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**Assessment of the Chemical and Biological Safety of Vermicompost
and Water Produced at Rulindo Faecal Sludge Treatment Plant in
Rwanda**

A dissertation submitted in partial fulfillment of the requirement for the award of master's degree in environmental chemistry program

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DECLARATION

I certify that this dissertation, entitled as "Assessment of chemical and biological safety of vermicompost and water produced from Rulindo faecal sludge treatment plant", submitted in part fulfillment of requirements for the Master of Environmental chemistry degree at the University of Rwanda, is my original work. All data, information, and idea sources have been adequately cited, and any assistance received while conducting the research has been clearly indicated. This work has not submitted either in part or in whole for any other degree or qualification at this university or any other.

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DEDICATION

This dissertation is dedicated to my loving family, whose ongoing inspiration, love, and support have kept me motivated throughout. To my parents, for believing in me and sacrificing for me; to my brothers and sisters, for their constant motivation; and to my friends, for providing me with unceasing encouragement and understanding. This work is also dedicated to my supervisors, whose guidance and wisdom have shaped my academic path. Thank you all for being the pillars that have made this achievement possible.

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ABSTRACT

Growing global concerns about environmental sustainability and public health have emphasized the need for safe and effective waste management solutions. Key challenges, such as insufficient faecal sludge treatment, inadequate policies and regulations, and weak institutional frameworks, hinder sustainable sanitation in developing countries. In low- and middle-income countries like Rwanda, where sewer networks are often lacking, on-site treatment and reuse of faecal sludge have emerged as critical strategies to address sanitation challenges while promoting resource recovery. Vermicomposting, a biological process that utilizes tiger worms to decompose organic waste, has gained attention as an eco-friendly method for converting faecal sludge into valuable products like compost and treated water. However, the chemical and biological safety of these by-products remains a significant concern, particularly regarding potential contamination of organic matter, nutrients, pathogens, heavy metals, or other harmful substances. The Rulindo Faecal Sludge Treatment Plant in Rwanda uses vermicomposting technology to treat faecal sludge. Its outputs, vermicompost and treated water, show potential for agricultural and non-potable applications, but their safety must be thoroughly assessed to ensure they pose no risks to the environment or human health. There have been fewer research works in sub-Saharan Africa focused on assessing FSTP wastewater, black soldier fly larvae use in FSTPs, faecal sludge valorization, and faecal sludge management technology and ultimate applications. Even as yet, there has been no discussion of chemical and biological safety assessment of vermicompost and water released from FSTPs in Rwanda. Therefore, the aim of this study is to assess the chemical and biological safety of vermicompost and water produced in Rulindo FSTP, offering useful information on their suitability for sustainable reuse. Physical, chemical, and biological parameters were extensively checked. Average results of the physical parameters in FSTP wastewater are as follows: pH = 7.83, turbidity at 13 NTU, total suspended solids (TSS) = 7.5 mg/L, and dissolved oxygen (DO) = 3.74 mg/L. Chemical parameters included chemical oxygen demand (COD) of 198.9 mg/L and removal efficiency of 94.34% and biochemical oxygen demand (BOD) of 38.5 mg/L and removal efficiency of 94.16%. In addition, the total nitrogen (TN) was 305.25 mg/L, nitrates (NO₃⁻) were 225.53 mg/L, total phosphorus (TP) was 8.38 mg/L, potassium (K) was 397.5 mg/L, calcium (Ca) was 12.83 mg/L, magnesium (Mg) was 103.03 mg/L, and sulfur (S) was 58.95 mg/L. Biological analysis of the wastewater indicated high levels of total coliforms, faecal coliforms, *Salmonella* and *Shigella* with a removal efficiency of 93.54%, 55%, 91.75% and 98.09% respectively, and the

absence of *E. coli* (about 100% removal efficiency). Although some physical and chemical parameters of the wastewater comply with Rwanda Utilities Regulatory Agency (RURA) and FAO guidelines, high nutrient levels and microbial contaminants, including total coliforms, faecal coliforms, *salmonella*, and *shigella* (exceeding 1,000 CFU/100 mL), raise concerns about its suitability for irrigation. To identify water quality, significant indices such as Sodium Adsorption Ratio (SAR), Kelly's Ratio (KR), Soluble Sodium Percentage (SSP), and Magnesium Adsorption Ratio (MAR) were ascertained. SAR, KR, and SSP values indicate the water as suitable for irrigation. However, the MAR value of 88.92% exceeding the tolerance limit of 50% due to the high content of magnesium suggests otherwise. In addition, the Wilcox diagram that classifies irrigation water in terms of salinity and sodium hazards positions produced effluent in class C4S1, which is not suitable for irrigation due to high salinity. These findings conclude that the wastewater from the Rulindo Faecal Sludge Treatment Plant is not suitable for irrigation unless further technical adjustments are made. On the other hand, the solid vermicompost produced at the Rulindo STP was analyzed using the Gravimetric Ignition method for proximate analysis. The results revealed a moisture content of 58.21%, total solids of 41.79%, volatile organic solids (VOS) of 39.18%, total organic carbon (TOC) of 22.73%, total nitrogen of 1.96%, and a carbon-to-nitrogen (C/N) ratio of 12:1. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used for the chemical analysis, identified macronutrients, micronutrients, and heavy metals. Detected macronutrients included phosphorus (P) at 1.747%, K at 0.248%, Ca at 2.203%, Mg at 0.392%, and S at 0.531%. Micronutrients such as iron (Fe) at 2.565% and manganese (Mn) at 0.058% were also identified. Heavy metals detected included lead (Pb) at 0.004%, titanium (Ti) at 0.043%, vanadium (V) at 0.004%, and zinc (Zn) at 0.052%. According to the RURA guidelines for faecal sludge management, FAO guidelines for organic fertilizers, and other national standards, the vermicompost produced at Rulindo FSTP is of good quality for agricultural use and is chemically safe due to the absence of toxic heavy metals. Taken together, the study found that the Rulindo FSTP effectively reduces most contaminants to acceptable levels. However, nutrient concentrations in the treated wastewater remain high, and biological pathogens require further disinfection to meet acceptable standards. To support sustainable waste management practices and contribute to Rwanda's environmental sustainability goals, additional research studies are recommended to complement this work.

Keywords: *faecal sludge, vermicompost, Vermifiltration, tiger worms and biofilter.*

LIST OF ABBREVIATION AND ACRONYMS

CFU: Colony Forming Unit

pH: Potential in Hydrogen

NTU: Nephelometric Turbidity Unit

EC: Electrical Conductivity

VOS: Volatile Organic Solids

TOC: Total Organic Carbon

IWQIs: Water Quality Indices for Irrigation

NAR: Sodium Adsorption Ratio

KR: Kelly's Ratio

SSP: Soluble Sodium Percentage

RSCC: Sodium Carbonate Content Residual

MAR: Magnesium Adsorption Ratio

PI: Index of Permeability

FSM: Faecal Sludge Management

FSTP: Faecal Sludge Treatment Plant

EPA: Environmental Protection Agency

RSB: Rwanda Standard Board

UNBS: National Bureau of Standards for Uganda

RWB: Rwanda Water Resource Board

RURA: Rwanda Utility Regulatory Authority

SDGs: Sustainable Development Goals

PPEs: Personnel Protective Equipment

RFS: Raw Faecal Sludge

TB: Tiger Biofilter

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CHAP 1. GENERAL INTRODUCTION

1.1. Background

Rising global concerns about environmental sustainability and public health have underscored the pressing need for safe and efficient waste management solutions. The Sustainable Development Goals (SDGs) aim for universal access to sustainable water and sanitation systems. However, over 1/3 of the world's population uses non-sewered onsite sanitation systems, which produce fecal sludge [1]. This problem is made worse by the fact that more than 80% of wastewater worldwide is discharged into the environment untreated [2]. Fecal sludge management (FSM) plays a vital role in on-site solid and wastewater management systems. With growing populations and accelerating urbanization, there is an increasing need for a strong FSM value chain to enhance sanitation systems worldwide ([3], [4]). This is particularly important in low- and middle-income nations like Rwanda, where centralized sewer networks are frequently lacking in quickly growing towns [4]. In these settings, treating and reusing fecal sludge on-site has become a key approach to tackle sanitation issues while advancing resource recovery [5]. On-site sanitation systems (OSS) generate fecal sludge (FS), which is composed of excreta and black water that are collected, stored, or treated, either separately or mixed with greywater [1]. The term "fecal sludge" describes any liquid, semi-liquid, or semi-solid waste that builds up in containment structures, vaults, or pits of on-site installations, such as septic tanks, public and private restrooms, aqua privies, and latrines [6].

Despite its significance, FSM encounters major obstacles in developing nations. These include inadequate treatment and maintenance of fecal sludge, insufficient policies and regulations, and fragile institutional frameworks [4]. Such challenges are particularly severe in low-income and fast-growing urban areas (e.g. Kigali), where untreated fecal sludge poses growing threats to water resources, public health, and ecosystems [7]. Pollution caused by improper fecal sludge management further intensifies these risks, underscoring the urgent need for sustainable solutions.

Organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), pesticides, pharmaceuticals, antibiotics, and personal care products (PPCPs) may be present in vermicompost and wastewater, along with microbial loads such as bacteria, viruses, parasites, and bacteria resistant to antibiotics [8], as well as

inorganic pollutants (e.g., heavy metals, salts and ions, non-metallic compounds, radioactive substances, and synthetic chemicals) and other contaminants [9].

The process of vermicomposting involves the cooperation of tiger worms and microbes to stabilize and bio-oxidize organic waste, with microorganisms playing a major role in the material's breakdown. The ultimate result is vermicompost, a nutrient-rich organic soil conditioner and fertilizer that offers a sustainable method of turning organic waste into a useful agricultural product. Vermicompost enriches soil by providing a high ratio of NPK (Nitrogen, Phosphorus, and Potassium), improving fertility, restoring degraded land, and enhancing farm productivity [8]. Tiger worms are essential for breaking up and conditioning the substrate, even though bacteria are mostly in charge of the biodegradation of organic waste [10]. However, exposure to pollutant mixtures, such as heavy metals, can challenge the vermicomposting process by affecting both earthworms and microflora [8]. Despite potential toxicity, certain earthworm species used in vermicomposting such as *Eisenia fetida*, *Lampito mauritii*, and *Perionyx excavatus* have been shown to significantly reduce heavy metals (e.g, Pb, Cd, Cu, and Cr) through bioaccumulation, particularly in household waste vermicompost [8].

The Rwandan government has adopted the WASH (Water, Sanitation, and Hygiene) concept, which focuses on improving public health by ensuring access to clean water, safe sanitation, and promoting good hygiene practices. The initiative aims to reduce waterborne diseases through the construction of sanitary infrastructure, such as toilets, sewage systems, and water sources like boreholes. Hygiene education, including proper handwashing and safe sanitation practices, plays a key role in empowering communities and preventing the spread of diseases. In collaboration with various partners, the government has made significant progress in improving WASH facilities, particularly in rural areas, contributing to better health outcomes and sustainable development. As part of this collaboration, Water for People, an NGO, constructed a fecal sludge treatment plant (FSTP) in Rulindo District, specifically a vermifiltration plant located at Base Sector, to address local needs. These FSTPs are intentionally designed to operate without energy inputs or chemicals, making them both environmentally friendly and cost-effective. It is crucial to understand the efficiency of removing various pollutants in order to assess the system's performance when handling mixed fecal sludge loads. This understanding aids in making operational and design improvements to the treatment system. By monitoring the removal of physical, chemical, and

microbial indicators, one can ensure compliance with valid standards and reveal any potential impact on human health as well as the environment.

Prior studies in the topic have mostly identified the physico-chemical characteristics of wastewater and water from homes, businesses, and public sources. For instance, [11] examined CHUB hospital effluents and discovered that treated effluents surpassed WHO permitted limits for environmentally hazardous parameters such as EC, TDS, TSS, BOD, COD, phosphates, and cadmium. Similarly, [12] analyzed industrial wastewater from KSEZ and reported high concentrations of EC, BOD₅, COD, PO₄³⁻, NO₃⁻, and heavy metals (Cu, Pb, Mn, and Zn), indicating significant pollution concerns. Furthermore, studies on fecal sludge treatment plants, wastewater, and vermicompost have explored nutrient recovery, contaminant reduction, microbial activity, system performance, and community health impacts globally and in Africa ([13], [14], [15],[16], [1]). Recently, a few reports in sub-Saharan Africa have focused on the pollution assessment of wastewater from FSTPs, black soldier fly larvae usage in FSTPs, the valorization of fecal sludge, and fecal sludge management technologies and their end uses ([17], [18], [19],[4]). For example, [20] reported that, under optimal conditions, fecal sludge from pit latrines with moisture content as high as 90% can be effectively treated using black soldier fly larvae. The study achieved a reduction efficiency of 50%. Furthermore, [20] showed that the mean lead (Pb) contents in the sludge samples in batches 1 and 2 were 11.912 mg/kg dry matter (dm) and 5.304 mg/kg dm, respectively. Compared to the Environmental Protection Agency's (EPA) maximum allowable dosage of 840 mg/kg for land application, these values are far lower.

1.2. Problem statement

The world, particularly developing countries, faces significant challenges such as water scarcity and the sanitation of waste, including faecal sludge, due to rapid population growth. Both human health and the environment are seriously at risk from these problems. The construction of treatment facilities has become a crucial answer to this problem, since it not only decreases trash accumulation but also improves soil fertility by producing fertilizers. Globally, efforts are now directed towards ensuring the safe management of faecal sludge to support sustainable management practices that are environmentally as well as general public and health-friendly. In Rwanda, at Rulindo Faecal Sludge Treatment Plant, vermicomposting and water recovery are some of the new treatments currently being used in order to treat fecal sludge in an effort to produce useful by-products like vermicompost and treated effluent. However, the chemical and biological safety of these by-products remains uncertain, raising critical questions about their suitability for agricultural use and environmental discharge. Comprehensive evaluation of the presence of heavy metals, dangerous pathogens, and other pollutants in the facility's treated effluent and vermicompost is lacking. Without rigorous evaluation, there is a risk of introducing hazardous substances into the environment or food chain, potentially compromising human health, soil quality, and water resources. Furthermore, inadequate safety standards for these by-products could hinder their acceptance and adoption by local communities and stakeholders. There aren't many studies in sub-Saharan Africa that concentrate on evaluating wastewater from FSTPs, using black soldier fly larvae in FSTPs, valuing fecal sludge, and fecal sludge management systems and their final applications [17] [20], [19] , [4]. However, there is still a lack of thorough research and understanding of the physico-chemical and biological safety of vermicompost and water discharged from FSTPs. Thus, the purpose of this study is to evaluate the biological and chemical safety of the water and vermicompost generated at Rulindo FSTP, providing important information on their potential for long-term reuse. Furthermore, this study seeks to address the following key issues:

What are the levels of chemical contaminants (e.g., organic/ inorganic compounds, and heavy metals) and biological pathogens (e.g. coliforms, E. coli, Salmonella, and Shigella) present in the vermicompost and treated water produced at the Rulindo Faecal Sludge Treatment Plant?

Do these by-products meet national and international safety standards for agricultural use and environmental discharge?

What measures can be implemented to enhance the safety and sustainability of vermicompost and treated water production at the plant?

By addressing these questions, this research aims to provide evidence-based recommendations to ensure the safe and sustainable utilization of vermicompost and treated water, thereby supporting public health, environmental protection, and the circular economy in Rwanda.

This problem statement clearly identifies the issue, outlines the gaps in knowledge, and sets the stage for the proposed assessment while emphasizing its relevance to public health, environmental safety, and sustainable resource management.

1.3. Significance of the study

For a number of reasons, it is crucial to evaluate the chemical and biological safety of the water and vermicompost generated at Rwanda's Rulindo Faecal Sludge Treatment Plant:

Public Health Protection: Evaluating the safety of vermicompost and treated water ensures that these by-products do not introduce harmful pathogens, heavy metals, or other contaminants into the environment or food chain. This is essential to safeguard human health, particularly for communities using these resources in agriculture or being exposed to discharged water.

Environmental Sustainability: By determining the levels of contaminants, this study helps prevent soil and water pollution, ensuring that the use of vermicompost and treated water supports sustainable environmental practices rather than contributing to ecological degradation.

Promotion of Circular Economy: By turning waste into useful goods like fertilizer and irrigation water, vermicompost and treated water can be efficiently used as valuable resources and support a circular economy if they are shown to be safe. This reduces waste accumulation and enhances resource recovery.

Policy Support and Standards: The outcomes of this study will provide evidence-based recommendations to guide policymakers and regulatory bodies in establishing or refining safety standards for faecal sludge-derived products in Rwanda and similar contexts.

Encouraging Community Acceptance: Assessing and confirming the safety of these by-products can build trust among local communities and stakeholders, encouraging the adoption of innovative waste management solutions and supporting sustainable agricultural practices.

Global Relevance and Replication: As many developing countries face similar challenges in managing faecal sludge, the outcomes of this study can serve as a model for other regions seeking sustainable and safe waste treatment solutions.

1.4. Objectives

1.4.1 General objective

This study aims to assess chemical and biological safety of vermicompost, and water produced at the Rulindo FSTP in Rwanda.

1.4.2 Specific Objectives:

1. To determine the levels of chemical constituents, including heavy metals and nutrients, in the vermicompost and water produced at the Rulindo FSTP.
2. To assess the levels of biological pathogens, such as bacteria (*E. coli*, coliforms, *Salmonella*, and *Shigella*) in the water produced at Rulindo FSTP.
3. To compare these levels with the acceptable limits for agricultural use.
4. To better understand the vermifiltration process at Rulindo and identify potential ways to optimize the faecal sludge treatment process there.

