AKENTEN APPIAH-MENKA UNIVERSITY OF SKILLS TRAINING AND ENTREPRENEURIAL DEVELOPMENT

ASSESSMENT OF HEAVY METAL CONCENTRATION AND MICROBIAL LOAD IN *FUFU* PROCESSED FROM MILLING MACHINES IN NSUTA, ASHANTI REGION OF GHANA.

CORNELIUS ESHUN

2025

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BY

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A thesis submitted to the School of Graduate Studies, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, in partial fulfilment of the requirements for the award of a Master of Philosophy degree in Public Health.

SEPTEMBER, 2025

DECLARATION

Candidate's Declaration

I hereby declare that this thesis, except for quotations and references contained in published works which have been duly acknowledged and cited, is the result of my own original work and that no part of it has been presented for another degree at this university or elsewhere.

Candidate's Name: CORNELIUS ESHUN

Signature: ……………………………… **Date:** ……………………...

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the Akenten Appiah Menka University of Skills Training and Entrepreneurial Development.

Principal Supervisor's Name: DR. KOFI SEKYERE BOATENG

Signature: …………………………. **Date:** ……………………......

Co-Supervisor's Name: DR. DENIS DEKUGMEN YAR

Signature: ………………………… **Date:** ………………………...

ACKNOWLEDGMENT

I wish to express my profound gratitude to Almighty God for His gracious love, care, protection, and direction throughout my entire educational journey. I want to express my appreciation to Dr Kofi Sekyere Boateng and Dr Denis Dekugmen Yar, my supervisors, for their excellent guidance, direction, and critical corrections, which enabled me to produce this thesis. I am also grateful to my parents for their unforgettable love, assistance and support throughout my entire education. And finally, to Ms Ama Asamaniwaa Attua for her support and inspiration towards the completion of this study.

DEDICATION

This research work is dedicated to Almighty God and my parents for all their wonderful support, sacrifices and contributions towards my education**.**

TABLE OF CONTENTS

Content **Page**

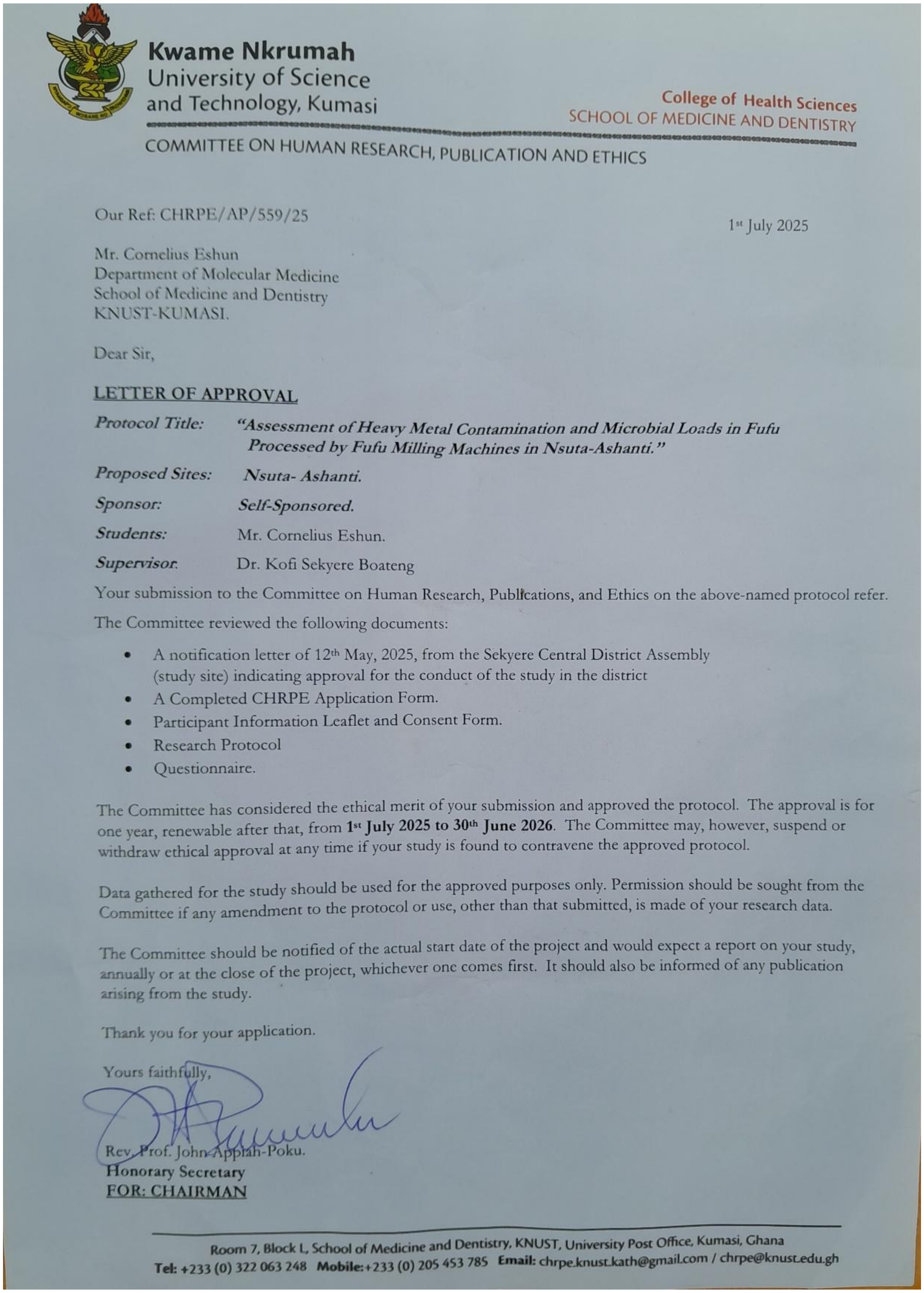
LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS (ABBREVIATIONS)

AAS - Atomic Absorption Spectrophotometry
AT - Averaging time
BPW- Buffered Peptone Water
BW - Body weight
CDI- Chronic Daily Intake
CFU - Colony Forming Unit
CR- Cancer Risk
ED - Exposure duration
EF - Exposure frequency
FAO - Food and Agriculture Organization
FDA - Food and Drugs Authority
GSA - Ghana Standards Authority
HACCP- Hazard Analysis and Critical Control Points
HQ- Hazard Quotient
IR - Intake rate of *fufu*
KNUST- Kwame Nkrumah University of Science and Technology
MCS- Monte Carlo Simulation
Pinf - Risk of infection
RSDs - Relative Standard Deviations
SD - Standard Deviation
SSA- *Salmonella Shigella* Agar
USEPA - United States Environmental Protection Agency
WHO- World Health Organization

ETHICAL APPROVAL



Kwame Nkrumah
University of Science
and Technology, Kumasi

College of Health Sciences
SCHOOL OF MEDICINE AND DENTISTRY

COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Our Ref: CHRPE/AP/559/25

1st July 2025

Mr. Cornelius Eshun
Department of Molecular Medicine
School of Medicine and Dentistry
KNUST-KUMASI.

Dear Sir,

LETTER OF APPROVAL

Protocol Title:	***"Assessment of Heavy Metal Contamination and Microbial Loads in Fufu Processed by Fufu Milling Machines in Nsuta-Ashanti."***
Proposed Sites:	***Nsuta- Ashanti.***
Sponsor:	***Self-Sponsored.***
Students:	Mr. Cornelius Eshun.
Supervisor.	Dr. Kofi Sekyere Boateng

Your submission to the Committee on Human Research, Publications, and Ethics on the above-named protocol refer.

The Committee reviewed the following documents:

- A notification letter of 12th May, 2025, from the Sekyere Central District Assembly (study site) indicating approval for the conduct of the study in the district
- A Completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Protocol
- Questionnaire.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for one year, renewable after that, from **1st July 2025 to 30th June 2026**. The Committee may, however, suspend or withdraw ethical approval at any time if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than that submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at the close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you for your application.

Yours faithfully,

Rev. Prof. John Appiah-Poku.
Honorary Secretary
FOR: CHAIRMAN

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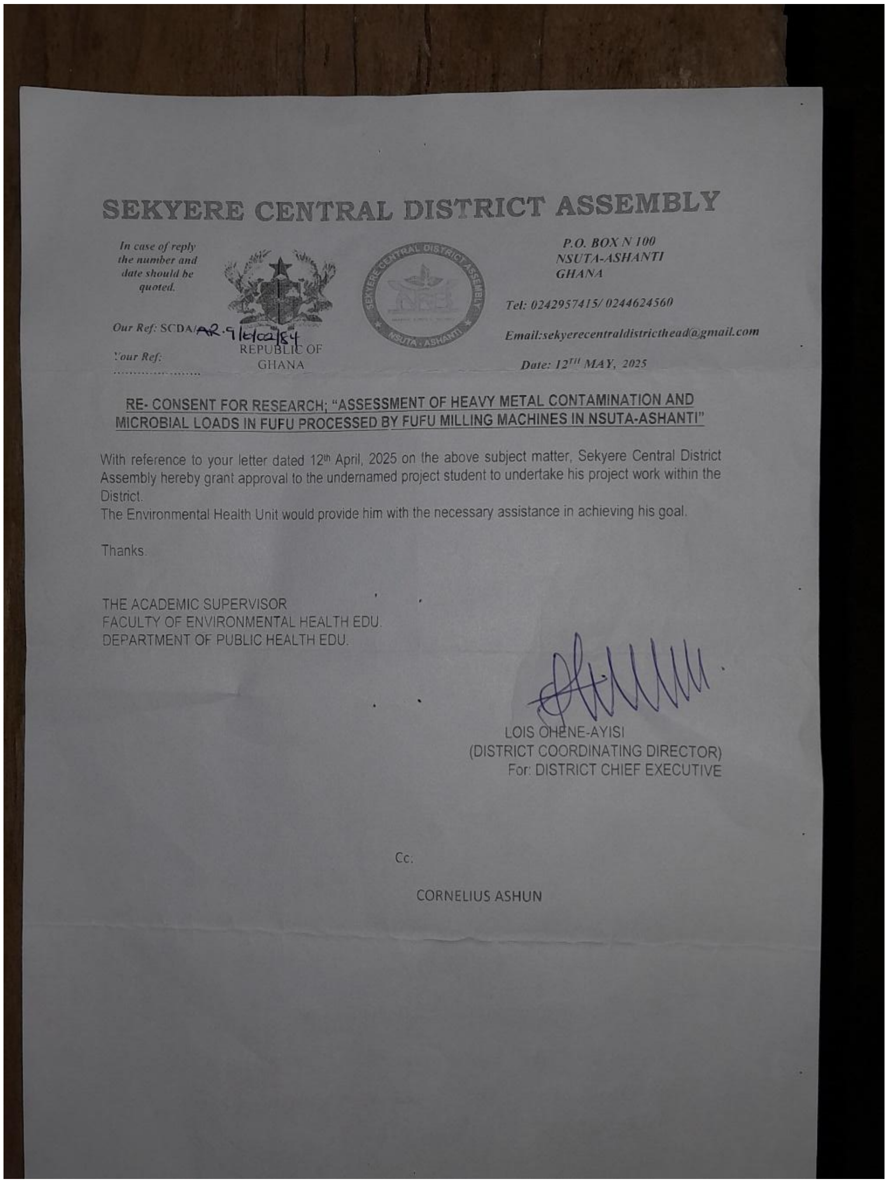
INSTITUTIONAL APPROVAL



SEKYERE CENTRAL DISTRICT ASSEMBLY

In case of reply the number and date should be quoted.

Our Ref: SCDA/AR.9/16/02/84

Your Ref: ……………………

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Date: 12TH MAY, 2025

RE- CONSENT FOR RESEARCH; "ASSESSMENT OF HEAVY METAL CONTAMINATION AND MICROBIAL LOADS IN FUFU PROCESSED BY FUFU MILLING MACHINES IN NSUTA-ASHANTI"

With reference to your letter dated 12th April, 2025 on the above subject matter, Sekyere Central District Assembly hereby grant approval to the undernamed project student to undertake his project work within the District.
The Environmental Health Unit would provide him with the necessary assistance in achieving his goal.

Thanks.

THE ACADEMIC SUPERVISOR
FACULTY OF ENVIRONMENTAL HEALTH EDU.
DEPARTMENT OF PUBLIC HEALTH EDU.

LOIS OHENE-AYISI
(DISTRICT COORDINATING DIRECTOR)
For: DISTRICT CHIEF EXECUTIVE

Cc.

CORNELIUS ASHUN

ABSTRACT

Mechanised milling of *fufu* improves efficiency. This study assessed the microbial quality and heavy metal concentrations of fufu processed in mechanised *fufu* milling machines. A longitudinal study design was employed for the study, where 60 samples (30 milled *fufu* and 30 unmilled ingredients) were collected from 10 milling sites, and 110 questionnaires were administered. Quantitative Microbial Risk Assessment (QMRA) and Monte Carlo simulation were employed to estimate the infection risks associated with contaminated *fufu*. After milling, *Salmonella*, *Shigella*, and Total Coliforms increased significantly, while *E. coli* levels varied ($p=0.008$) by location. T test to assess the impacts of milling on the heavy metals showed Arsenic and zinc being most impacted ($p <0.05$). Lead concentration was also significantly ($p = 0.01$) impacted but varied ($p = 0.00$) across the various milling locations. Notably, 90% of milling operators reported daily cleaning of their work environment. *Salmonella* posed an infection risk even at minimal exposure, with all simulations classifying it as a high risk (Pinf > 0.001). *Shigella* and *E. coli* showed consistently high infection probabilities, peaking between 0.6 and 0.75. Cadmium exposure remained within safe limits (HQ < 1), while lead presented a potential health risk, with some values exceeding 1.0. Arsenic posed minimal non-carcinogenic risk. The assessment of *fufu* processed by milling machines in Nsuta-Ashanti revealed significant microbial contamination, with *Salmonella*, *Shigella*, *E. coli*, and total coliforms increasing after milling due to poor hygiene and cross-contamination. At the same time, heavy metals such as lead and cadmium posed potential health risks, emphasizing the need for improved sanitation and regulatory measures to ensure food safety.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Fufu is a traditional West African dish made from boiled starchy staples such as cassava, plantain, or yam, which are pounded into a smooth, dough-like consistency (Asante-Donyinah et al., 2024; Gnagne et al., 2023). It is a popular dish in Ghana, where it is often served with a variety of sauces, such as light soup, groundnut soup, or palm nut soup (Sefah et al., 2024; Adu-Gyamfi, 2018). *Fufu* comes from Ghana and was introduced by the Akan people.

Fufu can be made with a variety of different starchy staples, but the most common types are cassava, plantain, and yams (Eyinla et al., 2021). Once the *fufu* is made, it is typically served with a variety of sauces; the most popular sauces to serve with *fufu* are light soup, groundnut soup, and palm nut soup. Light soup is a clear, light dish made with vegetables and often served with fish or meat. Groundnut soup is a thick, creamy soup made with peanuts, vegetables, and meat or fish (Bello & Ibrahim, 2023; Bassey et al., 2020).

Palm nut soup is a thick, oily soup made with palm nuts, vegetables, and meat or fish (Appiah et al., 2020; Clark, 2014). The majority of Ghanaian *fufu* dishes are made with cassava. Cassava is a typical food crop for carbohydrates that thrives in soils with poor nutrient content (Anikwe & Ikenganyia, 2018). The composition of fresh cassava root is characterized by a high moisture content ranging from 75-80%, ash content between 0.70-2.50%, approximately 1% fibre, about 0.1% fat, 2-3% protein, and 32-35% carbohydrates (Nkansah et al., 2021). Cassava roots additionally provide trace amounts of β-carotene, iron, and zinc (Nkansah et al., 2021). Due to its high moisture content, fresh cassava root is highly perishable and can deteriorate within 48 to 72 hours if not properly handled or preserved (Martin, 2020).

Cassava undergoes several processing stages, including starch extraction, crushing and boiling, roasting and toasting, drying and milling into flour, fermentation, and peeling (Shittu et al., 2016). In Ghana, cassava-based products such as *kokonte, gari,* and *fufu* are prepared everyday through drying and milling (Nkansah et al., 2021). Among these, *fufu* holds particular cultural significance as a staple traditional dish, especially cherished by the Ashanti people (Adu-Gyamfi, 2018). It is made by combining cooked cassava with plantains, cocoyam, or just cassava by pounding it in a mortar and pestle (Adu-Gyamfi, 2022).

Conventional food processing methods included kneading, grinding, frying, baking, and boiling, among other techniques, employing implements like pestles, and mortars (Yar et al., 2023; Serna-Saldivar & Carrillo, 2019). These traditional methods had several inherent drawbacks, such as being sluggish, heavy, time-consuming, and unsanitary (Panwar et al., 2022). As a result, cutting-edge techniques for processing food were created employing contemporary tools, techniques, and technology, such as blenders, mills, and crushers (Dubey et al., 2025; Xu et al., 2022).

Disk mills, which utilize friction or shear forces to reduce particle size, are a crucial component of current methods for fine grinding (Gao et al., 2020). The double-disk mill type, in which two discs rotate in opposition to one another, is frequently used in grinding mills (Flach et al., 2017). During the grinding process, these mills occasionally contaminate the foods with germs, metal residues, paints, corroded metal products, lubricants, and other foreign substances (Talabi et al., 2025; Yar et al., 2023; Agyarko, 2021; Jha, 2015). The introduction of locally manufactured milling machines has dramatically improved the efficiency of food processing by reducing the time required for traditional methods, while also enhancing the sanitary quality of the final products

(Adeyeye, 2017). Tomatoes and pepper were previously hand-ground, a labour-intensive process (Ampah et al., 2021; Annan et al., 2018)

The invention of milling machines more than ten years ago rendered human hammering or grinding obsolete (Dzikunoo et al., 2021). This has caused the development of commercially run mill outlets. Although this phenomenon has made food processing easier, operators' non-adherence to food safety regulations may compromise the benefits of the otherwise beneficial invention (POPA et al., 2019). Foodborne diseases remain a major public health challenge worldwide, with the food production and processing chain often identified as a key source of contamination (Astill et al., 2019).

Each year, millions of people are affected globally, and the World Health Organization (WHO) estimates that about 600 million individuals suffer from foodborne illnesses, leading to roughly 420,000 deaths annually (Adley & Ryan, 2025; Lee & Yoon, 2021). The ingestion of contaminated food is a primary cause of these illnesses, which may arise from factors such as unsafe food handling, inadequate preparation, contaminated water, and poor hygiene practices by food vendors (Adley & Ryan, 2025; Gourama, 2020).

The level of microbial contamination is closely associated with the microbiological quality of food; high contamination levels usually reflect poor storage or handling practices, increasing the likelihood of disease transmission (Ehuwa et al., 2021; Han et al., 2021). In Ghana, foodborne diseases are estimated to affect approximately 420,000 people annually, resulting in about 65,000 deaths and imposing an economic burden of nearly USD 69 million each year (Ahiabor et al., 2024).

The problem of foodborne infection in developing countries is mainly due to the failure of the street food vendors to comply with the standard guidelines during preparation of the food, and secondary contamination after preparation (ImathIu, 2017; Alimi, 2016). A study conducted by Bonah (2015) in Tamale on milled tomatoes identified several bacterial species, including *Bacillus* sp. and *Staphylococcus aureus*. The findings further indicated that more than 66% of the samples were positive for *Escherichia coli*, likely resulting from the use of contaminated processing water or cross-contamination between raw materials and cooked food through equipment or vendor handling. Additionally, all samples recorded bacterial counts exceeding acceptable safety limits, highlighting a potential health risk for the local population that frequently consumes these products (Bonah, 2015).

Heavy metal pollution is one of the major concerns for food safety and security due to its severe detrimental effects on human health and the environment (Sarker et al., 2022; Anani et al., 2020; Motesharrei et al., 2016). The presence of heavy metals results in plant accumulation, and the intake of contaminated food results in their biomagnification in the human body and is associated with an increased risk of neurological, kidney and cardiovascular diseases (Yang et al., 2020). As a result of the profound negative impact that heavy metal contamination has on both human health and the environment, it is one of the main concerns for food safety and security (Sarker et al., 2022).

Heavy metal contamination has emerged as a critical challenge to food safety and security because of its profound adverse effects on both human health and the environment (Sarker et al., 2022 Nkansah et al., 2021; Anani et al., 2020). These metals can enter the food chain through contaminated soils, irrigation water, and materials used during food processing (Nkansah et al., 2021; Huang et al., 2018; Islam et al., 2018). Once present, they accumulate in plants and subsequently biomagnify in humans through dietary intake, increasing the risk of neurological disorders, kidney damage, and cardiovascular diseases (Yang et al., 2020). Given these risks, heavy metal pollution remains a pressing concern in safeguarding food systems (Sarker et al., 2022).

Their persistence in the environment also leads to accumulation in plants; further amplifying exposure risks (Nkansah et al., 2021). Although technological advancements have significantly supported economic development, they have also contributed to the widespread release of heavy metals, making their contamination in food an almost unavoidable consequence of modern industry (Nkansah et al., 2021).

Evidence of this contamination has been reported in several staple foods across sub-Saharan Africa, including gari (Nkansah et al., 2021; Awoyale et al., 2018), dried cassava products from Nigeria (Abass et al., 2017), cassava tubers in Ogun State, Nigeria (Makanjuola, 2016), and *fufu* in Kumasi, Ghana (Nkansah et al., 2021; Ankar-Brewoo et al., 2020). Furthermore, studies on processing equipment have identified mercury, chromium, iron, manganese, lead, zinc, cobalt, nickel, and other metals as frequent contaminants in processed foods, though concentrations vary widely (Srivastava et al., 2024).

1.2 Problem statement

In many Ghanaian communities, including Nsuta, lifestyle changes have led to the commercialization and mechanization of *fufu* pounding into milling by *fufu* milling machines. These milling machines are typically made from steel (Addai, 2022). Despite the widespread consumption of *fufu* in Nsuta and the increasing reliance on milling machines, there is a lack of comprehensive research on the microbial loads and heavy metal contamination associated with mechanized *fufu*. This raises public health concerns, as studies have reported that milled or mechanized foods can become contaminated during the grinding process (Gao et al., 2020; Oniya et al., 2018; Jha, 2015).

Although several studies have examined microbial contamination in other milled food products, such as tomatoes, pepper, maize, millet, etc., these have also been reported to be contaminated (Yar et al., 2023; Nkansah et al., 2021; Bonah, 2015; Dzah, 2015)**.** However, there have been very few studies specifically focusing on the microbial contamination of milled *fufu*. One such study by Yar in 2023 reported microbial contamination levels in milled pepper, maize, and *fufu* (Yar et al., 2023). Despite the growing body of research on food contamination in sub-Saharan Africa, many existing studies have not explicitly examined the underlying sources or contributing factors of contamination, nor have they comprehensively evaluated the potential human health risks associated with consuming contaminated foods.

Inadequate hygiene practices and poor maintenance of milling equipment remain probable drivers of both microbial and heavy metal contamination in processed products, thereby posing significant threats to public health. Addressing this gap, the present study aims to systematically assess the quality of *fufu* produced by milling machines in Nsuta, with a particular focus on microbial contamination and heavy metal concentrations. The findings are expected to provide critical insights that can inform interventions to safeguard consumer health and promote food safety standards in the community.

