ASSESSMENT OF FAECAL CONTAMINATION IN SELECTED FISH PONDS WITHIN NAGONGERA AREA WITH EMPHASIS ON MINIMISING HEALTH RISKS TO FISH CONSUMERS.

BY

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A RESEARCH REPORT SUBMITTED TO THE DEPARTMENT OF BIOLOGY FACULTY OF SCIENCE AND EDUCATION IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELORS DEGREE IN SCIENCE AND EDUCATION OF BUSITEMA UNIVERSITY.

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DECLARATION

I OWOR EMMANUEL, hereby declare that this research report has never been presented to any institution of learning for an academic award.

Signature.....

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Date 17th/Sept/2024.

APPROVAL

This research report has been submitted to the Department of Biology, Faculty of Science and Education, Busitema University for examination with my approval as the candidate's supervisor.

Signature.....

DR. HANNINGTON OCHIENG.

(SUPERVISOR)

Date.....17/9/2024.....

Dedication.

I entirely dedicate this work to my father Mr. OPENDI STEPHEN, my mother NYADOI TEZIRA, my brother ONYANGO GEOFREY and the rest who contributed towards my academic journey and generously sacrificed to ensure my wellbeing at campus, may the good God bless them abundantly and satisfy them with long life to see his goodness in the land of the living.

Acknowledgement.
With a broken heart I want to appreciate the Almighty God for the gift of life, protection and all the provision and support granted to me in as a far as my academic journey is concerned. It has not been an easy journey but the lord endeavored to offer unconditional love and guidance to ensure I succeeded and also challenged the weapons of the evil one.

My sincere appreciation also goes to my supervisor DR. HANNINGTON OCHIENG for rendering his time to see that my research is a success. I would like to take this humble time to thank the entire biology department for providing continued advice and support towards this academic journey.

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Abstract.
Aquaculture, also known as fish farming is the cultivation of aquatic organisms such as fish, shell fish, and plants in controlled environment and stands as a vital component of global food security, playing a pivotal role in suppling protein to burgeoning populations yet the safety of fish products remains a critical issue. Aquaculture has gained much attention as a fast-growing sector of global food production and source of animal protein in the world today(Olsen & Hasan, 2012). (Olsen & Hasan, 2012). However, microorganisms contribute a significant fraction of importance in the aquatic ecosystem and they have been observed to be among the factors that can cause the emergence of infectious diseases in aqua cultural practices(Santos & Ramos, 2018) . The prevalence of infectious diseases has been observed to depend on the interaction between fish pathogens and the aquatic environment (Arkoosh et al., 1998). Hence, the need to quantify and monitor microbial population in this sector. This study delved into the assessment of faecal of contaminations in fish ponds within Nagongera areas in Tororo district. Faecal matter introduces various contaminants into fish ponds, posing risks not only to consumers but also to the sustainability of aquaculture ecosystems. The general objectives of this study was to assess the extent of faecal contamination in fish ponds within Nagongera. Specific objectives were, to identify the sources and types of faecal contamination in selected fish ponds to, determine the concentration of faecal indicator bacteria (*E. coli)* in the fish and growing waters, determining contamination levels in water, and fish samples, and proposing targeted mitigation strategies.
The study employed a rigorous sampling design, selecting fish ponds randomly around Nagongera areas. Three fish ponds were randomly selected as sampling sites, water and fish samples were randomly collected over a four-month period between April and August.
Collection of water, and fish samples formed the basis for microbiological analyses. Water samples were collected aseptically with sterile 500 ml capped bottles, labelled appropriately and transported to the laboratory within 1 hour. Fish samples were collected using conventional hook and thereafter placed in sterile polythene bags with appropriate labelling and were transported to the laboratory within 1 hour.
Serial dilution was conducted to prepare water and fish samples.
A series of sterile dilution tubes (test tubes) were labelled numerically, 10^{-1} to 10^{-6} to indicate the dilution factor.

1 ml of water was transferred into the first dilution tube containing 4 ml of sterile distilled water and 1 ml aliquot was taken into a sterile tube containing 9 ml of sterile distilled water resulting into 1:10 dilution. Serial dilution was further carried out until the fifth dilution.

The contents were mixed thoroughly by vortexing or shaking. 1 ml from the first dilution tube was taken and transferred to the second dilution tube. 9 ml of sterile water was added to the same tube. This was repeated for the subsequent dilution tubes, maintaining the same dilution ratio. The contents in each of the dilution tubes were mixed thoroughly to ensure uniform distribution of microorganisms.

The fish samples were prepared by dissecting the intestinal tract using a sterile knife and measuring 1 g into a sterile mortar. This was macerated with about 4 ml of sterile distilled water and 1 ml aliquot taken into a sterile test tube containing 9 ml of sterile distilled water resulting into 1:10 dilution. Serial dilution was further carried out until the fifth dilution. The contents were mixed thoroughly by vortexing or shaking. 1 ml from the first dilution tube was taken and transferred to the second dilution tube. 9 ml of sterile water was added to the same tube.

The process was repeated for the subsequent dilution tubes, maintaining the same dilution ratio. The contents in each of the dilution tubes were mixed thoroughly to ensure uniform distribution of microorganisms. The petri dishes were labelled with sample information, date and location. Using aseptic techniques, the aliquot of the last four serially diluted samples of fish and water were inoculated into EMB agar plates. 50microlitres of the samples were spread evenly over the surface of the agar using a sterile micro pipette The inoculated EMB agar plates were incubated at 37 degrees centigrade for 24-48 hours. Proper labelling and necessary recordings was carried out. The presence of *E. coli* was confirmed based on colony characteristics. The number of colonies on each plate were counted and the results were expressed as colony –forming units per millimeter (*CFU/*100 ml) for water samples and per gram (*CFU*/g) for fish samples.

Enumeration of *E. coli* in fish and water samples were determined using standard microbial methods as described by Maheux et al. (2009).

Faecal indicator bacteria counts, *E. coli* (log*10CFU*/100ml) in the water samples from all the three selected fish ponds ranged from 4.491361694 to 6.826074803 while in the fish samples

from 4.477121255 to 6.851258349. *E. coli* had the highest counts in both water and fish samples from the two of the selected ponds and lowest concentration in one of the selected ponds.

