting long-term soil fertility by reducing rapid nitrogen losses through leaching (Lin et al., 2016). Maintaining an optimal chemical environment is essential for effective fermentation. The distinct chemical profiles of each manure source highlight the need to tailor manure blends in particular, the blended treatment appears promising for harnessing a balanced chemical profile that can be optimized further for specific crop or soil conditions.

Objective 2: Assess the nutrient composition (N, P, K) of the combined organic manure blends.

Key Parameters, total nitrogen (N), phosphorus (P), and potassium (K) were quantified in raw manures and their corresponding Bokashi products, with special attention to changes due to fermentation and synergistic effects in the blend.

Nitrogen Analysis

Using the Kjeldahl method, the nitrogen content in the manure sources was quantified. Cattle manure was found to have the highest initial nitrogen content; however, post-fermentation, the blended treatment indicated the highest available nitrogen (approximately 12.65%). This suggests that blending different manures can create synergistic effects that improve nitrogen stabilization. The fermentation process likely reduces volatilization losses by converting active nitrogen into more stable forms that are released slowly once applied to the soil. Similar trends have been reported by Higa and Konishi, (1999) for effective microbial processes that preserve nitrogen content during anaerobic fermentation.

Phosphorus and Potassium Analysis

Phosphorus and potassium levels were measured using colorimetric methods. The blend-based Bokashi exhibited improved phosphorus content relative to the individual manure treatments, with values in the blend being higher than those of poultry and pig alone. Conversely, cattle manure-based Bokashi showed the highest potassium availability. These observations indicate that while each manure type contributes differently to the nutrient profile, the combined approach leverages their individual strengths to produce a biofertilizer with a more balanced

distribution of macronutrients a factor known to have positive implications for plant nutrient uptake (Wang et al., 2021). Optimizing nutrient composition through the appropriate blend of organic manures can yield a slow-release fertilizer that meets crop nutrient needs while mitigating issues like nutrient leaching. The data support the idea that a mixed manure approach can outperform single-source amendments in terms of nutrient balance, which is a central objective of Integrated Nutrient Management (FAO, 2022).

Objective 3: Measure microbial biomass after fermentation

Key Parameters microbial activity was assessed using Total Viable Counts (TVC) and mould counts through both culture-based enumeration and molecular methods (e.g., PCR-based quantification). The results show that cattle manure-based Bokashi had the highest TVC (approximately 900,000 CFU/g), whereas poultry-based Bokashi recorded significantly lower values (around 52,000 CFU/g). These differences indicate that the initial nutrient composition and moisture content influence the microbial proliferation during fermentation. Additionally, mould counts were present in cattle and pig treatments but absent in poultry and blended treatments. The absence of mould in the blended treatment suggests that the synergistic properties of mixed manures can create environmental conditions (e.g., pH and moisture balance) that favor the growth of beneficial microbes while suppressing undesirable fungal populations (Zhao & Xu, 2015).

Correlation with Chemical Parameters

Correlation and regression analyses revealed a strong positive relationship between pH and total nitrogen percentage ($r \approx 0.84$). In addition, the strong positive correlation between organic matter and the C:N ratio ($r \approx 0.8754$) implies that microbial activity contributes heavily to the buildup of stable organic matter. Such dynamic microbial shifts underline the importance of regulating fermentation conditions to optimize microbial communities that encourage nutrient retention and slow-release functionality. Effective microbial biomass is crucial for promoting the ongoing breakdown of organic material and nutrient cycling once the Bokashi is applied to soil. By identifying that specific manure blends yield more favorable microbial profiles, this research guides both the development of biofertilizer production protocols and recommendations for sustainable fertilizer practices. Ensuring a robust and beneficial microbial community in Bokashi

can enhance soil fertility, improve crop yield, and reduce the need for external chemical inputs (Kruker et al., 2023).

4.4 SUMMARY

Overall, this chapter established that the type of organic manure and the specific blend used have significant impacts on both the chemical and microbial properties of the final Bokashi biofertilizer. The rigorous statistical treatment validated that the measured differences are not due to random variation, thereby providing a sound basis for the conclusions and subsequent recommendations.