1.3 Research questions

1. What is the microbiological quality and sources of contamination of *fufu* from milling machines in Nsuta?

2. What are the concentrations of heavy metals (lead, cadmium, zinc, and iron) in *fufu* produced from milling machines and the sources of contamination in Nsuta?
3. What is the human health risk associated with the consumption of *fufu* contaminated with heavy metals and microbial loads?

1.4 Research Objectives

1.4.1 Main objective

The primary objective of this study was to evaluate the heavy metal concentration and microbial load in *fufu* produced using milling machines in Nsuta, Ashanti Region, Ghana.

1.4.2 Specific objectives

This study specifically sought to:

1. Determine the microbiological load and sources of contamination of *fufu* from milling machines in Nsuta.
2. Determine the concentrations of heavy metals (lead, cadmium, zinc, and iron) in *fufu* produced from milling machines and the sources of contamination in Nsuta.
3. Assess the human health risk associated with the consumption of *fufu* produced from milling machines contaminated with microorganisms.
4. Assess the human health risk associated with the consumption of *fufu* produced from milling machines contaminated with heavy metals.

1.5 Significance of the study

This study in Nsuta-Ashanti offers a practical contribution to both public health awareness and food safety monitoring in local communities. Our role in identifying and analyzing contaminants in staple foods, such as *fufu*, directly addresses a gap in food hygiene regulation at the point of processing, where oversight is often limited. The findings will provide valuable data that can inform local health authorities and policymakers about the risks associated with unregulated milling practices. This could support the creation or strengthening of food safety guidelines, routine inspection protocols, or localized interventions to improve the hygiene and material standards of processing equipment. For the population, especially those who rely on commercial milling for daily meals, this research has the potential to influence safer consumer choices and encourage mill operators to adopt better practices. For the scientific community, the study contributes to the growing body of literature on food contamination in informal processing environments, highlighting the intersection between traditional food practices and modern public health concerns. It lays the groundwork for further studies on contaminants in other commonly consumed local foods across Ghana and similar regions.

1.6 Justification

Several studies have highlighted the health risks associated with food contamination due to poor handling, environmental exposure, and unsafe processing methods. Research by Abdulai et al. (2024) and Adjei-Mensah et al. (2021) has documented the presence of heavy metals, such as lead, cadmium, and arsenic, in food products across Ghana, often linked to the use of substandard equipment or contaminated environments. Similarly, microbial assessments of locally processed foods, including *kenkey, banku, a*nd *fufu*, have shown elevated levels of coliforms and other pathogenic organisms (Dela, 2021; Yeleliere et al., 2018), underscoring the public health concern. Although awareness is increasing, little research has looked at how heavy metal and microbial contamination affect *fufu* processed with milling machines, especially in rural areas like Nsuta-Ashanti. Most existing studies focus only on pounded *fufu*, microbial quality of *fufu* produced by milling machines, or heavy metal levels (Yar et al., 2023; Nkansah et al., 2021; Samuela et al., 2019), leaving a gap in understanding the safety of *fufu* made produced from milling machines in less regulated settings. Therefore, this study is necessary to bridge that gap by generating empirical data on both the microbial quality and heavy metal concentration of *fufu* produced in Nsuta-Ashanti. By focusing on the local context, this research will highlight the specific risks faced by the community and serve as a reference for broader discussions on food safety in Ghana's informal food processing sector. The findings will support public health campaigns, help shape sanitation training for mill operators, and provide evidence to guide improvements in inspection and licensing standards.

1.7 Limitations of the study

The study did not include every *fufu* milling machine in the area, so the findings did not capture all possible variations in contamination. For microbial analysis, the focus was on general microbial presence, and not all organisms were identified to the genus level. This limited the detail and accuracy of the microbial risk assessment. Another limitation is the variation in hygiene practices and day-to-day operations among mill operators. Factors such as the age and condition of machines, as well as the frequency of cleaning, likely vary, which can affect contamination levels and introduce variability that's hard to measure or control. Additionally, since machine operation can vary from one individual to another, this operator-dependent variability may act as a confounding factor, making it more challenging to ensure consistency and comparability across samples. Concerns about confidentiality and trust may lead to underreporting or inaccuracies in the data collected. The research findings may not be readily applicable to other geographical areas or contexts, as microbial loads and heavy metal concentrations in *fufu* may vary due to local factors such as agricultural practices, water sources, and soil composition.

1.8 Organization of the study

The research was organized into five chapters. Chapter one consisted of the problem statement, research questions and research objectives of the study, justification of the study, scope, and organization of the study. The second chapter focused on the conceptual framework underpinning the study. An extensive review of the literature will be included in this chapter. The third chapter presented the research approach and methodology that will be employed in carrying out the study. Chapter four presented an analysis of the results and discussion. Finally, the fifth chapter presented the major findings of the study and provided recommendations to address the identified challenges.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The consumption of milled *fufu*, a staple food in Ghana, raises concerns about potential contamination from heavy metals and microbial loads due to processing methods, water quality, and environmental exposure. Understanding the sources, health risks, and regulatory standards for contaminants in food is crucial for ensuring public health and food safety. This chapter reviewed existing literature on heavy metal contamination in food, emphasizing common contaminants such as lead (Pb), cadmium (Cd), arsenic (As), and Zinc (Zn), their sources, and potential health effects. Additionally, it explored microbial contamination, focusing on *Escherichia coli* (E. coli), *Salmonella spp*., *Shigella spp.*, and Total Coliforms, which can compromise food safety and contribute to foodborne illnesses. The review also examined processing, handling, and storage practices that may influence contamination levels in milled *fufu*.

2.2 Conceptual framework

The conceptual framework was developed using the socioecological theory. The Socioecological theory is a framework for understanding the complex interactions between individuals, their environment, and their health (Bates et al., 2019). Urie Bronfenbrenner developed it in the 1970s, and it has been used in a variety of fields, including public health, education, and social work (Bronfenbrenner, 1986). Microbial and heavy metal contamination of *fufu* produced from *fufu* milling machines poses a risk to food safety and is associated with environmental sources and factors. This theory was used as it best demonstrates the relationship between individuals, their environment, and their health.

The microbial loads and heavy metal levels in *fufu* will vary depending on the factors that contribute to contamination. For example, *fufu* processed on a metal machine is more likely to have higher levels of heavy metals than *fufu* processed on a mortar and pestle made of wood. The microbial loads and heavy metal levels in *fufu* will also vary depending on the time and temperature at which the *fufu* is processed. *Fufu* that is processed for a more extended period of time or at a higher temperature is more likely to have higher levels of microbial contamination. Some *fufu* milling machines are more likely to be contaminated with microbes or heavy metals than others. For example, machines made of metal may be more likely to corrode and release heavy metals into the environment.

Improper hygienic practices among operators, such as inadequate hand washing and irregular cleaning of milling machines, are likely to increase the risk of microbial contamination in *fufu*. Furthermore, the environmental conditions in which milling machines are operated play a significant role; machines located in unsanitary or dust-prone areas are particularly vulnerable to microbial infiltration. The consumption of *fufu* with high microbial loads is a public health concern, as it can result in foodborne illnesses manifested through symptoms such as diarrhoea, vomiting, and nausea, and in severe cases, may lead to mortality.

Similarly, the ingestion of *fufu* contaminated with elevated concentrations of heavy metals poses serious health risks. Heavy metals have the potential to impair the nervous system, kidneys, and other vital organs, with prolonged or acute exposure sometimes proving fatal. While these factors present a conceptual framework for understanding contamination risks, the specific determinants of microbial and heavy metal contamination in *fufu*, as well as the associated health outcomes, require systematic investigation. This framework, however, provides a foundational basis for analyzing the problem and guiding the design of interventions aimed at improving the safety and quality of *fufu* produced by milling machines.

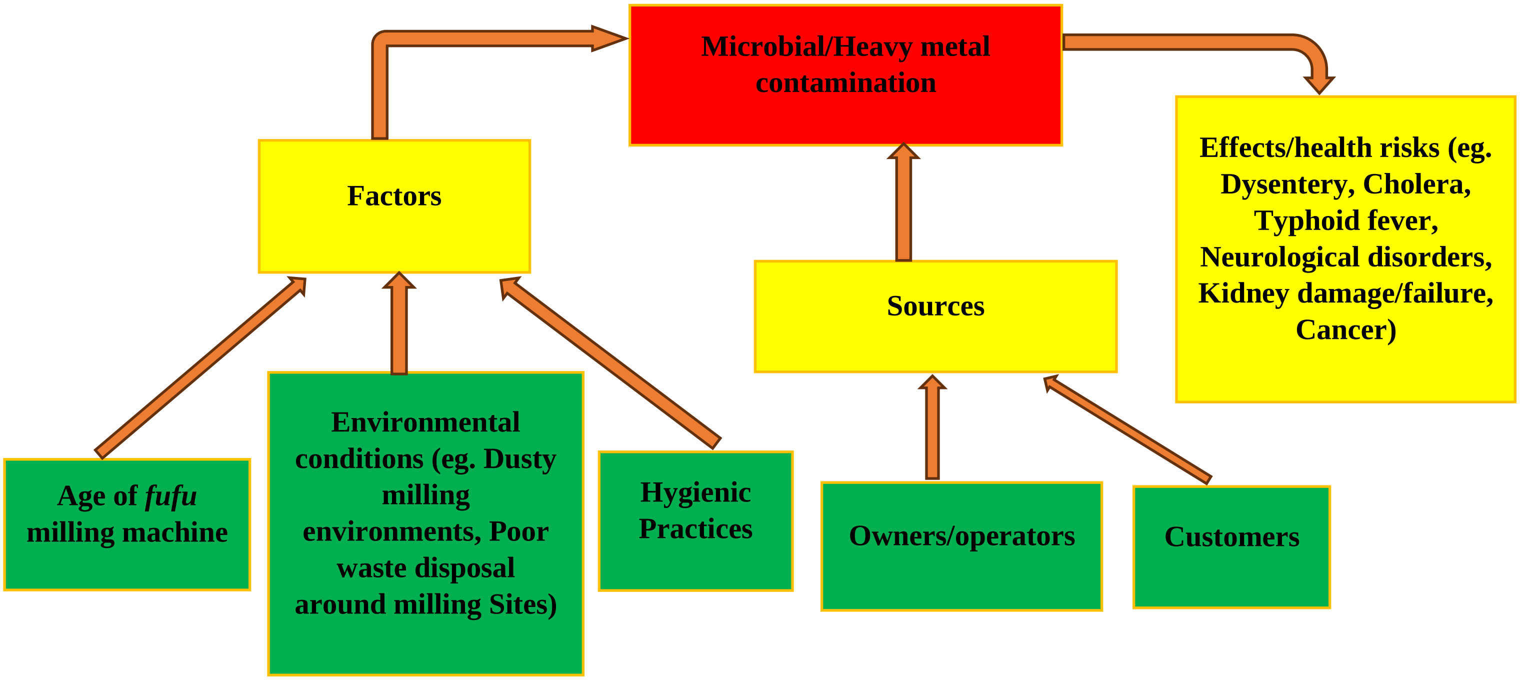

Figure 2. 1 Conceptual Framework (Author's own construct)

2.3 Microbial Contamination in Milled Food Processing

Microbial contamination in food processing is a significant public health concern globally. Several studies have documented the presence of pathogens such as *Escherichia coli*, *Salmonella*, *Shigella*, and total coliforms in various food products, including those processed by milling machines (Yar et al., 2023; Singh et al., 2016). Studies conducted in different regions have revealed that poor hygiene practices, inadequate cleaning of equipment, and environmental contamination are primary factors contributing to microbial contamination in food processing (Hasnan et al., 2022; Valero et al., 2016). Microbial contamination in milled food processing is a significant public health concern, as poor hygiene, water quality, and environmental exposure can introduce harmful microorganisms (Alum et al., 2016). Milled foods, such as *fufu*, are particularly vulnerable to contamination due to their multiple handling stages, from raw material preparation to milling, fermentation, and storage (Younge, 2022).

Common foodborne pathogens in milled foods include *Escherichia coli (E. coli), Salmonella spp., Shigella spp*., and Total Coliforms, which can lead to gastrointestinal infections and other health risks (Yar et al., 2023; Makinde et al., 2020). Contamination sources include contaminated water, unhygienic milling equipment, improper handling, and exposure to airborne microbes (Masotti et al., 2019). Studies have shown that traditional processing environments, especially those lacking strict sanitation measures, increase microbial loads in milled products (Yar et al., 2023; Sarkar & Fu, 2022).

2.3.1 Microbial contamination of milled Food Processing in Africa

Microbial contamination in milled food processing is a major food safety challenge in Africa, where traditional processing methods, inadequate sanitation, and poor water quality contribute to high microbial loads in staple foods (Mahunu et al., 2024; Makinde et al., 2020). Many milled foods, such as *fufu*, maize flour, cassava flour, and millet-based products, are susceptible to contamination at various stages, including harvesting, milling, storage, and distribution (Adesemoye et al., 2025; Onyango, 2019).

Studies across Africa have identified common foodborne pathogens in milled foods, including *Escherichia coli, Salmonella spp., Shigella spp.,* and *Staphylococcus aureus,* which are often introduced through contaminated water, poor hygiene practices, and unsanitary milling equipment (Yar et al., 2023; Oduori et al., 2022 ; Makinde et al., 2020 ; Adeyeye, 2017). Research in Nigeria, Ghana, Kenya, and South Africa has reported high microbial loads in milled foods, often exceeding

WHO and FAO safety standards, highlighting the need for improved food safety measures (Thumbi, 2020; Ahiaba, 2019; Olotu, 2018).

Traditional milling environments, particularly in open-air or communal processing facilities, further expose milled foods to airborne contaminants and cross-contamination (Jones et al., 2024; Mutungi et al., 2019). Additionally, improper post-processing storage and handling contribute to microbial proliferation (Biwas et al., 2018). While fermentation and heat treatments can reduce microbial loads, inadequate drying and packaging can lead to recontamination (Groenewald, 2023; Smith, 2020).

To address these concerns, African countries are increasingly adopting Good Manufacturing Practices (GMP), Hazard Analysis and Critical Control Points (HACCP), and food safety training for processors (Olaniran et al., 2023; Ngwa, 2017). Strengthening food safety regulations and promoting hygienic milling practices are essential to reducing microbial contamination risks in milled food processing across Africa (Adeyeye, 2017).

2.3.2 Microbial Contamination of Milled Food Processing in Ghana

Fufu, being a highly perishable food product, is susceptible to microbial contamination, especially during milling. The moist nature of the ingredients and the milling process itself can create an environment conducive to the growth of harmful microorganisms (Ndudi et al., 2024; Younge, 2022). Research found that traditional milling machines used for food processing could harbour a variety of microbial pathogens due to improper sanitation practices (Mahunu et al., 2024; Yar et al., 2023). These pathogens pose significant health risks, including gastrointestinal infections and foodborne illnesses, particularly in communities with limited access to clean water and sanitation (Asfaw et al., 2022; Bintsis, 2017). Research on milled cassava and maize-based foods emphasizes the crucial role of water and milling equipment sanitation in ensuring microbial safety (Bintsis, 2017).

Fermentation and heat processing can reduce microbial loads, but improper post-processing handling can reintroduce contaminants (Snyder, 2017). Regulatory standards by the WHO and FAO emphasize the need for good manufacturing practices (GMP), hazard analysis, and proper food handling techniques to ensure microbial safety in milled foods (Mahunu et al., 2024; Osei-Kwarteng et al., 2024). Microbial contamination in milled food processing poses a significant public health concern in Ghana, particularly in the production of staple foods such as *fufu*, maize flour, cassava flour, and millet-based products (Onyango, 2019; Tawiah, 2015). The widespread use of traditional milling methods, combined with inadequate hygiene practices and poor water quality, increases the risk of microbial contamination (Doddabematti Prakash et al., 2024).

Studies have reported the presence of *Escherichia coli, Salmonella spp., Shigella spp., Staphylococcus aureus*, and Total Coliforms in milled food products across Ghana (Yar et al., 2023; Aboagye et al., 2020; Tawiah, 2015). The primary sources of contamination include contaminated water, dirty milling equipment, and unhygienic handling by food processors (Oniya et al., 2018; Moerman & Mager, 2016). *Fufu* and maize flour samples from local mills contained microbial loads exceeding WHO safety limits, indicating poor hygiene standards (Ahiabor et al., 2024; Maduka & Ugbogu, 2024). Communal milling facilities, often lacking proper sanitation infrastructure, further expose milled foods to airborne microbes and cross-contamination (Walia et al., 2019; Bonah, 2015;).

Additionally, the use of untreated water during processing contributes significantly to microbial contamination (Balali et al., 2020; Uyttendaele et al., 2015). Although fermentation and boiling can reduce microbial loads in some milled foods, improper post-processing storage and handling can reintroduce contaminants (Mehany et al., 2024; Pilarski & Gerogiorgis, 2020). Despite the existence of Ghana Standards Authority (GSA) guidelines on food safety, enforcement remains inconsistent, especially in informal food markets (Agar, 2018; Ohene-Darko, 2018).

2.4 Heavy Metal Contamination in Milled Foods

Heavy metal contamination in milled food is a growing food safety concern due to the potential health risks associated with toxic metal accumulation (Nkansah et al., 2021). Milled foods such as cassava flour, maize flour, rice flour, and *fufu* can be contaminated with heavy metals during cultivation, processing, packaging, and storage (Ferraro et al., 2016; Lateef & Ojo, 2016). The presence of metals like lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), and chromium (Cr) is often linked to soil contamination, irrigation with polluted water, atmospheric deposition, and processing equipment (Khatun et al., 2022; Naja & Volesky, 2017).

Studies have shown that milled grains and root-based foods can absorb heavy metals from the environment, especially in areas near mining activities, industrial zones, and agricultural lands treated with chemical fertilizers and pesticides (Tang et al., 2024; Oladebeye, 2017). Research in Nigeria, Ghana, and South Africa has detected high levels of lead and cadmium in maize and cassava flour, exceeding WHO/FAO permissible limits (Kantanka, 2021; Addo et al., 2015). These contaminants pose serious health risks, including kidney damage, neurological disorders, and carcinogenic effects from prolonged exposure (Priyadarshanee et al., 2022; Rehman et al., 2018).

Additionally, traditional milling methods using metal grinders can introduce heavy metal residues into milled food, particularly when the equipment is corroded or improperly maintained (Daniel et al., 2021; Hinson & Darkwa, 2016). Packaging materials and storage conditions can also contribute to contamination if substandard materials are used (Adeyeye, 2017). To minimize heavy metal contamination in milled foods, food safety regulations emphasize Good Agricultural Practices (GAP), improved milling techniques, and routine monitoring of food products (Fărcaș, 2024; Sarkar & Fu, 2022; Alum et al., 2016). Strengthening enforcement of WHO and FAO safety standards, promoting environmentally sustainable farming, and ensuring the use of safe milling equipment are critical steps in reducing heavy metal exposure through milled food consumption (Shahnaz et al., 2024; Namubiru et al., 2022).

2.4.1 Heavy Metal Contamination in Milled Foods in Africa

Heavy metal contamination in milled food is a significant public health issue in Africa, where environmental pollution, industrial activities, and poor agricultural practices contribute to metal accumulation in food products (Nkansah et al., 2021; Adu et al., 2020). Milled staples such as cassava flour, maize flour, rice flour, and *fufu* are particularly vulnerable to contamination from sources like mining, industrial emissions, pesticide use, and irrigation with polluted water (Giupponi & Deppieri, 2022; Ezeonu & Ezeonu, 2016).

Studies across Africa have reported elevated levels of lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), and chromium (Cr) in milled grains and root-based foods, often exceeding WHO/FAO safety limits (Nkansah et al., 2021; Adeyeye, 2017; Bonah, 2015). Research in Nigeria, Ghana, and South Africa has found significant heavy metal contamination in maize and cassava flour, particularly in areas close to mining operations, landfills, and industrial zones (Nnaji & Akanno, 2022; Nwinanee & Lugbe, 2021; Ogbonna et al., 2020). Contaminated soil and water used for farming play a crucial role in the uptake of heavy metals by crops (Oladebeye, 2017).

Furthermore, traditional milling techniques using metal grinders can introduce heavy metals into food, especially when processing equipment is old, rusted, or poorly maintained (Oniya et al., 2018). Storage and packaging materials, if not properly regulated, can also contribute to contamination (Karanth et al., 2023). Prolonged consumption of heavy metal-contaminated food has been linked to serious health risks, including neurological disorders, kidney damage, and carcinogenic effects (Munir et al., 2021; Hembrom et al., 2020).

Despite the existence of food safety regulations in many African countries, enforcement remains weak due to limited monitoring capacity and informal food markets (Unnevehr, 2022; Grace, 2015). Addressing heavy metal contamination in milled food requires improved agricultural practices, stricter environmental regulations, regular food testing, and awareness programs to protect public health and food security (Alabi et al., 2024; Collado-López et al., 2022). Several studies have investigated the levels of heavy metals in food products, with a particular focus on foods processed using traditional or mechanized methods (Grace, 2015).

In the context of food processing, contamination by heavy metals can occur through various sources, including the raw materials, processing equipment, and the environment (Gautam et al., 2016; Sankhla et al., 2016). Lead contamination in food has been linked to the use of lead-based equipment or contaminated water sources during processing (Dignam et al., 2019; Obeng-Gyasi, 2019). Cadmium, another harmful metal, is often introduced through contaminated soil or water used in the cultivation of the raw materials (Hayat et al., 2019; Khan et al., 2017).

2.4.2 Heavy Metal Contamination in Milled Foods in Ghana

In Ghana, studies on heavy metal contamination in traditional foods have shown varying levels of contamination depending on the region and the type of food (Nkansah et al., 2021; Bonah, 2015).

Heavy metal contamination in milled food is a growing food safety concern in Ghana, particularly in staple foods such as cassava flour, maize flour, rice flour, and milled *fufu*. Contamination can occur at various stages, including crop cultivation, milling, storage, and packaging, due to exposure to polluted soil, water, air, and processing equipment (Younge, 2022; Adeyeye, 2017).

Studies in Ghana have detected elevated levels of lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) in milled food products, often exceeding WHO/FAO safety limits (Kantanka, 2021; Asamoa, 2016; Bonah, 2015).

Research conducted in mining areas such as Obuasi, Nsuta, and Tarkwa has revealed high concentrations of heavy metals in cassava and maize-based foods, attributed to soil contamination from mining waste, industrial emissions, and the use of untreated water for processing (Bortey-Sam et al., 2016, 2015). Additionally, heavy metal residues have been found in food processed with metal grinding machines, especially when equipment is old or corroded (Talabi et al., 2025; Ahmed et al., 2023).

Despite existing Ghana Standards Authority (GSA) and Food and Drugs Authority (FDA) regulations on food safety, challenges in enforcement and inadequate routine monitoring allow contaminated food to reach consumers (Yidana, 2023; Darko & Boateng, 2020). Heavy metal contamination in milled food is a growing public health concern in Ghana, particularly due to the increasing industrialization, mining activities, and agricultural practices that introduce toxic metals into food chains (Kantanka, 2021; Darko & Boateng, 2020). Staples such as cassava flour, maize flour, rice flour, and milled *fufu* are widely consumed and can become contaminated through exposure to polluted soil, water, air, and processing equipment (Ferraro et al., 2016; Lateef & Ojo, 2016). Given that these foods form a significant part of the Ghanaian diet, heavy metal contamination poses long-term health risks, necessitating continuous monitoring and regulation.