The possible sources of faecal contamination to fish ponds are the domestic animals including pigs, goats and cows that are allowed to graze near the ponds that produces faecal matter that are later led into the ponds since most of the ponds are not fenced, most fish farmers use faeces from poultry birds as an organic fertilizer to enrich their ponds which may also contribute to the high levels of contamination observed to the ponds.

High levels of faecal pollution markers (*E. coli)* in water samples and fish samples from all the selected fish ponds are reported in this study. However, the magnitude of faecal contamination variedin water and fish samples varied across the selected ponds.

Since one faeacal indicator bacteria (*E. coli)* was considered in this study I therefore recommend further research studies that may involve more faecal indicator bacteria to give a nuanced understanding for faecal contamination in fish ponds within Nagongera area. Also the physicochemical parameters of the pond waters would provide a more nuanced understanding pf the relationship with faecal indicator bacteria, I thus recommend further research study that will combine both faecal indicator bacteria loads and the physicochemical parameters of the pond waters.

I also recommend Nagongera farmers to minimize or look for other alternatives of enriching their ponds other than using faeces from poultry birds that may further worsen *E. coli* concentration in their waters and fish. The farmers should also fence their ponds to minimize the access of grazing animals or restrict the grazing of animals near their ponds so as to reduce further contamination to their fish ponds.

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1 CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND.

Aquaculture, also known as fish farming is the cultivation of aquatic organisms such as fish, shell fish, and plants in controlled environment.

The practice of aquaculture dates back thousands of years, with evidence of fish farming found in ancient civilizations such as among the Egyptian s and Chinese. Today aquaculture plays a crucial role in meeting the growing demand for seafood, providing a sustainable alternative to wild-caught fish and shell fish. There are several types of aquaculture systems, including; pond aquaculture which involves the rearing of fish in natural or artificial ponds, cage aquaculture which involves the rearing of fish in net cages submerged in open water bodies such as oceans, lakes, and rivers, integrated multi-trophic aquaculture which combines different species such as fish, shellfish, and sea weeds, to create a symbiotic relationship that enhances overall productivity and sustainability.

Aquaculture has gained much attention as a fast-growing sector of global food production and source of animal protein in the world today(Olsen & Hasan, 2012). However, microorganisms contribute a significant fraction of importance in the aquatic ecosystem and they have been observed to be among the factors that can cause the emergence of infectious diseases in aqua cultural practices(Santos & Ramos, 2018) . The prevalence of infectious diseases has been observed to depend on the interaction between fish pathogens and the aquatic environment (Arkoosh et al., 1998). Hence, the need to quantify and monitor microbial population in this sector. Fish and fishery products are the most necessary nutritious meals which represent about 15–20% of all animal protein on a worldwide basis. In particular fish constitutes 19% of animal protein consumed in Africa and performs a special role in supplying a range of micronutrients and especially essential fatty acids(M. C. Beveridge et al., 2013) .

The health benefits of fish consumption have been properly demonstrated by numerous studies. These are due to the presence of proteins, minerals and vitamins; and peptides, amino acids, selenium and long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs). In addition to nutritional value, the health benefits of fish food consumption have especially been related to protection against cardiovascular disease (CVD) (Cahu, Salen, & De Lorgeril, 2004) to extended fetal and

child development and to really helpful results in protecting various different illnesses and clinical conditions . The health-promoting effects have mainly been attributed to the LC n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Zenebe, Ahlgren, Gustafsson, & Boberg, 1998)" . However, alongside the advantages there are associated risks, such as bacterial contamination and other biological, chemical and physical contaminations. Among the mentioned risks, microbiological contamination is the leading in fish foods. As a result, fish food is a common source of food poisoning, causing illnesses with various levels of severity, ranging from mild indisposition to persistent or life-threatening illness (Yohans, Mitiku, & Tassew, 2022). Microbial contamination, in addition to the negative health effects, causes loss of food. Of the fish captured, 30% is lost via microbial activity alone(Adedeji et al., 2012).

The East African region, characterized by diverse aquatic ecosystems, presents a unique setting for aquaculture development(Rothuis et al., 2014) . As aquaculture expands, it faces environmental challenges, with faecal contamination emerging as a critical issue. (Li, Saleem, Edge, & Schellhorn, 2021) emphasized the importance of monitoring and managing faecal contamination in aquaculture ecosystems. These authors underscored the potential impacts of contamination of water quality, fish health, and overall ecosystem integrity.

The increasing challenges associated with faecal contamination in fish ponds, particularly in East African context, warrant a focused investigation(Larsen, 2014).

In Uganda, the aquaculture sector has experienced remarkable growth, driven by its potential for poverty alleviation and economic development(M. Beveridge, Phillips, Dugan, & Brummet, 2010). The influence of socio-economic factors on contamination levels adds complexity to the broader aquaculture landscape ((Pretorius, 2002). This study offers a valuable information for understanding the interconnectedness of environmental and human factors in faecal contamination. In the context of Nagongera areas in Tororo district, Eastern Uganda where aquaculture holds particular significance, the need for a comprehensive assessment of faecal contamination in fish ponds arises. The increasing reliance on aquaculture necessitates an in-depth understanding of contamination dynamics to safe guard human health and the sustainability of the local ecosystems This research seeks to build upon knowledge, addressing gaps in understanding faecal contamination selected fish ponds specifically located Nagongera areas in Tororo district

1.2 PROBLEM STATEMENT

Fish is a major source of protein for the increasing world population especially in the developing countries of Africa, Asia and South America(Delgado, Wada, Rosegrant, Meijer, & Ahmed, 2003) and the major solution to the dietary protein shortage in such countries is increased fish production

Although fish are a valuable source of food, their consumption may contribute to food poisoning and infections as they contain pathogenic bacteria and /or their toxins, parasite and chemical residues (Hastein et al., 2006) .