1.4. Research questions

1. To what extent do the water and vermicompost produced at the Rulindo FSTP contain heavy metals, pathogens, and other contaminants?
2. Does the vermicompost and water contain levels of heavy metals, pathogens, and other pollutants that are within permissible limit for use in agriculture and release into the environment?
3. To what extent are contaminants and pathogens removed throughout the treatment process at the Rulindo Faecal Sludge Treatment Plant?

CHAPTER 2. LITERATURE REVIEW

2.1. Introduction to faecal sludge treatment

Faecal sludge is partially digested excrement that originates from on-site sanitary facilities such as dry toilets, septic tanks, and pit latrines. It can vary greatly in terms of its qualities and consistency and might be more or less solid [21]. Faecal sludge often consists of the following "inputs": solid waste, chemicals, flush water, greywater, anal cleansing material, excreta, and anything else that might get into the container [22]. The major constituents of faecal sludge are suspended or dissolved solids, nutrients such as potassium, nitrogen and phosphorous from urine, faeces and pathogenic micro-organisms (helminths, parasitic protozoa, viruses and bacteria) [21]. Factors including solids content, chemical and biological oxygen demands (COD and BOD), nutrients, pathogens, and heavy metals should all be taken into account when characterizing faecal sludge (FS). These are the same parameters that are considered while analyzing domestic wastewater, albeit it should be noted that FS and residential wastewater are entirely different [23].

Faecal sludge can be recycled into fertilizer and soil amendments in agriculture since it is high in organic carbon and nutrients. Agriculture is also a significant agro-food sector of food production since it helps in feeding human beings as the demand for food and human population increase [21]. In September 2015, the United Nations General Assembly adopted the 2030 agenda, which contained a particular goal for sanitation and water (UN, 2015). The percentage of the population that uses safely managed sanitation services (SDG 6.2.1) is specifically mentioned in the Sustainable Development Goals 6 (SDG 6) targets. These goals involve safe management of fecal sludge throughout the value chain, which includes capture, containment, emptying, transportation, treatment, and disposal/reuse [24]. To stop the germs from reentering the environment, it is crucial to look beyond toilets. Therefore, it is critical to stop waste seepage or overflow from flawed sanitation systems. Prior to disposal, sludge should be treated, and also for the purpose of reducing the volume of wastes released in the environment, recovery of energy and nutrients and also to comply with a goal for water and sanitation, faecal sludge management should be prioritized through faecal sludge treatments plants [25].

Faecal sludge can be treated using different methods including physical, biological, and chemical treatment methods. Physical sludge treatment method is performed through dewatering process

where liquid phase is separated from solid phase. Dewatering the sludge reduces its mass, which is advantageous before transit and additional treatment, like composting for resource recovery. The active pathogens are also decreased by the decreased water content of the faecal sludge since microorganisms require water to survive. Dewatering treatment processes thus lower the quantity of germs that are active [21].

By biological treatment, they treat faecal sludge using microorganisms that are already present in the faeces through their metabolic processes. Microbes can produce the required effects, such as the breakdown of organic materials and reduction of smell and pathogens, under controlled conditions. Nutrients, oxygen, and temperature are significant factors that influence the microbial activity [21]. Furthermore, Black Soldier Flies (BSF) that are largely found in temperate climates are being used in faecal sludge degradation, as fly larvae degrade organic matter. Since BSF only feeds during the larval stage, there is little chance that it will act as a disease vector. The sludge is purified because BSF larval activity inactivates bacteria like *Salmonella* spp. and *E. coli*. However, extensive research on how BSF affects other bacteria *spp*, viruses, and parasites in faecal sludge is lacking [21].

2.2 Vermifiltration Technology in Faecal Sludge Treatment

Vermifiltration involves the use of tiger worms (*Eisenia fetida*) to filter and degrade organic waste and contaminants from faecal sludge, which can be hazardous if not properly treated. Vermifiltration has been shown in numerous studies to be an effective method of treating a variety of organic wastes, such as fecal, wastewater, and sewage sludge. According to the research done by (Agrawal et al., 2020) highlighted how vermifiltration technology has been successfully employed to treat faecal sludge, with composting worms significantly contributing to the reduction of pathogenic bacteria, such as *E. coli*, and other contaminants. Refer to the research conducted by Jadhav et al. (2018) as well. This study examined the chemical and biological safety of treated effluents and found that total suspended solids (TSS), chemical oxygen demand (COD), and biological oxygen demand (BOD) had decreased.

Vermicomposting (Vermifiltration technology) is an emerging biological technique for reducing the amount of organic waste by using tiger worms. The process involves passing the effluent through a filtration system containing tiger worms, which help in the breakdown and stabilization of organic material, thereby reducing contaminants. In faecal sludge, worms have been demonstrated to reduce coliforms and helminth eggs [21]. During vermicomposting, vermicompost and water are yielded, as the final products. Chemical faecal sludge treatment occurs by applying chemical substances such as lime, ammonia or urea. Microbial activity is impacted by raising the pH above 12 by adding an alkaline substance, such as lime. In turn, this lowers the sludge's pathogen content and odor. Though, a large dose of lime is needed to prevent the pH from dropping once more, which would allow pathogens to reappear [21]. The inactivation of microorganisms by ammonia treatment in sludge is successful, however the precise mechanisms are not well known. For instance, the ammonia may occur in form of urea, $\text{CO}(\text{NH}_2)_2$, or aqueous ammonia, $\text{NH}_3(\text{aq.})$ which is quickly converted to ammonia [26]. While the effectiveness of aqueous ammonia or urea-based disinfection has been established for the treatment of sewage sludge, compost, and urine, faecal sludge is still being studied. For the effective disinfection pH must be greater than 8.5. If the pH is stable, germs cannot proliferate again [21]. The main final products produced from the treatment of faecal sludge are water (leachate) which is rich in nutrients and some microorganisms, and solid organic matter known as vermicompost.

2.3 Sanitary service chain

The Sanitary Service Chain (SSC) is a framework that describes the management of sanitation services from the generation of waste through to its final disposal or reuse. The SSC approach considers the entire sanitation system and recognizes the interdependence and interactions between different components of the system. It is particularly relevant for the management of sewage and faecal sludge [27].

As well shown in **Figure 1**, appropriate FSM system comprises safe containment, sufficient and secure emptying of all on-site sanitation systems, proper transportation, appropriate treatment, and suitable disposal or reuse [3].

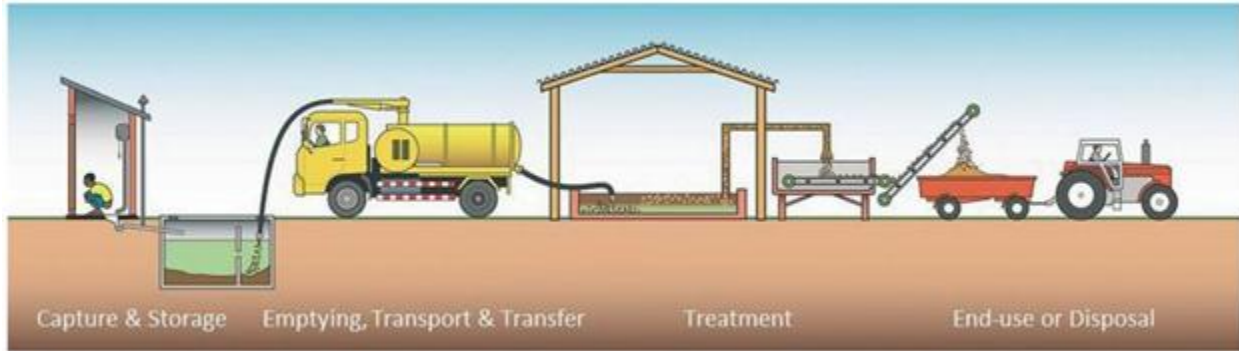


Figure 1: The chain of sanitary service [3].

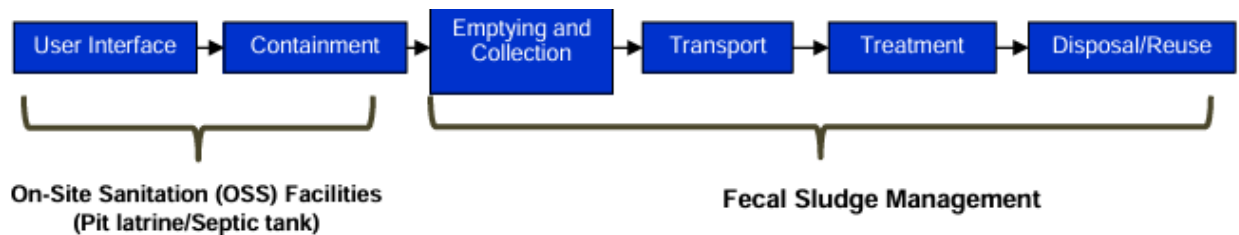


Figure 2:FSM System elements for OSS Facilities[28]

Containment: involves the provision of adequate and appropriate toilets, septic tanks, and other types of on-site sanitation facilities that are designed to safely contain and treat human waste.

Emptying followed by transport: entail taking the collected fecal sludge out of the containment facilities and moving it to treatment centers.

Treatment: involves the process of converting the faecal sludge into a safe and stable form that can be disposed of or reused.

Safe disposal or reuse involves the final disposal or beneficial use of the treated faecal sludge. This may include land application, use as fertilizer or soil conditioner, or energy recovery [27].

The SSC approach recognizes the importance of involving all stakeholders in the sanitation chain, from households and communities to service providers and regulatory authorities. This strategy encourages coordination and cooperation amongst stakeholders to guarantee the smooth and successful operation of the entire system [3].

2.3 Vermicompost

2.3.1 Vermicomposting process

Vermicomposting is a mesophilic method of decomposing organic waste that produces nutrient-rich vermicompost by using composting worms (**Figure 3**) and other microbes [29]. The common species of earthworms used in this decomposition process are: *Lumbricus rubellus* and *Eisenia fetida*, and also the presence of other microorganisms like bacteria, fungi and ciliates in the composting bin contribute in the breaking down of organic matter [30]. Vermicomposting research is being conducted most frequently in Asia (55%) and secondly in Africa (14%) [31]. Vermicast, also referred to as worm castings or vermicompost, is a nutrient-rich compost made from food scraps and other organic materials by the worm's unique digestive tract. It thrives in an aerobic (aerobic) atmosphere. It may quickly multiply in a small area and process a lot of food waste [32].

A number of steps are involved in the vermicomposting process, which is essential for the breakdown of faecal sludge and production of vermicompost that is high in nutrients.

Pre-composting stage: In this stage, the faecal sludge is usually mixed with a bulking agent, such as shredded paper or sawdust, to provide a porous structure that promotes aeration and drainage. This mixture is then placed in piles or in vermicomposting bins to allow for initial decomposition through aerobic processes [33]. In this phase, the mixture's pH and moisture level are monitored and adjusted to guarantee ideal circumstances for earthworm growth.

Vermicomposting stage: Once the pre-composting stage is complete, composting worms are introduced into the mixture to speed up the decomposition process. The worms feed on organic matter in the faecal sludge, breaking it down into smaller particles and releasing enzymes and microorganisms that further decompose the material. During this stage, temperature of the mixture is monitored to make sure that it is remained within the optimal range for earthworm activity [29].

Post-composting stage: Once the vermicomposting stage is complete, the vermicompost is allowed to develop for a few weeks to a few months. During this stage, the remaining organic matter is broken down into stable humus, and the vermicompost becomes rich in nutrients and microorganisms. The final product is usually screened to remove any large debris or undecomposed material and then stored for later use [29].

Generally, Vermicomposting is the process by which tiger worms consume toxic organic waste, break down sewage sludge biochemically, homogenize the material using intestinal muscles, and produce nutrient-rich vermicompost for plants and soil. The weight of *E. fetida* worms increases by 5–20g [34].

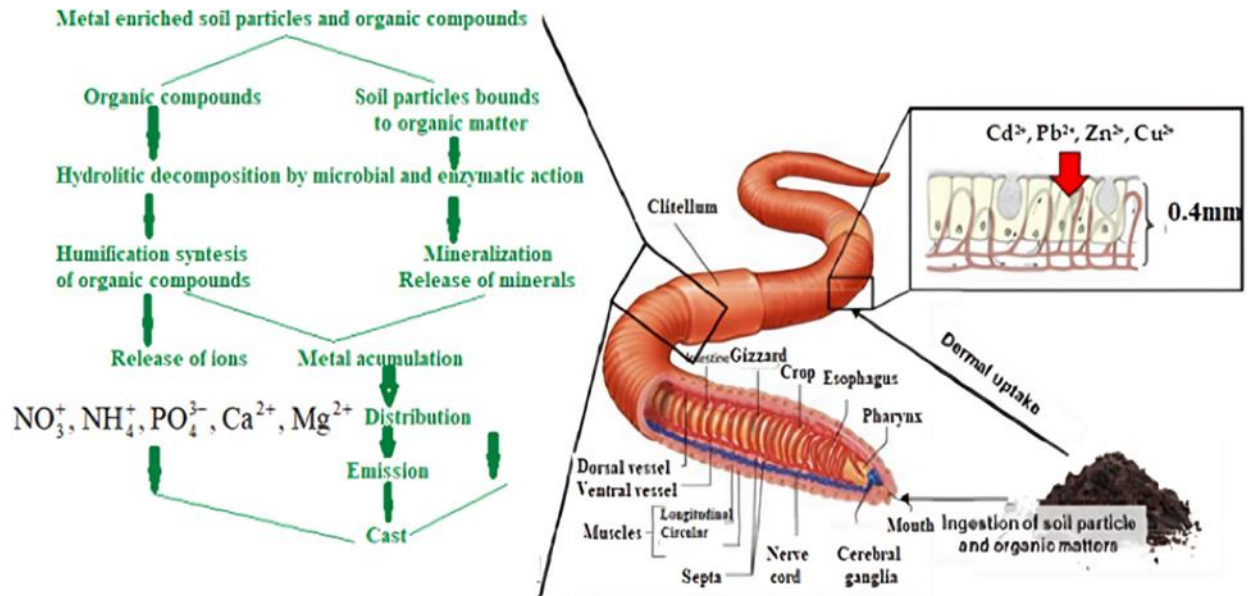


Figure 3: Vermicomposting process details via tiger worm physiology [34]

2.3.2 Worm health

The red wiggler worm, manure worm, red worm, or tiger worm (*Eisenia fetida*) is the best worm for vermicomposting in bins. Red wiggler worms are epigeic, which means they are surface-dwelling worms. They inhabit the top layers of very rich organic matter in decomposing waste heaps. They do not burrow like the common garden earthworm and do not occur in the deep subsoil. They are the features that adapt the red wiggler to worm bin composting. Earthworms would not be able to live in an indoor worm bin's conditions. [32].

Red wiggler worms can survive in temperatures between 40 and 90 °F, but they thrive between 55 and 77 °F. Between 71 and 89°F is the optimal range for composting and reproduction. The worms become less active and feed less frequently below 50°F. About 75 to 90 percent of the weight of red wiggler worms is water. Make sure the bedding has the proper amount of moisture to prevent the worms from drying out. These worms can survive in a pH range of 4 to 9, but they prefer a pH of about 5.5 [32].



Figure 4: Tiger worms (*Eisenia fetida*) taken from (https://www.originalorganics.co.uk/media/catalog/product/cache/d1ea054400a0fbd81fcea4bc589ecce6/t/i/tigerworms_2.jpg)

2.3.3 Vermifilter bedding

Some of the bedding materials that were utilized include clay pebbles (Cp), woodchips (Wc), and granular activated carbon (GAC). Woodchip was used as a control as prior studies had indicated that it was the most effective. GAC was utilized because, although it had never been utilized as bedding material, it is capable of absorbing nitrates and nitrites. Even though their ability to reduce suspended particles and chemical oxygen consumption has been demonstrated, clay pebbles have not yet been tried if they are capable of removing nitrogen [35]. According to practical set-up found in the research done by Enrique Hernández (2017), to construct six vermifilters (V1 to V6), two plastic boxes of 0.09 m² in surface area and 22.5 cm in depth were utilized. The upper box, which was supplied with holes in the bottom, held *E. fetida* and bedding layers. The lower box possessed a tap and served as an effluent sump. The bedding layers were assigned to the following matrices: V1 and V2 woodchip (Wc vermifilters); V3 and V4 woodchip and clay pebbles in equal volumetric proportions (Cp vermifilters); and V5 and V6 woodchip and GAC in equal volumetric proportions (Ac vermifilters) [35]. And also based on the research done by Furlong (2015) [36]. The analysis indicated that, when compared to the initial sludge data, both the amount of *Ascaris spp.* eggs and the number of faecal coliforms has dramatically decreased. It was believed that *Ascaris spp.* eggs would gather in the bedding layer before being consumed by worms [36].

2.3.4 Factors affecting composting process

Factors that influence biological degradation or composting are described below:

- **pH control**

Compost's pH varies during the composting process. Additionally, it is a trustworthy indicator of breakdown. The pH range that most bacteria prefer is 6.0 to 7.5. The pH levels in the final step increase to roughly 8.5 [37].

- **Moisture control**

During the composting process, moisture levels should range between 50% to 60%, with 55% being ideal. When waste particles are more than 65% moist, water starts to enter those spaces, limiting interstitial oxygen and resulting in anaerobic conditions. This leads to a sharp drop in temperature and the simultaneous development of unpleasant odors. The composting process becomes sluggish when the moisture concentrations fall below 50% [37].

- **Carbon-Nitrogen ratio**

The bacteria require a minimal amount of nutrients in order to survive, therefore an ideal balance between carbon (C) and nitrogen (N) is required. The bacteria cell relies on nitrogen as a key component. While bacteria get their energy from carbon, on the other hand. The initial carbon to nitrogen ratio has a significant impact on the rate of breakdown [37].