Ghana is a major gold-mining country, with large and small-scale mining operations in areas like Tarkwa, Obuasi, Prestea, and Nsuta (Koranteng, 2019; Kumah & Nyarko, 2018). These activities release high levels of arsenic (As), lead (Pb), mercury (Hg), and cadmium (Cd) into the environment.

Contaminated soil and water sources expose crops to heavy metals, which persist in food products even after milling. Processing plants, electronic waste sites, and vehicle emissions contribute to the deposition of toxic heavy metals in farming areas (Vanisree et al., 2022). The use of chemical fertilizers, pesticides, and untreated wastewater for irrigation introduces cadmium, lead, and arsenic into food crops. Some fertilizers contain trace heavy metals that accumulate in the soil over time, leading to bioaccumulation in food products (Soleimani et al., 2023; Rashmi et al., 2020). Most food milling in Ghana is done using metal grinders and locally fabricated milling machines, which can introduce metallic residues into food, especially when equipment is old, corroded, or poorly maintained (Talabi et al., 2025; Yar et al., 2023; Agyarko, 2021).

2.5 Sources and Factors of Contamination in Food Milling Machines

Milling machines play a crucial role in food processing across Ghana and Africa, particularly for staple foods such as maize, cassava, rice, millet, and sorghum (Kantanka, 2021; Adeyeye, 2017). However, contamination during milling poses serious food safety concerns, as heavy metals, microbial pathogens, and chemical residues can be introduced at different stages of the milling process (Doddabematti Prakash et al., 2024; Agyarko, 2021). Contamination can occur due to environmental factors, machine material composition, poor hygiene practices, and external pollutants (Parvin et al., 2020; Wang et al., 2020). The potential sources of microbial and heavy metal contamination in *fufu* milling machines are diverse and multifaceted. One major source of contamination is the milling machine itself, which, if not properly cleaned and maintained, can become a reservoir for pathogens and heavy metals (Priyanka et al., 2024; Agyarko, 2021).

Environmental factors, such as the location of the milling operation and the quality of water used during processing, also play a crucial role in the level of contamination (Asgharnejad et al., 2021; Rathi et al., 2021). Milling operations located near industrial areas or heavily trafficked roads are more likely to be exposed to airborne pollutants and heavy metals, which can then contaminate the food being processed (Dehkordi et al., 2024). The materials used in constructing the milling machines can also contribute to heavy metal contamination (Lin et al., 2022). Metals like lead and zinc can leach into food if the machine components are made from or coated with these metals (Deshwal & Panjagari, 2020). Additionally, the use of contaminated water for washing the raw materials or the equipment can introduce both microbial pathogens and heavy metals into the *fufu* (Suglo et al., 2022).

In Ghana and many parts of Africa, traditional milling machines are made from iron, steel, or aluminum, which can corrode over time, leading to metallic contamination in food products (Yar et al., 2023; Agyarko, 2021). Lead (Pb), cadmium (Cd), arsenic (As), and chromium (Cr) have been detected in milled food, often linked to the use of poor-quality or old milling equipment (Kaushik et al., 2023; Hamid et al., 2022). Milling machines that are not regularly cleaned or sanitized can harbor bacteria, molds, and yeasts, leading to microbial contamination (Sabillón et al., 2021). Studies in Ghana have shown that *Salmonella, Escherichia coli,* and *Staphylococcus aureus* are common contaminants in maize and cassava flour, often linked to unhygienic milling conditions (Ahiabor et al., 2024; Agyarko, 2021).

Many milling machines in Africa are located in open-air markets or roadside stalls, making them vulnerable to dust, vehicle emissions, and industrial pollutants (Ogwu et al., 2024; Wekoye, 2019).

Crops used for milling, such as maize, cassava, and millet, can already contain heavy metals and pesticides due to exposure to polluted soil, irrigation water, and agrochemicals (Gali et al., 2024; Barau et al., 2023). Studies in mining regions like Obuasi and Tarkwa in Ghana have shown that cassava and maize grown in contaminated soils often retain high levels of arsenic and mercury, which persist through the milling process (Mensah, 2022; Koranteng, 2019; Abubakar, 2015).

Some milling operations require water for wet milling or cooling, and when untreated water sources are used, they introduce pathogens, heavy metals, and chemical residues into the food (Asgharnejad et al., 2021). In many rural parts of Ghana and Africa, water sources are often contaminated with coliform bacteria, lead, and cadmium, contributing to post-milling contamination (Abanyie et al., 2020; Yeleliere et al., 2018; Affum et al., 2015). Many local milling machines are old and poorly maintained, increasing the likelihood of metal wear and corrosion (Lahiri, 2017; Chen & Thouas, 2015). Lack of regular machine servicing, cleaning, and replacement of worn-out parts exacerbates contamination risks (Sidashenko et al., 2017). Lack of proper cleaning protocols before and after milling increases the buildup of mold, bacteria, and metal particles in food (Moerman & Mager, 2016).

2.6 Health Risks Associated with Contaminated *Fufu* with Microbial Loads

Fufu, a widely consumed staple in West Africa, particularly in Ghana, is vulnerable to microbial contamination due to poor handling, unsanitary processing conditions, and exposure to contaminated water, equipment, and storage environments (Ahiabor et al., 2024; Adeyeye, 2017). The presence of high microbial loads in *fufu* can lead to foodborne illnesses, causing significant public health concerns, especially in communities where food safety regulations are weak or poorly enforced (Tanyitiku, 2024; JP, 2023). The consumption of *fufu* contaminated with microbial loads and heavy metals poses significant health risks to consumers. Microbial pathogens such as

E. coli, *Salmonella*, and *Shigella* are known to cause severe gastrointestinal diseases, which can lead to symptoms ranging from mild diarrhea to more severe conditions like dysentery and food poisoning (Sell & Dolan, 2018; Strockbine et al., 2015).

Studies have identified several microbial pathogens in *fufu*, often introduced during processing, milling, storage, or handling. The most frequently detected microbes include:

- *Escherichia coli* – Indicative of faecal contamination, often linked to diarrhoea, gastroenteritis, and haemolytic-uremic syndrome (HUS) in severe cases (Bystrom et al., 2017; Bitzan & Lapeyraque, 2016).
- *Salmonella spp*. – A major cause of food poisoning, leading to symptoms such as abdominal cramps, fever, and vomiting (Mohammad et al., 2018).
- *Staphylococcus aureus* – Produces heat-stable enterotoxins, leading to severe foodborne intoxications with symptoms like nausea, vomiting, and diarrhoea (Bhunia, 2018).
- *Clostridium perfringens* – Can cause gastrointestinal infections when consumed in high numbers, particularly when *fufu* is left at unsafe temperatures (Maduka & Ugbogu, 2024).
- Moulds and Yeasts – Some fungal species, such as *Aspergillus* and *Fusarium spp.*, can produce mycotoxins, which are linked to liver damage, immune suppression, and cancer (Awuchi et al., 2022; Ráduly et al., 2020).

The ingestion of pathogenic bacteria such as *E. coli* and *Salmonella* from contaminated *fufu* can lead to diarrhoeal diseases, which are among the leading causes of child mortality in sub-Saharan Africa (Christiana Cudjoe et al., 2022; Odo et al., 2021). Symptoms include vomiting, abdominal pain, nausea, and fever, which can lead to dehydration and electrolyte imbalance (Nadeem et al., 2023). *Staphylococcus aureus* and *Bacillus cereus* can produce heat-resistant toxins that cause rapid-onset food poisoning. Consumption of improperly stored or handled *fufu* increases the risk of vomiting and nausea within hours of ingestion (Nwadike et al., 2025).

Salmonella typhi, a strain of *Salmonella*, can be transmitted through contaminated *fufu*, leading to typhoid fever, which presents with high fever, weakness, abdominal pain, and potential intestinal perforation if left untreated (Njok et al., 2023; Odo et al., 2021). In areas where contaminated water is used in food preparation or milling, parasitic infections such as *Giardiasis* and *Amoebiasis* can occur, causing severe diarrhea, weight loss, and malabsorption issues (Siwila, 2023; Kristanti et al., 2022). Fungal-contaminated food may contain aflatoxins and fumonisins, which are linked to liver cancer, immune suppression, and growth retardation in children (Ráduly et al., 2020). Aflatoxin exposure is particularly concerning in Ghana, where high humidity promotes fungal growth on cassava and other raw materials used in *fufu* production (Abass et al., 2017; Adjovi et al., 2015).

2.7 Health Risks Associated with Contaminated Foods with Heavy Metals

Fufu, a widely consumed staple food in Ghana and West Africa, is at risk of heavy metal contamination due to exposure to contaminated soil, water, milling equipment, and processing conditions (Nkansah et al., 2021; Ankar-Brewoo et al., 2020; Adeyeye, 2017). Heavy metals such as lead, cadmium, arsenic, and mercury have been detected in various food products, including cassava-based foods like *fufu* (Balali-Mood et al., 2021; Hussain et al., 2019). Chronic exposure to these toxic metals poses severe health risks, affecting multiple organ systems and leading to both acute and long-term health complications (Rehman et al., 2018). Heavy metals, on the other hand, have long-term health implications due to their ability to accumulate in the body. Chronic exposure to heavy metals like lead and cadmium can result in neurological damage, kidney failure, and increased risk of cancer (Sankhla et al., 2016).

In Ghana, there is growing concern about the health risks associated with traditional food processing methods, particularly in rural areas where access to modern food safety practices is limited (Cudjoe et al., 2022). This underscores the need for comprehensive studies that assess the levels of microbial and heavy metal contamination in commonly consumed foods like *fufu*, as well as the development of interventions to reduce these risks.

Lead exposure from contaminated *fufu* can cause brain damage, memory loss, reduced IQ, and developmental delays in children (Dignam et al., 2019). Chronic lead poisoning can lead to neurotoxicity, mood disorders, and decreased motor function in adults (Dórea, 2019). Cadmium and mercury accumulate in the body, causing kidney failure, liver dysfunction, and metabolic disorders (Grace, 2015). Long-term cadmium exposure is linked to renal toxicity, proteinuria, and irreversible kidney damage (Huang et al., 2024). Arsenic contamination is strongly associated with skin, lung, bladder, and liver cancers due to its carcinogenic properties (Tchounwou et al., 2023; Palma-Lara et al., 2020). Cadmium has been classified as a Group 1 carcinogen by the WHO, increasing the risk of lung and prostate cancers (Rapisarda et al., 2018; Richter et al., 2017).

Heavy metals such as lead and mercury can cause congenital disabilities, miscarriages, and infertility in women. Pregnant women consuming contaminated *fufu* risk passing toxins to the fetus, leading to congenital abnormalities and developmental delays (Dutta et al., 2022; Rzymski et al., 2015). Heavy metals interfere with immune function, making individuals more susceptible to infections, autoimmune diseases, and chronic inflammation (Anka et al., 2022). Arsenic exposure can reduce the body's ability to fight infections, increasing the risk of tuberculosis and respiratory illnesses (Attreed et al., 2017). Heavy metal contamination in *fufu* is a serious public health concern in Ghana and other parts of Africa due to its long-term toxic effects on the brain, kidneys, liver, and immune system (Feruke-Bello, 2023; Nkansah et al., 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

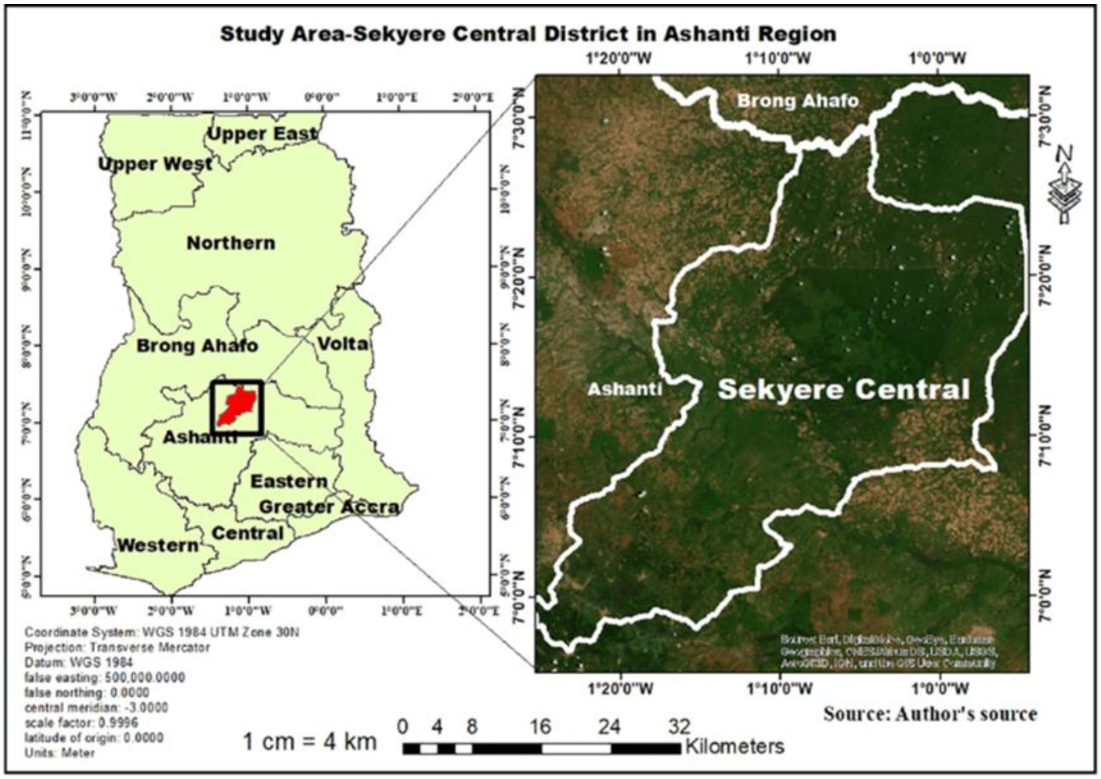

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Figure 3. 1 Map of Sekyere Central District (Akoto Sarfo et al., 2022)

The study was conducted in Nsuta communities within Sekyere Central District, located in the Ashanti Region of Ghana. Sekyere Central District, which is one of the 30 administrative districts in the Ashanti Region of Ghana, is located in the northern part of the region and shares boundaries with Mampong Municipal, Atebubu District, Sekyere East, Sekyere South, and Ejura Sekyeredumasi. The land size of the district is 1,631.1 sq. km, and it is located within longitudes 0.05 ° and 1.30 ° W and latitudes 6.55° and 7.30° N. It has about 150 settlements, with about 70% being rural. The rural areas are primarily found in the Afram Plains portion of the District, where communities with fewer than 50 people are primarily located (Ghana Statistical Services, 2014).

3.2 Study design

This study employed a longitudinal design to assess the current levels of heavy metal concentration and microbial quality of milled *fufu* in Nsuta-Ashanti. This study is essential for identifying potential health risks and guiding future studies or interventions aimed at improving food safety in the region. Heavy metal concentrations and microbial contaminants in milled *fufu* in Nsuta-Ashanti were determined by collecting samples of milled *fufu* from various locations within Nsuta-Ashanti.

3.3 Population

The population of the study consisted of all *fufu* milling machine operators and their customers in Nsuta townships.

3.4 Sampling and sample size

The study employed a combination of purposive and convenience techniques. Purposive sampling was used to select milling machines within the Nsuta-Ashanti community, as these sites are the main sources of *fufu* processing and are directly relevant to the research objectives. From each selected milling centre, samples of *fufu* were then collected. This approach enabled the study to obtain reliable data on the microbial load and heavy metal concentrations of *fufu* processed from different machines, while also reflecting variations in processing practices across the community.

3.4.1 Sample Size

A total of 60 samples (30 milled and 30 un-milled) were obtained from 10 *fufu* milling machine sites in Nsuta guided by Yar et al. (2023). The sites were selected based on the level of patronage by customers. This ranged from the sites with the highest to the lowest number of customers patronizing the services. This level was observed by the researcher between 1-3 weeks before samples were collected.

3.4.2 *Fufu* Sampling Procedures

Six samples were collected from each of the 10 *fufu* milling sites. One sample from each *fufu* milling machine was taken before milling, and the other after milling. The samples were collected into a sterile specimen container, stored in an ice-chest cooler, and transported under aseptic conditions to the Department of Science Laboratory, AAMUSTED, Asante Mampong for microbiological and heavy metal analysis. The sampling was carried out for a period of three months from November, 2024 to February, 2025.

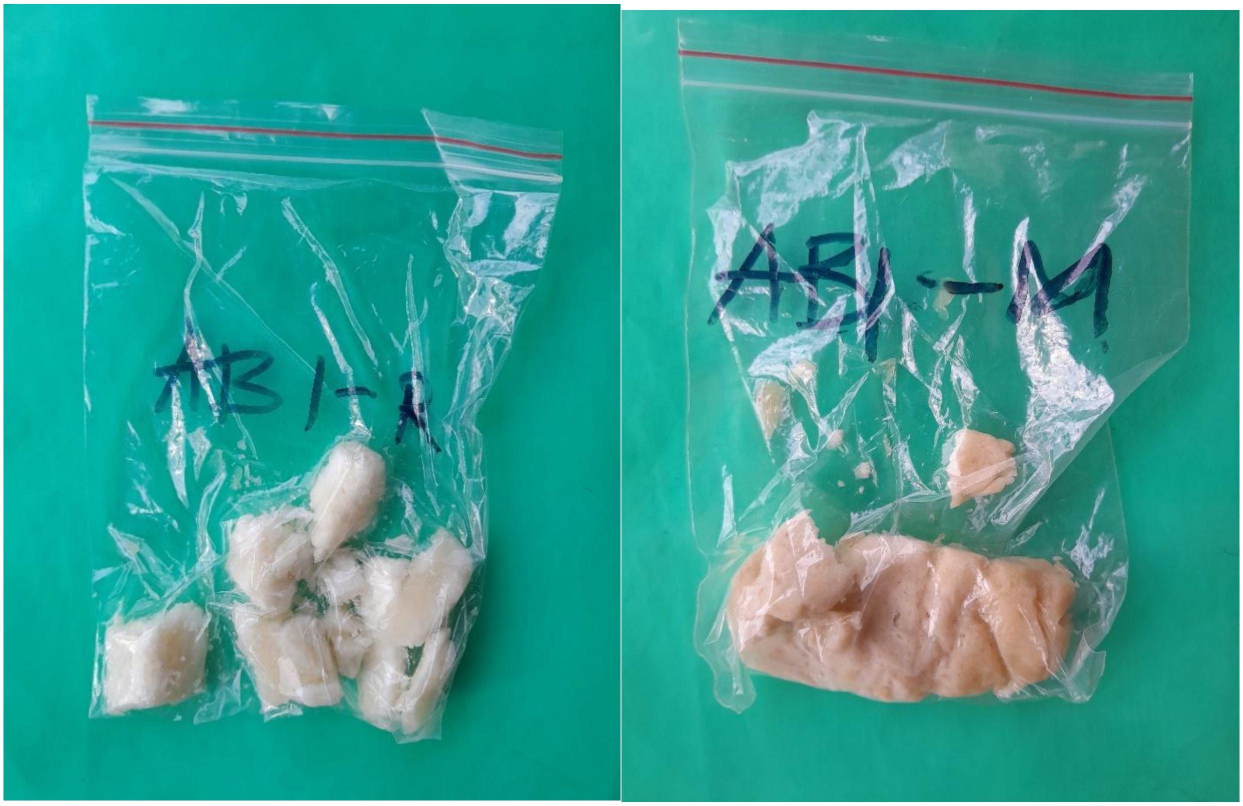

Figure 3. 2 Raw (R) and Milled (M) *fufu* sample

3.5 Data Collection and Tools

A well-structured questionnaire was used to collect data on operators' hygienic practices. It was sectioned into A, B, C, and D, where section A captured demographic data of the operators, and sections B, C, and D captured the hygiene of the milling environment, hygiene and maintenance of milling machines, and hygiene practices during operation, respectively. In all, 10 questionnaires were administered to the operators of the *fufu* milling sites in Nsuta.

Another questionnaire was used to collect data from customers who patronized the services of the *fufu* milling machines. The questionnaire for the customers was divided into three sections: A, B, and C. Section A captured the demographic data of the customers, and Section B also captured information on anthropometry. Finally, Section C focused on *fufu* consumption patterns. In all, one hundred and ten (110) questionnaires were administered; however, 6 persons opted out during the administration.

3.5.1 Data Collection Procedures

3.5.2 Questionnaire administration

The questionnaires were designed in English, but the questions were asked and explained in both English and Twi. This ensured a better understanding of *fufu* milling machine operators/owners and customers who had challenges with speaking the English language.

3.5.3 Laboratory Methods

3.5.3a Samples Preparation (*Fufu* for microbes)

Samples of *fufu* from the milling sites were collected and subjected to microbiological analysis to determine the presence and levels of contamination (ISO, 2017; Salfinger & Tortorello, 2015).

Un-milled samples were separated and homogenized using a porcelain mortar and pestle to serve as controls. A mass of 10 g of each *fufu* sample was put into a sterile stomacher bag containing 90 mL of sterile peptone water (0.1%) or Buffered Peptone Water (BPW) (Oxoid CM 009; Oxoid Ltd Basingstoke, Hamsphire, England). Homogenizing was done using a stomacher (Laboratory Paddle Blender, DW 04) for 1–2 minutes to ensure even distribution of microbes. Serial dilutions (10^{-1} to 10^{-6}) were done using sterile test tubes, each containing 9 mL of sterile diluent (peptone water). A sterile pipette was used to transfer 1 mL from the homogenized sample into the first tube (10^{-1}), mix, then transfer to the next and repeat.

Culture Media and Preparation

i. For Total coliforms and *Escherichia coli*

Using a sterile pipette, 0.1 mL from appropriate dilutions (10^{-2} to 10^{-6}) was transferred onto the surface of MacConkey Agar plates and spread evenly using a sterile glass spreader. The agar plate method was employed to determine the total coliforms and *Escherichia coli* in the *fufu* samples. One milliliter aliquot from each of the dilutions prepared were inoculated onto a sterile MacConkey Agar (Oxoid CM 009; Oxoid Ltd Basingstoke, Hamsphire, England) and incubated at 44°C (±1) for both total coliforms and *Escherichia coli* for 48h. The colonies that showed pink red non mucoid colonies were counted as *E. coli* and all colonies on the plate were counted as total coliforms. A triplicate replication was done for all samples (ISO, 2017; Salfinger & Tortorello, 2015).