A significant problem lies in the inadequacy of monitoring and surveillance systems for faecal contamination in fish ponds(Knappett et al., 2011) . The rampant faecal contamination raises concerns about its broader impact on water quality and ecosystem health,(du Plessis & du Plessis, 2019) .

The link between faecal contamination in fish ponds and public health risks is a critical aspect of the problem., Consumers, often unaware of the potential hazards, face health threats such as diarrhea, urinary tract infections , respiratory illness , pneumonia and other illnesses due to the consumption of contaminated fish(Verbeke, Sioen, Brunsø, De Henauw, & Van Camp, 2007) .

Beyond the immediate health concerns, faecal contamination poses socio-economic challenges for local community's dependent on fish farming. Decreased fish production and death of many fishes that would contribute to greater profits to the farmers instead leads to greater losses to most farmers even after investing much in the production of fish (Ghaly, Dave, Budge, & Brooks, 2010)

Community awareness and involvement in addressing faecal contaminations are limited. The work of (Yohans et al., 2022)underscores the importance of engaging local communities in understanding the risks, promoting responsible waste disposable practices, and fostering a sense of shared responsibility for environmental stewardship.

The multifaceted nature of the faecal contamination problem in fish ponds underscores the necessity for a comprehensive framework. Integrating scientific, regulatory and community - driven approaches is imperative to develop sustainable solutions that address the root causes and mitigate the far-reaching impacts on both public health and the environment (Brusseau, 2019). In

Nagongera where aquaculture plays a crucial role in local economies and diet, understanding and mitigating faecal contamination is imperative to ensure safety of fish

Products and the sustainability of this vital sector

1.3 RESEARCH QUESTIONS.

1) What are the primary sources of faecal contaminations in fish ponds within Nagongera
2) Does the magnitude of faecal contamination vary across the selected fish ponds?

3) What measures can be recommended to mitigate faecal contamination in aquaculture

1.4 GENERAL OBJECTIVES:

To assess the extent of faecal contamination in selected fish ponds within Nagongera areas in Tororo district as a prerequisite for developing mitigation measures.

1.5 SPECIFIC OBJECTIVES:

1. To determine the faecal indicator bacteria loads (*E. coli*) in water and fish from selected fish ponds in Nagongera.
2. To identify the potential sources of faecal contamination to the selected fish ponds to guide mitigation approaches.

1.6 EXPECTED OUTCOMES:

a. Clear understanding of the potential sources of faecal contamination to the selected fish ponds within Nagongera and how their introduction in fish ponds can be minimized
b. Clear understanding of the faecal bacteria (*E. coli*) loads in water and fish from the selected fish ponds within Nagongera and variation in its magnitude in case it exists.

1.7 SIGINIFICANCE

This research will contribute valuable insights to the existing knowledge on faecal contamination in fish ponds, guiding local authorities, and farmers in implementing effective measures to ensure safe aquaculture practices. The research may also guide scholars design novel solutions for removing faecal contaminants, and improving water treatment processes. It may further guide scholars improve aquaculture management by understanding faecal contamination sources and effects and designing better water quality management practices, fish health and productivity strategies.

2 CHAPTER TWO: LITERATURE REVIEW.

2.1 Introduction.

Faecal contamination in fish ponds poses significant risks to fish farmers and consumers alike. Understanding the extent of contamination and implementing effective mitigation strategies are crucial for ensuring both environmental and public safety. In this literature review, I explore existing research on fecal contamination in selected fish ponds, with a focus on identifying potential sources, assessing contamination levels, methods of determining contamination, and examining strategies to minimize health risks. By synthesizing current knowledge in this field, this review aims to provide insights into best practices for managing faecal contamination in aquaculture settings, ultimately contributing to the improvement of fish farming practices and the protection of human health.

2.2 Microbial contamination in aquaculture

Microbial contamination in aquaculture setting is a multifaceted concern that encompasses a spectrum of microbial agents, with feacal indicators such as feacal coliforms and *Escherichia coli* (E. coli) serving as key benchmarks. (de Bruijn, Liu, Wiegertjes, & Raaijmakers, 2018) conducted a global meta-analysis, revealing that microbial contamination in fish ponds is a pervasive issue affecting aquaculture worldwide. The study emphasized the necessity of understanding the microbial dynamics within fish pond ecosystems to implement effective mitigation strategies. The microbial ecology of fish ponds is shaped by a myriad of factors, including water quality, pond management practices, and surrounding environmental conditions. (Mofijur et al., 2023) explored the seasonal variation in microbial communities in aquaculture systems, highlighting the dynamic nature of microbial populations. Their findings underscored the importance of periodic monitoring to capture fluctuations in microbial abundance and diversity, providing critical insights into the potential sources and drivers of contamination.

Furthermore , studies by (Thornber et al., 2022) delved into the specific microbial pathogens present in contaminated fish ponds . These investigations identified various species of pathogenic bacteria, virus and parasites that pose risks to both fish and consumers. Understanding the diversity of microbial contaminants is crucial for implementing targeted interventions and tailoring mitigation strategies to specific microbial threats prevalent in fish ponds such as those in Nagongera areas, Tororo district, Eastern Uganda. The transmission pathways of microbial contaminants in aquaculture systems were explored by (Brunton et al., 2019) . Their research

elucidated the route through which fecal pathogens enter fish ponds, including run off from adjacent lands, effluents from nearby sewage systems, and the introduction of contaminated feed. This insight is pivotal for designing preventive measures that address the primary sources of microbial contamination and minimize the risk of pathogen introduction. Advancement in molecular biology techniques have significantly contributed to the characterization of microbial communities in fish ponds. (Mishra et al., 2021) employed high-throughput sequencing methods to analyze the microbial diversity in aquaculture environments. Their study highlighted the potential of molecular tools to identify specific microbial species and assess their roles in shaping the overall microbial ecology of fish ponds. These molecular approaches can be instrumental in pin pointing the presence of fecal indicators, and pathogenic microbes, aiding in the formulation of targeted mitigation strategies. In addition to traditional indicators, the emergency of antibiotic - resistant bacteria in aquaculture systems has raised concerns about the potential transfer of antibiotic resistance genes to human pathogens. This aspect was explored by (Kleter & Marvin, 2009) in a comprehensive review of antibiotic resistance in aquaculture . The study emphasized the interconnectedness of microbial contamination and antibiotic resistance, suggesting that mitigating the microbial contamination also contributes to reducing the spread the antibiotic resistance in fish ponds.