- **Oxygen requirement**

The aerobic composting process depends on the presence of air. However, because it depends on so many factors, including temperature, moisture content, and the availability of nutrients, it is very challenging to pinpoint the precise amount of oxygen needed. Checking for bad scents in the compost is a rough way to make sure there is enough oxygen available. Bad odors are a sign that there is not enough oxygen in the air [37].

- **Temperature**

Additionally, a significant element influencing biological activity is temperature. Microorganisms can only begin to take part in the composting process when the temperature is rapidly raised. Because of thermophilic bacterial activity, this temperature is very high. In order to completely eliminate the disease-causing germs, a high temperature is required. For the purpose of destroying pathogens, higher temperatures should be maintained, such as 60–70°C for roughly 24 hours [37].

2.3.5 Constituents of vermicompost from faecal sludge

Solids

The solids of the faecal sludge are suspended or dissolved and organic (volatile) or inorganic (fixed). The dissolved solids are dissolved, while suspended solids are floating, settleable, and colloidal material. If transportation or additional treatment is to be carried out, the solids in the faecal sludge should be concentrated and reduced as much as possible, i.e., the water fraction, i.e., the percentage of the total solids must be high [21]. If faecal sludge is treated chemically, the amount and type of chemicals employed will determine how much solid particles are introduced. To establish the amount of solids and organic content percentage in faecal sludge, two parameters known as total solids (TS) and volatile solids (VS) are periodically monitored. [38].

Nutrients

The majority of nutrients found in home wastewater, including potassium, nitrogen, and phosphorus, come from urine and feces. Eutrophication and environmental contamination may happen when excreta are released into the environment carelessly. The recovery of nutrients from human waste reduces environmental pollutants and makes it possible to reuse it for agricultural uses [21]. These nutrients include of micronutrients like calcium (Ca), magnesium (Mg), and iron (Fe), as well as macronutrients like nitrogen (N), phosphorus (P), and potassium (K). Vermicompost is a nutritive organic fertilizer that contains micronutrients, helpful soil microbes like nitrogen-fixing bacteria, and mycorrhizal fungi in addition to NPK (nitrogen 2-3%, potassium 1.85-2.25%, and phosphorus 1.55-2.25%) [30]. Earthworms break down organic waste products to create vermicompost, a nutrient-rich organic fertilizer. The feedstock utilized and the vermicomposting conditions affect the chemical makeup of vermicompost. Compared to conventional compost, vermicompost typically has higher concentrations of organic matter,

nutrients, and beneficial microbes. Vermicompost made from fecal sludge has been found to have significant concentrations of potassium, phosphorus, and nitrogen in addition to other micronutrients like calcium, magnesium, and iron [39].

Pathogenic microorganisms

The treatment of faecal sludge through vermicomposting can result in a significant reduction in the concentration of pathogenic microorganisms. However, some pathogenic microorganisms may still be present in the final product, including vermicompost and water produced from the faecal sludge treatment process [40]. Some of the pathogenic microorganisms that may be found in vermicompost, and water produced from faecal sludge treatment like:

Escherichia coli (E. coli): *E. coli* is a bacterium commonly found in the human gut, and its presence in water or vermicompost may indicate the presence of faecal contamination. Some strains of *E. coli* can cause gastrointestinal illnesses [40].

Salmonella spp.: *Salmonella* is a bacterium commonly found in the environment and can cause foodborne illness. It can survive for extended periods in soil and water, making it a potential hazard in vermicompost and water produced from faecal sludge treatment [40].

Helminth eggs: Helminth eggs are commonly found in faecal sludge, and they can survive for extended periods in the environment. They can cause various gastrointestinal illnesses and parasitic infections in humans [40].

Enterococcus spp.: *Enterococcus* is a group of bacteria commonly found in the guts of humans and animals. They can cause urinary tract infections, wound infections, and other infections in humans. Regular testing for harmful microorganisms and monitoring of vermicompost and water generated from faecal sludge treatment are crucial for ensuring their safety. It is advised by the World Health Organization (WHO) to evaluate the microbiological quality of vermicompost and water using indicator species as enterococci or *E. coli*. In addition, the use of proper hygiene and sanitation practices during handling and application of vermicompost can help to minimize the risk of pathogenic exposure [41].

2.3. 6. Characterization of faecal sludge and vermicompost

Assessing and measuring the characteristics of faecal sludge is known as characterization. Faecal sludge management solution research, design, building, and operation depend on the material characterization of faecal sludge as well as information on its physical, biological, and chemical characteristics. Characterizing faecal sludge serves a variety of general objectives, including determining the best technology for sludge emptying from onsite holdups, monitoring treatment effectiveness and pathogen removal, calculating loadings for treatment plant design and operation, and assessing the potential for resource recovery [42]. The process of vermicomposting yields vermicompost, an organic fertilizer that is rich in nutrients. It is a beneficial soil additive that enhances plant growth, soil fertility, and water-holding capacity [43]. The characterization of vermicompost involves the assessment of its physical, chemical, and biological properties. The characteristics of faecal sludge and vermicompost from septic tanks and public toilets are mainly determined by the following parameters: total solids (TS), BOD₅, COD, total Nitrogen, NH₄-N, NO₃-N, Ascaris eggs, Total P (%), Total K (%), electrical conductivity (EC) and pH [44].

Physical properties of vermicompost

The physical properties of vermicompost include texture, color, moisture content, and particle size. The texture of vermicompost is typically loose and crumbly, with a dark brown to black color. Vermicompost should have a moisture content of 30% to 60%, as this is optimal for plant growth. Vermicompost's particle size can vary from fine to coarse, contingent on the feedstock type and vermicomposting time [45]. The physical characteristics of vermicompost indicated by its: **texture**: where vermicompost has a granular texture, which is usually fine and crumbly. The texture of vermicompost produced from faecal sludge treatment process is similar to that of regular vermicompost, **Color**; the color of vermicompost produced from faecal sludge treatment process is usually dark brown to black. The color is an indication of the organic matter content in compost, the darker the color, the higher the organic matter content, **Odor**; vermicompost produced from faecal sludge treatment process usually has a mild earthy smell, which is an indication of a stable composting process. Any offensive odors should be absent if the composting process is properly managed, and **moisture content**: vermicompost produced from faecal sludge treatment process typically has a moisture content of between 30% and 60%. A moisture content of around 40% is considered ideal for vermicomposting. Moisture content can affect the texture and quality of vermicompost.

Chemical properties of vermicompost

According to Das et al, 2022 [46], this study highlighted that vermicompost can provide essential nutrients, but certain chemical contaminants, such as heavy metals, should be regularly monitored, Eliyan et al, 2023 [47] this research emphasized the need for rigorous screening of faecal sludge to minimize the risk of contaminants in the final product. The chemical properties of vermicompost include pH, nutrient content, and organic matter content. The pH of vermicompost should be between 6.5 and 8.0, which is optimal for plant growth. Nitrogen, phosphorus, potassium, calcium, and magnesium are among the nutrients that vermicompost is abundant in and are critical for plant growth. Vermicompost often has a high organic matter content, which enhances the soil's structure and ability to retain water.

Nutrient content: Important plant nutrients including nitrogen (N), phosphorus (P), and potassium (K) as well as micronutrients like calcium (Ca), magnesium (Mg), and sulfur (S) are abundant in vermicompost made from fecal sludge treatment. The quality of the initial material and the way the composting process is managed determine how many nutrients the compost contains [48].

Carbon-to-nitrogen ratio: Vermicompost made from fecal sludge treatment typically has a carbon-to-nitrogen (C:N) ratio of 10:1 to 20:1. For vermicomposting, a C:N ratio of about 15:1 is thought to be optimal. The compost's nutrient availability and rate of decomposition are influenced by the C:N ratio [37].

Organic matter content: The amount of organic matter in vermicompost made from fecal sludge treatment is usually high, ranging from 30% to 60%. Since organic matter enhances soil structure, water retention, and nutrient cycling, it is crucial for soil health.

pH: The pH of vermicompost produced from faecal sludge treatment process ranges from 6.5 to 8.0, which is regarded as neutral to slightly alkaline. The pH can impact the compost's nutrient availability as well as the pH of the soil when applied as a soil amendment [37].

Heavy metals content: If the initial material is contaminated, heavy metals including lead, cadmium, and arsenic may be present in the vermicompost made from the fecal sludge treatment process. Nevertheless, research indicates that vermicomposting can lower the compost's heavy metal content by as much as 90% [49].

Biological Safety of Vermicompost and Effluent produced at Rulindo FSTP.

The term "biological safety" describes the existence and survival of harmful microorganisms in both the treated water and vermicompost. The key biological concerns include the survival of pathogens such as bacteria, viruses, and protozoa, which could pose health risks when used in agriculture or irrigation. Vermicompost's biological characteristics include the presence of helpful microorganisms such as actinomycetes, fungus, and bacteria. These microbes release nutrients, aid in the breakdown of organic matter in the compost, and inhibit pests and dangerous pathogens [50]. These micro-organisms can also increase plant growth, soil fertility, and disease and insect resistance [51]. Vermifiltration is known to reduce pathogens due to the natural filtering and digestive action of earthworms. However, the degree of pathogen reduction depends on factors such as the type of earthworm species used, retention time, and initial contamination levels. According to (Manya et al., 2020) this study assessed the biological safety of both treated effluent and vermicompost, concluding that earthworm activity significantly reduces fecal coliforms and other pathogens. Studies have also reported a significant reduction in fecal coliforms and other enteric pathogens in treated faecal sludge, making the vermicompost and water potentially safe for agricultural use when managed properly, based to the research done by (Chakravarty et al., 2021) this research reviewed the microbial safety of vermicompost produced from faecal sludge, concluding that with adequate treatment, pathogen levels could be reduced to safe levels for agricultural purposes.

According to Guidelines of Rwanda Standards Board (RSB), the following Table 1, Table 2, Table 3, and Table 4 indicate the specific requirements, nutrients and pathogens concentration limit in the organic fertilizers [52].

Table 1: Specific requirements of Organic fertilizers according to RSB standard

S/N	Parameter	Requirements
1	pH	6 – 9
2	Carbon: Nitrogen ratio	$\leq 20:1$
3	Moisture content, (solid) %, m/m	10-35
4	Total Nitrogen, %, m/m, min	>1
5	Dry matter content (solid), %, m/m, min.	≥ 70
6	Organic carbon, %, m/m, min.	12
7	Total primary nutrients: N-P ₂ O ₅ -K ₂ O (solid and liquid organic fertilizer), %, m/m, min	5
8	Stones >5 mm, %, m/m, max	5
9	Foreign matter > 2 mm, % m/m, max	0.5
10	Seed, number/kg, max	5
11	Soluble salts (conductivity), $\mu\text{Sm}1$ max.	5

Table 2: Lowest percentage guarantee of nutrients for Organic fertilizers according to RSB standard.

S/N	Nutrients	Limit
1	Calcium, as Ca, %, m/m,	≥ 1.0000
2	Magnesium (%)	≥ 0.5000
3	Sulphur (%)	≥ 1.0000
4	Boron mg/kg	20-140
5	Cobalt (mg/kg)	0.5-1.0
6	Copper (mg/kg)	8-300
7	Iron (mg/kg)	1000-2500
8	Manganese (mg/kg)	200-800
9	Molybdenum (mg/kg)	0.5-1.0
10	Zinc (mg/kg)	40-1000

Table 3: Heavy metals contaminants in organic fertilizers according to RSB standard.

S/N	Heavy metals	Acceptable maximum limits (mg/kg, dry weight)
1	Arsenic (As)	10
2	Lead (Pb)	30
3	Chromium (Cr)	50
4	Nickel (Ni)	50
5	Mercury (Hg)	0.5
6	Cadmium (Cd)	5

Table 4: Minimum pathogens in Organic fertilizers according to RSB standard

S/N	Microorganisms	Acceptable limit
1	<i>E. coli</i>	1000 cfu/g
2	<i>Salmonella spp</i>	Absent in 25 g fresh mass
3	Faecal streptococci	<500cfu/g
4	Total coliforms	Nil
5	Ascaris eggs	Nil

2.4 Vermicompost leachate or Water produced from treatment process

2.4.1 Toxicity of vermicompost leachate

Vermicompost improves the soil's ability to hold water, boosts plant resistance by out-competing helpful bacteria in the soil's composition, is non-toxic, controls the pH level of the soil, and has a favorable impact on a number of metrics, including plant fresh and dry weights and yield [53].

Even though there is potential benefit to using vermicompost leachate, it is also very important to consider its potential toxicity depending on its applied concentration. Many studies show that undiluted leachate can cause the damages effect in germination and growth of plant, that is why many researchers recommend to apply only diluted vermicompost leachate [54]. The potential toxicity of undiluted leachate is attributed to high salt or pH, and dilutions are recommended but this inhibition of germination and growth may vary depending to plant species applied because some species are salt tolerant [54].

Although some species used in vermicomposting like *Lumbricus rubellus* composting worms detoxify heavy metals by binding and storing them in metallothionein and metal binding proteins in order to survive and this process leads to the accumulation of metals in their tissue, and the rate of remediation correlate with the accumulation level, it is possible that non-detoxified heavy metals found in leachate may potentially contaminate soil, groundwater and impact the quality of surface water which can cause the problems to the exposed organisms including humans [55].

Food chain contamination

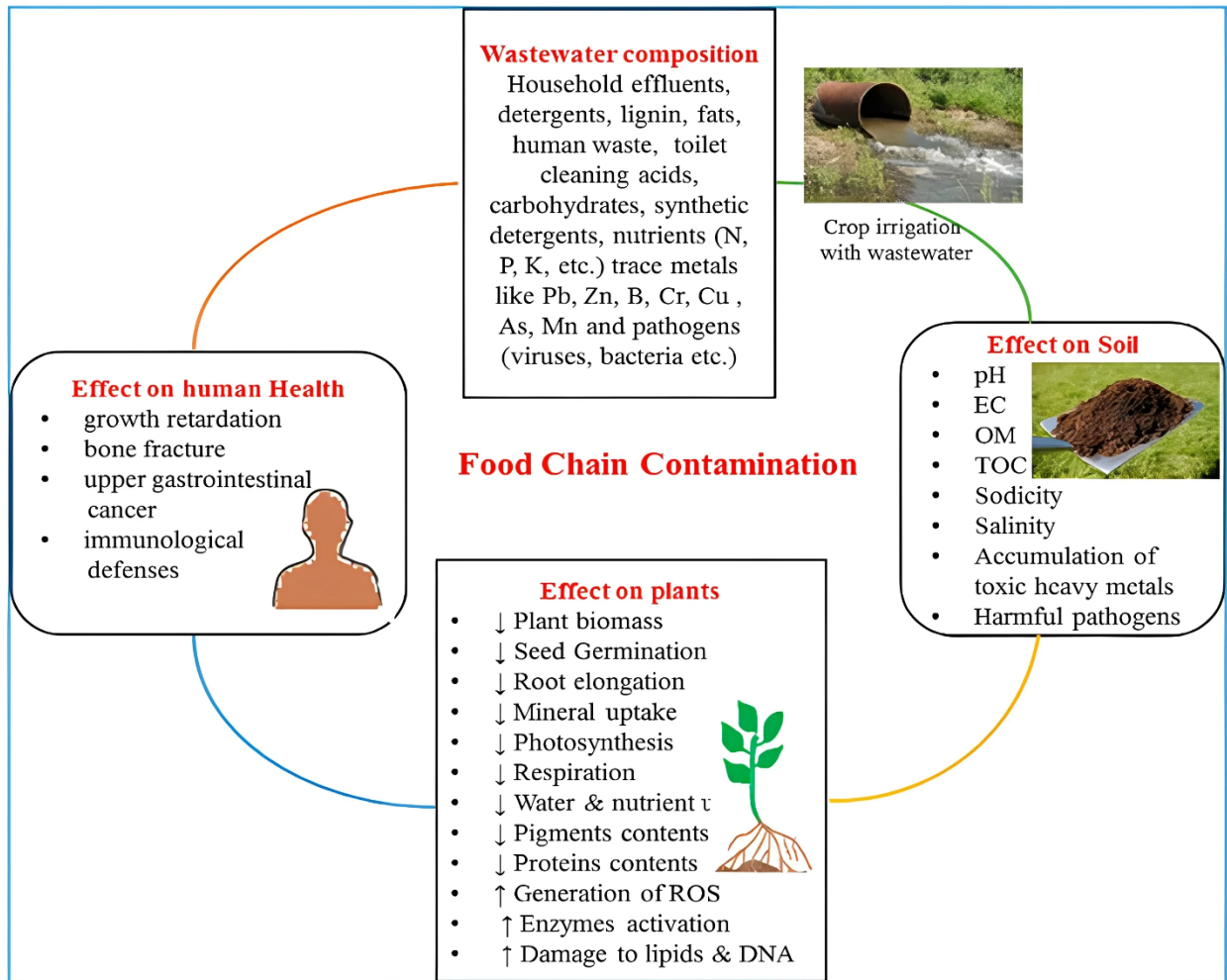


Figure 5: probable food chain contamination by reuse of treated wastewater for agricultural irrigation originated from [56].

According to Guidelines of Rwanda standard board published in 2020 for water quality management, the following table shows the permissible limits for water effluents to be discharge in water bodies as well as in the environment [57].