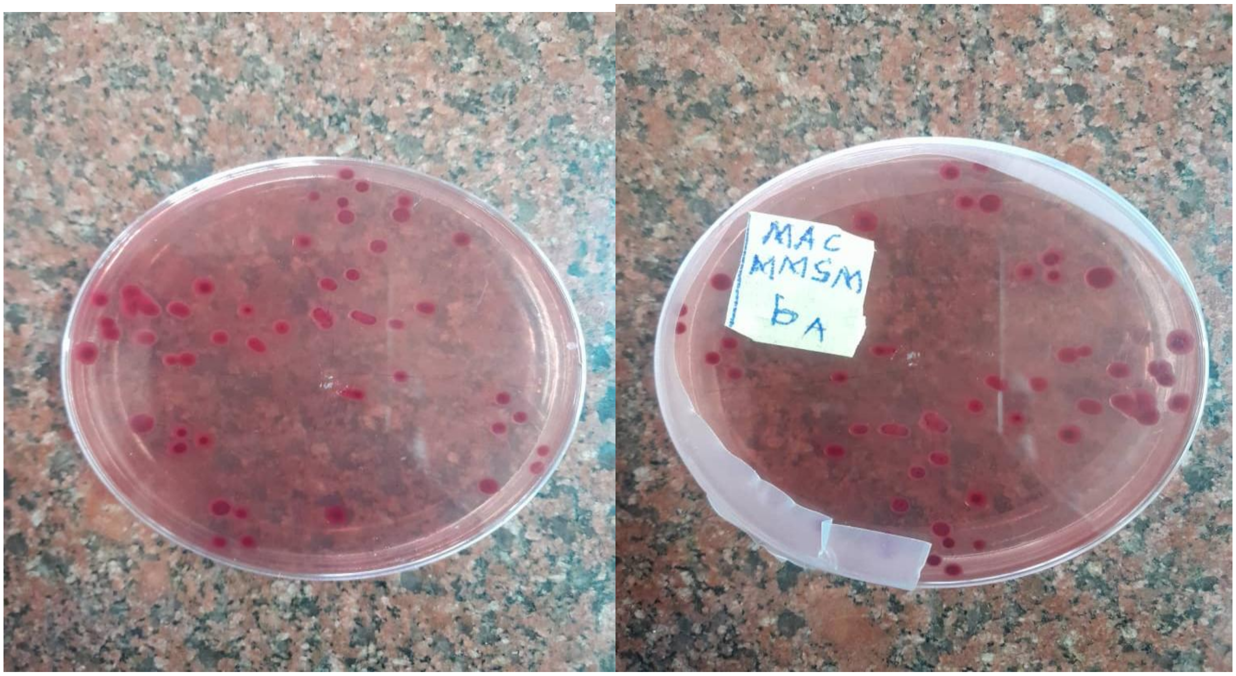

Figure 3. 3 MacConkey Agar Plate; *E coli*-small pink/red non-mucoid colonies.

ii. For *Salmonella* and *Shigella*

Salmonella-Shigella Agar (SS Agar) is a selective and differential agar used for the isolation and identification of *Salmonella* and some strains of *Shigella* from clinical and non-clinical specimens (ISO, 2017; Salfinger & Tortorello, 2015). Suspend the components, dehydrated powder, in water (63 grams in 1000 ml of purified/distilled water). Boiling and stirring frequently was done to completely dissolve the medium. The autoclave was not overheated, as overheating could destroy the selectivity of the medium. It was cooled to 50°C and poured into the Petri dishes. A volume of 0.1 mL of the sample was inoculated onto SSA (Oxoid CM 009; Oxoid Ltd Basingstoke, Hamsphire, England) plates, spread uniformly to obtain isolated colonies, and labelled accordingly. Selective and non-selective media were inoculated to increase the potential for isolation of enteric pathogens. Streaking the plate for isolation was done using a sterile inoculating loop. The methodology involved inoculating the sample onto the SS Agar plates and incubating them at 35-37°C for 18-24 hours in an inverted position. Salmonella colonies appeared as black or dark, while Shigella appeared as colourless colonies. This was done via a triplicate replication (ISO, 2017; Salfinger & Tortorello, 2015).

Figure 3. 4 *Salmonella-Shigella* Agar (SSA): *Salmonella*- cream colonies, *Shigella*-pink colonies.

3.5.3b Samples Preparation (*Fufu* for heavy metals)

Un-milled samples were separated and homogenized using a porcelain mortar and pestle to serve as controls. Samples were transported and stored in a cool, dry environment. Un-milled samples were separated and homogenized using a porcelain mortar and pestle to serve as controls. Samples were transported and stored in a cool, dry environment. A portion (about 50-100 g) of each fresh *fufu* sample was weighed and then subjected to wet acid digestion.

Wet acid digestion of *fufu* samples

Wet acid digestion was employed to isolate the metals in the food samples from their complex matrices before assessing them with the Atomic Absorption Spectroscopy (AAS). Each 1.0 g of food sample was digested by heating with a digestion mixture containing concentrated nitric, perchloric and sulphuric acids in a ratio of 2:2:5 in a 50 mL digestion tube. Each sample mixture was evaporated on a hot plate in a fume hood at 200 °C for 30 min until the brown fumes disappeared, leaving the white fumes.

The clear digests were cooled and diluted with distilled water to the 50 mL mark. Digestion of a reagent blank was carried out in parallel with the *fufu* samples, with the same digestion parameters. Appropriate dilutions were made with deionized water. The heavy metals were analyzed using a Flame Atomic Absorption Spectrophotometer (Model: novAA400P, Germany). The instrument was equipped with a deuterium lamp corrector and standard air acetylene flame. The mean and relative standard deviations (RSDs) of the spike recoveries for the triplicate analyses were determined.

3.6 Quality Assurance/Quality Control

To ensure the reliability, accuracy, and reproducibility of results obtained for Cd, As, Pb, and Zn using the novAA400P Atomic Absorption Spectrophotometer (Germany), a comprehensive quality assurance and quality control (QA/QC) protocol was implemented. This protocol encompassed calibration verification, contamination control, precision and accuracy checks, detection limit confirmation, and instrument performance monitoring.

Calibration Verification

Certified reference solutions (1000 mg/L) of Cd, As, Pb, and Zn were used to prepare working standards by serial dilution with ultrapure water. Calibration curves were constructed using at least five concentration points spanning the linear range of each element (Cd: 0.0005–2 mg/kg; As: 0.001–5 mg/kg; Pb: 0.001–5 mg/kg; Zn: 0.0005–5 mg/kg). The correlation coefficient (R^2) was required to be ≥ 0.999 to confirm linearity. Initial calibration verification (ICV) was performed using independent standards, with acceptable recovery between 90–110%. Continuing calibration verification (CCV) standards were analyzed every ten samples, and recalibration was performed if recoveries fell outside the acceptance range.

Blanks and Contamination Control

Reagent blanks and method blanks were included in each analytical batch to monitor contamination. Method blanks were processed alongside samples, with acceptable responses below the limit of detection (LOD). All reagents used were of trace-metal grade, and ultrapure water was employed throughout. Laboratory glassware and sample containers were acid-washed to minimize contamination. Carryover was checked by inserting blanks after high-concentration samples, with acceptable blank responses <5% of the preceding sample.

Precision and Accuracy

Precision was assessed by duplicate analysis of at least 10% of samples, with relative standard deviation (RSD) required to be $\leq 10\%$. Accuracy was evaluated through spike recovery experiments, where known concentrations of Cd, As, Pb, and Zn were added to representative matrices. Acceptable recovery ranges were 90–110% for Cd, Pb, and Zn, and 85–110% for As due to potential matrix interferences. Certified reference materials (CRMs) appropriate to the sample type (*fufu*) were analyzed in each batch, with results required to fall within the certified uncertainty or 90–110% of the stated value.

Detection and Quantification Limits

Table 3. 1 Linear range, linearity, recovery, limit of detection (LOD) and limit of quantification (LOQ) for the target heavy metals.

Element	Linear Range (mg/kg)	R^2	Recovery ± RSD (%)	LOD (mg/kg)	LOQ (mg/kg)
Zn	0.0005–5	0.9998	92.36 ± 6.90	0.27	0.39
Cd	0.0005–2	0.9990	90.15 ± 10	0.05	0.15
As	0.001–5	0.9990	85.01 ± 10	0.20	0.35
Pb	0.001–5	0.9990	90.48 ± 10	0.10	0.25

Limits of detection (LOD) and quantification (LOQ) were determined based on blank measurements and calibration slope, using the criteria of three times and ten times the standard deviation of blank signals, respectively. Typical values obtained were: Cd (LOD 0.05 mg/kg; LOQ 0.15mg/kg), As (LOD 0.20 mg/kg; LOQ 0.35mg/kg), Pb (LOD 0.10 mg/kg; LOQ 0.25 mg/kg), and Zn (LOD 0.27 mg/kg; LOQ 0.39 mg/kg). Results below LOQ were reported as “detected but not quantified,” while those below LOD were reported as “not detected.”

Instrument Performance Monitoring

Instrument performance was verified daily by checking lamp current, wavelength alignment, and baseline stability. Background correction using a deuterium lamp was applied to minimize matrix interferences. Nebulizer and burner components were cleaned regularly, and consumables were replaced according to manufacturer recommendations. Drift was monitored through CCV standards, with recalibration required if drift exceeded 10%.

3.7 Health Risk Assessment

Risk assessment is a tool for assessing the likelihood of health conditions or diseases in living organisms exposed to a toxic medium over time (Gerba, 2019; Bakand & Hayes, 2016). The probability of getting cancer (not the probability of dying of cancer) and the associated dose consist of an average taken over an assumed 70-year human lifetime (Grant et al., 2017). This dose is called the lifetime average daily dose or chronic daily intake.

3.7.1 Microbial Risk Assessment (Quantitative Microbial Risk Assessment (QMRA))

Quantitative Microbial Risk Assessment (QMRA) is used to estimate the risk of infection or illness from consuming contaminated food (in this case, *fufu*) (Alegbeleye & Sant'Ana, 2021; Kundu et al., 2018). Quantitative Microbial Risk Assessment (QMRA) in R Studio follows four main steps: hazard identification, exposure assessment, dose-response assessment, and risk characterization (Monte Carlo Simulation). The risk of infection (Pinf) was calculated using the Beta-Poisson model.

Dose (D)

The dose (D) is the number of pathogens ingested per meal:

$$\mathbf{D = C \times IR}$$

Where:

- C = Pathogen concentration (CFU/g)
- IR = Intake rate (g/day)

Beta-Poisson Model:

$$\mathbf{Pinf = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha}}$$

Where:

- **α, β** = Pathogen-specific parameters
- **D** = Dose of pathogen consumed

To account for variability and uncertainty, we run 10,000 simulations using a Monte Carlo simulation and analyze the risk distribution.

3.7.2 Risk Assessment for Heavy Metals

To account for the uncertainties inherent in the calculation process, the health risks to the local population resulting from metal exposure were assessed using a Monte Carlo simulation technique, implemented in R Studio software and involving 10,000 iterations. Before this process, the distribution characteristics of each exposure parameter were examined based on the exposure results. A probabilistic distribution of the exposure dose was then obtained as a simulation result.

The use of Monte Carlo simulations in risk assessment provides an understanding of the degree of uncertainty and variability around a risk estimate that single-point estimates of risk cannot provide (EPA, 1994b). The following Assumption was made for the ED = 70-year lifetime for carcinogenic effects (70 years × 365 days/year). The 70-year exposure duration was not meant to suggest that a person starts eating *fufu* from birth and continues every single day until death. It is a standard lifetime value recommended for carcinogenic risk assessment. Agencies such as the U.S. EPA use 70 years as a default assumption to represent an average human lifespan when estimating long-term exposure to contaminants.

In risk assessment, this value helps maintain consistency with established guidelines and allows results to be compared with other studies. It does not reflect actual daily consumption across all ages. Instead, it provides a conservative framework for estimating potential lifetime cancer risks when specific age-dependent consumption data are unavailable. Exposure duration was throughout the year, as consumption occurs every day of the year. The mean exposure concentration of contaminants was used in conjunction with exposed population variables, and the assessment identified variables to estimate contaminant intake.

The risk of heavy metals was evaluated using the Hazard Quotient (HQ) and Cancer Risk (CR) models.

Key Equations:

Chronic Daily Intake (CDI) (mg/kg-day):

CDI = $\frac{\mathrm{C} \times \mathrm{IR} \times \mathrm{EF} \times \mathrm{ED}}{BW \times \mathrm{AT}}$, **Equation 1**

Where:

- C = Heavy metal concentration in *fufu* (mg/kg)
- IR = Intake rate of *fufu* (kg/day)
- EF = Exposure frequency (days/year)
- ED = Exposure duration (years)
- BW = Body weight (kg)
- AT = Averaging time (days)

Hazard Quotient (HQ):

HQ = $\frac{CDI}{RFD}$, **Equation 2**

RfD = Reference dose (mg/kg-day) for each heavy metal

HQ > 1 suggests a **potential health risk**

Monte Carlo Simulation was run by clicking to simulate (10,000 trials). Analysis of the mean, the 95th percentile, and the probability of HQ > 1 was done with output presented on a histogram.

3.8 Statistical Analysis

The data obtained were analyzed using Microsoft Excel (2016 version), SPSS (Version 22) and R Statistical Software (version 4.5.1). The means of bacterial load (MPN) from samples collected at various sites were compared using One-Way Analysis of Variance (ANOVA), with significant differences determined at a 5% level of significance ($p = 0.05$). The impacts of milling on both microbial loads and heavy metal concentrations were assessed using the paired T-test. Additionally, the data were analyzed using descriptive statistics, T-tests, and One-Way ANOVA.

Descriptive statistics were used to summarize the levels of microbial contamination in the food samples. The microbial quality of the food samples was evaluated in accordance with Food and Agriculture Organization (FAO) and World Health Organization (WHO) guidelines to identify potential hazards and sources of contamination. Furthermore, the results from the observations and questionnaires were analyzed to identify potential sources and risk factors and to make recommendations for improving the microbial and heavy metal quality of *fufu* produced by milling machines.

3.9 Ethical considerations

Prior to participation in the study, the researcher ensured that all participants were fully informed about the purpose, procedures, potential risks, and benefits of the research. Informed consent was obtained from each participant, confirming their voluntary agreement to take part in the study with a clear understanding of their rights, including the option to withdraw at any stage without penalty. Ethical approval and clearance were obtained at KNUST (CHRPE/AP/559/25).

CHAPTER FOUR

RESULTS

4.1 Introduction

This chapter presents the findings on microbial and heavy metal contamination, followed by an in-depth discussion of their implications. The results include an analysis of microbial contamination levels, focusing on the presence of coliform bacteria, *E. coli, Salmonella, and Shigella*, as well as their associated infection probabilities. Additionally, the chapter examines the concentrations of heavy metals, including arsenic, lead, zinc, and cadmium, and compares them with established regulatory standards.

4.2 Level of microbial counts in *fufu* produced from *fufu* milling machines in Nsuta.

Table 4.1 presents data on microbial contamination levels of *fufu* samples from 10 different areas, focusing on *Salmonella, Shigella*, *E. coli,* and total coliforms. The data is compared against FAO/WHO limits for microbial contamination

Table 4. 1 Microbial levels of raw and milled *fufu* (cassava and plantain)

Sample /	***Salmonella* (×10^{-6}) (CFU/g)**		***Shigella* (×10^{-6}) (CFU/g)**		***E-coli* (×10^{-6}) (CFU/g)**		**Total coliform (×10^{-4}) (CFU/g)**	
FAO/WHO limits	**<2.0**		**<2.0**		**<2.0**		**<2.0**	
	Raw	**Milled**	**Raw**	**Milled**	**Raw**	**Milled**	**Raw**	**Milled**
Abonkosu-1	2.19	2.78	1.19	2.78	Nil	Nil	2.90	3.76
Abonkosu-2	Nil	1.61	Nil	2.50	Nil	Nil	2.52	3.30
Abonkosu-3	1.50	3.05	1.65	3.37	Nil	Nil	1.60	4.00
Powerhouse-1	1. 34	2.59	1.43	3.60	Nil	Nil	1.36	3.30
Powerhouse-2	3.00	6.80	4.20	7.93	Nil	Nil	5.20	9.80
Powerhouse-3	1.09	2.88	2.19	2.92	Nil	Nil	2.90	3.76
Anansu-1	Nil	1.61	Nil	2.66	Nil	Nil	Nil	3.70
Anansu-2	1.50	3.05	1.68	3.33	Nil	Nil	1.90	3.76
Anansu-3	1. 34	2.59	1. 78	2.71	Nil	Nil	2.00	3.30
Hemang-1	3.30	4.82	3.17	4.42	Nil	Nil	2.80	3.36
Hemang-2	2.20	3.89	2.23	4.19	1.27	2.53	3.63	5.80
Hemang-3	Nil	1.61	Nil	1.78	Nil	Nil	1.20	2.76
Saamete-1	1.50	3.05	1.45	3.16	Nil	Nil	2.25	4.36
Saamete-2	1. 34	2.59	1. 43	2.89	Nil	Nil	2.22	4.30
Saamete-3	2.19	5.81	2.28	5.95	Nil	Nil	3.33	7.38

Doku-1	2.29	3.78	2.30	3.83	Nil	Nil	1.42	4.88
Doku-2	Nil	4.61	Nil	4.34	Nil	Nil	1.22	5.36
Doku-3	1.50	3.25	1.66	3.39	Nil	Nil	1.41	5.66
Zongo-1	1. 35	3.59	2. 34	4.49	1.90	3.76	1.33	6.56
Zongo-2	2.10	4.80	1.05	3.68	2.00	3.30	3.34	6.38
Zongo-3	1.49	2.68	2.29	3.79	1.60	4.00	3.15	5.26
Bonkro-1	Nil	3.61	Nil	2.74	1.20	3.30	1.47	6.67
Bonkro-2	1.50	4.05	1.32	3.45	Nil	2.80	2.35	6.38
Bonkro-3	1. 34	2.59	1. 78	3.87	Nil	Nil	2.46	7.46
Asokwa-1	2.00	5.80	1.80	4.46	Nil	Nil	2.45	6.76
Asokwa-2	1.19	2.78	2.21	4.92	Nil	Nil	2.50	7.86
Asokwa-3	Nil	1.61	Nil	2.14	Nil	Nil	1.28	4.38
Asuafo-1	1.50	4.15	1.75	3.25	Nil	Nil	2.63	5.56
Asuafo-2	1. 34	2.59	2. 37	2.45	Nil	Nil	1.32	3.37
Asuafo-3	3.00	5.80	2.20	6.50	Nil	Nil	4.24	7.36

Salmonella and Shigella

There was an indication that, in the unmilled cassava and plantain samples analyzed, no contamination was detected (referred to as “Nil”) in some locations. In contrast, the maximum level of contamination recorded was 3.00 CFU/g. The CFU/g (colony-forming units per gram) measurement is a standard unit used to quantify the number of viable bacteria in a sample. Prior to milling, half of the raw samples contained *Salmonella* levels below the acceptable limit, while the other half had contamination levels that exceeded safe limits. In contrast to the raw samples, the milled *fufu* samples showed a range of *Salmonella* contamination between 1.61 and 6.80 CFU/g. This indicates that the milling process may contribute to higher levels of contamination compared to the unprocessed (raw) samples. Following milling, all samples exhibited an increase in *Salmonella* levels, with several exceeding the FAO/WHO limits, particularly in Powerhouse-2 (6.80 CFU/g), Saamete-2 (5.81 CFU/g), and Asuafa-3 (5.80 CFU/g).

It is worth noting that certain regions, such as Abonkosu-2, Hemang-3, Bonkro-1, and Asokwa-3, had no detectable *Salmonella* in the raw samples but exhibited contamination after the milling process. Like *Salmonella, Shigella* levels rose after milling across all sites. Raw samples ranged from “nil - 4.20 CFU/g”, which indicated that *Shigella* was either absent (Nil) or present in varying concentrations in raw samples, with the highest recorded level being 4.20 CFU/g. This suggests that while some raw samples were uncontaminated, others had notable levels of contamination. Many raw samples showed no detectable *Shigella*, but the levels post-milling were notably elevated. Milled samples ranged from 1.78 to 7.93 CFU/g, indicating that contamination levels increased significantly after the milling process. The lowest milled sample contained 1.78 CFU/g of *Shigella,* while the highest reached 7.93 CFU/g (Powerhouse-2).

E. coli and Total coliform

Raw samples did not reveal any detectable *E. coli*, except for Hemang-2, Zongo-1, Zongo-2, Zongo-3 and Bonkro-1, which had a presence of 1.27 CFU/g, 1.90 CFU/g, 2.0 CFU/g, 1.6 CFU/g and 1.20 CFU/g, respectively. After milling, only the sites stated to have had the presence of it showed *E. coli* contamination, indicating sporadic yet considerable cross-contamination. This indicates that the presence of E. coli in the *fufu* samples from the processing locations is largely absent E. coli is largely absent in the *fufu* samples from the processing locations. This is a positive finding, as it suggests that most samples did not show detectable levels of this harmful bacterium, which is significant in food safety assessments.

For total coliforms, the raw samples ranged between 0 and 5.20 CFU/g, indicating that microbial contamination levels were not detected (0) up to a maximum of 5.20 CFU/g in the unmilled cassava and plantain samples studied. This suggests that Un-milled cassava and plantain are generally low in contamination, falling within the safety limits outlined by FAO/WHO, which is set at <2.0 CFU/g for microbial presence. Milled samples ranged from 2.76 to 9.87 CFU/g, highlighting that the milling process significantly increased contamination levels, with values ranging from 1.30 to 7.87 CFU/g. This increase is concerning as it exceeds the safety limit in some cases. The highest levels detected post-milling were in Zongo-1, at 3.76 CFU/g, and in Asokwa-1, at 4.46 CFU/g. Every sample indicated an increase in total coliforms after milling. The highest contamination was noted in Powerhouse-2 (9.80 CFU/g), Asokwa-2 (7.86 CFU/g), and Bonkro-3 (7.46 CFU/g). Even sites with comparatively lower contamination in the raw samples (such as Hemang-2 and Zongo-3) exhibited coliform levels that were 2 to 3 times higher post-milling.

4.2.1 Impacts of milling on microbial load contamination levels

The study examined the impact of milling on microbial load contamination, as revealed in Table 4.2.

Table 4. 2 Impacts of milling on microbial contamination

Microorganism	Pearson Correlation	t-Statistic	P-value
Salmonella	0.464	-11.567	**0.00**
Shigella	0.631	-13.992	**0.00**
E. coli	0.95	-1.72	**0.061**
Total Coliforms	0.224	-4.446	**0.001**

This paired t-test (Table 4.2) examined whether the milling process significantly alters microbial contamination levels in *fufu* by comparing pre- and post-milling data for *Salmonella, Shigella, E. coli*, and Total Coliforms. Statistical analysis revealed a significant increase in the levels of *Salmonella*, *Shigella*, and Total Coliforms after milling. The p-values for these bacteria are close to zero, indicating a strong rejection of the null hypothesis of no change in contamination levels. This suggests that milling plays a crucial role in enhancing their presence. In contrast, the data show no significant change in *E. coli* levels post-milling. With a p-value of 0.061 above the 0.05 threshold, there is insufficient evidence to reject the null hypothesis, suggesting that milling does not substantially affect *E. coli* contamination. Correlation analysis further highlighted varying trends. *E. coli* levels before and after milling exhibit a strong correlation (0.95), indicating consistency in their presence. Meanwhile, Total Coliforms displayed a weak correlation (r = 0.224), reflecting considerable variability in contamination levels after milling. This suggests that while some bacteria remain stable, others are more susceptible to significant fluctuations due to the milling process.

4.2.2 Difference in contamination levels across various locations.

Table 4.3 presents the statistical differences in microbial contamination across locations, focusing on four key pathogenic microorganisms*: Salmonella, Shigella, Escherichia coli (E. coli),* and total coliforms.

Table 4. 3 Difference in Microbial Contamination Across Locations.