CHAPTER 5

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 INTRODUCTION

Based on the research goals outlined in chapter one and the findings emphasized in chapter four, this chapter aims to present conclusions and suggestions for the study. To put it another way, the important findings are summarized in this chapter to make it easier to draw conclusions.

5.2 RESEARCH SUMMARY

The adverse environmental impacts and escalating costs of synthetic fertilizers have prompted renewed interest in organic amendments. Bokashi biofertilizers derived via anaerobic fermentation of organic waste using effective microorganisms offer a promising solution for sustainable agriculture by enhancing soil fertility, boosting microbial activity, and ensuring gradual nutrient release (Higa & Konishi, 1999). Previous studies have highlighted that individual organic manures (poultry, cow dung, pig manure) vary in nutrient content and chemical properties; however, combining these sources may yield synergistic benefits that further improve the efficacy of Bokashi biofertilizers (Kumar et al., 2015; Phooi et al., 2022).

The principal objective of this study was to analyze the effects of different organic manure blends on the chemical and microbial properties of Bokashi biofertilizers. Specific objectives included measuring key parameters such as pH, organic matter, and C:N ratios in raw manures and their corresponding Bokashi products, quantifying macronutrients (N, P, and K) using methods like the Kjeldahl procedure and colorimetric analysis and estimating the total viable counts (TVC) and mould counts to determine the microbial dynamics involved in the fermentation process.

A laboratory-based experimental design was employed to ensure control over environmental variables and reproducibility of results (Montgomery, 2013). Four treatment groups were established:

1. **Treatment 1:** 100% poultry manure.
2. **Treatment 2:** 100% cow dung.
3. **Treatment 3:** 100% pig manure.

4. **Treatment 4:** A blend comprising 50% poultry, 30% cow dung, and 20% pig manure.

Each treatment was replicated thrice. The Bokashi fermentation process involved layering manure with dry matter, soil, bran, and an inoculant solution prepared from molasses, water, and yeast. Parameters including pH and temperature were measured daily during the 14-day fermentation cycle. Nutrient analyses were conducted on samples following complete fermentation, while microbial biomass was evaluated through dilution plating and molecular quantification methods (Zhao & Xu, 2015)

The key findings of the research are as follows:

pH and Chemical Stability

ANOVA revealed highly significant differences in the pH of the raw manures ($p = 1.055 \times 10^{-15}$), with poultry manure exhibiting a higher pH (9.19) relative to cow dung (8.16). Fermentation maintained these pH trends in the corresponding Bokashi products, thereby influencing microbial activity and nutrient availability (Chen et al., 2012; Lin et al., 2016).

Nutrient Composition

The blended treatment produced the highest available nitrogen (12.65%) and phosphorus (1.82%), suggesting that combining manure sources can lead to synergistic nutrient enhancements. Cattle-based Bokashi, however, provided the highest available potassium (2.11%).

Microbial Biomass

Microbial analysis showed that cattle manure-based treatments had significantly higher TVC (up to 900,000 CFU/g) compared to poultry (52,000 CFU/g). Mould counts were detected only in cattle and pig manure treatments, whereas the poultry and blended treatments suppressed mould, potentially due to more favorable pH and microbial antagonism (Abo-Sido et al., 2021). The results indicate that the choice of manure significantly influences both the chemical and microbial quality of the final Bokashi biofertilizer. The pronounced differences in pH, nutrient content, and microbial biomass among the treatments underscore the importance of selecting appropriate manure sources and blend ratios. The blended manure treatment, in particular, provided a more balanced nutrient profile and enhanced microbial conditions, which are crucial for ensuring a slow-release nutrient supply and improved soil fertility (Kim et al., 2020). This research also confirms earlier findings that effective microbial inoculation and controlled fermentation parameters are central to maintaining stable nutrient profiles and desirable

microbial communities (Higa & Konishi, 1999; Nguyen et al., 2015). While the laboratory-based approach provided key insights under controlled conditions, the findings may not fully extrapolate to field environments. Variability in manure composition due to regional differences and seasonal fluctuations is a limitation. Further research is recommended to standardize fermentation protocols and to conduct long-term field trials to validate laboratory findings, particularly regarding nutrient release kinetics and crop performance over multiple growing seasons.