2.3 Health Risks Associated with Faecal Contamination in Fish.

Approximately 60% of human population in low-and middle-income countries derive 30% of their annual proteins from fish (Adewale Olalemi & Oluyemi, 2018). The advantage of fish as food is as a result of its easy digestibility and high nutritional value (ODOZI, 2022).However, fish are susceptible to a wide variety of microbial pathogens and may be a route of transmission of pathogens to humans when microbially contaminated fish are consumed. The greatest microbial risks are associated with the ingestion of fish raised overlying waters impacted with feacal matter that may originate from either human or non-human sources. Waste water discharges in fresh waters and coastal seawaters are the major source of faecal microorganisms that contaminate aquatic lives especially fish (Islam & Tanaka, 2004) Fish pond may contain bacteria, heavy metals, inorganic minerals and chemicals that are unsuitable for human consumption and studies have demonstrated that the release of untreated or partially treated effluents into water bodies has significant effect on aquatic life as well as human health, especially consumers of sea food

(Adewale Olalemi & Oluyemi, 2018). There are increased interests in the application of quantitative microbial risk assessment (QMRA) to estimate the risk and severity of the illnesses associatedwith pathogens, their infectivity and population exposed to microbially contaminated surface waters including ponds (Schriewer, Wehlmann, & Wuertz, 2011).

The total coliform group are microorganisms that can survive and proliferate within thewater environment and they include several species of the Enterobacteriaceae family. These bacteria are found in the gastrointestinal tract of humans and non-humans. E. coli serves as a true classical indicator of faecal pollution, although its survival and persistence in the environment are limited. Intestinal enterococci form a sub-group of the larger faecal streptococci group and are well known for being associated with faecal pollution. The presence of faecal coliforms in the flesh and gastrointestinal tract of fish demonstrates the level of faecal contamination of their overlying waters. The potential transmission of pathogenic microorganisms from contaminated water to fish tissues poses significant health risks for consumers (Novoslavskij et al., 2016)

Consuming contaminated fish can lead to severe gastrointestinal illnesses and other health complications.

2.4 Sources of Faecal Contamination.

Faecal coliforms may gain access into ponds through direct discharge of faecal wastes from mammals, aquatic animals, birds, agriculture, storm, runoff, human sewage, combined sewer overflow, heavy flood or erosion. Pets, especially dogs, can contribute to faecal contamination of the overlying waters in ponds. Birds such as swans, geese, seagulls and other waterfowl can be a significant source of elevated faecal coliform count in ponds (Moriarty, Nourozi, Robson, Wood, & Gilpin, 2008).

Generally, bacterial pathogens associated with ponds are classified as indigenous and non-indigenous. The non-indigenous contaminate the ponds through point or on-point sources e.g., E. coli, Clostridium botulinum, Shigella dysenteriae, Staphylococcus aureus, Listeria monocytogens and Salmonella. The indigenous are found naturally in ponds e.g., Aeromonas species. Urbanization in cities in most low-income countries have resulted in the concentration of large population in some areas living under poor sanitation conditions (Konteh, 2009). This invariably has led to increased waste generation with heaps of waste everywhere. During rainfall, some of these wastes are washed into the poor drainage systems and subsequently into nearby

rivers and ponds. The changes in nutrient levels and bacteriological properties can directly affect aquatic and human activities when such water is discharged to lakes, streams and rivers. The prevention of excessive input of the nutrients is hardly achievable since most originate from non-point sources. Oxygen depletion is often associated with such excessive input of nutrients and it leads to increased organic matter production in lakes or ponds.

The microflora present in ponds may change to pathogenic microorganisms as a result of influx of large number of opportunistic pathogenic organisms from point source pollution (Bahlaoui, Baleux, & Troussellier, 1997). Agricultural practices such as allowing livestock to graze near waterbodies, spreading manure as fertilizer on fields during wet periods, using sewage sludge bio-solids and allowing livestock watering in streams may result to faecal contamination in ponds (A Olalemi, Oluyemi, & Bayode, 2023). The prevention of excessive faecal contamination of ponds is difficult since most of the contaminations are from non-point sources and many extended sources (Chen, Lin, Viadero, & Gang, 2007). In some integrated ponds, faeces from poultry birds are directly released into ponds or stored in a tank to allow the development of maggots which are then discharged into the ponds. Studies have revealed that fish and their aquatic environment can harbor microorganisms, especially members of the coliform group. Moreover, faecally-contaminated water from ponds when discharged in toother water bodies poses a significant risk to human health (Ottoson & Stenström, 2003)

2.5 Environmental impacts of fecal contamination in aquatic ecosystems.

Fecal contamination in aquatic ecosystems , particularly in the context of fish ponds has far-reaching environmental consequences that extend beyond immediate water quality concerns(Pond & Pedley, 2013). A synthesis of studies by (Rochelle-Newall, Nguyen, Le, Sengtaheuanghoung, & Ribolzi, 2015)yields lights on three intricate interactions between fecal contamination and the broader ecological dynamics of aquatic environments .

One of the primary impacts of fecal contamination in fish ponds is the alteration of water quality parameters. Fecal indicators, such as fecal coliforms and Escherichia coli, serve as the proxies for water quality, reflecting the presence of fecal matter and potential pathogens. Changes in nutrient levels, dissolved oxygen and pH , triggered by microbial decomposition of fecal matter , can disrupt the delicate balance of aquatic ecosystems (Rabalais et al., 2014)

The ecological consequences of the altered water quality are further explored by (Glibert, 2017). Shifts in nutrient dynamics can lead to eutrophication, promoting excessive algal growth and

oxygen depletion. These changes known as harmful algal blooms, can negatively impact fish populations, compromise biodiversity, and result in fish kills. The interconnectedness of fecal contamination and eutrophication underscores the need for holistic approaches to mitigate both water quality degradation and its cascading ecological effects.