Micro-organism	F	P-value
Salmonella	0.217	**0.985**
Shigella	0.213	**0.985**
E-coli	5.205	**0.008**
Total coliform	0.355	**0.933**

The analysis of variance (ANOVA) results indicated that among the tested microorganisms, *E. coli* exhibited a statistically significant difference across locations (F = 5.205, p = 0.008). At the same time, *Salmonella*, *Shigella*, and total coliforms showed no significant variations (p-values > 0.05). The analysis showed that *Salmonella* (F = 0.217, p = 0.985), *Shigella* (F = 0.213, p = 0.985), and total coliforms (F = 0.355, p = 0.933) did not exhibit significant variations across locations. The extremely high p-values suggest that contamination levels of these pathogens were relatively uniform across different sampling sites, indicating that their presence may be widespread rather than site-specific.

4.3 Levels of heavy metal contamination in *fufu* produced from *fufu* milling machines in Nsuta.

The presence of lead (Pb), cadmium (Cd), arsenic (As), and zinc (Zn) in food poses significant health risks, particularly with prolonged exposure. This study evaluated heavy metal contamination in raw and milled *fufu* across ten locations in Nsuta-Ashanti, comparing results with FAO/WHO safety limits to assess potential health implications. Table 4.4 revealed the levels of heavy metal contamination of raw and milled *fufu* (cassava and plantain).

Table 4. 4 Levels of heavy metal concentration (*mg/Kg*) of raw and milled *fufu* (cassava and plantain)

Sample	**Lead (Pb)**		**Cadmium (Cd)**		**Arsenic (As)**		**Zinc (Zn)**	
FAO /WHO	**0.3**		**0.2**		**0.5**		**3.3**	
limits	**Raw**	**Milled**	**Raw**	**Milled**	**Raw**	**Milled**	**Raw**	**Milled**
Abonkosu-1	0.3246	0.3337	0.2041	0.2407	0.0021	0.0024	0.0013	0.0014
Abonkosu-2	0.3831	0.3924	0.1017	0.1022	0.0018	0.0021	0.0017	0.0018
Powerhouse-1	0.2136	0.2448	0.1013	0.1019	0.0015	0.0019	0.0012	0.0014
Anansu-1	0.2063	0.2072	0.0137	0.0155	0.0017	0.0023	0.0015	0.0016
Anansu-2	0.2836	0.2859	0.1024	0.1036	0.0019	0.0022	0.0017	0.0018
Hemang-1	0.5142	0.5163	0.2038	0.2055	0.0018	0.0024	0.0014	0.0015
Saamete-1	0.3957	0.3965	0.1026	0.1032	0.0017	0.0021	0.0012	0.0015
Saamete-2	0.1742	0.1761	0.2015	0.2019	0.0020	0.0023	0.0015	0.0016
Doku-1	0.2261	0.2272	0.0218	0.0222	0.0018	0.0021	0.0012	0.0015
Zongo-1	0.5683	0.5695	0.2048	0.2155	0.0019	0.0024	0.0013	0.0016
Zongo-2	0.5278	0.5367	0.2022	0.2134	0.0012	0.0015	0.0014	0.0014
Bonkro-1	0.4131	0.4549	0.1031	0.2038	0.0014	0.0017	0.0012	0.0015
Bonkro-2	0.0723	0.0891	0.2012	0.2814	0.0021	0.0024	0.0015	0.0016
Asokwa-1	0.4014	0.4134	0.1913	0.2321	0.0015	0.0019	0.0016	0.0017
Asuafo-1	0.2267	0.2477	0.1021	0.2028	0.0026	0.0031	0.0015	0.0018

Lead (Pb) Concentration

Surprisingly, 6 out of 15 samples surpassed the FAO/WHO threshold for lead in unprocessed samples. The highest levels of concentration were found in Zongo-1 (0.5683 mg/kg) and Zongo-2 (0.5278 mg/kg), which are nearly double the safety standard. After milling, lead concentrations showed a slight rise in most samples, indicating potential contamination from the milling equipment. Furthermore, locations that initially had lower lead levels in their raw samples, such as Powerhouse-1 and Anansu-1, also experienced slight increases in lead levels following the milling process.

Cadmium (Cd) Concentration

Four out of fifteen samples surpassed the cadmium limit in their raw samples: Abonkosu-1 (0.2041 mg/kg), Hemang-1 (0.2038 mg/kg), Zongo-1 (0.2155 mg/kg), and Zongo-2 (0.2134 mg/kg). After milling, cadmium levels showed a slight increase in most sites, suggesting possible contamination during the milling process. The most significant rise was observed in Doku-1, with cadmium levels escalating from 0.2018 mg/kg (raw) to 0.222 mg/kg (milled).

Arsenic (As) Concentration

All samples adhered to the FAO/WHO safety standards for arsenic. The highest recorded arsenic level was in Asuafa-1 (0.0031 mg/kg post-milling), which remains significantly below the allowable limit. Specific locations (such as Bonkro-2) exhibited no rise in arsenic levels following milling, indicating that the milling process does not lead to arsenic contamination.

Zinc (Zn) Concentration

All samples were significantly below the safety threshold for zinc set by FAO/WHO. Although zinc levels exhibited minor increases after milling, the amounts stayed extremely low, ranging from 0.0012 to 0.0018 mg/kg. The zinc levels showed slight increases post-milling. This suggests that the milling process may have introduced a minor amount of zinc into the samples or that the milling could have caused re-distribution of existing zinc within the *fufu* samples. The values mentioned (between 0.0012 and 0.0018 mg/kg) are extremely low, reinforcing the point that zinc contamination in these *fufu* samples is negligible.

4.3.1 Impacts of Milling on Levels of Heavy Metal Concentration

This study investigated whether the milling process had a significant impact on the heavy metal concentration, as shown in Table 4.5.

Table 4. 5 t-Test: Paired Two-Sample for Means

Heavy metal	Pearson Correlation	t-Statistic	P-value
Lead	0.996	-2.810	**0.011**
Cadmium	0.862	-2.024	**0.039**
Arsenic	0.970	-12.833	**0.000**
Zinc	0.847	-6.957	**0.000**

The results presented in Table 4.5 showed a significant difference in the concentration of heavy metals (Lead, Cadmium, Arsenic, and Zinc) before and after milling. The results indicated a consistent relationship between heavy metal contamination in raw and milled *fufu*, as shown by the strong to very strong correlations across all tested metals (Pb, Cd, As, and Zn). This suggests that contamination levels in Un-milled cassava and plantain largely determine the extent of contamination in the milled product. However, significant reductions were observed in several metals after milling, indicating that processing plays a role in reducing contamination, likely through the removal of surface-bound particles.

Among the metals analyzed, **arsenic (As) and zinc (Zn)** showed the most significant reductions, with extremely low p-values ($p < 0.001$). This suggests that milling effectively reduces arsenic contamination, likely due to its surface accumulation, making the removal of arsenic during processing crucial for food safety. Similarly, zinc levels decreased substantially, which, although beneficial in removing excess contamination, raises concern about potential nutrient loss, as zinc is an essential trace element. **Lead (Pb) and cadmium (Cd)** also exhibited strong correlations between raw and milled samples. While lead levels significantly declined after milling ($p < 0.05$), their strong correlation suggests that contamination remains relatively stable throughout processing.

Cadmium reduction was statistically significant ($p = 0.039$), indicating that milling reduces cadmium levels to some extent. Overall, these findings emphasize the importance of milling as a contamination control measure in *fufu* processing. While it effectively reduces heavy metal contaminants, particularly arsenic and lead, the extent of reduction varies by metal. This highlights the need for additional interventions, such as pre-processing washing, stricter raw material sourcing, and improved milling practices, to further minimize exposure to harmful contaminants while preserving essential nutrients.

4.3.2 Difference in heavy metal concentration levels across various locations.

The results (Table 4.6) from the 10 sampled locations revealed critical insights into the presence and statistical significance of heavy metal concentration in *fufu*.

Table 4. 6 Difference in Heavy Metal Concentration Across Locations.

Heavy Metal	F	P-value
Lead	233.956	0.000
Cadmium	8.612	0.001
Arsenic	5.205	0.073
Zinc	1.779	0.191

The F-values indicate the strength of variability in concentration levels across locations, while the p-values determine the statistical significance of these variations. The extremely high F-value (233.956) and p-value (0.000) indicate a highly significant variation in lead concentration across locations. This suggests that lead levels in *fufu* vary considerably based on location, likely due to differences Sin environmental contamination, water quality, and the type of processing equipment used. The strong statistical significance ($p < 0.001$) confirms that these variations are not due to random chance.

The F-value (8.612) and p-value (0.001) indicate significant variability in cadmium contamination across locations. While lower than lead, cadmium concentration remains a concern, as it is known to accumulate in root crops like cassava, from which *fufu* is made. This suggests that environmental factors, such as soil contamination, water used for processing, and milling equipment, could influence cadmium levels. The F-value (5.205) and p-value (0.073) suggest moderate variation in arsenic levels across locations, though the statistical significance is borderline ($p > 0.05$). While this does not confirm substantial differences in arsenic concentration between locations, the F-value indicates some degree of variability, warranting further investigation.

Zinc presents the lowest F-value (1.779) and a non-significant p-value (0.191), indicating that its levels remain relatively consistent across locations, with no statistically significant variation. Unlike toxic metals like lead and cadmium, zinc is a vital trace element essential for human health. However, excessive zinc concentration can still pose risks if it results from industrial pollution.

4.4 Factors of microbial quality and heavy metal concentration in the milling machines in Nsuta.

4.4.1 Demographic data of Operators

Table 4.7 revealed the demography of the *fufu* milling machine operators in the ten areas in Nsuta. The majority (70%) of the operators are between 20 and 30 years old, while 30% fall within the 31-40 years' bracket. Notably, no operators are under 20 or above 40 years old. This suggests that *fufu* milling is primarily a profession for young adults, possibly due to the physical demands of operating the machine. The data reveals a significant gender disparity, with 80% male and only 20% female operators. Most machines (60%) are between 1 and 3 years old, while 30% are 4 and 6 years old, and 10% are less than a year old. Machine age is a critical determinant of mechanical wear, contamination risk, and operational efficiency. The operators have diverse educational backgrounds, with 50% having primary education, while none reported having no formal education.

Table 4. 7 Demographic Data of Operators

Parameter	n(%)
Age (Years)	
Under 20	0
20–30	7(70)
31–40	3(30)
41-50	0
Gender	
Male	8(80)
Female	2(20)
Age of the Milling Machine	
Under 1 year	1(10)
1-3 years	6(60)
4-6 years	3(30)
Level of Education	
Primary Education	5(50)
Secondary Education	3(30)
Tertiary Education	2(20)
No Formal Education	0

4.4.2 Hygiene and Maintenance of Milling Machines

Table 4. 8 Maintenance of Milling Machines

Parameter	n(%)
The regularity of cleaning the milling machine	
After every use	0
Daily	10 (100)
Weekly	0
Cleaning materials used	
Water only	8(80)
Water and detergent/soap	2(20)
Visibility of signs of rust or wear and tear on the milling machine.	
Yes	6 (60)
No	4 (40)
Visibility of signs of dirt or food residue in the milling area	
Yes	2(20)
No	8(80)
How often is the milling machine serviced or maintained	
Weekly	0
Monthly	1(10)
Quarterly	4(40)
Rarely	5(50)
Who is responsible for maintaining the machine?	
Operator (myself)	7(70)
Technician	3(30)
Manufacturer’s representative	0
Total	**10(100)**

Table 4.8 reveals the hygiene and maintenance of the milling machines, as well as the frequency with which they are cleaned. It is encouraging that all operators (100%) clean their milling machines daily. However, the absence of after-use cleaning is alarming (Table 4.8). Rust and wear on food-processing equipment are not just aesthetic issues; they pose real health hazards. A significant 60% of operators reported visible rust and wear on their milling machines, indicating that these machines may leach heavy metals into the *fufu*. The data reveal that 80% of milling environments appear clean, with only 20% showing visible food residue or dirt. While this may seem reassuring, it does not necessarily indicate a safe environment. Perhaps the most concerning aspect of the findings is the maintenance frequency. A staggering 50% of operators "rarely" service their machines, and 40% do so only quarterly. The fact that 70% of operators perform their own machine maintenance, while only 30% rely on technicians, raises questions about the quality of maintenance.

4.4.3 Hygiene Practices During Operation

The study also examined the hygienic practices of the milling machine operators, and the summary is revealed in Table 4.9. The lack of protective clothing among *fufu* mill operators is concerning, as revealed in Table 4.9, as no operators always wear it, only 20% wear it sometimes, 10% wear it rarely, and 70% never wear it. Hand hygiene is a simple yet effective measure to prevent the spread of contamination. However, only 10% of operators always wash their hands before and after milling, while 90% do so only sometimes, and none do so rarely or never. Training is crucial in shaping hygiene behaviour, yet only 40% of operators have received hygiene training, while 60% have never had any form of hygiene education.

Table 4. 9 Hygiene Practices during Operation

Parameter	n(%)
The regularity of wearing protective clothing during milling	
Always	0
Sometimes	2 (20)
Rarely	1 (10)
Never	7 (70)
Handwashing before and after operating the machine	
Always	1 (10)
Sometimes	9 (90)
Rarely	0
Never	0
Receiver of any hygiene training or guidance related to operations	
Yes	4 (40)
No	6 (60)
Total	**10 (100)**

4.5 Risk Assessment

4.5.1 Biodata of customers

The demographic results show that the majority of respondents were female, accounting for 86% (89 respondents), while males represented 14% (15 respondents). In terms of age distribution, most respondents were between 18 and 30 years old (37%), followed by those aged 41 to 50 years (26%), and 10% between 31 and 40 years. Regarding educational attainment, 41% of respondents had secondary education, 26% had tertiary education, and 18% had no formal education. These results indicate that the study population was largely composed of young to middle-aged individuals with varying levels of formal education, dominated by males.

Table 4. 10 Summary of relevant demographics

Parameter	n(%)
Gender	
Male	15 (14)
Female	89 (86)
Age (Years)	
Under 18	13 (13)
18–30	38 (37)
31–40	10 (10)
41-50	27 (26)
Above 50	16 (15)
Level of Education	
Primary Education	15 (15)
Secondary Education	43 (41)
Tertiary Education	27 (26)
No Formal Education	19 (18)

4.5.2 Estimation of dietary intake/exposure

The data from the questionnaire were categorized and converted to show the mean daily consumption of the milled *fufu* in grams per person per day (Table 4.11). The average *fufu* consumption per meal is 0.4612 kg (461.2 g), indicating that most individuals consume nearly half a kilogram of *fufu* per meal. However, there is considerable variation in consumption, as shown by the standard deviation of 0.1060 kg. The 95th percentile value of 0.6 kg suggests that only 5% of individuals consume more than 0.6 kg per meal.

Table 4. 11 Distribution of mean daily consumption (kg/person/day)

Statistic	
Mean Consumption	0.4612
Standard Deviation	0.106
Minimum Consumption	0.2
Maximum Consumption	0.7
95th Percentile	0.6

4.5.3 Weight distribution

The weights of 104 respondents were measured in the survey (Tables 4.12 and 4.13).

Table 4. 12 Weight Distribution

Weight (kg)	Frequency (n)	Percent (%)	Cumulative Percent(%)	95% CI Lower (%)	95% CI Upper (%)
41-45	2	2	2	0.24	7.03
46-55	11	11	13	5.54	18.83
56-60	11	11	24	5.54	18.83
61-70	54	52	76	42.27	61.53
>70	26	25	100	17.05	34.42
Total	104	10%	100	-	-

Table 4.12 indicated that 52% of individuals weighed between 61-70 kg, while 25% weighed more than 70 kg. Smaller portions fall within the 46-55 kg (11%) and 56-60 kg (11%) ranges, with only 2% in the 41-45 kg category, making it the least represented.

Table 4. 13 Body weight (kg) of customers

Statistic	
Mean weight	65.19
Standard Deviation	8.06
Minimum weight	43.00
Maximum weight	75.50
95th Percentile	75.50

Table 4.13 revealed that the average body weight was 65.19 kg, with a standard deviation of 8.06 kg, indicating some variation around this mean. Weights range from a minimum of 43 kg to a maximum of 75.5 kg, indicating a balanced distribution with no significant outliers. The 95th percentile is 75.5 kg, meaning only 5% of individuals exceed this weight.

4.6 Human health risk associated with the consumption of *fufu* contaminated with microbial loads.

4.6.1 *Salmonella*

Figure 4.1 (histogram) illustrates the distribution of *Salmonella* infection probabilities based on a Monte Carlo simulation, highlighting the variability in infection risk across different exposure scenarios.

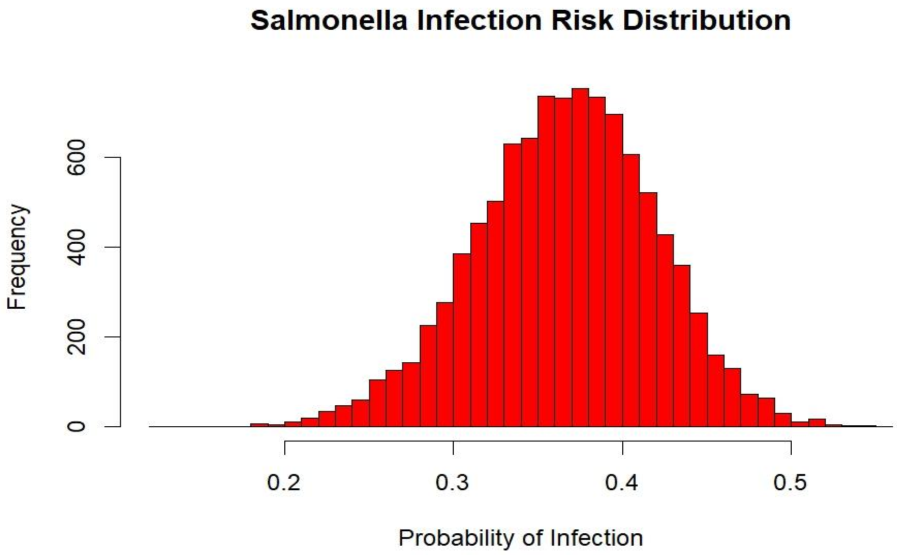

Figure 4. 1 Salmonella Infection Risk Distribution.

The bell-shaped distribution, centred around a probability of infection between 0.35 and 0.40, suggests that most simulated cases fall within this range, with fewer cases exhibiting extreme probabilities below 0.2 or above 0.5.

The plot (Figure 4.2) illustrates the relationship between *Salmonella* dose (CFU) and the probability of infection, following a dose-response model. Even at very low doses (near 0 CFU), there is a non-zero probability of infection (~0.1-0.2), suggesting that *Salmonella* can still cause infection at minimal exposure levels. When the dose exceeds 5,000 CFU, the infection probability approaches 50%, and beyond 10,000 CFU, it continues rising but at a slower rate, aligning with the Beta-Poisson dose-response model. The observed dose-response relationship for *Salmonella* in the plot aligns with findings from established microbial risk assessment studies.

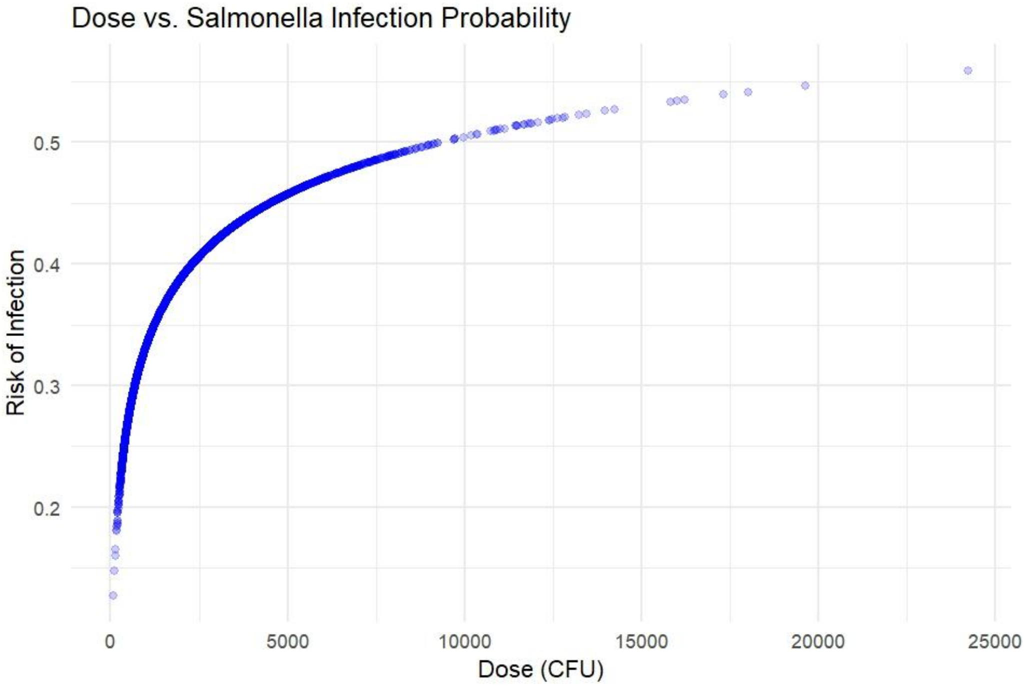

Figure 4. 2 Dose vs *Salmonella* infection probability

The bar chart (Figure 4.3) titled “Proportion of High vs. Acceptable Risk Cases” visually represents the percentage of simulations that fall under either High Risk (> 0.001) or Acceptable Risk (< 0.0001) categories.

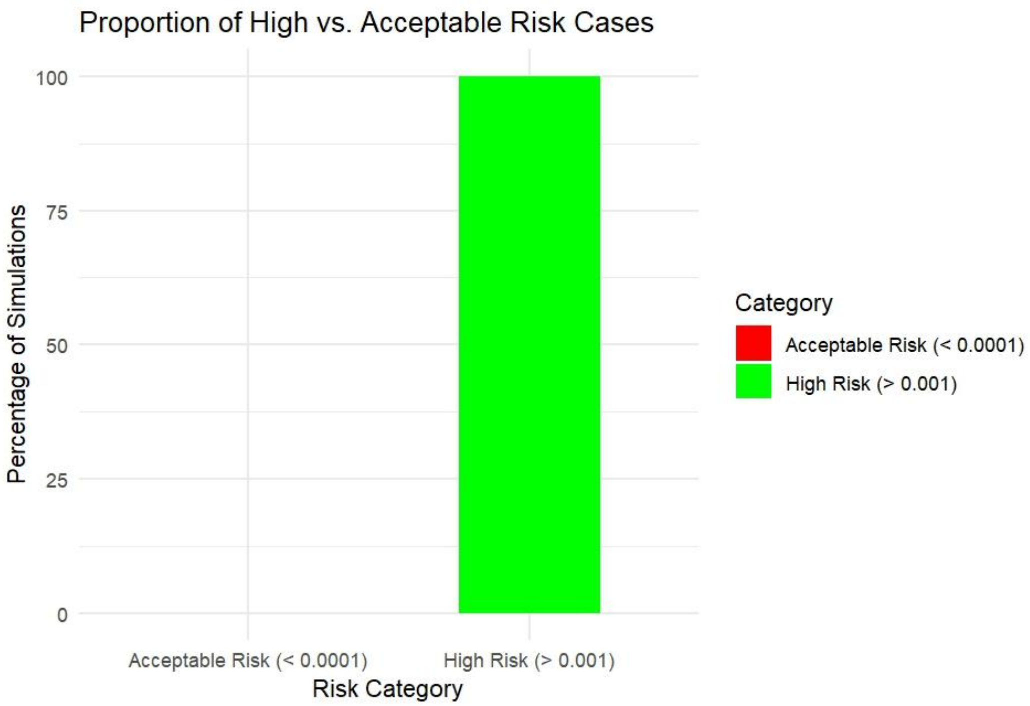

Figure 4. 3 Proportion of High vs. Acceptable Risk Cases

The chart reveals that 100% of the simulations resulted in a high-risk classification (Pinf > 0.001), indicating a consistently significant health threat due to microbial contamination across all scenarios. The lack of a red bar (which would represent acceptable risk cases) confirms that none of the simulated scenarios met the safety threshold (Pinf < 0.0001). This raises a serious concern, as every analyzed case exceeds regulatory safety limits, highlighting the urgent need for intervention to mitigate microbial contamination risks.