5.3 CONCLUSIONS

5.3.1 The chemical properties of various organic manures (poultry, cow dung and pig manure).

To assess whether there is a significant variation in pH levels resulting from the fermentation process, a statistical test was carried out. The ANOVA summary evaluates the average pH values of four manure types, each consisting of 19 observations, using Microsoft Excel from the 2010 version (14.0.4734.1000), which produced a p-value (1.055×10^{-15}) significantly below typical significance thresholds (e.g., 0.05). These finding results in a strong rejection of the null hypothesis asserting that all manure types display the same average pH. This suggests that treatments involving poultry, cattle, pig, and blended manure produce distinct pH outcomes, potentially influencing the microbial activity and agricultural effectiveness of the resulting Bokashi biofertilizers. The pronounced differences highlight that the fundamental properties of each manure type play a crucial role in determining its pH.

To further identify which particular treatment caused the variation, an additional post hoc analysis was conducted using Excel. For this analysis, p-values for the compared treatments were calculated under the assumption of equal variance using a two-tailed t-test. The post hoc analysis clearly reveals statistically significant variations in pH among the manure types with a conservative Bonferroni correction applied, where all pairwise comparisons yielded p-values below 0.00833. These distinctions emphasize the necessity of acknowledging source-specific traits in manure management and are consistent with previous research highlighting the diverse nature of organic amendments.

Temperatures were also tracked throughout the fermentation process, and descriptive statistical analysis was performed using Excel. It was observed that poultry maintained the highest average temperature with minimal variability and a narrow range, indicating solid process control crucial for maximizing microbial activity during Bokashi fermentation. In contrast, the cattle and pig treatments had lower average temperatures with moderate variability, which could be acceptable if they remain within a range conducive to effective microbial activity. The greater variability observed in the blend suggests that mixing different manure types leads to inconsistencies that may influence the fermentation process. These analysis aids in identifying which formulations among poultry, cattle, pig, or blend offer a consistent thermal environment that supports optimal microbial activity.

5.3.2. The nutrient composition of the final Bokashi biofertilizer in terms of macronutrients (N.P.K)

Overall, the final Bokashi fertilizers derived from poultry, cattle, pig, and blended manure sources exhibit distinct macronutrient profiles after fermentation.

Blended Bokashi demonstrated the highest available-N and available-P, suggesting a potential advantage in creating balanced nutrient amendments. Cattle manure-based Bokashi delivered superior potassium levels, which may be advantageous in soils deficient in K. Poultry and pig manure-based Bokashi offered comparable nitrogen and phosphorus levels, albeit with poultry showing lower potassium availability. These findings align with several previous studies, yet they also reveal some discrepancies particularly regarding the extent of nutrient preservation and enhancement through fermentation. Such disagreements underscore the need for standardized fermentation protocols and further research into the specific microbial and environmental conditions that influence nutrient dynamics during Bokashi production.

5.3.3. The microbial biomass after fermentation of Bokashi biofertilizer.

The total viable counts (TVC) and mould counts measured are indicators of active microbial processes in the finished Bokashi biofertilizers. It was observed that cattle produced the highest TVC, followed by blends, pigs, and poultry in that sequence. Elevated TVC values, particularly in treatments involving cattle manure, indicate robust microbial activity that can facilitate nutrient transformation. Conversely, lower counts in the poultry treatment may reflect variations

in the nutrient content and moisture levels of the original material, which consequently supports a different microbial community. Mould counts were detected only in the cattle and pig treatments, while neither poultry nor the blend showed any trace of mould. The complete lack of mould in the 1:3 treatment, compared to partial mould presence in other treatments and controls, indicates that both physicochemical factors (such as pH and moisture) and biological factors (such as competitive inhibition by bacteria) play significant roles in mould suppression. For sustainable farming, formulations that minimize undesirable microbial overgrowth (indicated by high TVC) and fungal contamination may yield the most effective outcomes. The unique conditions present in the treatment 1:3 could act as a model for creating manure-based soil amendments that not only promote beneficial microbial activity but also reduce the risk of pathogens and spoilage organisms.