Table 5: Maximum permissible limit for discharged industrial effluent in water bodies according to RWB/RURA guidelines

	Parameters	Permissible limits
1	Total suspended solids (mg/L)	50.0
2	Total Dissolved Solids (mg/L)	2000.0
3	Oil and grease (mg/L)	10.0
4	BOD5 (mg/l) (20°C)	50.0
5	COD (mg/l)	250.0
6	Faecal Coliforms (MPN/100 mL)	400
7	Hexavalent Chromium (mg/L)	0.05
8	Copper (mg/L)	3.0
9	Lead (mg/L)	0.1
10	Sulphide (mg/L)	1.0
11	Zinc (mg/L)	5.0
12	pH	5-9

2.4.2 Irrigation water quality indices (IWQIs)

Different types and amounts of dissolved salts can be found in irrigation water. The salt content of irrigation water may have a significant effect on the salt content of soil, hence regulating primary production and plant growth. To assess the appropriateness of irrigation water, specific hydro-chemical water indices must be determined [58]. Literature frequently uses a variety of parameters to calculate irrigation water quality indices, such as residual sodium carbonate (RSC), permeability index (PI%), soluble sodium percentage (SSP), total hardness (TH), sodium adsorption ratio (SAR), Kelly's ratio (KR), magnesium hazard (MH%), and salinity hazard (EC, TDS). These IWQ indicators provide crucial assistance in identifying crop and soil problems that may be caused by

the quality of irrigation water used. Based on certain agronomic considerations pertaining to soil permeability, water/soil salinity, crop toxicity and ultimately, agricultural output, these irrigation indices were chosen [58].

Sodium Adsorption Ratio (SAR)

Sodium Adsorption Ratio (SAR) is a key parameter used to assess the suitability of irrigation water. It indicates the relative concentration of sodium (Na^+) ions compared to calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions in water [56]. SAR is calculated using the following equation [59].

$$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$$

High sodium concentrations in irrigation water raise the water's salinity, which influences soil classification and turns natural water into salt water, lowering the soil's hydraulic conductivity and infiltration rate [60]. According to WHO guidelines water with **SAR < 6 mg/L**: is safe for most soils. Maintaining a balanced SAR is crucial for sustainable irrigation and soil health [61]. If SAR falls between 0 and 10, the sodium rate is minimal and there is no soil disturbance, according to the classification provided by the US Salinity Laboratory. If the SAR is between 10 and 18, the sodium rate is moderate, making long-term irrigation with this water unsuitable; if the SAR is between 18 and 26, the sodium rate is intensive, making irrigation unsuitable. At some point, if SAR is between 26 and 30, the sodium rate is quite high, and using the designated water for irrigation is both unlawful and extremely risky[62].

Magnesium Adsorption Ratio (MAR)

MAR is dependent on the levels of magnesium and calcium. A higher ratio indicates a higher magnesium content, which raises moisture and degrades soil structure. A risk index is defined as a value greater than 50 [62], and is calculated using the following equation:

$$MAR = \frac{Mg}{Ca + Mg} \times 100$$

Kelly's Ratio

KR is also an additional parameter used in irrigation and agriculture to evaluate the quality of water. It is influenced by the contents of calcium, magnesium, and sodium and is comparable to the magnesium adsorption ratio. This criterion divides water into three categories: appropriate ($KR < 1$), marginal ($1 < KR < 2$), and inappropriate ($KR > 2$) [62].

$$\text{Calculated via the formula: } KR = \frac{Na}{(Ca+Mg)}$$

Residual sodium carbonate contents (RSC)

Actually, RSC is the difference between the sum of calcium and magnesium cations and the sum of carbonate and bicarbonate anions. If RSC is less than 1.25 mEq/L (66.25 mg/L), using the intended water is safe; if it is between 1.25 and 2.5 mEq/L (66.25–132.5 mg/L), using the intended water is questionable; and if it is 2.5 mEq/L (132.5 mg/L), using the intended water for irrigation is inappropriate [62].

$$RSC = (CO_3^{2-} + HCO_3^-) - (Ca^{2+} + Mg^{2+})$$

Soluble sodium percentage (SSP)

Soluble sodium rate was used to categorize irrigation water and display the amount and concentration of sodium in a water sample. Typically, there is a linear relationship between the proportion of sodium exchange and the increase in SAR. It may result in a number of issues with the soil, including bogging, increased evaporation and salinity, decreased permeability and leaching, and dispersion and rupture of the soil structure [62]. It is calculated by using the following equation:

$$SSP = \frac{(Na) \times 100}{(Ca+Mg+Na+K)}$$

Permeability index (PI %)

The permeability issues of the natural infiltration rate are expressed by the PI parameter. The quality of water used for agriculture and irrigation was expressed using a permeability index in addition to the other indexes. Classifications based on PI values are as follows: Class 1: $PI > 75$,

Class 2: $25 > PI > 50$, and Class 3: $PI < 25$. In this categorization, $PI > 75$ is not suitable for irrigation [62].

$$PI = \left(\frac{Na^+ + \sqrt{HCO_3^-}}{Ca^{2+} + Mg^{2+} + Na^+} \right) \times 100$$

Wilcox Diagram

A graphical tool for assessing irrigation water quality based on Electrical Conductivity (EC) and Sodium Adsorption Ratio (SAR) is the Wilcox Diagram. It categorizes water into different suitability classes ranging from "Excellent" to "Unsuitable" based on salinity and sodium hazards. The Wilcox diagram is utilized in irrigation, agricultural, and water categorization. Based on sodium hazard (SAR) and salinity hazard (EC), this graph divides water into 16 categories: low, medium, high, and extremely high. C is the salinity indicator, while S is the sodium level [62].

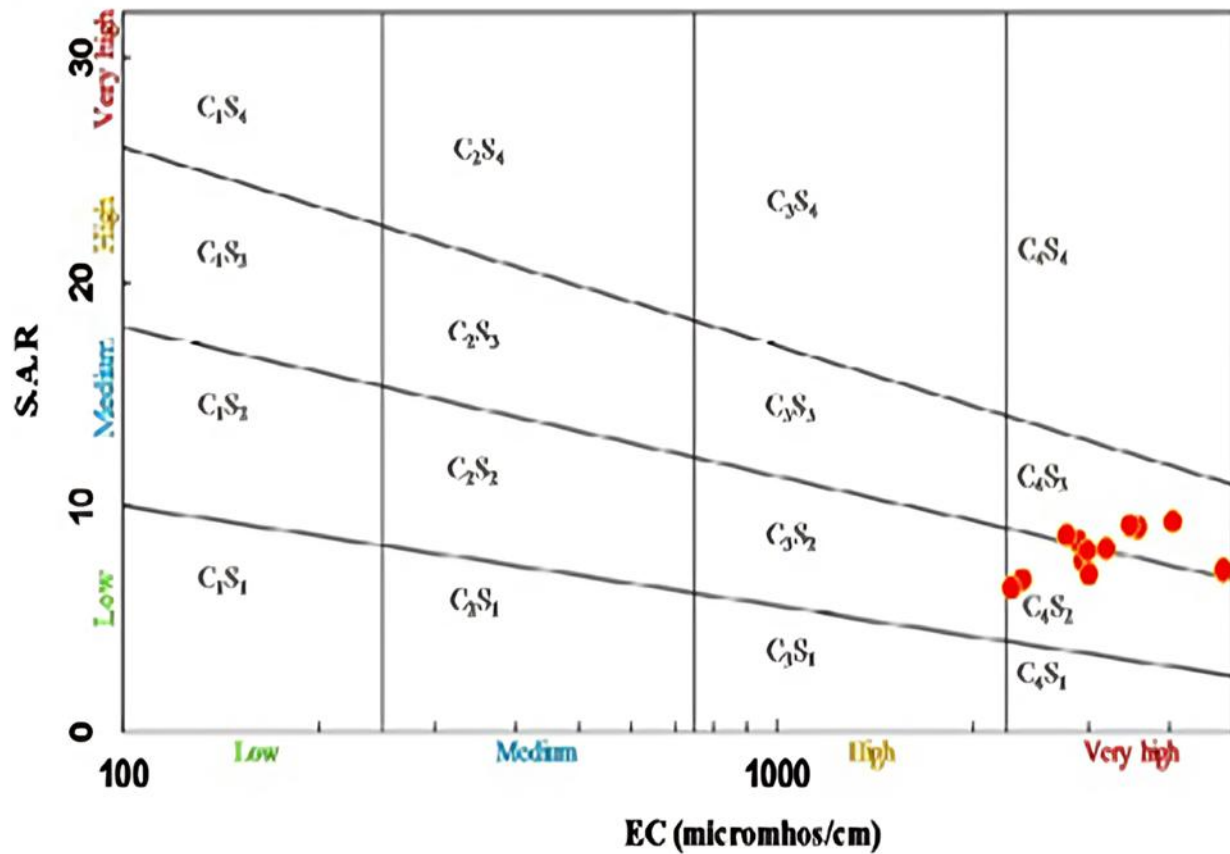


Figure 6: Wilcox diagram of samples collected in TWW in research done by [62]

Table 6: Water quality classes based on wilcox diagram [62]

EC ($\mu\text{S}/\text{cm}$)	Water Salinity Class	SAR Range	Sodium Hazard Class
< 250	C1: Low salinity (excellent)	0–10	S1 (Low Sodium)
250–750	C2: Medium salinity (Good)	10–18	S2 (Medium Sodium)
750–2250	C3: High salinity (Permissible)	18–26	S3 (High Sodium)
> 2250	C4: Very high salinity (Unsuitable)	> 26	S4 (Very High Sodium)

To classify irrigation water based on the mentioned indices above done using ranking as described in the table below.

Table 7: Classification of Irrigation water based on the standard value intervals for irrigation water quality standards [63].

Parameter	Rank	Water classification
Electrical conductivity (EC) ($\mu\text{S}/\text{cm}$)	<250	Excellent
	250 - 750	Good
	750 - 2000	Permissible
	2000 - 3000	Doubtful
	>3000	Unsuitable
TDS (mg/L)	0 - 1000	Fresh water
	>1000	Blackish water
TH (mg/L)	0 - 75	Soft
	75 - 150	Moderately hard
	150 - 300	Hard
	>300	Very hard
SAR	0 - 6	Good
	6 - 9	Doubtful
	>9	Unsuitable
KR	<1	Suitable
	>1	Unsuitable

SSP	<20	Excellent
	20 - 40	Good
	40 - 60	Permissible
	60 - 80	Doubtful
	>80	Unsuitable
PI (%)	>75	Good
	25 - 75	Suitable
	<25	Unsuitable
RSC (meq/L)	<1.25	Good
	1.25 – 2.5	Doubtful
	>2.5	Unsuitable
MH (%)	<50	Suitable
	>50	Unsuitable

2.5 Safety and Regulatory Standards.

To ensure the chemical and biological safety of vermicompost and water produced through vermifiltration, it is essential to adhere to regulatory standards. Local environmental protection organizations and international organizations like the World Health Organization (WHO) have established standards about the acceptable limits for heavy metals, pathogens, and other contaminants in agricultural by-products. As mentioned in the above tables indicate FAO/WHO guidelines for wastewater reuse in agriculture provide thresholds for pathogen levels and chemical contaminants that must be adhered to in treated water and organic fertilizers (WHO, 2018). Also according to report of European commission (European Commission, 2021) this report discusses the safety standards for compost and organic fertilizers, including limits on heavy metals and pathogens, relevant for assessing the safety of vermicompost, that why when working with faecal sludge, vermicompost or its leachate in the lab or at the field, pathogens and other toxic substances present threats to human health that call for stringent health and safety protocols. Precautions must be taken by everyone participating in the sampling and testing process at every stage. The extent of exposure to feces determine the need of vaccines to the regionally frequent diseases (such as cholera, tetanus, polio, typhoid fever, and hepatitis) [42]. Among the measures to fight against the risks associated with the exposure to faecal sludge and vermicompost from faecal sludge is to use properly the following precautions: Appropriate Personnel Protective Equipment (PPEs) to create a barrier between human and causal agents of diseases should be used correctly, those PPEs include: Laboratory coat and/or safety overalls, safety shoes, eye protection, gloves and masks [42]. Always carry out the TS analysis in a space with adequate ventilation and an exhaust system. When inserting and retrieving crucibles from the oven, put on gloves made to endure high temperatures. After laboratory analyses the remaining solid samples will be disposed in the appropriate waste container while liquid samples will be poured in the sink with tap water and the container will be cleaned by using bleaching detergent [64]. The microbiological wastes (used petri-dishes containing grown bacteria) will be disinfected before disposal using bleaching agent (at least 5% Chlorine bleach liquid).

CHAPTER 3. MATERIALS AND METHODS

3.0. Description of the study area

Figure 7 shows geographical location of Rulindo faecal sludge treatment plant, located in Northern province of Rwanda, Rulindo district, Base sector, Gitare cell, Nyamugari village constructed in September 2022 by an international non-profit organization known as Water for People. The Water for People in Rwanda began in 2008, with an overall objective to help all communities, schools and health facilities gain access to sustainable water, sanitation, and hygiene services. It is in this regard that vermifiltration plant was constructed to help to achieve basic sanitation services by 2024 National Strategy for Transformation (NST1) where households will be using improved facilities (latrines) that are not shared with other households and to contribute to the achievement of SDG#6: that consists of “Use of safely managed sanitation services” by 2030. Water for people constructed this FSTP at Rulindo district targeting the following potential users; 12,000 people of Base sector and other surrounding institutions.

Table 8: Coordinates of the study area

Sampling points	GNSS Viewer Coordinates
Raw faecal sludge (RFS)	Latitude: -1.65750
	Longitude: 29.88727
Tiger Biofilter I (TB I)	Latitude: -1.65737
	Longitude: 29.88739
Liquid storage tank (LST)	Latitude: -1.65736
	Longitude: 29.88729
Tiger Biofilter II outlet (TB II)	Latitude: -1.657328
	Longitude: 29.88725
Treated water tank (final effluent) (TRWT)	Latitude: -1.65720
	Longitude: 29.88730

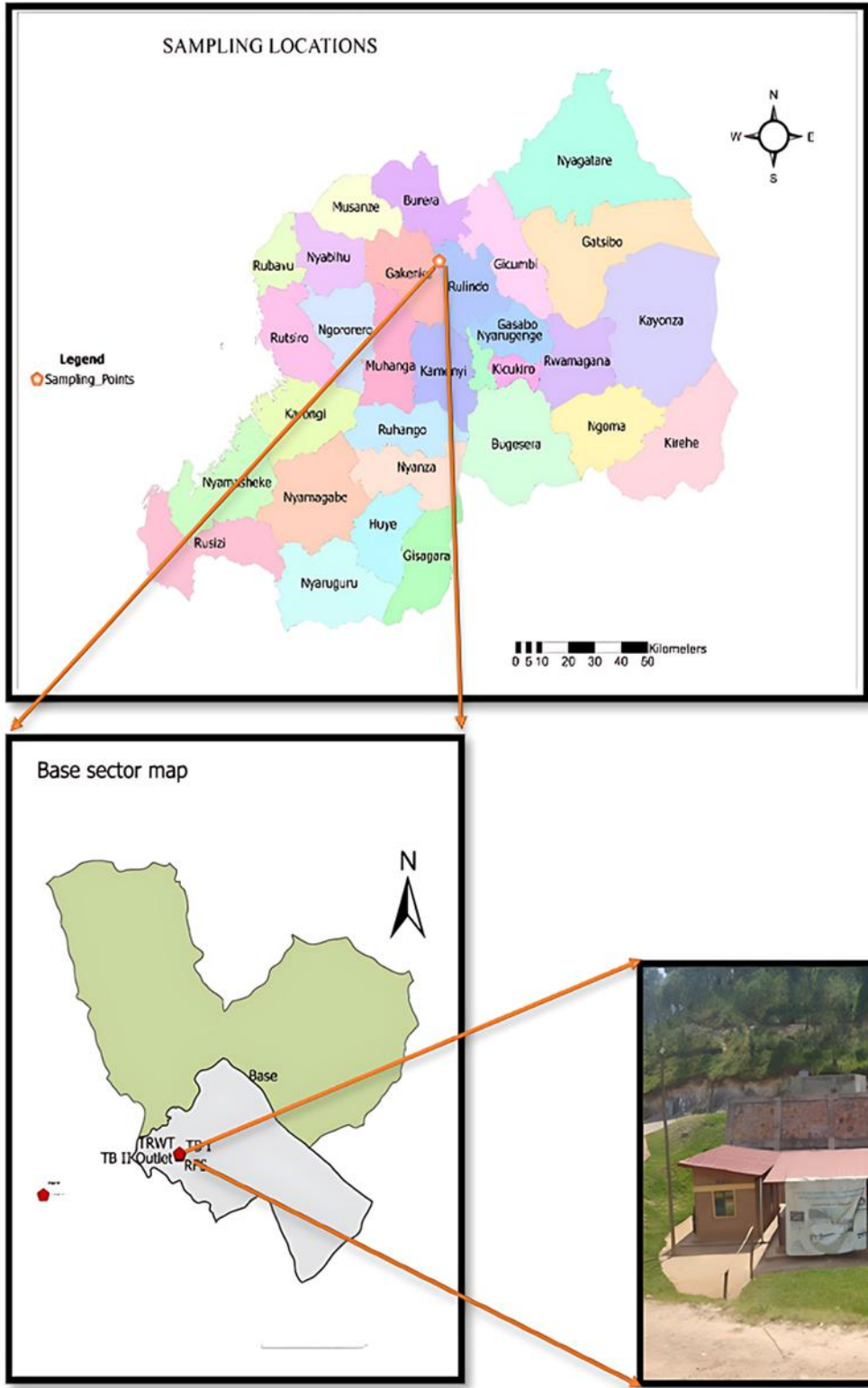


Figure 7: Maps indicating sampling location at Rulindo FSTP

3.1 Description of the project and technical data of the technology

The plant has the design flow of 10 cum per day (10KLD), the fecal sludge treatment process consists of three main steps, including a pretreatment stage (screening) to retain larger items such as rags, sticks, and other large debris that may damage the treatment plant equipment. After screening, the sludge is stored in the sludge storage tank (SST) which has 32m³ with retention time of 48hours then, by gravity, it is transferred to the anaerobic stabilization reactor (ASR) for further treatment. In the ASR, bacterial communities anaerobically biodegrade the organic compounds from the fecal sludge. These tanks are used to reduce organic load from Fecal Sludge. This tank contains specially formulated bacterial culture which consumes organics from Fecal sludge with 2 stages: Mixing chamber which has 7.5m³ with retention time 18hours, and Digestion chamber which has 9m³ with retention time 60hours, Furthermore, at the Flow rate of 1.75m³/day, the thickened sludge is spread on biofilters (tiger biofilter I) composed of 6 beds with 10kg of *Eisenia fetida* (tiger worms) that feed on trapped solids or residual solids and convert them into vermicompost. From the bottom of biofilters, liquid is collected and stored in the liquid storage tank of 6m³ with expected retention time of 12hours. This is a collection tank of liquid separated from the sludge at the biofilter I level. Notably, the bacteria provide a favorable condition and respiration zone for tiger worms growth and reproduction, thereby removing organic loads and bad odors. The wastewater from the storage tank is spread by gravity on tiger biofilter II beds, where it undergoes the aeration process coupled with biodegradation by tiger worms attached to eucalyptus barks. This is a modular vermifiltration unit designed to reduce the residual BOD from fecal sludge tiger biofilter II made by 1 bed and 32 crates with 5kg of worms and the retention time should be 8hours before entering into the next stage, the treated effluent coming out from TBF II is treated with horizontal planted gravel filter. This is polishing unit, provided for removal of nutrients and color from treated water [65]. Canna plants are used to consume nutrients and grow rapidly. Total Kjeldahl Nitrogen (TKN) and Nitrates are good nutrients and are efficient for plants which results in reduction of TKN and Nitrate. The treated effluent is stored into a treated water tank of 6m³ and expected to be used for gardening, irrigation, or other land applications, and the retention time of treated effluent in treated tank planned to be 12hours. The water treated after HPGF is supposed to be disinfected using chlorine dosing system or UV lamp.