4.6.2 *Shigella*

The histogram (Figure 4.4) presents the distribution of infection probabilities (Pinf) for *Shigella*, illustrating the variability in infection risk across different exposure conditions. The distribution is roughly bell-shaped, with most infection probabilities concentrated between 0.6 and 0.75, peaking around 0.7.

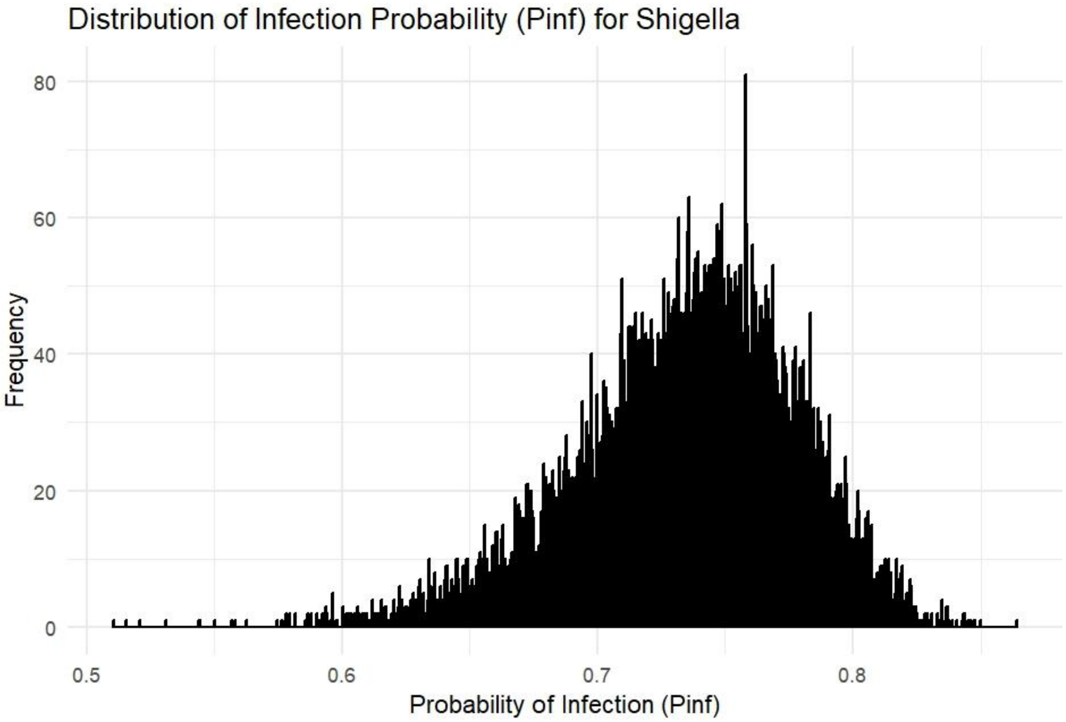

Figure 4. 4 Distribution of Infection Probability for *Shigella*

4.6.3 *Escherichia coli*

Figure 4.5 (histogram) displays the probability distribution of *E. coli* infection, with the majority of infection probabilities concentrated between 0.4 and 0.75. This pattern suggests that E. coli has a relatively high infectious potential, albeit with a slightly lower probability of infection compared to highly virulent pathogens, such as *Shigella*. The peak infection probability around 0.6–0.75

suggests that while exposure to *E. coli* does not always lead to infection, the likelihood increases significantly at moderate to high doses.

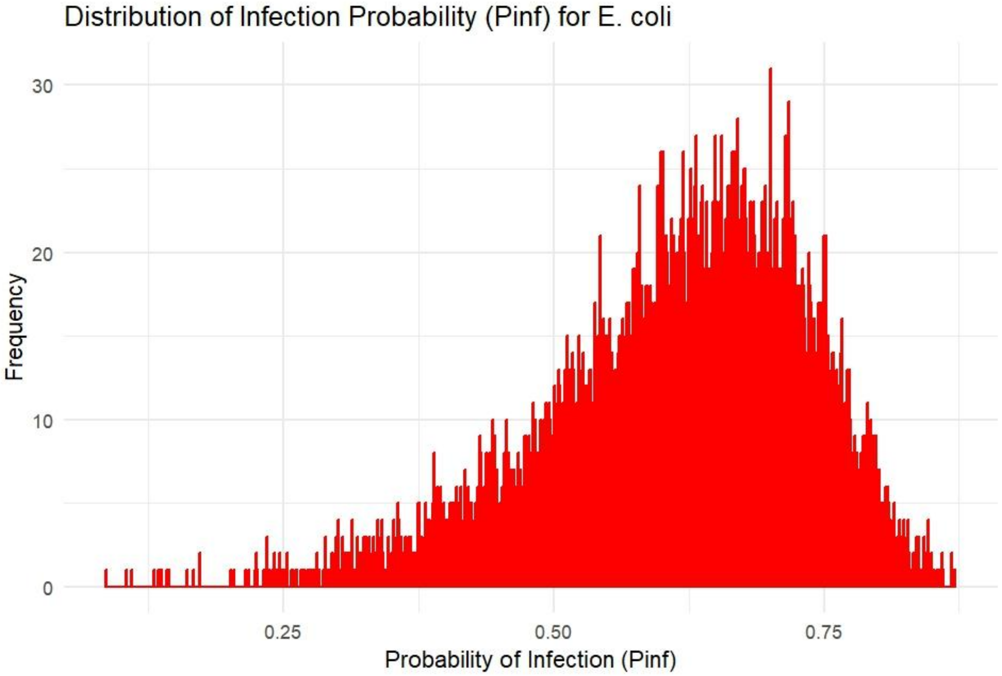

Figure 4. 5 Distribution of Infection Probability of *E coli*.

4.6.4 Total coliform

The histogram presented in Figure 4.6 revealed the probability distribution of infection for total coliform bacteria.

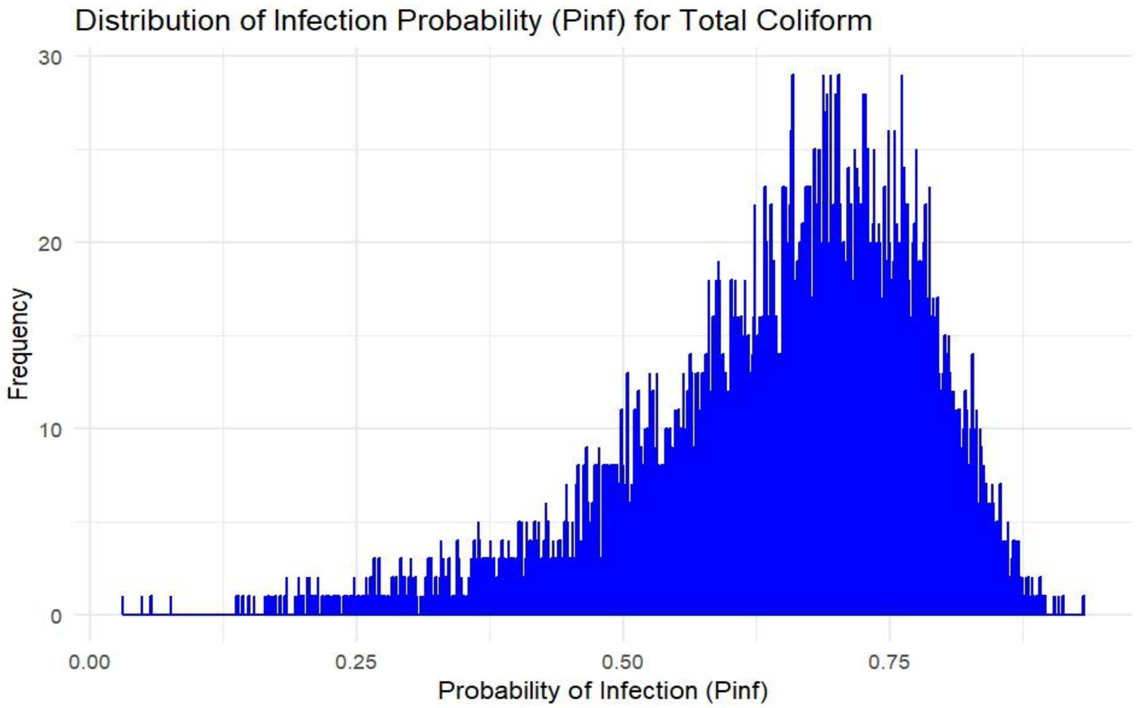

Figure 4. 6 Distribution of Infection Probability for total coliform.

The histogram presented revealed the probability distribution of infection for total coliform bacteria, with most infection probabilities clustering between 0.4 and 0.8. The peak infection probability is around 0.7, indicating that coliform bacteria have a moderate to high potential for causing infections, though with a broader range of probabilities compared to highly virulent pathogens like *Shigella*. The mean probability was 0.659. This suggests that while exposure to coliform bacteria can lead to infection, the probability of infection depends on factors such as the virulence of the strain, host immunity, and the exposure dose. Coliform bacteria are commonly used as indicators of faecal contamination in water quality assessments rather than direct causes of illness.

4.7 Risk Assessment of Heavy Metal Contamination

4.7.1 Cadmium (Cd)

The Monte Carlo Simulation (MCS) histogram (Figure 4.7) provides a probabilistic risk assessment of cadmium exposure through milled *fufu*, a staple food essential in many West African communities. The HQ values predominantly range between 0.2 and 0.4, with a peak near 0.3. This indicates that the majority of simulated cadmium exposure scenarios remain below the safety threshold (HQ < 1) set by regulatory agencies such as the USEPA and WHO. Since HQ < 1 in most cases, acute toxicity is unlikely to occur in the general population.

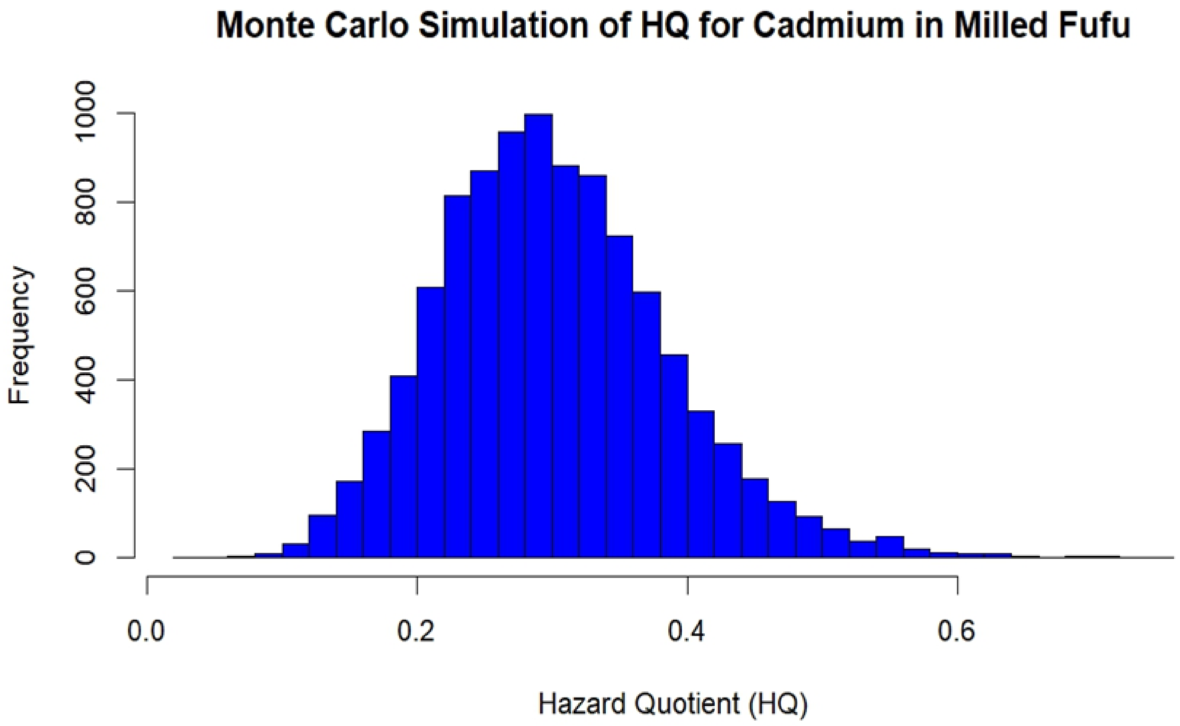

Figure 4. 7 Monte Carlo simulation of HQ for Cadmium in Milled *Fufu*

4.7.2 Lead (Pb)

The histogram (Figure 4.8) presents the Monte Carlo simulation of the Hazard Quotient (HQ) for lead (Pb) in milled *fufu*, offering critical insight into the potential health risks associated with chronic lead exposure from this staple food. Lead's HQ distribution raises concerns, as a significant portion of values exceed 0.5, approaching and even surpassing the risk threshold of 1.0. Lead exhibits a higher HQ spread, with values extending beyond 1.0, which suggests potential non-carcinogenic health risks (USEPA, 2017).

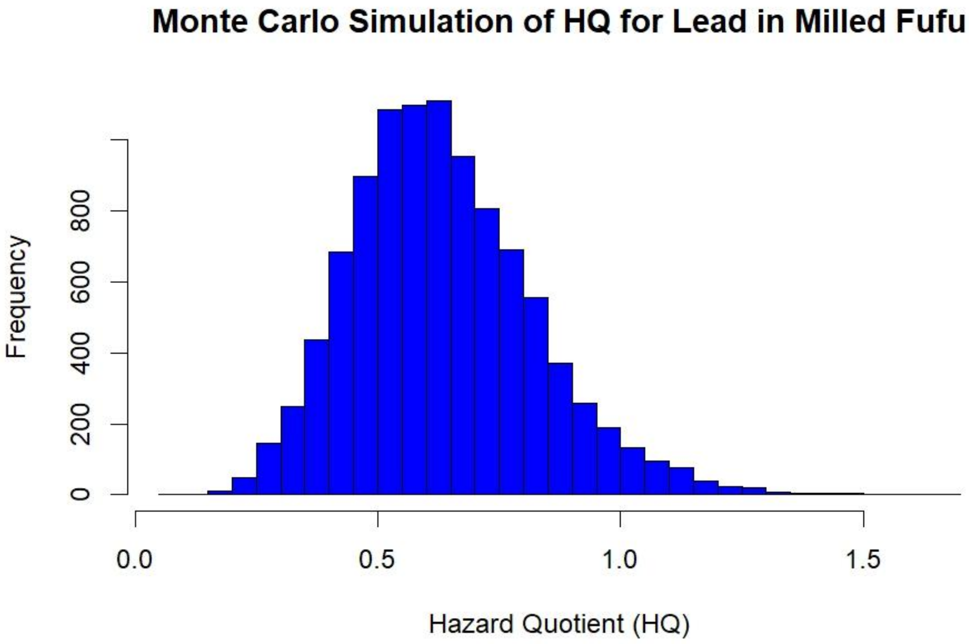

Figure 4. 8 Monte Carlo simulation of HQ for Lead in Milled *Fufu*

4.7.3 Arsenic (As)

The histogram (Figure 4.9) illustrates the Monte Carlo simulation of the Hazard Quotient (HQ) for arsenic (As) in milled *fufu*, providing insight into the potential health risks associated with arsenic exposure through dietary intake.

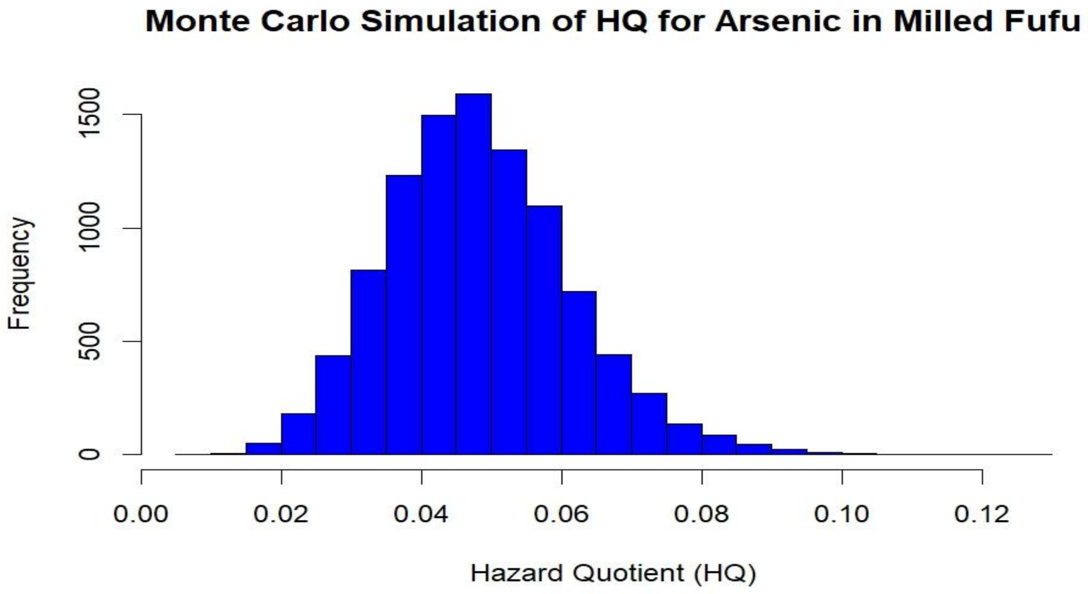

Figure 4. 9 Monte Carlo simulation of HQ for Arsenic in Milled *Fufu*

Arsenic's HQ values remain far below the risk threshold of 1.0, indicating a relatively low non-carcinogenic health risk. The most frequent HQ values are between 0.04 and 0.06, with a peak around 0.05. The entire distribution remains well below 0.1, confirming that exposure to arsenic in milled *fufu* is significantly below the risk threshold (HQ = 1.0).

4.7.4 Zinc (Zn)

Figure 4.10 (histogram) represents the Monte Carlo simulation of the Hazard Quotient (HQ) for zinc (Zn) in milled *fufu*, offering insights into the potential health risks associated with zinc intake from this food source.

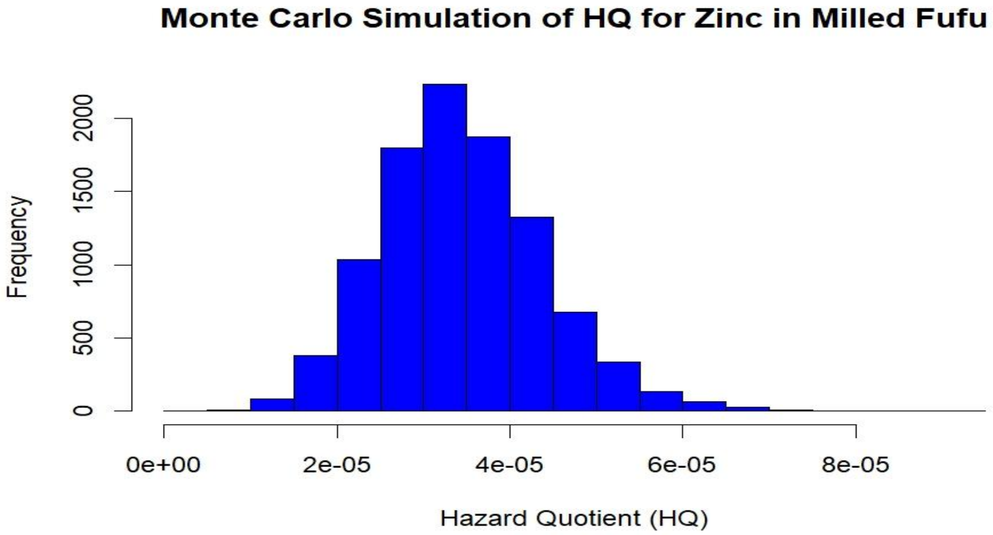

Figure 4. 10 Monte Carlo simulation of HQ for Zinc in Milled *Fufu*

Zinc is an essential micronutrient, meaning its presence in food is beneficial at appropriate levels. However, excessive intake can lead to toxicity. The histogram follows a normal-like distribution, with the most frequent HQ values around 4.0×10^{-5}. The entire range of HQ values remains far below 1.0, indicating an insignificant non-carcinogenic health risk from zinc exposure. The highest observed HQ values barely reach 8.0×10^{-5}, which is negligible compared to toxic thresholds.

4.8 Human health risk associated with the consumption of *fufu* contaminated with heavy metals.

Table 4.14 presents a comparative assessment of chronic daily intake (CDI) and hazard quotient (HQ) after Monte Carlo simulation for cadmium, lead, arsenic, and zinc, benchmarked against FAO/WHO/USEPA reference values.

Table 4. 14 Comparison of CDI and HQ values with the FAO/WHO/USEPA reference values.

Parameter	**CDI (mg/kg-day)**	**FAO/WHO/USEPA RfD (mg/kg-day)**	**HQ (Provided)**	**HQ (Reference)**
Cadmium	0.0008	0.0005 (USEPA)	0.279	<1 (Safe)
Lead	0.0018	No safe threshold (USEPA)	0.5	-
Arsenic	1.15×10^{-5}	3.0×10^{-4}	0.0383	<1 (Safe)
Zinc	6.29×10^{-7}	0.3 (USEPA)	2.10×10−6	<1 (Safe)

The chronic daily intake (CDI) for cadmium in milled *fufu* was 0.0008 mg/kg-day, which exceeds the USEPA Reference Dose (RfD) of 0.0005 mg/kg-day. The hazard quotient (HQ) was calculated to be 0.279, which is below 1, indicating that acute toxicity is unlikely. Unlike other metals, lead has no safe threshold according to USEPA, meaning any level of exposure is hazardous. The CDI for lead in milled *fufu* was 0.0018 mg/kg/day, and the HQ was 0.5, indicating a moderate risk. The CDI for arsenic in milled *fufu* was 1.15×10^{-5} mg/kg-day, which is below the USEPA RfD of 3.0×10^{-4} mg/kg-day. The HQ was 0.0383, indicating a low but non-negligible risk. The CDI for zinc in milled *fufu* was 6.29×10^{-7} mg/kg/day, significantly lower than the USEPA RfD of 0.3 mg/kg/day. The HQ was 2.10×10^{-6}, indicating no risk of toxicity.

CHAPTER FIVE

DISCUSSION

5.1 Level of microbial counts in *fufu* produced from *fufu* milling machines in Nsuta.

Salmonella and Shigella

The rise in *Salmonella* levels following the milling process indicates possible cross-contamination from the milling equipment. Research indicates that inadequate machine cleanliness and regular reuse without sufficient cleaning lead to the ongoing presence of bacteria (Wang et al., 2020; Rutala & Weber, 2016). In Ghana, foodborne pathogens found in street-vended foods typically stem from processing tools rather than the raw materials themselves (Bani, 2022; Amedewonu, 2020; Aglidza, 2019).