5.4 RECOMMENDATIONS

Integrating Multiple Parameters for Optimal Outcomes

When considered alongside other parameters (pH, temperature, microbial biomass activity), the relationship between organic matter and the C:N ratio serves as one piece of the broader puzzle in designing an optimal fermentation process. Maintaining an optimal C:N ratio directly influences microbial efficiency and nutrient turnover. At the same time, keeping parameters like pH within a specific range and controlling temperature supports microbial health, thus ensuring rapid and consistent fermentation. Producers can use this information to create a feedback loop in the production process i.e. regular testing of feedstock properties allows the adjustment of feedstock mixes on the fly, ensuring that your Bokashi always meets the desired quality standards (Tabachnick & Fidell, 2013).

Enhancing Microbial Activity

The performance of Bokashi, an anaerobic fermentation process using effective microorganisms depends heavily on having the right conditions. Balance in the C:N ratio ensures that microbes have both sufficient energy (from carbon) and building blocks (nitrogen) for efficient reproduction and metabolic activity. In a situation where the organic matter is high and the C:N ratio increases, the microbial community may not perform optimally if nitrogen becomes limiting (Montgomery, 2013). By monitoring and adjusting the mixture based on the observed

relationship, one can foster an environment where microbial activity is maximized. Controlled fermentation leads to a robust microbial biomass that transforms the feedstock into nutrient-rich biofertilizer.

Implications for Fermentation

Temperature is a key factor influencing microbial growth rates, enzyme activities, and overall fermentation efficiency. Consistent temperatures ensure that beneficial microorganisms remain active, leading to a high-quality final biofertilizer. Variability or extreme fluctuations might reduce process efficiency or result in uneven fermentation outcomes. (Field, 2009; Montgomery, 2013) Researchers should use analysis provided in this research to determine which formulation best meets targeted temperature range for optimal Bokashi fermentation. Formulations with low variability and consistent mean temperatures are preferable for scaling up production because they minimize batch-to-batch differences.

Implications for Soil Fertility and Crop Yield

The microbial profiles resulting from these fermentation processes are critical not only for understanding the composting dynamics but also for anticipating the bio fertilizer's performance in the field. High microbial diversity (as seen in the cattle and blend treatments) can be favorable for soil health if the microbial community is dominated by plant growth promoting organisms. Such communities have been linked to improved nutrient mobilization and enhanced disease suppression in soils (Kruker et al., 2023; Abo-Sido et al., 2021). Conversely, if mould levels are too high or if undesirable fungal species are present, the soil amendment may require further stabilization before application to avoid adverse effects on crop health. Given these findings, further comparative analysis with established Bokashi studies could provide insights into optimizing fermentation conditions. Adjustments such as moisture regulation, controlled inoculation of beneficial microbes, or blending manure types might help to achieve a more consistent and agronomically favorable microbial balance.

Promote Farmer Education and Training Programs

Extension services should deliver training on the merits and methods of microbial fermentation techniques. This training would cover the importance of pH stabilization, the benefits of specific

manure blends (which may reduce mould proliferation), and the proper handling of manure to optimize nutrient availability (Nguyen et al., 2015; Garcia et al., 2018). Educational programs can help farmers make informed decisions and adopt innovations proven in previous research. Extension services should provide training on both the benefits and limitations of using Bokashi fertilizers. Workshops, demonstration projects, and on-farm trials can help farmers understand how to effectively integrate Bokashi into their nutrient management practices and adjust application rates based on soil testing. This will ensure that the advantages of high organic matter and improved soil structure are fully realized in the field (Nguyen et al., 2015).

Adopt On-Farm Demonstration Projects and Field Trials

Establish pilot projects and demonstration farms to showcase the benefits of adopting standardized fermentation protocols. On-farm trials can help refine the blending and inoculation techniques, validate laboratory findings under variable field conditions, and provide tangible examples of improved soil health and crop performance (Kim et al., 2020). This real-world evidence is critical for broader adoption.