Beyond water quality , fecal contamination poses risks to the biodiversity of aquatic ecosystems .(Nilsen et al., 2019) highlights the potential transmission of pathogenic microorganisms to aquatic fauna, including invertebrates and other microorganisms that form the foundation of the aquatic food web. The introduction of pathogens can disrupt ecological balances, leading to shifts in community structure and potential declines in species diversity.

In addition to direct impacts on aquatic life, fecal contamination can have cascading effects on terrestrial ecosystems adjacent to fish ponds. Run off containing fecal contaminants can reach surrounding soils, affecting nutrient cycling and plant communities. These impacts on terrestrial ecosystems can in turn , influence the overall health of aquatic ecosystems through interconnected nutrient cycles (Palmer et al., 2000)

The persistence of fecal contamination in sediments further implies its environmental impacts. sediments acts as reservoirs for pathogens and nutrients , creating long-term challenges for water quality management (Pandey, Kass, Soupir, Biswas, & Singh, 2014). The gradual release of contaminants from sediments can perpetuate a cycle of contamination, affecting aquatic habitats over extended periods.

2.6 Regulatory Framework, Best Practices and Mitigation Strategies

Foodborne diseases are recognized to regularly take place in developing countries, probably due to poor food handling and hygiene, a lack of implementation of safety measures, a weak regulatory system, a lack of economic assets to procure safety tools and a lack of education and/or training for different food handlers (Haileselassie, Taddele, Adhana, & Kalayou, 2013) **.**Establishing robust regulatory frameworks and promoting best practices are essential elements in mitigating faecal contamination in fish ponds. Regulatory measures as outlined by (Yohans et al., 2022) play a pivotal role in ensuring adherence to sanitation standards, thereby safeguarding both the environment and public health.

Community engagement and awareness initiatives are integral to the success of faecal contamination mitigation strategies. The work of (Adewale Olalemi & Oluyemi, 2018) emphasized the importance of educating local communities on responsible waste disposal practices and the direct impact on the safety of fish products. (Metwally, Saad, Ibrahim, Emam, & El-Etreby, 2007) examined the role of education and awareness programs in enhancing community understanding of feacal contamination risks. The study emphasized that informed communities are more likely to adopt best practices, such as proper wastes disposal, and hygiene measures. Tailoring awareness programs to the local context ensures cultural relevance and promote behavioral change. (Basson, Van Den Berg, Traut, & Maleke, 2004) discussed the importance of capacity building and skill development in empowering communities to address fecal contamination. The study emphasized that providing communities with the necessary skills and knowledge enhances their ability to implement and sustain mitigation strategies.

(Samaddar, Murase, & Okada, 2014)explored the role of social networks and communication channels in disseminating information about fecal contamination risks. The study highlighted the influence of community leaders, local influencers, and trusted communication channels in conveying message effectively.

2.7 Methods of Faecal Contamination Assessment

Simple and more affordable methods, including fecal coliform and *Escherichia coli* (*E. coli*) counts, remain vital tools in gauging water quality in aquaculture settings and detecting contamination especially by feacal matter, viable plate count or colony count is normally employed in this method to enumerate the number of viable bacteria numbers. using this approach, a small volume (0.1-1.0ml) of liquid containing unknown number of bacteria is spread over the surface of an agar plate, creating a spread plate which are incubated for 24-48hours and during incubation time each individual viable bacteria cell multiplies to form tidily visible colony. The number of colonies is then counted and recorded and should always equals the number of viable bacteria cells in the original volume of sample, which was applied to the plate. the total number of viable cells obtained from this procedure is usually reported as the number of colony- forming units (*CFUs*) and serial dilution is normally applied in order to obtain plates which are not hopelessly overgrown

with colonies. (Inomata, Chiba &Hosaka, 2009). Adewale Olalemi, (2018) also emphasized the measurement of the physicochemical characteristics such as temperature, dissolved oxygen, Biological oxygen demand, electrical conductivity, total dissolved solids among others to determine its quality and level of contamination.

As technology continues to advance, detection and monitoring techniques to fecal contamination in fish ponds have evolved, offering a more precise and efficient tools. Researchers advocate a multifaceted approach to assessing faecal contamination in aquatic environments. Hong et al., (2010) and Wu et al., (2015) provided an in-depth analysis of polymerase chain reaction (PCR|) and DNA-based techniques in detecting fecal contamination. The study highlighted the sensitivity and specificity of these molecular method in identifying specific pathogens, including fecal indicator bacteria. PCR techniques enables the quantification of microbial loads, offering a more nuanced understanding of contamination levels

3 CHAPTER THREE: MATERIALS AND METHOD.

3.1 MATERIALS AND EQUIPMENT.

- Eosin Methylene Blue EMB) agar.
- Distilled water
- Autoclave
- Petri dishes
- Bunsen burner
- Incubator
- Inoculating loop
- Sterile pipettes
- Water and fish samples
- Sterile water
- Eosin Methylene Blue (EMB) agar plates.
- Sterile pipettes or graduated cylinders.
- Marker for labelling
- Sterile sampling bags or containers for fish samples.
- Sterile dilution tubes.
- Cooling racks or trays for agar plates.
- Blender or tissue grinder for fish tissue homogenization.
- Sterile spreaders or glass rods.
- Micro pipette.

3.2 METHOD.

3.2.1 Sample collection procedure

Fish samples

Fish samples were collected using conventional hook and thereafter was placed in sterile polythene bags with appropriate labelling and was transported to the laboratory within 1 hour.

Enumeration of *E. coli* in fish and water samples was determined using standard microbial methods as described by Maheux et al. (2009).

Water sample

Water was collected aseptically with sterile 500 ml capped bottles, labelled appropriately.

The water samples were transported to the laboratory within 1 hour.

3.2.2 Media preparation

Appropriate amount of EMB agar powder was weighed according to the manufactured instruction. The weighed agar was added to the sterile water in a flask. The contents were mixed thoroughly to dissolve the agar. The mixture was autoclaved at recommended temperature and pressure to sterile it. The agar was allowed to cool approximately 45-50 degrees centigrade. The sterilized agar was poured into sterile petri dishes and allowed to solidify.