Shigella, a significant instigator of dysentery, is transmitted via inadequate sanitation and faecal contamination (Sadek et al., 2023; Thavaththurai & Amarasekara, 2021). A study conducted by Addo et al. (2020) in Ghana revealed that communal food processing facilities, such as grinding and milling machines, play a considerable role in the spread of bacteria. The notable increase observed after milling indicates the formation of biofilms on the surfaces of machines, enabling bacteria to survive even with sporadic cleaning.

***E. coli and* Total coliform**

E. coli contamination in food often arises from inadequate hand hygiene, tainted water, or unsanitary surfaces. The observation that most sites did not show *E. coli* in raw samples indicates that contamination is probably introduced during the milling process rather than originating from the cassava or plantain itself. Research conducted by Yar et al. (2023) highlights that the use of contaminated water for cleaning milling machines is a significant source of *E. coli* in food within

Ghana. Coliform bacteria indicate inadequate sanitation and hygiene in the environment. Their detection after milling supports the notion that milling machines are a significant source of contamination. Research conducted on fermented African foods has highlighted that food processing equipment, if not adequately sanitized, significantly increases coliform levels (Oyedeji et al., 2023; Owusu-Kwarteng et al., 2020; Byakika et al., 2019; Olotu, 2018).

The average rise in contamination after milling is in line with the findings of Oyeyipo et al. (2023), which indicate that processing equipment can serve as a source of contamination. Locations recognized as powerhouses consistently display elevated levels of contamination, which aligns with findings from urban processing environment research (Kumar et al., 2019). The steady rise in microbial levels following the milling process strongly indicates that the source of contamination is the milling machines rather than the raw cassava and plantain.

This is consistent with research by Yar et al. (2023) and Dzikunoo et al. (2021), which revealed that inadequately maintained processing equipment was responsible for 70% of bacterial contamination in traditional Ghanaian foods. The detection of *Salmonella and Shigella* heightens the likelihood of gastrointestinal infections, diarrhoea, and foodborne illnesses. *E. coli* presence, especially in areas such as Zongo-1 and Asokwa-1, raises alarms about waterborne pathogens and contamination from faeces. Elevated total coliform counts suggest inadequate hygiene, emphasizing the necessity for enhanced sanitation measures in food processing environments. This indicates a significant food safety concern.

5.1.1 Impacts of milling on microbial load contamination levels.

Milling, while essential for food processing, can also introduce harmful contaminants through poor hygiene, unclean machine surfaces, and environmental exposure (Moerman & Mager, 2016). Research has consistently highlighted that bacterial contamination in street-vended foods is often traced back to unsanitary processing equipment, emphasizing the importance of proper cleaning and maintenance (Rohith, 2021; Crocker, 2020). Pathogens like *Salmonella and Shigella* thrive in food-processing environments and tend to increase after handling, making hygiene protocols crucial in preventing outbreaks (Akinsemolu & Onyeaka, 2024; Sharif et al., 2024).

A significant rise in Total Coliform levels suggests a high risk of faecal contamination, commonly linked to contaminated water, improper handling, or poorly maintained machinery (World Health Organization, 2020). Unlike *Salmonella* and *Shigella, E. coli* levels remained relatively unchanged, possibly due to the thermal or environmental resistance of the specific *E. coli* strains present. Another potential explanation is bacterial competition, where the increased presence of Salmonella and Shigella may have suppressed *E. coli* growth (Leung et al., 2024; McMahon et al., 2022; Anderson et al., 2017).

5.1.2 Difference in contamination levels across various locations.

The significant variation in *E. coli* aligns with findings by JP (2023), who reported that *fufu* processing environments with poor water quality and unhygienic handling had higher *E. coli* loads compared to those with improved sanitary conditions. The presence of *E. coli* in processed foods is often linked to faecal contamination, inadequate washing of cassava before milling, or poor personal hygiene among handlers (Makinde et al., 2020). In some studies, *E. coli* levels have been used as an indicator of overall microbial safety in fermented foods, with the World Health

Organization (2024) emphasizing that its presence in ready-to-eat foods poses significant health risks.

The significant variation in *E. coli* levels suggests that specific locations have higher contamination levels, likely due to differences in sanitation practices, water quality, and hygiene standards at milling sites. Studies have consistently highlighted *E. coli* as a primary faecal contamination indicator, often linked to improper handling, cross-contamination, and unsanitary water sources (World Health Organization, 2024). This finding aligns with research by Ghartey (2019), who reported significant disparities in *E. coli* contamination across street-vended foods in Ghana, attributing the variation to inconsistent hygiene practices among vendors.

Similarly, Graefe et al. (2019) demonstrated that water used in food processing exhibited location-dependent *E. coli* contamination, reinforcing the argument that local environmental and handling conditions significantly impact microbial loads. The significant variation in *E. coli* underscores an urgent need for targeted interventions to improve hygiene at locations with higher contamination. Given that *E. coli* is a direct faecal contamination indicator and can cause severe gastrointestinal illnesses (CDC, 2024), its presence at unsafe levels raises significant public health concerns.

The findings align with studies that have highlighted the persistence of Salmonella and Shigella in food processing environments, primarily due to poor hygiene and cross-contamination during food handling. Research by Ahiabor et al. (2024) on street-vended foods in Ghana found that Salmonella and Shigella were present at similar levels across multiple locations, attributed to shared risk factors such as contaminated water sources and improper sanitation practices. Additionally, total coliforms are common indicators of faecal contamination (Khan & Gupta, 2020; Martin et al., 2016), and their uniform presence across locations suggests that contamination sources, such as water used in *fufu* processing, are consistent across sites.

Prior studies have reported *Salmonella* contamination in Ghanaian street foods and water sources, but noted that its distribution is often associated with seasonal fluctuations and sporadic contamination events rather than consistent environmental factors (Cudjoe et al., 2022). Previous research by World Health Organization (2021) highlighted that total coliform presence in food-processing environments is often persistent but may not always translate into location-specific variations unless there are major differences in infrastructure, hygiene enforcement, or contamination sources. The lack of significant variation in *Salmonella*, *Shigella*, and total coliforms does not imply their absence. Instead, it suggests that contamination control measures should be consistently applied across all locations rather than focusing on specific sites.

5.2 Levels of heavy metal contamination in *fufu* produced from *fufu* milling machines in Nsuta.

Lead is a neurotoxin that can lead to brain damage, kidney failure, and developmental delays in children. Its occurrence in food is frequently associated with contaminated soil, water, or equipment. Research conducted on heavy metal contamination in street foods in Ghana suggests that exposure to lead often originates from processing equipment rather than the raw ingredients themselves (Mensah, 2023; Ankar-Brewoo et al., 2020). The significant rise in lead levels after milling implies cross-contamination from the milling machines, likely due to the deterioration of metallic parts. Studies in Ghana and Nigeria have revealed that traditional food processing tools, such as grinders and milling machines, release lead into food gradually, especially in areas with inadequate industrial regulations (Olawore, 2023; Juuna, 2017; Ikechukwu & Lorreta, 2015). Since *fufu* is a typical staple food, long-term exposure to these lead levels could represent serious public health concerns.

Cadmium is a hazardous heavy metal that has no recognized biological role in human health. Prolonged exposure can result in kidney disorders, bone demineralization, and cancer. Research conducted indicated that the presence of cadmium in food is generally due to industrial pollution, fertilizers, and machinery used in metal processing (Khatun et al., 2022; Njoga et al., 2021). In Ghana, a study by Mensah (2023) revealed that cadmium contamination in traditional foods was mainly associated with outdated milling machines that had deteriorated metal components. The rise in cadmium levels following the milling process implies that some of the equipment utilized in Nsuta-Ashanti might be causing metal leaching into the food.

Arsenic contamination in food usually comes from polluted water, soil, or agricultural chemicals, rather than from processing equipment. Research conducted in Ghana indicated that arsenic levels in cassava-based foods were typically low, unless the cassava was cultivated in contaminated soil (Akyereko et al., 2024; Adjei et al., 2023). The finding of minimal arsenic contamination in this study is reassuring, implying that the cassava and plantain used for *fufu* are cultivated in areas free from arsenic contamination. Zinc is a vital nutrient necessary for immune system performance, wound repair, and development (Yang et al., 2022; Wessels et al., 2017). In contrast to lead, cadmium, and arsenic, zinc is not significantly toxic except when ingested in vast quantities.

The minor rise after milling might be attributed to zinc seeping from metal machine components; however, the concentrations remain too low to pose any health hazards. The results paint an intriguing but concerning picture. While arsenic and zinc levels are within safe limits, the lead and cadmium levels in several locations require immediate attention. The variation between locations suggests different factors at play, perhaps the age of equipment, maintenance practices, or environmental contamination. What is particularly interesting is how the milling process affects contamination levels. The consistent slight increases in heavy metal content after milling suggest that the processing equipment itself might be contributing to contamination. This finding aligns with research by Emurotu et al. (2024) and Adewoyin et al. (2023) who also reported similar patterns in cassava processing equipment.

5.2.1 Impacts of Milling on Levels of Heavy Metal Contamination.

Heavy metal contamination in food is a well-documented global concern, especially in developing countries where processing techniques and environmental contamination can influence food safety (Onyeaka et al., 2024; Lebelo et al., 2021). Milling is known to reduce certain heavy metals because contaminants often accumulate in the outer layers of food crops (Wei et al., 2022). Studies on cereals and grains, and some tubers have demonstrated that polishing and milling significantly reduce lead and cadmium levels due to the removal of outer husks (Song et al., 2023; Hassan et al., 2021). The findings in this study align with these observations, as lead and cadmium show statistically significant reductions after milling.

During milling, mechanical friction and physical separation may remove heavy metal-contaminated particles, thereby lowering contamination (Wei et al., 2022). A study by Abass et al. (2019) on cassava processing found that milling and sieving significantly reduced the total heavy metal content in the final product, which is similar to the observed decreases in arsenic and zinc in our results. Although milling generally leads to reductions, some studies warn that contamination may persist or even increase if milling equipment is contaminated (Wachira, 2017; Alum et al., 2016). Milling machines made from non-stainless steel materials can introduce heavy metals such as lead and cadmium into the food (Linauskienė, 2022; Schmidt & Piotter, 2020).

The drastic decline in arsenic levels aligns with research showing that milling and washing can remove surface-bound contaminants, which are often the primary source of arsenic in agricultural products (Fang et al., 2025; Menon et al., 2021). Cadmium is known to accumulate within plant tissues rather than just on the surface (Sterckeman & Thomine, 2020), which might explain why its levels remain relatively persistent even after processing. Zinc, unlike the other metals, is an essential nutrient, and its significant reduction after milling ($p < 0.001$) raises potential concerns regarding nutritional loss.

5.2.2 Difference in heavy metal contamination levels across various locations.

Studies on heavy metal contamination in food have consistently reported lead as a major public health risk due to its persistence in the environment and potential sources such as contaminated water, soil, and processing tools. Research by Mensah (2023) on cassava-based foods led to finding that lead levels exceeding WHO/FAO safety limits were found in urban areas with high industrial activity. The strong variability observed in this study aligns with similar findings, emphasizing the need for stricter contamination controls, especially in urban and industrial regions. Research by Adekola et al. (2024) reported cadmium accumulation in cassava products sourced from regions with intensive agricultural activities, especially where phosphate fertilizers and wastewater irrigation are common.

The significant p-value here reinforces the idea that environmental exposure influences the presence of cadmium in *fufu*. Long-term exposure to cadmium is linked to kidney damage and bone demineralization, making its presence in food a critical concern (Fatima et al., 2019; Satarug, 2018). Arsenic contamination in food is often linked to contaminated irrigation water and soil conditions, particularly in areas with a history of mining activity or heavy pesticide use. A study by Shaji et al. (2021) found arsenic in foods at concerning levels, particularly in regions with groundwater contamination. While this study’s findings do not confirm a substantial statistical variation in arsenic levels, the presence of variability suggests potential environmental exposure that requires monitoring.

Previous studies highlighted that zinc in cassava-based products is typically influenced by soil composition rather than external contamination sources (Byju & Suja, 2020; Ferraro et al., 2016). Since the variations in this study are statistically insignificant, it suggests that zinc contamination in *fufu* is not a widespread concern; however, monitoring is still necessary to ensure an optimal nutrient balance.

5.3 Factors of microbial and heavy metal contamination in the milling machines in Nsuta.

5.3.1 Demographic data of Machine Operators

Comparatively, studies on small-scale food processing businesses in Ghana indicate that younger operators dominate due to higher adaptability, willingness to work long hours, and openness to technology adoption (Osei & Cheng, 2024; Dzisi et al., 2023). However, the absence of older workers (41+) could mean a lack of mentorship and long-term experience, which might affect machine handling skills and hygiene practices, factors crucial for food safety (Ravichandran et al., 2015). The data revealed a significant gender disparity, with 80% of operators being male and only 20% female. This aligns with findings that physical labour-intensive sectors in Ghana, such as food milling and mechanical work, remain male-dominated (Takyi et al., 2021).

However, studies indicate that women often play key roles in food safety management, particularly in domestic and small-scale food processing (Njuki et al., 2023). Female involvement in food handling businesses reduces microbial contamination risks due to greater adherence to hygiene practices (Ababio & Lovatt, 2015; Baluka et al., 2015). The low representation of women in Nsuta’s milling industry could therefore raise concerns about sanitation and risks of cross-contamination. Studies have shown that older milling machines are more prone to wear and tear, leading to heavy metal leaching into milled products (Adeniran et al., 2023; Nkansah et al., 2021). If proper maintenance is not conducted, metal parts in older machines can release lead (Pb), cadmium (Cd), and arsenic (As) into the milled *fufu*.

The 30% share of machines aged 4-6 years could therefore pose a higher contamination risk, especially if regular servicing and component replacements are not practiced. A study by Kantanka (2021) in Accra's maize milling sector, highlighted that poorly maintained machines contributed to elevated metal contamination levels, exceeding WHO food safety limits. This suggests an urgent need for routine maintenance schedules and contamination monitoring in Nsuta's *fufu* milling industry.

This educational distribution is encouraging, as higher education levels are correlated with better hygiene practices, food safety knowledge, and an understanding of contamination risks (Mullan et al., 2015; Webb & Morancie, 2015). However, with half (50%) of the operators having only primary education, there may be gaps in their knowledge regarding proper sanitation, machine maintenance, and contamination prevention. Food processing workers with at least secondary education were twice as likely to follow proper hygiene protocols compared to those with only primary education (Wandolo, 2016). In Nsuta, having 30% of operators with secondary education and 20% with tertiary education may help drive improved food safety practices if they are engaged adequately through training programs.

5.3.2 Hygiene of the Milling Environment

The fact that 90% of operators engage in daily cleaning is commendable, as regular sanitation helps minimize microbial contamination and foodborne illness risks. Food-processing environments should undergo frequent sanitation to reduce bacterial proliferation, cross-contamination, and biofilm formation on surfaces (DeFlorio et al., 2021; Abebe, 2020). In Ghana, studies on food vending and processing businesses have consistently shown that routine cleaning plays a significant role in ensuring food safety compliance. Studies by Lah et al. (2022) and Amedewonu (2020) on street food hygiene revealed that vendors who cleaned their equipment daily had 50% fewer bacterial contaminants compared to those who cleaned sporadically. This supports the argument that Nsuta's daily cleaning practice is a crucial preventive measure against microbial contamination in *fufu* milling.

However, the efficacy of daily cleaning depends on how thoroughly it is performed. If operators clean only visible dirt without using appropriate disinfectants or scrubbing hard-to-reach areas, microbial residues could accumulate over time. Research by Alum et al. (2016) found that food processing environments cleaned with only water and cloth wiping still harboured harmful pathogens, such as *Salmonella* and *Escherichia coli*, which persisted in milling machines even after cleaning. Thus, while the high percentage of daily cleaning is a promising trend, there is a need to assess the quality of cleaning practices to ensure they effectively remove contaminants, biofilms, and potential heavy metal residues from machines.

Only 10% of operators clean their milling environment after every use, which is the most effective hygiene practice. Cleaning after every milling session is recommended by food safety authorities to prevent cross-contamination between different batches of food (Reelfs, 2022). A comparative study by Owusu et al. (2021), on maize milling hygiene, it was found that mills cleaned after every use had 70% lower microbial load than those cleaned once daily. The study highlighted that residual organic matter left in milling machines serves as a nutrient source for bacterial growth, making after-use cleaning the best strategy for preventing contamination.

The low percentage (10%) of Nsuta’s operators following this best-practice cleaning routine raises concerns about food safety risks, particularly in high-volume milling operations where multiple customers use the same machine within short timeframes. Without after-use cleaning, there is a high likelihood of microbial carryover from one batch to another, which can potentially increase public health risks. Food processing hygiene in Ghana found that equipment cleaned after every use had 80% lower heavy metal residues and bacterial contamination compared to those cleaned once per day (Yar et al., 2023; Agyei-Mensah, 2021). This suggests that more frequent and thorough cleaning practices are necessary for optimal food safety.

5.3.3 Hygiene and Maintenance of Milling Machines

Ideally, cleaning should occur immediately after milling each batch to prevent the buildup of food residue, which serves as a breeding ground for bacteria (Moerman & Mager, 2016). The lack of after-use cleaning suggests that microbial cross-contamination between different batches of *fufu* is likely. A study by Owusu et al. (2021) on maize milling hygiene found that machines cleaned after every use had 60% fewer microbial contaminants than those cleaned only once daily. Thus, while Nsuta's daily cleaning practice is better than sporadic cleaning, it is not sufficient to eliminate contamination risks. The risk is further compounded by the cleaning materials used. A worrying 80% of operators rely solely on water, with only 20% using detergent or soap. Research indicates that water alone is insufficient for removing microbial biofilms, oils, and food residues (Abebe, 2020; González-Rivas et al., 2018). The absence of proper disinfectants raises serious concerns about hygiene standards, as pathogens like *Escherichia coli* and *Salmonella* can persist even after basic rinsing (Artasensi et al., 2021; Davies & Wales, 2019).

Studies show that prolonged exposure to rusted metal components increases the risk of iron contamination and potential ingestion of hazardous metal particles (Raffo et al., 2016; Sankhla et al., 2016). Given that heavy metal accumulation in food is linked to long-term health complications such as kidney damage and neurotoxicity (Engwa et al., 2019), the high incidence of rust in Nsuta's milling machines is a major public health concern.

The data reveal that 80% of milling environments appear clean, with only 20% showing visible food residue or dirt. While this may seem reassuring, it does not necessarily indicate a safe environment. Studies have shown that bacterial contamination can persist even on visually clean surfaces, particularly when improper cleaning methods are used (Owusu et al., 2021). A study on food-processing hygiene in Ghana found that machines that appeared clean still had high microbial loads due to improper cleaning techniques (Mensah et al., 2020). This suggests that Nsuta’s *fufu* milling machines could harbour invisible microbial hazards, even if they appear clean.

5.3.4 Hygiene Practices During Operation

In food processing, protective clothing such as gloves, aprons, and hairnets serves as a critical barrier against cross-contamination (World Health Organization, 2019). The failure of 70% of operators to wear any protective gear increases the likelihood of microbial and physical contaminants entering the *fufu* during processing. Mensah et al. (2020) found that food handlers without protective gear were 4.3 times more likely to introduce microbial contaminants into food than those who adhered to safety protocols.

Moreover, exposure to sweat, dust, and hair particles from operators poses additional health risks. Research by Amedewonu (2020) on street food vendors in Ghana highlighted that the lack of protective gear was directly correlated with higher incidences of foodborne illnesses among consumers. The prevalence of foodborne diseases in Ghana, particularly in informal food processing settings, is already high (Ahiabor et al., 2024; Christiana Cudjoe et al., 2022). The situation in Nsuta's *fufu* mills suggests that the risk is even more pronounced.

None of the operators completely neglects handwashing, but the fact that 90% do not consistently wash their hands before and after milling is deeply concerning. Studies show that hands are the primary vectors for foodborne pathogens such as *Salmonella*, *E. coli*, and *Listeria* (World Health Organization, 2021). A study found that poor handwashing practices among food handlers in Ghanaian markets contributed to 65% of detected bacterial contaminations in processed foods (Amegah et al., 2020). Moreover, in food environments where moisture and starch residues are present (such as *fufu* milling), bacteria thrive. Research shows that even a single unwashed hand can transfer millions of bacteria onto food surfaces (Singh et al., 2023).

This is a significant public health failure. Studies show that food processors who receive hygiene training are 75% more likely to adopt safer practices (FAO, 2021). In contrast, a lack of knowledge often leads to negligence and ignorance of contamination risks (Mensah & Osei, 2020). A study on cassava and other food processing in Ghana found that food processors with hygiene training demonstrated better cleaning practices, used protective clothing, and followed handwashing protocols more consistently than those without training (Omari et al., 2020; Thumbi, 2020). This aligns with findings from Nigeria, where hygiene training among garri processors led to a 65% reduction in microbial contamination rates (Izekor et al., 2023). In the context of Nsuta, the fact that the majority of operators (60%) lack hygiene training directly correlates with the poor hygiene behaviours observed (lack of protective clothing and inconsistent handwashing). This indicates that education and awareness campaigns are urgently needed.

5.4 Risk Assessment

5.4.1 Estimation of dietary intake/exposure

The reported mean daily *fufu* consumption of 461.2 g per person per meal aligns with studies on West African dietary patterns, where cassava-based dishes are a primary staple. The observed variability (SD = 106.0 g) suggests differing portion sizes based on age, gender, and physical activity levels, consistent with findings by Shokunbi (2017), who noted similar intake variations in Ghanaian households. The minimum (200 g) and maximum (700 g) consumption levels reflect diverse eating habits, supporting Sengar (2022), who states that cassava products contribute up to 60% of daily caloric intake in some regions. The 95th percentile value (600 g) reinforces that a minority of heavy consumers exist, likely influenced by economic factors, availability, and cultural preferences. Compared to global staple consumption, *fufu* intake parallels rice consumption in Asia, where portion sizes vary significantly based on socio-demographics (Talhelm & English, 2020; Wang et al., 2020).

5.4.2 Weight distribution

The average body weight (65.19 kg, SD = 8.06 kg) among respondents is consistent with regional anthropometric studies, where adult body weights typically range between 60-70 kg in West African populations (Agbo et al., 2020). The minimum (43 kg) and maximum (75.5 kg) weights suggest a balanced distribution, with no extreme outliers. The 95th percentile (75.5 kg) implies that only 5% of individuals exceed this threshold, which aligns with WHO BMI trends in Sub-Saharan Africa, where a shift toward higher body weights is noted due to urbanization and changing dietary habits (Ozodiegwu, 2019). Compared to global data, the weight distribution is similar to adult weight patterns in rural Asian communities, but lower than urban populations in developed regions (Bixby et al., 2019). This supports existing evidence that dietary habits, physical activity, and economic conditions influence body weight variability (López-Moreno et al., 2020).