On-Farm Quality Assurance

Encourage farmers to perform routine soil analyses to monitor changes in organic matter content, C:N ratios, and N availability after applying Bokashi . On-farm testing allows for adaptive management practices, ensuring that amendments are enhancing soil fertility as expected. This hands-on approach supports precision agriculture and minimizes environmental impacts such as nutrient leaching.

Utilize Integrated Soil Fertility Management Strategies

Farmers should integrate treated manure products into a broader soil fertility management plan. This involves combining these amendments with other practices such as crop rotation and conservation tillage to bolster soil organic matter, enhance nutrient use efficiency, and promote sustainable yield improvements while minimizing environmental impacts (Chen et al., 2012). Although Bokashi products show lower total N % (for example, cattle Bokashi has 0.87% compared to 2.11% in raw cattle manure), their high organic matter and elevated C:N ratios suggest that they provide a slow-release nutrient profile and improve soil structure. Farmers can

balance immediate crop nutrient needs by supplementing Bokashi with targeted nitrogen sources when required (Lin et al., 2016).

Develop and Implement Standardized Manure Treatment Protocols

Regulatory agencies should work with agricultural researchers to develop standardized protocols for manure treatment and fermentation. This includes guidelines on the appropriate blending ratios and microbial inoculation techniques (Higa & Konishi, 1999; Lin et al., 2016). Such standards can help ensure that properties like pH and organic matter are optimized, thereby enhancing soil amendment quality and minimizing environmental risks (Chen et al., 2012). Given that the Bokashi fertilizers show a dramatic increase in organic matter (e.g., poultry Bokashi : 16.23 vs 1.34 in poultry manure) and improved stabilization (as evidenced by higher C:N ratios), policies should support the development of standardized protocols for manure-to-Bokashi conversion. These standards should define optimal microbial inoculants, substrate ratios, and fermentation conditions to ensure consistent product quality. Standardization can help maintain soil amendment quality while reducing risks such as nutrient leaching (Higa & Konishi, 1999; Chen et al., 2012).

Incentivize Adoption of Sustainable Fermentation Practices

Policymakers can introduce subsidies or tax incentives for producers who adopt sustainable manure management techniques such as Bokashi fermentation, which has been shown to suppress undesirable microbial growth (including mould) and improve nutrient stabilization (Nguyen et al., 2015; Garcia et al., 2018). These measures encourage the transition from raw manure to higher-value Bokashi fertilizers, which benefit soil health through increased organic matter content and enhanced soil structure (Nguyen et al., 2015). In addition, grants for research and training in composting and fermentation techniques can facilitate wider adoption and innovation in nutrient management.

Integrate Manure Management into Broader Environmental and Agricultural Policies

Given that proper manure management can reduce nutrient run-off and promote soil health, it is advisable to integrate these practices into wider sustainable agriculture and environmental

protection policies. Support for research, extension programs, and demonstration projects can help bridge the gap between laboratory findings and on-the-ground practices (Kim et al., 2020).

Establish Quality-Control and Monitoring Programs

Policies should require routine quality control and monitoring of manure treatment. Parameters such as pH, nutrient content, and microbial profiles (including the absence of harmful mould) could be standardized to ensure the safety and efficacy of manure-based amendments. This systematic approach could also help resolve disagreements in the literature by providing consistent, reproducible data for policy decisions (Chen et al., 2012). Such programs help build confidence among end users and support data-driven policy decisions, enabling certification of product performance before market release (Garcia et al., 2018). Manure management practices that lead to improved Bokashi fertilizers should be integrated into broader sustainability policies. Such policies might encompass nutrient management plans, water-quality protection measures, and climate change mitigation strategies. By promoting the use of high-organic matter fertilizers, governments can enhance soil carbon sequestration and reduce reliance on synthetic inputs (Kim et al., 2020).

5.5 AREAS FOR FURTHER RESEARCH

Below is a set of potential avenues for further research each addressing gaps in our current understanding of manure-to-Bokashi conversion and its implications for agronomy and environmental sustainability. These areas build on existing findings and linked literature.

Exploring new organic manure sources

Future studies might look at additional organic waste items like food scraps, compost, or plant residues in addition to more conventional manures like pig, cow, and poultry manure. This may increase the scope of its use. Exploring new organic manure sources offers a dual benefit: it not only diversifies the portfolio of sustainable fertilizers but also provides an opportunity to valorize locally available waste, thereby enhancing overall agricultural sustainability.