(A) General overview of the plant



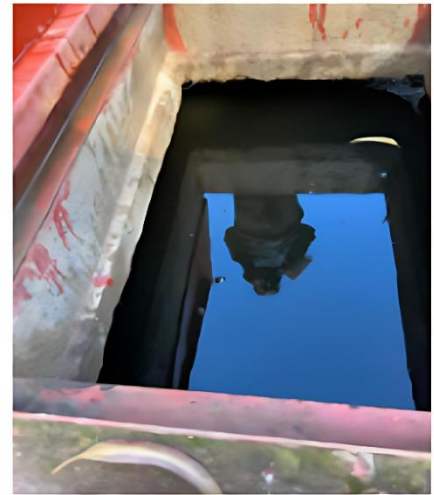
(B) Inlet point and screening chambers



(C) Anaerobic stabilization reactors



(D) Tiger biofilter I



(E) Liquid storage tank (LST)



(F) Tiger biofilter II



(G) Horizontal planted gravel



(H) Treated water tank (TRWT)

Figure 8: Images showing details of Rulindo faecal sludge treatment plant

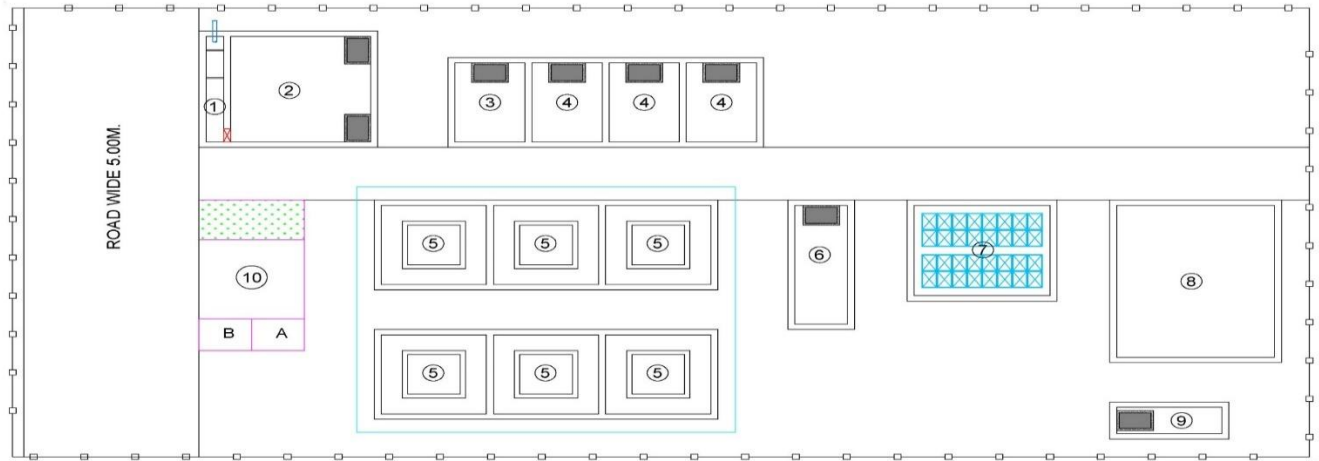


Figure 9: Schematic of 10KLD capacity FSTP based on tiger biofilter technology

Legend:

1	Screen Chamber	5	Tiger Biofilter I	9	Treated Water tank
2	Sludge Storage Tank	6	Liquid storage tank	10	Control panel room
3	ASR Mixing Chamber	7	Tiger Biofilter II	A	Male Toilet
4	ASR Digestion Chamber	8	Horizontal Planted gravel filter	B	Female Toilet

3.2 Sample collection and preservation

Vermicompost and water samples were collected twice at Rulindo faecal sludge treatment plant, the first collection conducted on 21st November 2023 and the second on 12th February, 2024. The sample of untreated faecal sludge aseptically taken from the settling tank of raw faecal sludge, and other samples collected at every stage of treatment process. For the water samples collection, the sterilized glass bottles used for microbiological samples while 1-L HDPE (High Density Polyethylene) plastic containers used for physical-chemical samples where (1 L each) collected from each sampling point [66]. Vermicompost samples (10g for each layer) collected from different six bio-filters considering the depth (top layer, middle layer and bottom layer) to make 30g for each bio-filter and different sampling points in a bio-filter had been taken into consideration to make sure that a collected sample is a good representative of a bi-filter as composite sample. The collection process conducted aseptically, and some physical parameters like pH, Electric conductivity, temperature and Dissolved Oxygen (DO) for water sample analyzed

in situ directly at the site by using portable HACH 440d multi-meter ([67], [58]). Then after all the collected samples preserved and kept cool in cooler box and transported directly to the Chemistry and Microbiology Laboratories of the University of Rwanda (UR) for further analyses and then kept in a refrigerator at 4°C before further analyses [66].



Compost sampling(A)



Compost kept in sealable bags(B)



TB II outlet sampling point (C)



Data recording (D)



Treated water collection (E)



Analysis at site (F)

Figure 10: Sampling activity at site

3.3. Physicochemical and biological analysis

3.3.1. Physicochemical parameter analysis

Analysis of liquid water (leachate)

Using the HQ40D portable multi-parameter meter (HACH Instrument, Loveland, Colorado, USA), the following parameters were measured in situ: pH, temperature, dissolved oxygen (DO), total dissolved solids (TDS), and electrical conductivity (EC). Following BOD (5 days, 20°C) EPA Method 4051 yielded the biochemical oxygen demand (BOD5), which measures the quantity of oxygen used during the biological breakdown of organic matter in a certain volume of water [66]. This method consists of completely filling a 300 ml sealed bottle and letting it sit at 20 °C for five days. Using a luminescent dissolved oxygen (LDO) device, BOD5 was calculated as the difference between the initial dissolved oxygen and the dissolved oxygen measured after five days of incubation. The difference between the amount of oxygen before and after the incubation procedure at 20 °C in the dark was used to calculate the BOD5. $[\text{DO}]_{\text{initial}} - [\text{DO}]_{\text{final}} = \text{BOD5 (20 °C)}$ [66].

While the chemical oxygen demand (COD) determined using colorimetric (EPA Method 4104), where a sample is exposed to a strong chemical oxidant (digestion solution), COD is measured as the oxygen equivalent of its organic matter content, to oxidize all organic materials, potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) was utilized as a powerful oxidant. The solution turns green-blue as Cr^{4+} is reduced to Cr^{3+} , indicating the presence of organic materials in the water. In this investigation, Ag_2SO_4 and H_2SO_4 were employed as catalysts to oxidize bio-organic materials. A UV spectrophotometer was used the following day to measure the absorbance at 600 nm [66].

Total nitrogen, Nitrate (NO_3^-), and Nitrite were analyzed by using cadmium reduction (EPA Method 353.3), while Turbidity measured by turbidity meter (HACH 2100Q). Meanwhile heavy metals and some inorganic nutrient (Na, K, and Ca) determined by utilizing Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES AVIO 500) [68]. To determine the indices that indicate the suitability of produced water to be used for irrigation the following formula in the table below were used [62].

Table 9: Irrigation water quality suitability indices

Indices	formula
Sodium Adsorption Ratio (SAR)	$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$
Kelly's Ratio (KR)	$KR = \frac{Na}{(Ca + Mg)}$
Soluble Sodium Percentage (SSP)	$SSP = \frac{(Na) \times 100}{(Ca + Mg + Na + K)}$
Permeability Index (PI %)	$PI = \left(\frac{Na^+ + \sqrt{HCO_3^-}}{Ca^{2+} + Mg^{2+} + Na^+} \right) \times 100$
Residual sodium carbonate (RSC)	$RSC = (CO_3^{2-} + HCO_3^-) - (Ca^{2+} + Mg^{2+})$
Magnesium hazard (MH%) or MAR	$MH\% \text{ or } MAR = \frac{Mg}{Ca + Mg} \times 100$

Analysis of solid faecal sludge and vermicompost

Physical parameters including Total solids and moisture content of the faecal sludge will be determined by using Gravimetric Dried at 103-105°C (EPA Method 1603), while Volatile and fixed solid and Total Organic Carbon (TOC) measured by Gravimetric Ignition at 550°C (EPA Method 1604) [42]. Similar to leachate heavy metals and inorganic nutrients (Na, K and Ca) measured by using Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES AVIO 500) after acidic digestion using EPA standard Method3050B [68].

3.3.2 Biological characteristics

Biological examinations include identifying specific pathogens, but due to the difficulties in their detections in the laboratory tests, the indicator organisms with the same environmental surviving conditions and easy detection are selected to be used. In this study the biological contaminants of both vermicompost and produced water identified aseptically as where coliforms bacteria analysed by using Plate count method (EPA Method 9215C), lactose Agar culture medium used for total coliforms and faecal coliforms while TBX Agar for *E. coli* as culture media. And also another kind of bacteria called *Salmonella typhi* which causes typhoid fever measured in this study by using EPA standard Method 1200 [69].

3.3.3 Data Analysis

Microsoft Excel 2010 was used to analyze the data. The characteristics of FS, vermicompost and effluent produced from Rulindo FSTP were described using descriptive statistics (means and standard deviation) [[17], [11]]. The graphs were drawn using ORIGIN PRO software (version 9.0)

CHAPTER 4. RESULTS AND DISCUSSION

4.1. Characterization of water produced from Rulindo faecal sludge treatment plant

4.1.1. Physical parameters for water sample

The physical parameters of pH, conductivity, and dissolved oxygen (DO), Turbidity, total suspended solids (TSS), chemical oxygen demand (COD) and biological oxygen demand (BOD) are crucial indicators of the effectiveness of the faecal sludge treatment process [62]. The results provide insights into the changes that occur during treatment and the quality of the treated effluent with comparison to Rwanda Water Resource Board (RWB) and Rwanda Utility Regulatory Authority (RURA) guidelines for industrial discharge [70], and by comparison with the Food and Agriculture Organization (FAO) guidelines on irrigation water quality [71].

Variation in pH and Electrical conductivity (EC)

In Rulindo FSTP, the fecal sludge has a pH of 8.19, which is slightly alkaline. This alkalinity may result from microbial activity, the breakdown of organic matter, or the presence of ammonia and other nitrogenous compounds. After treatment, the effluent's pH decreases to 7.83, closer to neutral (Fig. 11). This reduction indicates that the treatment process effectively neutralized some basic compounds, likely through microbial metabolism and chemical reactions that produce acidic by-products. A near-neutral pH is advantageous for agricultural and environmental use, as it minimizes harm to plants and aquatic life. According to FAO guidelines, the acceptable pH range for irrigation water is 6.5–8.4 [72]. Similarly, standards for effluent discharge suggest a pH range of 6.5–9.0 [73]. Deviations from this range may lead to nutritional imbalances or introduce toxic ions that could harm crops and soil [74].

Assessing electrical conductivity (EC) is crucial when monitoring wastewater, as it provides insights into salinity levels and reflects the extent of mineralization in a given sample [75]. There is a decrease in EC, where the raw faecal sludge had an EC of 8.68 mS/cm, while the effluent showed an EC of 5.17 mS/cm (5170 μ S/cm), consistent with Peter et al. [76]. Electrical conductivity dropped as a result of earthworm (composting worm) activity and the breakdown of organic matter. According to the results, this can be explained by certain minerals that biologically accumulate in the bodies of the composting worms. However, the permissible range for irrigation water varies between 750 and 2250 μ S/cm, according to WHO (2005). This indicates that the water produced at Rulindo FSTP has a very high EC, which requires dilution before use. As the research

indicates, the main consequence of high EC is that it reduces plant osmotic activity, hindering the uptake of nutrients and water from the soil [74].

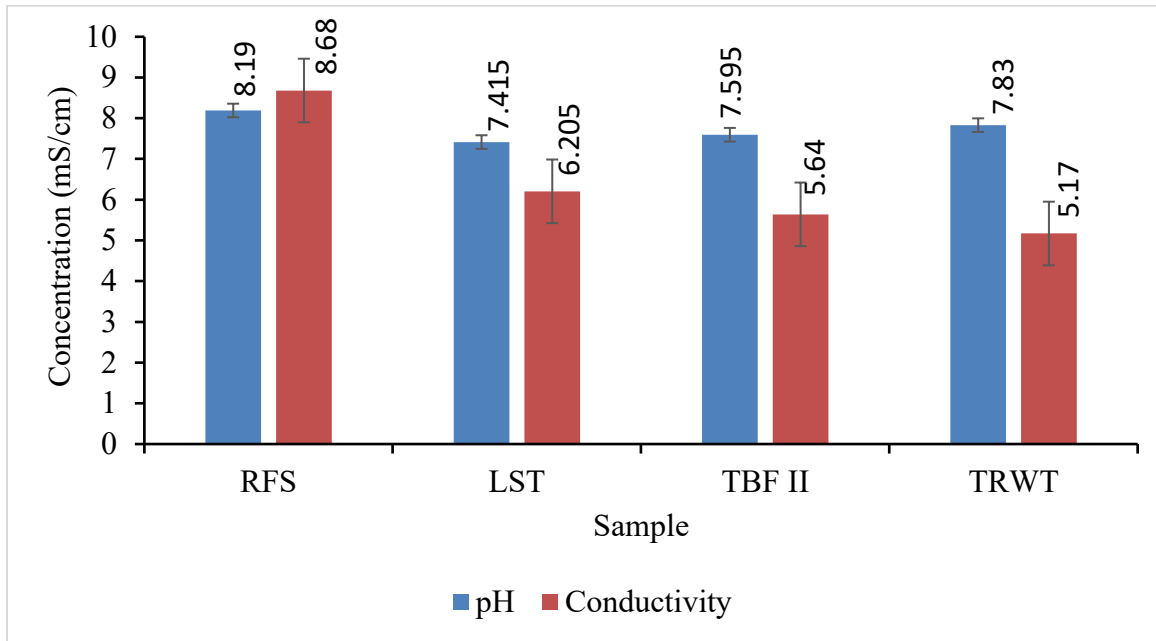


Figure 11: pH and Electrical conductivity changes in effluent collected at Rulindo FSTP

(RFS: Raw faecal sludge, LST: Liquid storage tank, TBF II: Tiger biofilter II, TRWT: Treated water tank)

Turbidity and Total Suspended solids (TSS) variation

The results show a gradual reduction in turbidity and TSS throughout the treatment process. Turbidity decreased from 148.5 NTU in the liquid storage tank (LST) to 24.5 NTU at the tiger biofilter II outlet (BF II), and further to 13 NTU in the treated water tank (TRWT). Correspondingly, the mean concentration of TSS dropped from 75 mg/L in the LST to 13.5 mg/L at the BF II outlet, and finally to 7.5 mg/L in the TRWT. These reductions demonstrate the treatment process effectively lowers turbidity and TSS to acceptable levels. According to RURA guidelines, effluent turbidity should not exceed 30 NTU, and TSS should be below 50 mg/L. Furthermore, FAO guidelines recommend TSS levels of ≤ 10.0 mg/L for irrigation [71]. Sasanka et al. [73] explored the management of faecal sludge and showed that for discharge into water bodies, land disposal, or groundwater recharge, total suspended solids (TSS) should remain below 100 mg/L.