5.5 Human health risk associated with the consumption of *fufu* contaminated with microbial loads.

5.5.1 *Salmonella*

The findings aligned with established microbial risk assessment studies, such as those by Koyama et al. (2017a, 2017b), which demonstrated that the risk of Salmonella infection is probabilistic rather than deterministic. The Beta-Poisson dose-response model, widely applied in microbial risk assessments, effectively captures this variability, explaining why individuals exposed to the same pathogen dose may experience different infection outcomes (Uwimanayantumye, 2024). The presence of infection probabilities above 0.5 in some cases is particularly concerning, as it implies that under certain conditions, such as higher doses or increased host susceptibility, more than half of exposed individuals could become infected. Given that *Salmonella* is a leading cause of foodborne illness worldwide (Ehuwa et al., 2021; Popa & Papa, 2021; Hoque et al., 2019; Chai et al., 2017), this analysis reinforces the urgent need for rigorous food safety measures. Even when the mean infection probability appears moderate, the tail end of the distribution suggests that a subset of the population is at much higher risk, warranting protective policies, strict hygiene protocols, and effective interventions to minimize contamination.

Even at very low doses (near 0 CFU), there is a non-zero probability of infection (~0.1-0.2), suggesting that *Salmonella* can still cause infection at minimal exposure levels. When the dose exceeds 5,000 CFU, the infection probability approaches 50%, and beyond 10,000 CFU, it continues rising but at a slower rate, aligning with the Beta-Poisson dose-response model. The observed dose-response relationship for *Salmonella* in the plot aligns with findings from established microbial risk assessment studies. According to the U.S. Environmental Protection Agency (EPA)(USEPA, 2017) and research by Karanth (2021) and Gurman (2016), *Salmonella* follows a Beta-Poisson dose-response model, which captures the non-linear increase in infection probability with rising doses. The steep increase in infection probability at low doses is well-documented in food safety literature, where studies indicate that ingestion of as few as 10–100 CFU can cause infection, particularly in vulnerable populations (Schoder et al., 2023; Rushing & Selim, (2019).

The infection probability nearing 50% at doses around 5,000 CFU is consistent with previous experimental data, such as studies by Choy et al. (2022) and Mendoza Jr et al. (2022), which show that human challenge studies often report a median infectious dose (ID50) between 1,000 and 10,000 CFU, depending on the strain and host susceptibility. Furthermore, the plateauing effect observed at high doses is characteristic of the Beta-Poisson model, reflecting saturation of infection probability as host susceptibility reaches a limit. These findings highlight the high infectivity of *Salmonella*, even at low exposure levels, underscoring the need for stringent food safety regulations and preventive measures to reduce contamination in food and water sources. Regulatory bodies like the EPA, WHO, and FDA typically set acceptable risk limits at Pinf < 0.0001 for drinking water and foodborne pathogens. Studies on microbial risk assessment indicate that risks exceeding Pinf > 0.001 pose a significant public health concern (Irakoze, 2022; Unruh, 2018).

5.5.2 *Shigella*

The distribution is roughly bell-shaped, with most infection probabilities concentrated between 0.6 and 0.75, peaking around 0.7. This suggests that under typical exposure scenarios, the probability of infection remains significantly high, with few cases showing lower infection risks below 0.6 or exceeding 0.8. These findings align with established dose-response studies, such as those by Frenck et al. (2020), Kotloff et al. (2018) and Soffer et al. (2017), which highlights the low infectious dose of *Shigella,* as few as 10–100 CFU can cause infection. The shape of the probability distribution also matches the characteristics of microbial dose-response models, particularly the Beta-Poisson model, which has been widely used for *Shigella* (Prasad & Haas, 2017; Haas, 2015).

The Beta-Poisson model, commonly applied in microbial risk assessments, captures the variability seen in infection probabilities, demonstrating that even small doses can lead to a relatively high risk of infection. The high infectivity of *Shigella* is well-documented in epidemiological studies, where outbreaks are often linked to contaminated food, water, and person-to-person transmission due to poor sanitation and hygiene (Harland, 2020). Given *Shigella's* resistance to acidic conditions in the stomach and its ability to cause severe dysentery, even minor lapses in hygiene can lead to widespread outbreaks (Yada, 2023; Riddle et al., 2016). These results underscore the urgency of interventions such as access to safe drinking water, handwashing campaigns, and food safety regulations to mitigate the substantial risk posed by *Shigella* contamination.

5.5.3 *Escherichia coli*

The peak infection probability around 0.6-0.75 suggests that while exposure to *E. coli* does not always lead to infection, the likelihood increases significantly at moderate to high doses. These findings are consistent with established dose-response models for *Escherichia coli,* particularly pathogenic strains like *E. coli* O157:H7. Haas et al. (2015) describe *E. coli* dose-response relationships as variable, depending on the strain and host susceptibility. Studies found that the probability of infection from *E. coli* O157:H7 increases significantly with ingestion of 100-1,000 CFU (Chapman et al., 2018; Pang et al., 2017), supporting the trend observed in this dataset, where infection probabilities predominantly fall within the mid-to-high range.

E. coli typically requires a higher dose to achieve similar infection rates (Jang et al., 2017; Van den Bergh et al., 2016). The broader range of infection probabilities observed here reflects the varying virulence among *E. coli* strains. Moreover, epidemiological studies highlight that *E. coli* infections are often associated with foodborne outbreaks linked to undercooked food, unpasteurized dairy, and contaminated water (Singha et al., 2023; Mesele & Abunna, 2019). This further emphasizes the need for stringent food safety regulations and public health interventions to mitigate exposure risks.

5.5.4 Total coliform

The peak infection probability is around 0.7, indicating that coliform bacteria have a moderate to high potential for causing infections, though with a broader range of probabilities compared to highly virulent pathogens like *Shigella*. The mean probability was 0.659. This suggests that while exposure to coliform bacteria can lead to infection, the probability of infection depends on factors such as the virulence of the strain, host immunity, and the exposure dose. Coliform bacteria are commonly used as indicators of fecal contamination in water quality assessments rather than direct causes of illness. According to the World Health Organization (2021), total coliforms alone are not necessarily pathogenic. However, their presence signals the potential for contamination by more virulent microorganisms, such as *E. coli* O157:H7 or *Salmonella*.

Literature suggests that infection risks associated with coliform bacteria vary widely; studies by da Silva et al. (2024) highlight that while most coliforms are harmless, certain strains, particularly those in the *Klebsiella* and *Enterobacter* genera, can be opportunistic pathogens. When compared to *E. coli*, which has a more defined pathogenic profile (Messner et al., 2017), coliform bacteria as a group demonstrate a broader and less concentrated infection probability. The variability in infection risk seen in the distribution aligns with findings by Sarowska et al. (2019), who noted that the role of coliform bacteria in disease transmission depends on the presence of specific virulence factors.

5.6 Risk Assessment of Heavy Metal Contamination

5.6.1 Cadmium (Cd)

The HQ values predominantly range between 0.2 and 0.4, with a peak near 0.3. This indicates that the majority of simulated cadmium exposure scenarios remain below the safety threshold (HQ < 1) set by regulatory agencies such as the USEPA and WHO. Since HQ < 1 in most cases, acute toxicity is unlikely to occur in the general population. However, long-term exposure to cadmium, even at HQ values below 1, has been linked to kidney dysfunction, bone demineralization, and cardiovascular diseases (Huang et al., 2024; Teschke, 2024). Doccioli et al. (2024) highlighted that chronic cadmium intake, even at low doses, leads to nephrotoxicity, a finding that aligns with the concern that long-term exposure accumulates over decades.

Cadmium is a heavy metal with no known biological function in humans. It enters food chains primarily through contaminated soils, irrigation water, and atmospheric deposition from industrial sources (Bouida et al., 2022; Genchi et al., 2020). The FAO/WHO has flagged root crops like cassava as particularly vulnerable due to high soil absorption rates (Aberman et al., 2015). Cassava tubers grown in mining-affected areas had cadmium levels above WHO limits, suggesting localized contamination hotspots contribute to dietary exposure (Brouziotis et al., 2022; Doso et al., 2016).

5.6.2 Lead (Pb)

Lead's HQ distribution raises concerns, as a significant portion of values exceed 0.5, approaching and even surpassing the risk threshold of 1.0. Lead exhibits a higher HQ spread, with values extending beyond 1.0, which suggests potential non-carcinogenic health risks (USEPA, 2017). According to the United States Environmental Protection Agency (USEPA), an HQ >1.0 indicates potential for adverse health effects, especially in vulnerable populations such as children and pregnant women. Lead toxicity is well-documented in food contamination studies. Research has linked chronic lead exposure to neurodevelopmental disorders, kidney damage, and cardiovascular issues (World Health Organization, 2023). The FAO/WHO reports that there is no known safe threshold for lead exposure because it bioaccumulates and disrupts essential bodily functions, especially in children (Thompson, 2024). Similar studies in Nigeria and Ghana have found lead contamination in staple foods due to industrial pollution, contaminated irrigation water, and improper food processing methods (Christiana Cudjoe et al., 2022; Nkansah et al., 2021; Asamoa, 2016).

5.6.3 Arsenic (As)

Arsenic's HQ values remain far below the risk threshold of 1.0, indicating a relatively low non-carcinogenic health risk. The most frequent HQ values are between 0.04 and 0.06, with a peak around 0.05. The entire distribution remains well below 0.1, confirming that exposure to arsenic in milled *fufu* is significantly below the risk threshold (HQ = 1.0). However, chronic exposure to even low arsenic levels has been linked to long-term health effects, including skin lesions, cardiovascular diseases, and weakened immune function (WHO, 2018). The FAO/WHO reference dose for arsenic (3.0×10^{-4} mg/kg/day) suggests that occasional exposure within these limits is unlikely to cause immediate harm. Studies in West Africa have reported low-to-moderate arsenic levels in root crops, including cassava, which is the main ingredient in *fufu* (Chijioke et al., 2024; Nkansah et al., 2021). Studies found that arsenic in staple foods like rice and cassava varies by region, with contamination often linked to the use of arsenic-contaminated irrigation water (Liu et al., 2023; Sabbagh, 2023). The WHO and USEPA classify arsenic as a Group 1 carcinogen, meaning long-term exposure, even at low levels, is associated with an increased risk of cancer, particularly skin, lung, and bladder cancers (Demissie et al., 2024; Tsuji et al., 2019).

5.6.4 Zinc (Zn)

Zinc is an essential trace element required for immune function, growth, and enzymatic activities (World Health Organization, 2023). Unlike toxic heavy metals like lead, cadmium, or arsenic, zinc deficiency is more concerning than excess intake in many populations, particularly in sub-Saharan Africa, where diets are often deficient in zinc (Gupta et al., 2020). The reference dose (RfD) for zinc, set by the U.S. EPA (USEPA, 2017), is 0.3 mg/kg/day, meaning that even high-end HQ values in this simulation are well within safe consumption limits. Chronic overexposure to zinc can cause gastrointestinal distress, immune dysfunction, and interference with copper metabolism, but this is highly unlikely given the observed HQ values. Studies on cassava-based diets in West Africa suggest that the zinc content is often low, necessitating efforts to fortify these diets (Lawal, 2022). According to FAO/WHO guidelines, zinc intake from staple foods should be monitored, but deficiencies pose a greater concern than toxicity (FAO, 2020). Studies found that traditional cassava-based diets in Africa often lack sufficient zinc, contributing to malnutrition in children (Ouma, 2019).

5.7 Human health risk associated with the consumption of *fufu* contaminated with heavy metals.

Cadmium exposure is primarily linked to kidney damage, bone demineralization, and carcinogenic effects (Ma et al., 2022; Genchi et al., 2020). However, chronic exposure, even at low levels, can cause renal dysfunction and oxidative stress, leading to severe long-term health effects (Matović et al., 2015). Studies indicate that cadmium contamination in food crops originates from soil contamination due to phosphate fertilizers, industrial effluents, and atmospheric deposition (World Health Organization, 2023). In regions where cassava (the main ingredient of *fufu*) is grown in contaminated soils, cadmium can accumulate in the plant and subsequently enter the food chain (Ferraro et al., 2016; Abubakar, 2015). A study by Okereke et al. (2020) in Nigeria, found that cadmium levels in cassava products exceed WHO standards, indicating that root crops like cassava readily absorb cadmium from contaminated soil.

Chronic lead ingestion is particularly alarming due to its irreversible effects on the nervous system, cognitive development in children, cardiovascular health, and kidney function. Given that lead exposure is cumulative over a lifetime, even low concentrations in food can contribute to elevated blood lead levels (BLLs) over time. Long-term exposure to lead is linked to neurological damage, cognitive impairment, anaemia, and cardiovascular issues (Santa Maria et al., 2019). In West Africa, studies indicate that cassava processing in areas with high vehicular emissions and mining activities contributes to lead accumulation in staple foods (Mombo et al., 2017). Chronic lead exposure in adults contributes to hypertension, renal dysfunction, and cardiovascular diseases (Satarug et al., 2020; Nigra et al., 2016).

Arsenic is a well-known carcinogen with exposure linked to skin lesions, cardiovascular diseases, and cancers of the lung, bladder, and kidney (Ozturk et al., 2022; Palma-Lara et al., 2020). Arsenic contamination in cassava products can result from contaminated groundwater used for irrigation, pesticide residues, and industrial emissions (World Health Organization, 2021). Ozturk et al. (2022) reported that chronic arsenic exposure through food contributes to oxidative stress and DNA damage, increasing cancer risk. Low-dose arsenic exposure affects cardiovascular health even when HQ values are below 1 (Nong et al., 2016). Zinc is an essential trace element involved in immune function, wound healing, and enzyme activity (Lin et al., 2017). Khan et al. (2022) emphasize that zinc deficiency is a greater concern in many African populations than toxicity. Nishito & Kambe, (2018) stated that while excess zinc can interfere with copper absorption, the levels in *fufu* are too low to pose any risk.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.0 Introduction

This chapter presents the conclusion of the study, which examined the microbial quality and levels of heavy metal contamination in *fufu* processed from milling machines within the Nsuta-Ashanti area. It consolidates the key findings in relation to the research objectives and reflects on the implications of these results for public health and food safety within the community. The discussion not only highlights the identified risks but also emphasizes the importance of safe food processing practices. Building on these insights, the chapter provides practical recommendations to guide key stakeholders, including local authorities, milling machine operators, and community members.

6.1 Summary of key findings

The study found that *fufu* processed by milling machines in Nsuta-Ashanti was contaminated with *Salmonella, Shigella, E. coli,* and total coliforms, with the milling machines identified as the primary source rather than the raw cassava and plantain. *E. coli* levels varied significantly across sites, reflecting differences in hygiene and sanitation, while *Salmonella, Shigella,* and total coliforms showed no major variation. Quantitative microbial risk assessment revealed high infection probabilities for all three pathogens, with Shigella posing the most significant risk even at very low doses. Heavy metal analysis detected arsenic, lead, zinc, and cadmium, with arsenic and zinc within safe limits; however, lead and cadmium levels increased slightly after milling, likely due to machine contamination. A health risk assessment indicated minimal risks from cadmium and zinc, a very low risk from arsenic, but potential health concerns from lead in some cases.

6.2 Conclusion

The microbial and heavy metal assessment of *fufu* processed in Nsuta-Ashanti showed serious contamination risks. Milling machines were identified as the primary source of microbial contamination, with significant increases in *Salmonella, Shigella,* and total coliforms after milling. While *E. coli* levels varied across sites, other microbes did not show location-based differences. QMRA results confirmed high infection probabilities for all pathogens, especially Shigella, which can cause illness even at very low doses.

Heavy metal analysis detected arsenic, lead, zinc, and cadmium in the samples. Although arsenic and zinc levels remained within safe limits, lead and cadmium levels increased slightly after milling, likely due to equipment contamination. Zinc increases were minor and below risk thresholds, while cadmium exposures were mainly within safe limits. However, some lead exposures exceeded the safety threshold, posing non-carcinogenic health risks. Overall, the study highlighted serious microbial hazards and potential heavy metal risks associated with *fufu* processed by milling machines in the area.

6.3 Recommendations

The following recommendations were made after the successful completion of the study, directed to the following institutions and stakeholders:

1. **Municipal /District Environmental Health Unit/Assembly**

   The Environmental Health Unit under the Assembly should strengthen regular monitoring and inspection of milling machines to ensure compliance with hygiene standards. They should also organize periodic training programs for mill operators on proper sanitation practices, machine maintenance, and safe food handling.

2. **Food and Drugs Authority (FDA)**

   The FDA should intensify its regulatory oversight by conducting routine quality checks on processed food products from milling machines. Clear guidelines on acceptable microbial and heavy metal levels should be established, and penalties should be introduced for non-compliance. The FDA can also collaborate with local authorities to provide technical support and food safety education.

3. **Researchers**

   Further research should focus on the long-term health effects of consuming contaminated *fufu* and on developing innovative interventions that promote safer food processing practices.

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APPENDIX 1

QUESTIONNAIRE FOR CUSTOMERS ASSESSMENT OF HEAVY METAL CONTAMINATION AND MICROBIAL LOADS IN *FUFU* PROCESSED BY *FUFU* MILLING MACHINES IN NSUTA-ASHANTI.

Hello Sir/Madam,

My name is **________________________________**, I am a final year student of Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development and as part of my thesis, I am to undertake a study on Assessment of Heavy metal contamination and Microbial loads in *fufu* processed by *fufu* milling machines in Nsuta-Ashanti.

SECTION A: DEMOGRAPHIC INFORMATION

1. Age
   ( ) Under 18 ^{1} ( )18–30 ^{2} ( ) 31–40 ^{3} ( ) 41–50 ^{4}

2. Gender
   ( ) male ^{1} ( ) Female ^{2}

3. Occupation
   ( ) Farmer ^{1} ( ) Trader ^{2} ( ) Student ^{3} ( ) Civil servant^{4}

4. What is your level of education?

   ( ) No formal education ^{1} () Primary ^{2} ( ) Secondary ^{3} ( ) Tertiary ^{4}

5. Household size
   ( ) 1-3 members ^{1} ( ) 4-6 members ^{2} ( ) 7-9 members ^{3} ( ) 10 or more members^{4}

SECTION B : *FUFU* CONSUMPTION PATTERNS

6. How often do you consume *fufu*?

   ( ) Daily ^{1} ( ) 2-3 times per week ^{2} ( ) Once a week ^{3} ( ) Occasionally ^{4}

7. What is your Average quantity of *fufu* consumed per meal (in grams)?

   ……………………

8. What is your body weight?

   ----

9. Whether the *fufu* is consumed hot, warm, or cold (affecting microbial survival)

   ( ) Yes ^{1} ( ) No ^{2}

10. How do you transport the milled *fufu* home?

    ( ) In a covered container ^{1} () In an open container ^{2} ( ) Plastic bag ^{3}

11. Storage method and duration before consumption (if not eaten immediately)

    ( ) Less than a day ^{1} () 1-2 days ^{2} ( ) More than 2 days ^{3}

SECTION C: AWARENESS OF FOOD SAFETY AND HEAVY METAL CONTAMINATION

12. Have you or any household member experienced food poisoning (e.g., diarrhea, stomach pain, vomiting) after consuming *fufu*?

    ( ) Yes ^{1} ( ) No ^{2}

13. If yes, how often?

    ( ) Often ^{1} ( ) Sometimes ^{2} ( ) Rarely ^{3}

14. Handwashing practices before and after food preparation and eating

    ( ) Always ^{1} ( ) Sometimes ^{2} ( ) Rarely ^{3} ( ) Never ^{4}

15. Are you aware that *fufu* can be contaminated with heavy metals and harmful microbes?

    ( ) Yes ^{1} ( ) No ^{2}

16. Do you believe milling machines can contribute to contamination?

    ( ) Yes ^{1} ( ) No ^{2}

17. Have you noticed any unusual taste, smell, or color in your milled *fufu*?

    ( ) Yes ^{1} ( ) No ^{2}

APPENDIX 2

QUESTIONNAIRE FOR ASSESSMENT OF HEAVY METAL CONTAMINATION AND MICROBIAL LOADS IN *FUFU* PROCESSED BY *FUFU* MILLING MACHINES IN NSUTA-ASHANTI. (FOR MACHINE OPERATORS ONLY)

Hello Sir/Madam,

My name is **________________________________**, I am a final year student of Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development and as part of my thesis, I am to undertake a study on Assessment of Heavy metal contamination and Microbial loads in *fufu* processed by *fufu* milling machines in Nsuta-Ashanti.

SECTION A: DEMOGRAPHIC INFORMATION OF MACHINE OPERATORS/OWNERS

1. Name of Area:
2. Age:

   ( ) Under 20 $^{\mathbf{1}}$ ( )20–30 $^{\mathbf{2}}$ ( ) 31–40 $^{\mathbf{3}}$ ( ) 41–50 $^{\mathbf{4}}$
3. Gender

   ( ) male $^{\mathbf{1}}$ ( ) Female $^{\mathbf{2}}$
4. How long have you been operating *fufu* milling machines?

   ( ) Less than 1 year$^{\mathbf{1}}$ ( ) 1–3 years $^{\mathbf{2}}$ ( ) 4–6 years $^{\mathbf{3}}$ ( ) More than 6 years $^{\mathbf{4}}$
5. What is your level of education?

   ( ) No formal education $^{\mathbf{1}}$ () Primary $^{\mathbf{2}}$ ( ) Secondary $^{\mathbf{3}}$ ( ) Tertiary $^{\mathbf{4}}$

SECTION B: HYGIENE OF THE MILLING ENVIRONMENT

6. How often do you clean the milling environment?

   ( ) After every use $^{\mathbf{1}}$ ( ) Daily $^{\mathbf{2}}$ ( ) Weekly $^{\mathbf{3}}$ ( ) Rarely $^{\mathbf{4}}$

7. Do you allow pets or animals in the milling area?

   ( ) Yes ^{1} ( ) No ^{2}

8. Do customers wash their ingredients (e.g., cassava, plantain) before milling?

   ( ) Yes ^{1} ( ) No ^{2}

SECTION C: HYGIENE AND MAINTENANCE OF MILLING MACHINES

9. How often do you clean the milling machine?

   ( ) After every use ^{1} ( ) Daily ^{2} ( ) Weekly ^{3} ( ) Rarely ^{4}

10. What cleaning materials do you use for the machine?

    ( ) Water only ^{1} ( ) Water and detergent/soap ^{2} ( ) Disinfectants ^{3}

11. Are there any visible signs of rust or wear and tear on the milling machine?

    ( ) Yes ^{1} ( ) No ^{2}

12. Are there visible signs of dirt or food residue in the milling area?

    ( ) Yes ^{1} ( ) No ^{2}

13. How often is the milling machine serviced or maintained?

    ( ) Weekly ^{1} ( ) Monthly ^{2} ( ) Quarterly ^{3} ( ) Rarely ^{4}

14. Who conducts the maintenance of the machine?

    ( ) Operator (myself) ^{1} ( ) Technician ^{2} ( ) Manufacturer’s representative ^{3}

SECTION D: HYGIENE PRACTICES DURING OPERATION

15. Do you wear protective gear (e.g., gloves, aprons) while operating the machine?

    ( ) Always ^{1} ( ) Sometimes ^{2} ( ) Rarely ^{3} ( ) Never ^{4}

16. Do you wash your hands before and after operating the machine?

    ( ) Always ^{1} ( ) Sometimes ^{2} ( ) Rarely ^{3} ( ) Never ^{4}

17. How do you dispose of waste generated during milling? H

( ) Dump it nearby **[1]** ( ) Dispose of it in a designated waste bin **[2]** ( ) Burry it **[3]**

18. Do you experience any challenges in maintaining hygiene during milling operations?

( ) Yes (please specify): ______________________________ **[1]** ( ) No **[2]**

19. Do you receive any hygiene training or guidance related to your operations?

( ) Yes **[1]** ( ) No **[2]**