Microbial inoculants and bio-augmentation

Examining the application of bio-augmentation or targeted microbial inoculants in Bokashi production may increase its microbial diversity, hence enhancing soil health and nutrient cycling.

The integration of microbial inoculants and bio-augmentation in manure fermentation offers multiple benefits from enhanced nutrient stabilization and consistent product quality to improved pathogen suppression. Policy measures that incentivize these practices, combined with rigorous on-farm implementation and further research on microbial dynamics, can create a robust system for producing high-quality Bokashi fertilizers.

Optimization of Fermentation Conditions

Identifying the optimal conditions such as moisture, temperature, inoculant types and manure blending ratios that maximize the benefits of Bokashi fermentation. Experimental designs can include factorial trials to determine interactive effects of these parameters on nutritional composition and pathogen suppression (including mould). The observations point to significant changes in pH and nutrient profiles due, in part, to the fermentation process. However, variability in these outcomes suggests that further research is needed to fine-tune the process.

Investigating Pathogen and Mould Suppression Mechanisms

Explore the mechanisms behind the observed absence of mould in certain treatments (e.g., poultry and blend treatments). This could involve controlled experiments to study antifungal compound production by beneficial bacteria and to test the influence of manure pH and other physicochemical parameters on fungal growth. While some studies have noted mould suppression under alkaline conditions and through microbial antagonism (Higa & Konishi, 1999; Nguyen et al., 2015), the precise biochemical pathways remain largely speculative. Detailed understanding here could lead to targeted strategies for ensuring pathogen-free organic amendments.

Microbial community profiling

Future studies might focus on microbial community profiling to pinpoint which organisms are most responsible for these observed changes. In addition, field trials assessing the impact of Bokashi on crop performance and nutrient cycling are needed to fully validate the benefits indicated by these chemical parameters.

Microbial Community Dynamics and Fermentation Kinetics

Investigate the taxonomic and functional composition of microbial communities during the fermentation process. Such studies can employ high-throughput sequencing and metagenomics to track microbial succession from raw manure to final Bokashi fertilizer and assess how these shifts influence physicochemical parameters like pH, nutrient stabilization, and organic matter conversion. Previous work (Higa & Konishi, 1999; Nguyen et al., 2015) has highlighted the critical role of microbes in organic matter transformation. However, detailed characterization of microbial consortia throughout the fermentation cycle remains underexplored. Understanding these dynamics could help optimize inoculation strategies and fermentation conditions for consistent product quality.

Nutrient Release Kinetics and Soil-Crop Interactions

Examine the rate and pattern of nutrient release (particularly nitrogen, phosphorus, and potassium) from Bokashi fertilizers after soil application. Long-term field experiments should assess how the fertilizer's elevated C:N ratios and organic matter content translate into nutrient availability, crop uptake, and overall yield. Determining how these factors affect plant nutrition can guide recommendations for integrated fertility management.

Environmental Impacts and Greenhouse Gas Emissions

Evaluate the environmental footprint of converting manure to Bokashi, focusing on greenhouse gas emissions (e.g., methane and ammonia volatilization) and nutrient leaching. This research should compare conventional manure management practices with those involving Bokashi processing, under controlled and field conditions. Assessing how Bokashi treatments mitigate emissions and leaching will help integrate manure management into broader climate change and water-quality strategies.

Long-Term Agronomic and Economic Assessments

Conduct extended field trials that track soil health indicators, crop performance, and economic returns over several growing seasons. This holistic evaluation should include an economic cost benefit analysis that compares Bokashi to other organic fertilizer options. These research directions not only build on the current study's findings regarding nutritional composition and microbial activity but also address practical challenges associated with sustainable manure

management. Integrating molecular techniques with long-term field assessments and economic analyses will provide a comprehensive foundation for both policy development and refined agronomic practices.

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APPENDICES

APPENDIX 1

Bokashi fermentation experimental setup



APPENDIX 2

Macro-nutrients for Bokashi biofertilizer



APPENDIX 3

Microbial biomass