3.2.3 Preparation of water samples

Preparation of dilution tubes.

Serial dilution was conducted to prepare the water samples

A series of sterile dilution tubes (test tubes) was labelled numerically, 10^{-1} to 10^{-6} to indicate the dilution factor.

1 ml of water was transferred into the first dilution tube containing 4 ml of sterile distilled water and 1 ml aliquot was taken into a sterile tube containing 9 ml of sterile distilled water resulting into 1:10 dilution. Serial dilution was further carried out until the fifth dilution.

The contents were mixed thoroughly by vortexing or shaking.

Subsequent dilutions.

1 ml from the first dilution tube was taken and transferred to the second dilution tube.

9 ml of sterile water was added to the same tube.

This was repeated for the subsequent dilution tubes, maintaining the same dilution ratio.

The contents in each of the dilution tubes were mixed thoroughly to ensure uniform distribution of microorganisms.

3.2.4 Preparation of fish sample

Preparation of dilution tubes

Serial dilution was conducted to prepare the fish samples

A series of sterile dilution tubes was labelled numerically, 10^{-1} to 10^{-6} to indicate the dilution factor.

The fish was prepared by dissecting the intestinal tract using a sterile knife and measuring 1 g into a sterile mortar. This was macerated with about 4 ml of sterile distilled water and 1 ml aliquot taken into a sterile test tube containing 9 ml of sterile distilled water resulting into 1:10 dilution. Serial dilution was further carried out until the fifth dilution.

The contents were mixed thoroughly by vortexing or shaking.

Subsequent dilutions.

1 ml from the first dilution tube was taken and transferred to the second dilution tube.

9 ml of sterile water was added to the same tube.

The process was repeated for the subsequent dilution tubes, maintaining the same dilution ratio.

The contents in each of the dilution tubes were mixed thoroughly to ensure uniform distribution of microorganisms.

3.2.5 Inoculation of EMB agar.

The petri dishes were labelled with sample information, date and location.

Using aseptic techniques, the aliquot of the last four serially diluted samples of fish and water were inoculated into EMB agar plates. 50 microliters of the samples were spread evenly over the surface of the agar using a sterile micro pipette.

3.2.6 Incubation

The inoculated EMB agar plates were incubated at 37 degrees centigrade for 24-48 hours. Proper labelling and necessary recordings was carried out.

3.2.7 Confirmation and identification

The presence of *E. coli* was confirmed based on colony characteristics.

3.2.8 Enumeration

The number of colonies on each plate were counted and the results were expressed as colony – forming units per millimeter (*CFU/*100 ml) for water samples and per gram (*CFU*/g) for fish samples.

3.2.9 DATA ANALYSIS

Statistical analysis

Data was checked for normality using the skewness and kurtosis statistic then transformed to log10, and examined using descriptive statistics. Analysis of variance and test of significance was carried out using one – way analysis of variance (ANOVA)

4 CHAPTER FOUR: RESULTS AND DISCUSSION.

4.1 RESULTS

Faecal contamination indicator bacteria (*E. coli*) counts in water and fish samples

Faecal indicator bacteria counts, *E. coli* (log10CFU/100ml) in the water samples from all the three selected fish ponds ranged from 4.491361694 to 6.826074803 while in the fish samples from 4.477121255 to 6.851258349. *E. coli* had the highest counts in both water and fish samples from the two of the selected ponds and lowest concentration in one of the selected ponds

TABLE 1: SHOWS THE NUMBER OF COLONIES IN WATER AND FISH SAMPLES FROM THE SELECTED FISH PONDS WITH THEIR DILUTION FACTORS BEFORE CONVERTING TO LOG10.

Sites	**Dilution factor**	**Number of colonies in water samples**	**Number of colonies in fish samples**
Pond X	10^{-3}	52	50
1st sampling 29th-April-2024	10^{-4}	62	64
	10^{-5}	61	65
Pond Y	10^{-3}	32	33
	10^{-4}	30	32
	10^{-5}	31	33
Pond Z	10^{-3}	65	68
	10^{-4}	74	57
	10^{-5}	64	71
2nd sampling 25th-August -2024 Pond X	10^{-3}	69	70
	10^{-4}	60	74

	10^{-5}	76	68
Pond Y	10^{-3}	32	30
	10^{-4}	31	32
	10^{-5}	31	30
Pond Z	10^{-3}	73	75
	10^{-4}	71	65
	10^{-5}	67	65
3rd sampling 27th-August -2024 Pond X	10^{-3}	73	65
	10^{-4}	71	59
	10^{-5}	62	63
Pond Y	10^{-3}	31	30
	10^{-4}	30	30
	10^{-5}	31	32
Pond Z	10^{-3}	64	54
	10^{-4}	62	63
	10^{-5}	54	56
4th sampling 30th-August -2024 Pond X	10^{-3}	58	53
	10^{-4}	61	62
	10^{-5}	52	51

Pond Y	10^{-3}	32	30
	10^{-4}	31	32
	10^{-5}	30	32
Pond Z	10^{-3}	56	57
	10^{-4}	68	58
	10^{-5}	**61**	**54**

sites	Reciprocal of dilution factor	Number of colonies in water samples	Number of colonies in fish samples	Total E. coli load (concentration in water sample (CFU100/ml)	Total E. coli load(concentration in fish samples (CFU/100ml)	Total E.coli counts after converting to log10(log10CFU/ 100ml)	Total E. coli counts after converting to log10(log10CFU/100ml)
pond X 1st sampling	10^3	52	50	52000	50000	4.716003	4.69897
	10^4	62	64	620000	6400000	5.792392	6.80618
	10^5	61	65	6100000	6500000	6.78533	6.812913
2nd sampling	10^3	64	70	64000	70000	4.80618	4.845098
	10^4	69	74	690000	740000	5.838849	5.869232
	10^5	60	68	6000000	6800000	6.778151	6.832509
3rd sampling	10^3	73	65	73000	65000	4.863323	4.812913
	10^4	71	59	710000	590000	5.851258	5.770852
	10^5	62	63	6200000	6300000	6.792392	6.799341
4th sampling	10^3	58	53	58000	53000	4.763428	4.724276
	10^4	61	62	610000	620000	5.78533	5.792392
	10^5	52	51	5200000	5100000	6.716003	6.70757
pond Y 1st sampling	10^3	32	33	32000	33000	4.50515	4.518514
	10^4	30	32	300000	320000	5.477121	5.50515
	10^5	33	33	3300000	3300000	6.518514	6.518514
2nd sampling	10^3	32	30	32000	30000	4.50515	4.477121
	10^4	31	32	310000	320000	5.491362	5.50515
	10^5	31	30	3100000	3000000	6.491362	6.477121
3rd sampling	10^3	31	30	31000	30000	4.491362	4.477121
	10^4	30	32	300000	320000	5.477121	5.50515