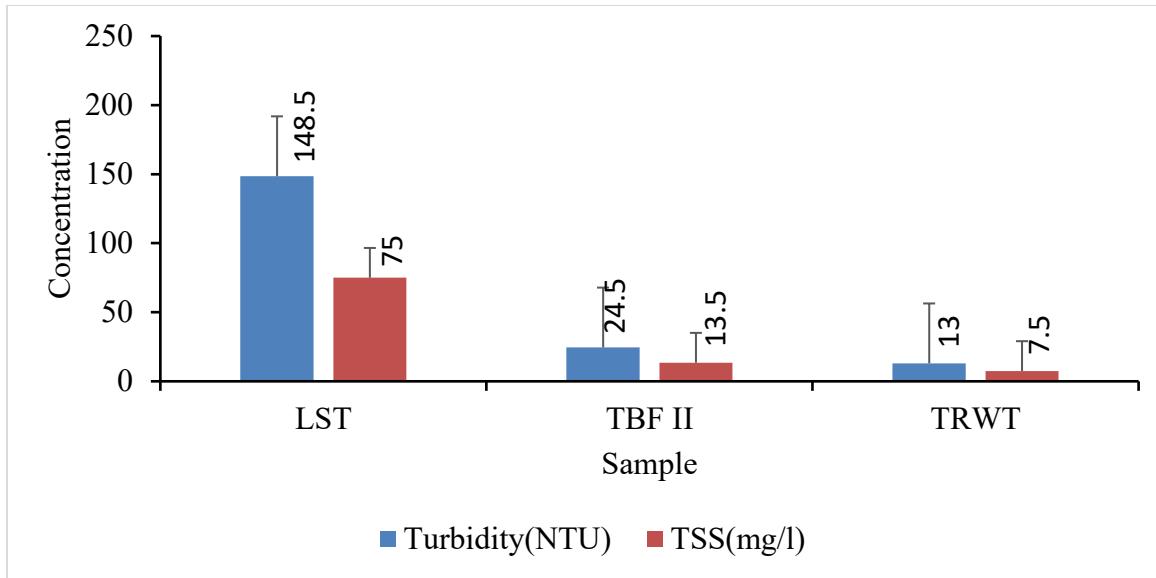


Figure 12: Turbidity and Total Suspended Solid changes of effluent collected at Rulindo FSTP (LST: Liquid storage tank, TBF II: Tiger biofilter II, TRWT: Treated water tank)

4.1.2. Chemical parameters of effluent from Rulindo FSTP

The analysis of chemical parameters in the water produced by the faecal sludge treatment plant provides valuable insights into its chemical safety and suitability for various applications, such as irrigation or environmental discharge.

COD and BOD₅ variation in the effluents collected at Rulindo FSTP

COD and BOD₅ are critical indicators used to evaluate organic pollution in wastewater effluent ([77]; [78]). COD measures the amount of oxygen required to chemically oxidize the organic content in a sample using a strong oxidizing agent, providing insight into the extent of water pollution caused by organic matter [79]. In contrast, BOD assesses the concentration of organic matter in water that can be broken down by microorganisms [77]. Specifically, BOD₅ quantifies the oxygen consumed by microorganisms over a five-day period as they decompose organic compounds in the wastewater [80]. This measurement is particularly important as it highlights the potential for oxygen depletion, which is essential for sustaining aquatic life [81].

In this study, the COD and BOD₅ values for raw faecal sludge were recorded at 3,514 mg/L and 659 mg/L, respectively. The elevated COD value signifies a significant presence of both organic and inorganic substances that consume oxygen during chemical oxidation. This indicates that the raw faecal sludge carries a substantial organic load, which includes both biodegradable and non-biodegradable components. Meanwhile, the BOD₅ value represents the portion of organic matter that can be broken down through biological processes [82]. The significantly lower BOD₅ value compared to COD suggests that a considerable proportion of the organic load is not easily biodegradable. Notably, the BOD₅/COD ratio is commonly used as an indicator of the biodegradability index of organic pollutants in wastewater ([83], [84]) For effective biodegradability, the BOD₅/COD ratio should ideally exceed 0.5 [85]. With a COD/BOD ratio of approximately 5:1, it is clear that the raw faecal sludge contains a significant amount of non-biodegradable or slowly biodegradable organic matter [82]. Generally, a ratio greater than 3:1 indicates that the sludge may present challenges for biological treatment, potentially requiring more intensive or prolonged treatment processes to achieve efficient degradation.

On the other hand, the treated water effluent, COD and BOD were found to be 198.995 mg/L and 38.5 mg/L, respectively. The substantial reduction in COD to 198.995 mg/L, with a removal efficiency of 94.34%, indicates that the treatment process has effectively removed the majority of organic and inorganic pollutants. Comparatively, a study by [35] on the optimization of faecal sludge treatment via vermifiltration reported a reduction efficiency range of 96–99%. Likewise, the BOD value in the treated effluent significantly decreased to 38.5 mg/L, reflecting a removal efficiency of 94.16%. These results suggest that the water produced at Rulindo FSTP is safe for discharge into the environment, as per RURA guidelines for industrial wastewater, which specify that COD and BOD should not exceed 250 mg/L and 50 mg/L, respectively [70]. However, the BOD result is slightly higher compared to the FAO and WHO guidelines, which stipulate that BOD₅ should not exceed 25 mg/L [86]. Besides, the BOD level is somewhat higher than the standard discharge values reported by [70] regarding quality in faecal sludge management, which recommends that BOD for effluent discharged into water bodies, land disposal, or groundwater recharge should be below 30 mg/L. Moreover, treated effluents from faecal sludge and wastewater often contain relatively higher microbial contaminants compared to the WHO (2006) guideline of BOD = 25 mg/L. For instance, a study on treated wastewater reuse for irrigation in a semi-arid region reported a BOD of 27.33 mg/L, which also exceeds the WHO guideline [86].

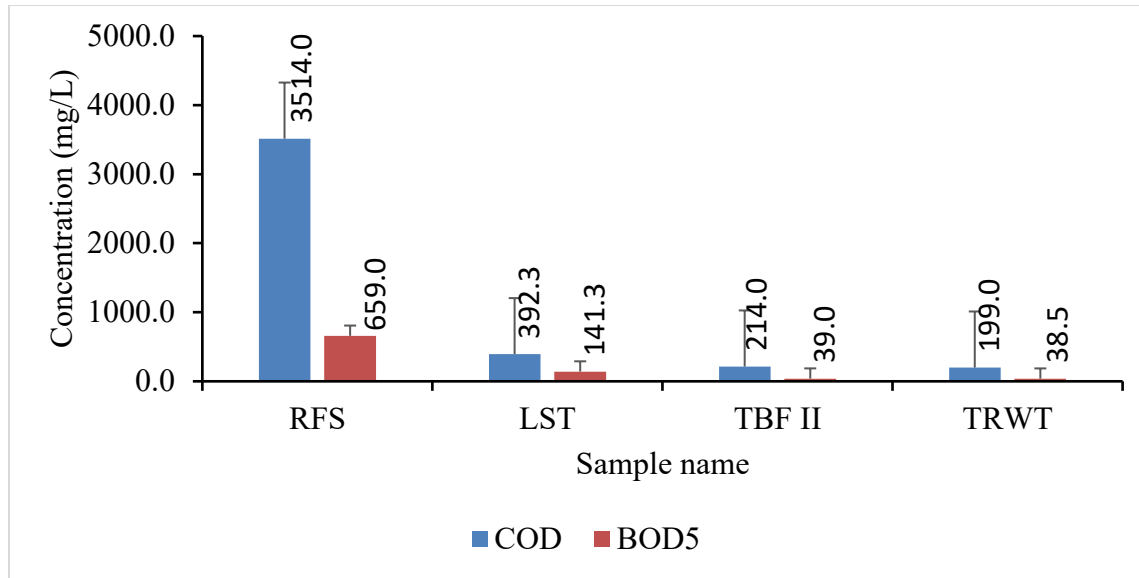


Figure 13: COD and BOD5 changes of effluent collected at Rulindo FSTP

(RFS: Raw faecal sludge, LST: Liquid storage tank, TBF II: Tiger biofilter II, TRWT: Treated water tank)

Nutrients found in the effluent produced at Rulindo FSTP

According to FAO (2015), nutrient depletion and soil fertility can have a detrimental effect on plant yield. The importance of the nutrients provided by treated sewage can be seen in the morphological development of plants. Utilizing wastewater for irrigation has a substantial effect on the soil since it supplies macro- and micronutrients such as nitrogen (N), phosphorus (P), potassium (K), iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) [56].

Primary macro-nutrients determined in the effluent from Rulindo FSTP

The TN and nitrates of effluent were 305.75mg/L and 225.53mg/L, respectively. Using this water for agricultural irrigation can significantly increase soil nutrients. According to a study by [56], using treated wastewater as irrigation water over a period of six years increased the nitrate concentration in the soil from 269–321 mg/L to 910–2,271 mg/L. These results suggest that the effluent produced is rich in nitrates, which could lead to eutrophication if discharged into water bodies. According to RURA guidelines for faecal sludge management, nitrate levels should be below 20 mg/L, and total nitrogen (TN) should not exceed 30 mg/L [24]. Besides, the nitrate concentration exceeds the maximum permissible limits set by Tanzania's standards for effluent

from faecal sludge treatment facilities, which specify that nitrates should remain below 20 mg/L for irrigation use and municipal and industrial wastewater discharge [87]. Moreover, other research studies indicate that, based on the Bureau of Indian Water Quality Standards (BIWQS) for irrigation water, nitrate levels should be less than 5 mg/L for zero impact and between 5–30 mg/L for moderate impact [88].

Phosphorus is an essential nutrient for plant growth; however, excessive levels can lead to algal blooms and disrupt aquatic ecosystems if discharged into natural water bodies. The mean phosphorus concentration was approximately 8.38 mg/L, suggesting that the effluent from Rulindo FSTP may be safe for irrigation purposes, as per the Environmental Protection Authority's guidelines for effluent use in irrigation, which stipulate that total phosphorus (TP) should not exceed 10 mg/L [89]. However, this TP value is slightly higher than the permissible limits outlined in both the Rwanda Utilities Regulatory Authority (2020) guidelines for faecal sludge management, which set a threshold of TP < 5 mg/L [14], and the Tanzanian Standard (2021) for faecal sludge management in Dar es Salaam, which specifies that TP should not exceed 6 mg/L [87].

The research also revealed that the effluent produced at Rulindo FSTP is rich in potassium (K), with a concentration of 397.5 mg/L. The high K level suggests that the treated water could be advantageous for agricultural use, as K plays a vital role in promoting plant health. However, this K concentration in the produced effluent far exceeds the FAO (2010) acceptable limit of 2 mg/L for irrigation water [86]. It also greater than the toralable limit of 100 mg/L set by RURA guidelines for industrial discharge. Furthermore, this elevated K level may have a significant impact on agricultural soils. A previous study by Peter et al. [56] demonstrated that K levels in soil increased from 59 mg/L to 195 mg/L after six years of using treated wastewater for irrigation.

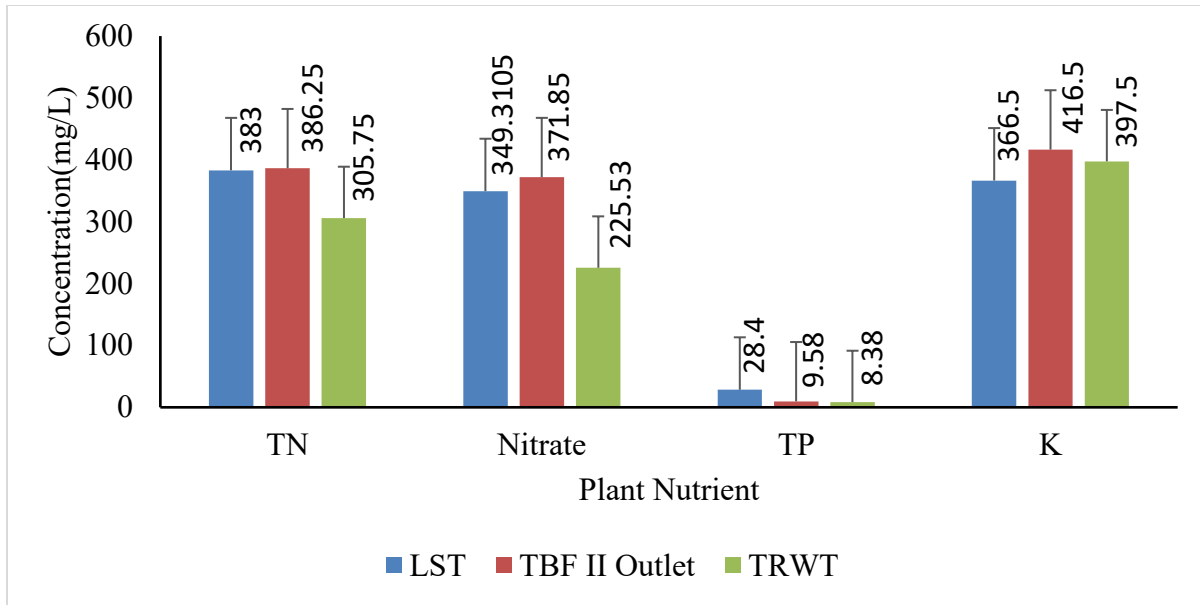


Figure 14: Plant macro-nutrients detected in effluent collected at Rulindo FSTP

(LST: Liquid storage tank, TBF II: Tiger biofilter II, TRWT: Treated water tank)

Secondary macronutrients, and other key parameters for irrigation water

The treated water effluent contains major ions such as sodium (Na), calcium (Ca), magnesium (Mg), sulfur (S), silicon (Si), and antimony (Sb), with concentrations of 8.36 mg/L, 12.83 mg/L, 103.03 mg/L, 58.95 mg/L, 2.512 mg/L, and 2.1 mg/L, respectively (Fig. 13). The relatively low levels of Na and Ca are positive indicators, as excessive Na can lead to soil salinization, while high Ca levels may affect soil structure and plant nutrient uptake. Mg content is significant and could be beneficial for agricultural use, as Mg is a critical component of chlorophyll production in plants. However, elevated Mg levels can contribute to soil hardness, necessitating careful monitoring. S, an essential plant nutrient, is present at a moderate concentration, suggesting that the water could help meet the S requirements of crops without causing adverse effects. Si, which is generally beneficial for plants, strengthens cell walls and enhances stress resistance. The observed Si level is safe and has the potential to improve crop resilience. Trace metal concentrations are generally low, except for Sb, which is relatively higher. Although Sb is less commonly discussed in environmental contexts, its elevated levels may raise concerns depending on regulatory standards

and the intended use of the water. Therefore, higher Sb concentration warrants further investigation to ensure compliance with safety guidelines and to mitigate any potential risks.

Sodium Adsorption Ratio (SAR)

The Sodium Adsorption Ratio (SAR) is a key parameter used to evaluate the suitability of irrigation water. It reflects the relative concentration of sodium (Na^+) ions compared to calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions in water [59]. Using the concentrations of Na, Ca, and Mg obtained above and applying the relevant formula, the SAR value in this study was found to be 1.09 mg/L. This indicates that the water produced at Rulindo FSTP is suitable for irrigation and safe for most soil types, as per WHO guidelines, which specify that SAR should be less than 6 mg/L [60].

Kelly's Ratio (KR) and Soluble Sodium Percentage (SSP)

By applying the following formula, $\text{KR} = \frac{\text{Na}}{\text{Ca} + \text{Mg}}$, and $\text{SSP} = \frac{(\text{Na}) \times 100}{(\text{Ca} + \text{Mg} + \text{Na} + \text{K})}$ the Kelly Ratio (KR) and Soluble Sodium Percentage (SSP) of the produced water were calculated based on the concentrations of sodium, potassium, calcium, and magnesium. The results were 0.072 for KR and 1.6% for SSP, respectively. These values indicate that the treated water is safe for irrigation, as good-quality irrigation water should have a KR of less than 1 and an SSP <20 [88].

Magnesium Adsorption ratio (MAR)

According to the findings of this research, the mean concentration of calcium (Ca) in the treated water effluent is 12.834 mg/L, while magnesium (Mg) is 103.030 mg/L. Using the formula: $\text{MAR} = \frac{\text{Mg}}{\text{Ca} + \text{Mg}} \times 100$, The MAR was found to be 88.9%, and these results indicate an increase in magnesium content, which may lead to increased hydration and potential destruction of soil structure. Additionally, this parameter suggests that the water produced at Rulindo FSTP is not suitable for irrigation unless additional techniques are applied to adjust this key parameter. Most research indicates that MAR values above 50 are considered a risk index for irrigation suitability [62]

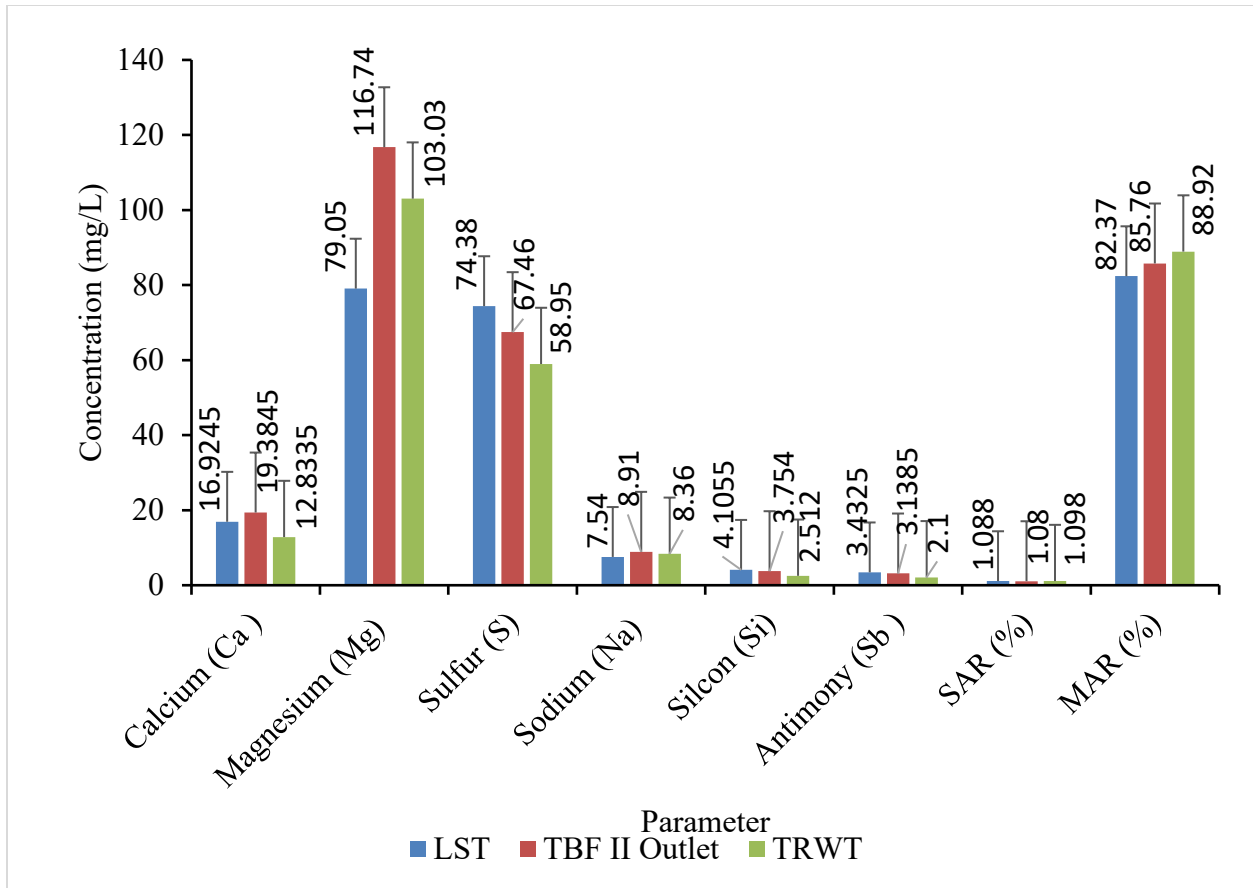


Figure 15: Major ions contents, SAR and MAR obtained in effluent collected at Rulindo FSTP (LST: Liquid storage tank, TBF II: Tiger biofilter II, TRWT: Treated water tank)

Wilcox Diagram

In order to determine if irrigation water is suitable for agricultural use, the Wilcox Diagram classifies it according to its Electrical Conductivity (EC) and Sodium Adsorption Ratio (SAR). According to the values of final effluent produced; with SAR = 1.09 mg/L (very low sodium hazard) and EC = 5170 $\mu\text{S}/\text{cm}$ (or 5.17 dS/m, indicating high salinity)

Analysis Based on Wilcox Classification:

1. Salinity Hazard (EC = 5.17 dS/m), water with EC above 2250 $\mu\text{S}/\text{cm}$ (2.25 dS/m) falls in the "Very High Salinity" (C4) category. Such water is generally unsuitable for irrigation

unless salt-tolerant crops are used, and proper soil management (such as leaching and drainage) is applied.

2. Sodium Hazard (SAR = 1.09), SAR of 1.09 is considered low (S1), meaning there is minimal risk of sodium-induced soil degradation. However, the high salinity of the water is a bigger concern.

Therefore, this water is classified as high-salinity but low-sodium water (**C4-S1 in Wilcox classification**). It is unsuitable for most crops without special management techniques, such as: Using salt-tolerant crops, improving soil drainage to prevent salt accumulation or dilution before use

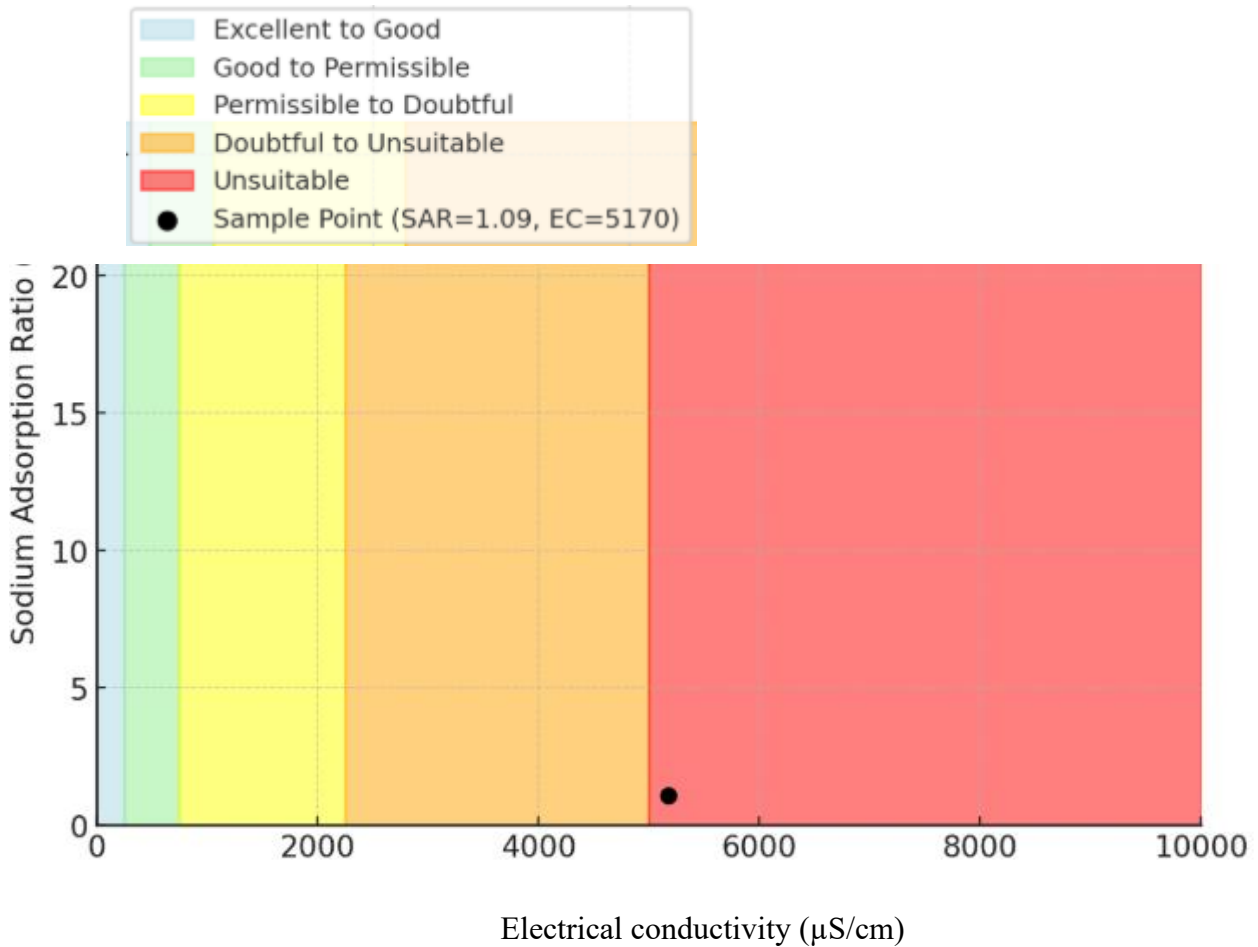


Figure 16: Wilcox diagram of treated effluent produced at Rulindo FSTP

Trace elements and heavy metals in effluent collected at Rulindo FSTP

Trace elements and heavy metals detected in the produced water include Iron (Fe) (0.0265 mg/L), Manganese (Mg) (0.539 mg/L), Boron (B) (0.315 mg/L), Strontium (Sr) (0.128 mg/L), and Vanadium (V) (0.133 mg/L) (**Fig. 17**). According to a 2023 report published by the Food and Agriculture Organization of the United Nations and the International Water Management Institute (FAO & IWMI, Rome) on water quality in agriculture, the maximum permissible concentrations of trace elements in irrigation water are as follows: B (<0.7 mg/L), Fe (5.0 mg/L), Sr and Li (2.5 mg/L), Mg (0.2 mg/L), and V (0.1 mg/L) [90]. By comparing these standards with the findings of this study, it is evident that B and Sr levels fall within the safe range. However, Mg and V concentrations are slightly elevated and somewhat exceed the maximum acceptable limits, raising some concerns about their potential impact.

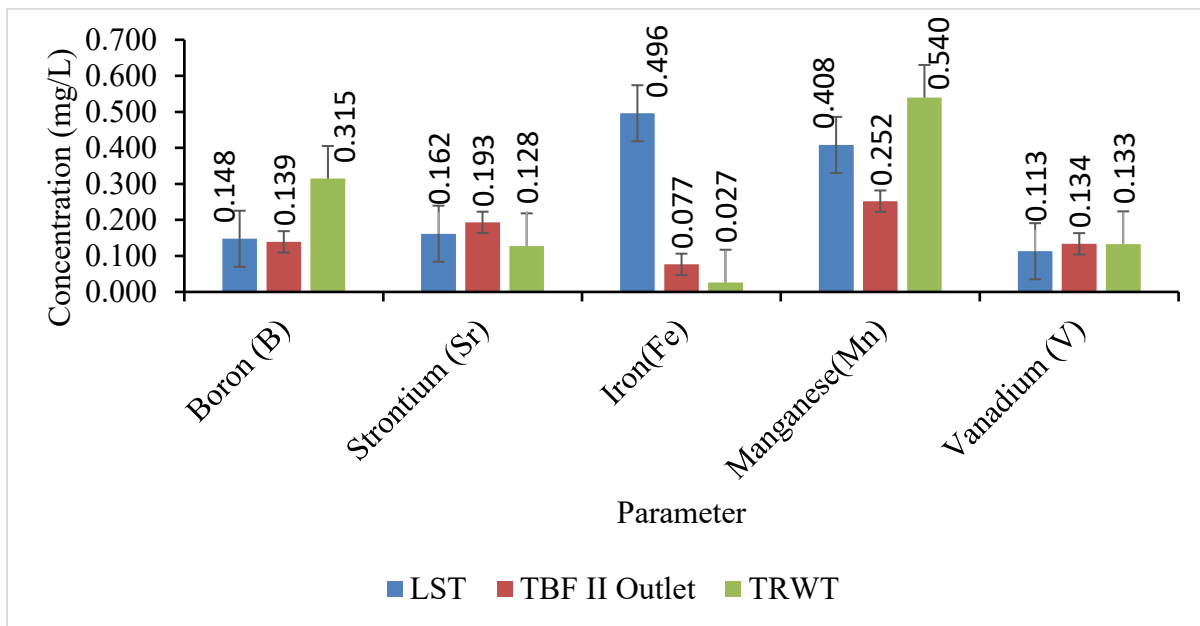


Figure 17: Trace elements and heavy metals detected in effluent collected at Rulindo FSTP

(LST: Liquid storage tank, TBF II: Tiger biofilter II, TRWT: Treated water tank)

4.1.3. Biological parameters in effluents from Rulindo FSTP

The biological safety of the effluent produced at the Rulindo faecal sludge treatment plant focuses on the determination of pathogenic microorganisms, including total coliforms, faecal coliforms, *Escherichia coli*, *Salmonella*, and *Shigella* bacteria. The results demonstrate the effectiveness of the treatment process in reducing the microbial load, which is crucial for ensuring the safety of the treated water.

The total coliform count in raw faecal sludge was measured at 20,145,000 cfu/100ml, while the effluent showed a significantly reduced count of 1,302,000 cfu/100ml after treatment. This represents a notable reduction of 93.54% during the treatment process. However, despite this substantial decrease, the remaining coliform levels in the treated water (1,302,000 cfu/100ml) still exceed the World Health Organization (WHO) guideline value of 1,000 cfu/100ml. This indicates that additional treatment or disinfection steps may be necessary to meet strict water quality standards, particularly if the water is intended for potable use [91]. Furthermore, the total coliform results from this study surpass the permissible limit set by Tanzanian standards for faecal sludge management. According to these standards, municipal and industrial wastewater discharge must have total coliform counts below 10,000 cfu/100ml [87]. The findings suggest that further improvements in the treatment process are needed to align with both international and local regulatory requirements.

This research demonstrated a significant reduction in faecal coliform levels, with counts decreasing from 510,000 cfu/100ml in raw faecal sludge to 229,500 cfu/100ml in the treated effluent. This corresponds to a removal efficiency of 55%, which is relatively lower compared to the reduction rates observed for other pathogens. This suggests that faecal coliforms are more resistant to the treatment process employed in this study. In contrast, the treatment process proved highly effective against *E. coli*, reducing its concentration from 251,000 cfu/100ml in raw faecal sludge to less than 1 cfu/100ml in the treated effluent. This represents an impressive removal efficiency of approximately 100%, indicating that *E. coli* was almost entirely eliminated during the treatment process. However, despite the high efficacy against specific pathogens like *E. coli*, the remaining levels of total coliforms and faecal coliforms in the treated water still exceed the

maximum permissible limits set by the World Health Organization (2006) [92]. According to WHO guidelines, total coliforms and faecal coliforms should not exceed 1,000 cfu/100ml for the irrigation of all crops using treated wastewater [86]. Additionally, reference [90] highlights that bacterial counts in irrigation water should remain below 10,000 cfu/ml to prevent adverse impacts on agriculture. These findings indicate that while the treatment process shows promise, further improvements are necessary to ensure compliance with both international and agricultural safety standards.

The research findings indicate that the water produced at the Rulindo Faecal Sludge Treatment Plant (FSTP) is safe from waterborne diseases related to *E. coli* contamination, in accordance with (WHO 2006) guidelines. These guidelines specify that *E. coli* levels should not exceed 200 cfu/250ml for irrigation of fruit trees and crops or vegetables that are eaten cooked (Category I). For irrigation of fruit trees only (Category II), the acceptable limit is slightly higher, at 1000 cfu/250mL [93]. This suggests that the treated water meets the standards for these specific agricultural uses.

However, effluent from wastewater treatment plants often contain significant levels of microbial contaminants, as highlighted by other studies. For instance, research conducted on "treated wastewater reuse for irrigation in semi-arid regions" focused on two wastewater treatment plants Zahle and Ablah. The findings revealed high microbial contamination levels: total coliform counts of 77,615 cfu/100 mL and 121,500.25 cfu/100 mL, faecal coliform counts of 30,467.83 cfu/100 mL and 103,788 cfu/100 mL, and *E. coli* levels of 60,250.33 cfu/250 mL and 2,517.67 cfu/100 mL, respectively. All these values far exceed the maximum limits recommended by WHO (2006) [86], underscoring the need for more stringent treatment processes to ensure compliance with health and safety standards.

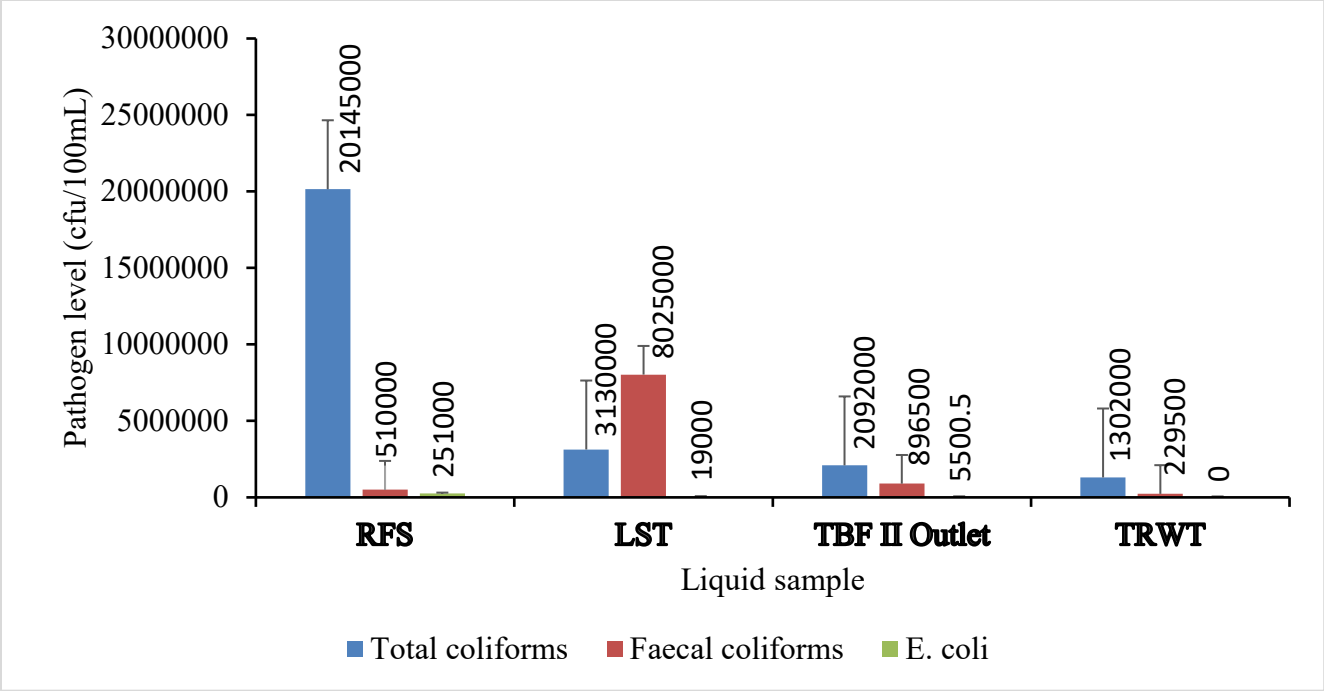


Figure 18: Total coliforms, faecal coliforms, and *E. coli* counts in effluent from Rulindo FSTP

(RFS: Raw faecal sludge, LST: Liquid storage tank, TBF II: Tiger biofilter II, TRWT: Treated water tank).