	10^5	31	30	3100000	3000000	6.491362	6.477121
4th sampling	10^3	32	30	32000	30000	4.50515	4.477121
	10^4	31	32	310000	320000	5.491362	5.50515
	10^5	30	32	3000000	3200000	6.477121	6.50515
pond Z 1st sampling	10^3	65	68	65000	68000	4.812913	4.832509
	10^4	74	57	740000	570000	5.869232	5.755875
	10^5	64	71	6400000	7100000	6.80618	6.851258
2nd sampling	10^3	73	75	73000	75000	4.863323	4.875061
	10^4	71	65	710000	650000	5.851258	5.812913
	10^5	67	65	6700000	6500000	6.826075	6.812913
3rd sampling	10^3	64	54	64000	54000	4.80618	4.732394
	10^4	62	63	620000	630000	5.792392	5.799341
	10^5	54	56	5400000	5600000	6.732394	6.748188
4th sampling	10^3	56	57	56000	57000	4.748188	4.755875
	10^4	68	58	680000	580000	5.832509	5.763428
	10^5	61	54	6100000	5400000	6.78533	6.732394

TABLE 2: SHOWS THE LOG CONCENTRATION OF E. COLI IN WATER AND FISH SAMPLES FROM THE SELECTED FISH PONDS WITH THEIR RECIPROCAL OF DILUTION.

Fish pond X

Log concentration of *E. coli* in water samples (log10*CFU*/100ml)	**Log concentration of *E.coli* in fish samples (log10*CFU*/100ml)**
4.716003344	4.698970004
5.792391689	6.806179974
6.785329835	6.812913357
4.806179974	4.84509804
5.838849091	5.86923172
6.77815125	6.832508913
4.86332286	4.812913357
5.851258349	5.770852012
6.792391689	6.799340549
4.763427994	4.72427587
5.785329835	5.792391689
6.716003344	6.707570176

Table 3: shows the log concentration of *E. coli* in water and fish samples from fish pond x

FISH POND Y

Log concentration of *E. coli* in water samples(Log10*CFU*/100ml)	Log concentration of *E. coli* in fish samples(Log10*CFU*/100ml)
4.505149978	4.51851394
5.477121255	5.505149978
6.51851394	6.51851394
4.505149978	4.477121255
5.491361694	5.505149978
6.491361694	6.477121255
6.491361694	4.477121255
5.477121255	5.505149978
6.491361694	6.477121255
4.505149978	4.477121255
5.491361694	5.505149978
6.477121255	6.505149978

Table 4: shows the log concentration of *E coli* in water and fish samples from fish pond y

FISH POND Z

Log concentration of *E. coli* in water samples(Log10*CFU*/100ml)	**Log concentration of *E. coli* in fish samples (log10*CFU*/100ml)**
4.812913357	4.832508913
5.86923172	5.755874856
6.806179974	6.851258349
4.86332286	4.875061263
5.851258349	5.812913357
6.826074803	6.812913357
4.806179974	4.73239376
5.792391689	5.799340549
6.73239376	6.748188027
4.748188027	4.755874856
5.832508913	5.763427994
6.785329835	6.73239376

Table 5: shows the log concentration of *E. coli* in water and fish samples from fish pond z

sites	mean concentration of E. coli in water	mean concentration of E. coli in fish
pond X	5.790719938	5.872687138
pond Y	5.790719938	5.49569867
pond Z	5.810497772	5.789345753

Table 6: shows the mean concentration of *E. coli* in water and fish samples from the selected fish ponds.

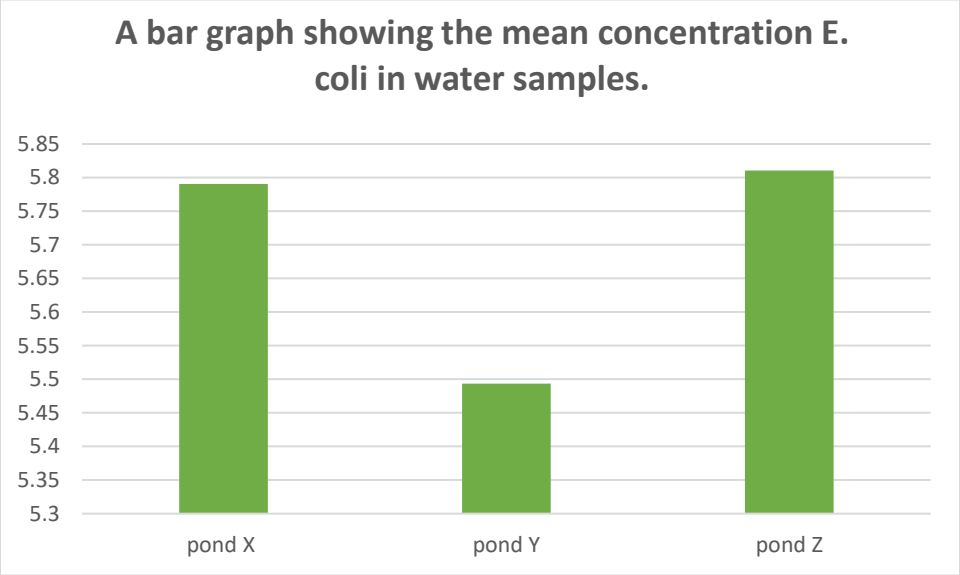

FIGURE 1: SHOWS THE MEAN CONCENTRATION *OF E. COLI* IN WATER SAMPLES FROM THE SELECTED FISH PONDS

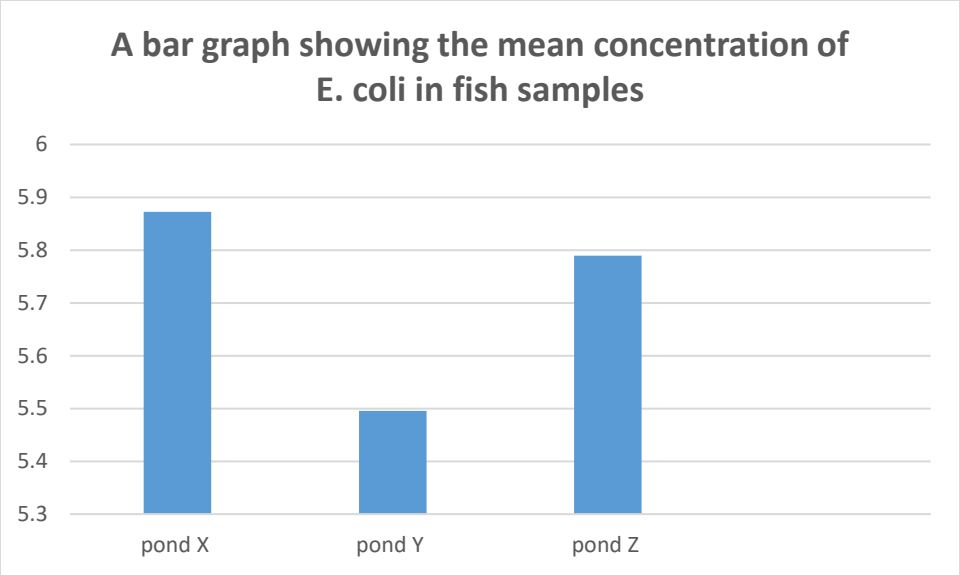

FIGURE 2: SHOWS THE MEAN CONCENTRATION *OF E. COLI* IN FISH SAMPLES FROM THE SELECTED FISH PONDS.

Statistical analysis of data (descriptive statistical analysis)

Log concentration of E. coli in water samples from the selected fish ponds		*Log concentration of E. coli in fish samples from the selected fish ponds*	
Mean	5.698243017	Mean	5.719243854
Standard Error	0.139242975	Standard Error	0.1437822
Median	5.792391689	Median	5.767140003
Mode	4.505149978	Mode	5.505149978
Standard Deviation	0.83545785	Standard Deviation	0.862693202
Sample Variance	0.697989819	Sample Variance	0.74423956

Kurtosis	-1.457324016	Kurtosis	-1.507989945
Skewness	-0.034066966	Skewness	-0.055391987
Range	2.334713109	Range	2.374137094
Minimum	4.491361694	Minimum	4.477121255
Maximum	6.826074803	Maximum	6.851258349
Sum	205.1367486	Sum	205.8927787
Count	36	Count	36
Confidence Level(95.0%)	0.282678267	Confidence Level(95.0%)	0.291893385

Table 7: shows the statistical analysis of the log concentration of *E. coli* in water and fish samples from the selected fish ponds

Distribution pattern of E. coli in water and fish samples from the ponds.

The frequency distribution patterns of *E. coli* from both water and fish samples from the selected fish ponds were not normally distributed. it significantly deviated from normal distribution based on the negative kurtosis values, water samples **(**-1.457324016), and fish samples (-1.507989945).

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	***Variance***		
log concentration in water	36	205.1367486	5.698243017	**0.697989819**		
log concentration in fish	36	205.8927787	5.719243854	**0.74423956**		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	***P-value***	*F crit*
Between Groups	0.007938633	1	0.007938633	0.011008835	**0.916737**	3.977779
Within Groups	50.47802827	70	0.72111469			
Total	50.4859669	71				

Table 8: shows the analysis of variance and p-values of the log concentration of *E.coli* from water and fish samples

4.2 DISCUSSION.

The assessment of feacal contamination in selected fish ponds was investigated in this study. The load of faecal indicator bacteria in water and fish samples were in agreement with (Njoku, Agwa, & Ibiene, 2015) who observed that the load of heterotrophic bacteria in pond water fluctuates between 0.01 and $8.7*10^5$ CFU/ml, which was consistent with the load of faecal indicator bacteria *(E. coli*) in water and fish samples The deviation in the distribution pattern of *E. coli* in both water and fish samples from all the selected fish ponds may be as a result of change in varying environmental conditions.

The distribution pattern of *E. coli* in all the selected fish ponds in this study agrees with the study of (Fernandes, Castro, Cunha Neto, & Figueiredo, 2018) who reported that the divergence in the distribution pattern of heterotrophic bacteria and total aquatic bacteria within the water layers could be linked to the distribution pattern of bacteria in fish ponds.

5 CHAPTER FIVE. CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION.

The findings of this study revealed high levels of faecal pollution in water and fish from all the selected fish ponds within Nagongera area. Based on the statistical analysis the magnitude of the faecal indicator bacteria in water and fish varies across the selected ponds.

5.2 RECOMMENDATIONS.

Since one faeacal indicator bacteria (*E. coli)* was considered in this study I therefore recommend further research studies that may involve more faecal indicator bacteria to give a nuanced understanding for faecal contamination in fish ponds within Nagongera area. Also the physicochemical parameters of the pond waters would provide a more nuanced understanding pf the relationshionship with faecal indicator bacteria, I thus recommend further research study that will combine both faecal indicator bacteria loads and the physicochemical parameters of the pond waters.

I also recommend Nagongera farmers to minimize or look for other alternatives of enriching their ponds other than using faeces from poultry birds that may further worsen *E. coli* concentration in their waters and fish. The farmers should also fence their ponds to minimize the access of grazing animals or restrict the grazing of animals near their ponds so as to reduce further contamination to their fish ponds.

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