The results reveal a significant reduction in *Salmonella* contamination levels, decreasing from 103,000 cfu/100 mL in raw faecal sludge to 8,500 cfu/100 mL in the treated effluent. This corresponds to a removal efficiency of 91.75%. Despite this substantial decrease, the presence of 8,500 cfu/100 mL in the treated effluent still poses a potential health risk, especially for applications involving human contact or consumption [94]. These findings exceed the maximum permissible limit for *Salmonella spp.*, which is set at 1,000 cfu/100 mL according to regulatory guidelines [95]. Further evidence of *Salmonella* persistence in treated wastewater is highlighted in a study conducted by [96]. This research focused on *Salmonella* contamination in recycled effluent from treated sewage and urban wastewater. The study found that 65% of raw sewage samples tested positive for *Salmonella*, while 52% of tank effluent samples also tested positive [96]. These results underscore the challenges of eliminating *Salmonella* during wastewater treatment. Using water with such contamination levels can lead to serious health consequences, as *Salmonella* is a well-known causative agent of gastroenteritis and other infectious diseases [97].

A significant reduction in *Shigella spp.* contamination was observed during the treatment process, with levels decreasing from 156,500,000 cfu/100 mL in raw faecal sludge to 2,992,000 cfu/100 mL in the final treated effluent. This represents a removal efficiency of 98.09%, which is remarkable given the extremely high initial concentration. However, despite this substantial reduction, the residual *Shigella* count of 2,992,000 cfu/100 mL in the treated water remains elevated and could pose serious health risks if the water is not subjected to further treatment or managed carefully [95]. Considering the high residual counts of certain pathogens, including faecal coliforms, *Salmonella*, and *Shigella*, it is evident that additional treatment steps are necessary to ensure the biological safety of the treated water. Advanced disinfection methods, such as ultraviolet (UV) systems, should be explored to further reduce pathogen levels. This is particularly critical if the water is intended for applications involving human exposure, such as irrigation or recreational purposes [86].

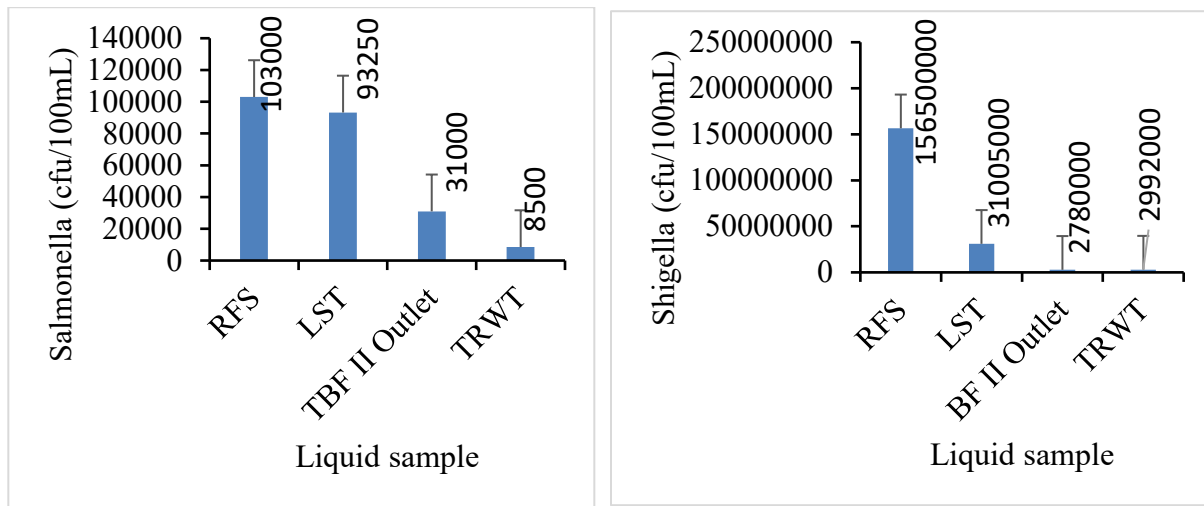


Figure 19: Salmonella (A) and Shigella (B) changes in effluent samples taken at Rulindo FSTP (RFS: Raw faecal sludge, LST: Liquid storage tank, TBF II: Tiger biofilter II, TRWT: Treated water tank).

4.2. Characterization of Solid vermicompost produced from treatment process

4.2.1. Proximate analysis results

The proximate analysis of vermicompost produced from faecal sludge offers key insights into the quality and stability of the final product. The parameters assessed are; moisture content, volatile organic matter, and total organic carbon, providing a comprehensive understanding of the vermicompost's physical and chemical properties

Moisture content

The average moisture content of raw faecal sludge, which was semi-solid, was found to be 94.18%, while the vermicompost produced from it had an average moisture content of 58.21%, based on data collected from six biofilter beds. This significant reduction in moisture content—from 94.18% in raw faecal sludge to 58.21% in the final vermicompost—demonstrates effective water loss during the composting process. Lower moisture levels in vermicompost are desirable, as they indicate greater stability, reduced microbial activity, and improved handling characteristics. For comparison, another study conducted on paunch manure and cattle manure reported moisture contents of 77.28% and 65.67%, respectively [98]. Vermicompost with a moisture level of around 58.21% is more manageable, easier to store, and less likely to produce odors or promote pathogenic growth. However, the moisture content of 58.21% in the final vermicompost still exceeds the recommended range of 15–25% by weight for mature organic compost, as outlined in the RURA guidelines for faecal sludge management [24]. This suggests that additional drying is necessary before the vermicompost can be stored or used effectively.

Organic matter

The results for Volatile Organic Matter (VOM) indicate an average of 61.09% in raw faecal sludge, which decreased to 39.18% in the final vermicompost. This reduction from 61.09% to 39.18% demonstrates that a significant portion of the easily decomposable organic material was broken during the vermicomposting process. The decline in volatile organic matter suggests that the vermicompost has become more stabilized, with reduced levels of readily degradable organic content. As a result, the product is more mature and suitable as a stable organic amendment for soil application. This concentration of organic matter falls within the range reported in other studies, which found that finished compost typically contains organic matter levels between 25%

and 50%. Such findings further validate the quality and stability of the vermicompost produced in this process [6].

Total Organic Carbon (TOC)

The Total Organic Carbon (TOC) content was estimated to be 35.43% in untreated faecal sludge, decreasing to an average of 22.73% in the final vermicompost. This reduction from 35.43% in raw faecal sludge to 22.73% in vermicompost indicates that a significant portion of organic matter was converted into humus and other stable forms during the vermicomposting process. The TOC value of 22.73% in the final vermicompost confirms its suitability for agricultural use, as it meets or exceeds the minimum threshold set by regulatory guidelines. For instance, the RURA guidelines specify that organic compost should contain at least 12% TOC by weight [24]. Similarly, research conducted by [6] reported that carbon concentrations in finished compost typically range between 8% and 50%. Additionally, the Uganda National Bureau of Standards (UNBS) also stipulates that the minimum TOC concentration in organic fertilizers should be 12% [99]. The reduction in TOC is a positive indicator of the compost's maturity and quality, as it reflects the transformation of raw organic matter into a more stable and nutrient-rich material suitable for enhancing soil fertility. For comparison, studies on animal manure fertilizers have reported carbon concentrations of 29.7% and 28.4% for pig and goat manures, respectively [100]. Furthermore, another study on the recovery of vermicompost from sewage sludge in agriculture reported TOC values ranging from 25% to 368% [34]. These findings highlight the potential of vermicompost as a valuable organic amendment, with TOC levels comparable to or exceeding those of other organic fertilizers.

Carbon/Nitrogen ratio (C: N ratio)

The data indicates that the carbon-to-nitrogen (C/N) ratio of the vermicompost produced at Rulindo FSTP is 12:1. This confirms that the vermicompost is of high quality, as it aligns with the RURA guidelines for faecal sludge management, which specify that the C/N ratio should be less than 20 [24]. Additionally, the vermicompost meets the standards set by the Uganda National Bureau of Standards (UNBS) for organic fertilizers and soil conditioners, which recommend a C/N ratio ranging between 12:1 and 15:1 [99]. The decrease in the C/N ratio during the vermicomposting process can be attributed to the breakdown of organic materials [101]. This reduction is further explained by an increase in mineralized nitrogen concentration and a decline in carbon content, as microorganisms utilize carbon as an energy source during decomposition

[102]. These changes not only highlight the efficiency of the vermicomposting process but also underscore the suitability of the final product for agricultural use.

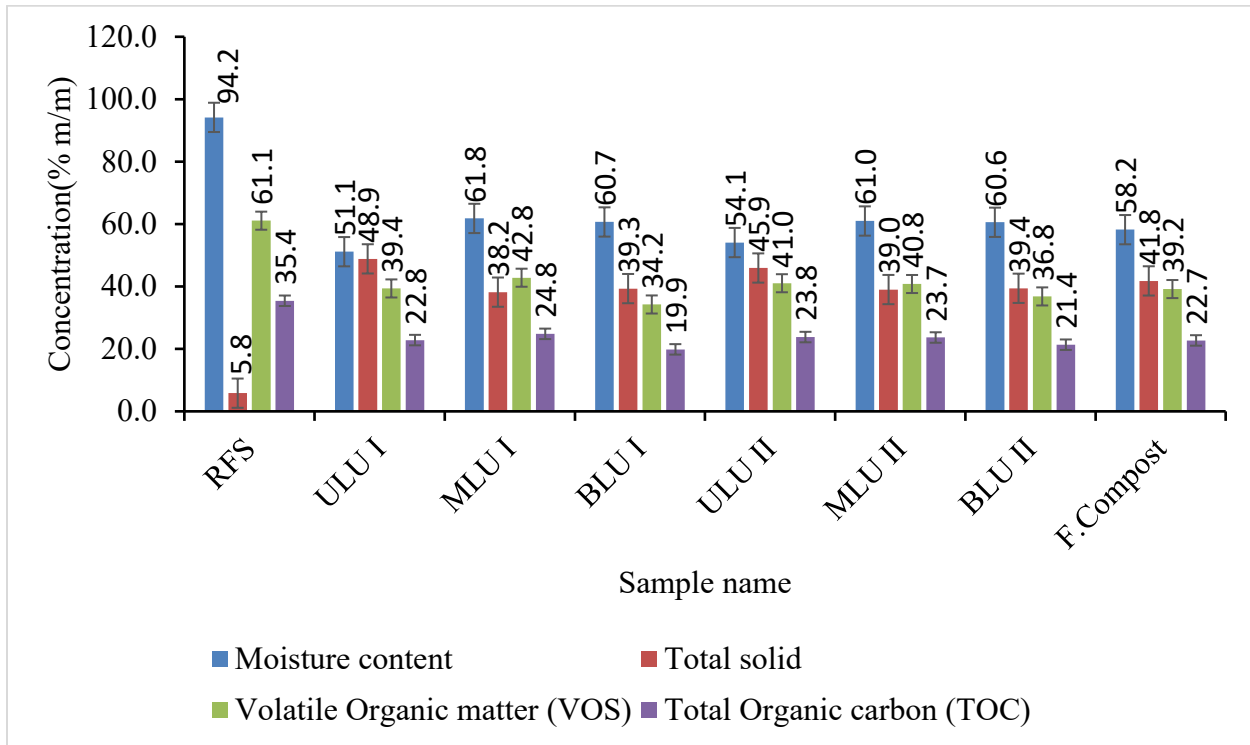


Figure 20: Proximate analysis results comparison of raw faecal sludge and vermicompost produced at Rulindo FSTP

(RFS: Raw faecal sludge, ULU I: Upper layer unit I, MLU I: Middle layer unit I, BLU I: Bottom layer unit I, ULU II: Upper layer unit II, MLU II: Middle layer Unit II, BLU II: Bottom layer unit II, F. compost: Final compost)

4.2.2. Chemical analysis results of vermicompost sample

The chemical analysis of vermicompost produced from faecal sludge reveals important information about its nutrient content and potential safety for use in agriculture. The levels of essential nutrients and trace elements determine their value as a soil amendment, while the concentrations of heavy metals provide insights into its safety and compliance with environmental standards.

Macro-nutrients determined in solid vermicompost at Rulindo FSTP

Total Nitrogen (TN)

From the results of the organic matter obtained above, TN was calculated using the formula: Total Nitrogen (%) = %OM (organic matter percentage) × 0.05, as per the research conducted by [103]. Using this formula, the total nitrogen value in raw faecal sludge was determined to be 3.054%, while the average value for the final vermicompost was 1.959%. This value for the final vermicompost indicates that it is of good quality for use in agriculture as a fertilizer. According to the RURA guidelines for faecal sludge management, organic compost used as fertilizer for food crops should contain a minimum nitrogen concentration of 0.8% by weight [24]. Additionally, the Uganda National Bureau of Standards (UNBS) specifies that organic fertilizers and soil conditioners must have a minimum total nitrogen content of 1% by weight [99]. The final vermicompost meets both these standards, confirming its suitability for agricultural use.

Phosphorus (P)

The results indicate a mean phosphorus concentration of 2.928% and 1.747% for raw faecal sludge and final vermicompost, respectively. The produced vermicompost is of high quality and suitable for use as fertilizer due to its higher phosphorus content, although it slightly exceeds the typical upper threshold range of 0.4–1.1% reported in the literature [104]. However, the phosphorus value of P = 1.747% indicates good-quality fertilizer according to French standards (P < 3%) and WHO (1993) guidelines, which recommend that phosphorus levels should range between 0.1% and 1.7%, as reported by [105] in a study on the agronomic value of compost made from faecal sludge and household waste and its effect on maize production in Dschang. Moreover, the phosphorus level in the vermicompost produced at the Rulindo Faecal Sludge Treatment Plant (FSTP) aligns with findings from research conducted on faecal sludge composting processes in Uganda. In this study, Water for People (an NGO) and its partner UNICEF Uganda sought to convert faecal sludge into compost in Kitgum Municipality at the DEFAST facility. Their research found phosphorus levels of 1.29% in crates and 1.24% in compost pellets [99].

Potassium (K)

Potassium is a vital plant nutrient commonly found in vermicompost derived from faecal sludge. Studies show that vermicompost produced from treated faecal sludge typically contains moderate to high levels of potassium, making it beneficial for improving soil fertility and promoting plant growth, particularly for crops requiring higher potassium inputs. The potassium in vermicompost is usually present in a readily available form, allowing for efficient uptake by plants. The results indicate a significant decrease in potassium concentration throughout the treatment process, from 0.947% in raw faecal sludge to 0.248% in the final vermicompost produced. According to the standard norms of WHO (1993), the potassium concentration in organic fertilizers derived from faecal sludge should range between 0.1% and 2.3% [105]. These standards confirm that the vermicompost produced at the Rulindo Faecal Sludge Treatment Plant (FSTP) falls within this acceptable range.

Secondary macronutrients; Calcium, Magnesium and Sulfur

A comparatively higher concentration of calcium, magnesium, and sulfur is essential for healthy crop growth. For many plant species, the demand for calcium exceeds that of phosphorus, while plants require sulfur and magnesium in nearly equal amounts. The use of high-analysis fertilizers, such as urea, in multiple cropping systems with elevated fertilizer inputs, but without secondary and micronutrients can reduce the availability of secondary nutrients like calcium, magnesium, and sulfur in the soil, leading to nutritional imbalances [106]. Through the analysis of the chemical composition of vermicompost, other major nutrients identified include calcium, magnesium, and sulfur. Generally, the concentrations of these nutrients decreased throughout the treatment process, with the exception of calcium. The results showed concentrations of 2.161%, 0.943%, and 1.174% for calcium, magnesium, and sulfur, respectively, in raw faecal sludge, and 2.203%, 0.392%, and 0.531%, respectively, in the final vermicompost.

Calcium is crucial for soil health and plant growth, as it aids in cell wall formation and stabilizes soil structure [107]. The high calcium content in the vermicompost suggests that it can improve soil quality, particularly in acidic soils. Studies have shown that composting faecal sludge with bulking agents like sawdust or coffee husks can influence the calcium content in the final compost. For instance, research indicates that the choice of bulking agent significantly affects the mineralization and stabilization of organic matter, which in turn impacts nutrient content, including calcium [107].

Magnesium, on the other hand, is vital for chlorophyll production. The presence of these nutrients at moderate levels in the vermicompost suggests that it can support balanced plant nutrition. Furthermore, the calcium and magnesium contents of the vermicompost produced at the Rulindo Faecal Sludge Treatment Plant (FSTP) fall within the range reported by [108], who indicated that calcium and magnesium in compost were 2.90% and 0.5%, respectively, in their research. Additionally, magnesium may range between 0.36% and 1.41%, according to previous studies.

Sulfur is necessary for protein synthesis and enzyme function in plants. It also forms strong associations with other macro-elements, such as P-N, K-N, K-P, S-P, and S-K [85]. The adequate sulfur content in the vermicompost makes it beneficial for crop growth, particularly in sulfur-deficient soils [76]. Based on the results obtained, the sulfur content in the vermicompost was 0.531%, indicating that the produced vermicompost is rich in sulfur compared to findings reported by [86], who determined sulfur content in compost generated from faecal sludge to be 0.17%. However, according to the Compost Standards in Bangladesh, sulfur content should range between 0.1% and 0.5% [109]. This confirms that the produced vermicompost approximately falls within this range. Additionally, it aligns with observations reported by [110], who found sulfur concentrations in biomass samples on a dry basis ranging between $1.3 \pm 0.1\%$ and $15 \pm 0.1\%$.

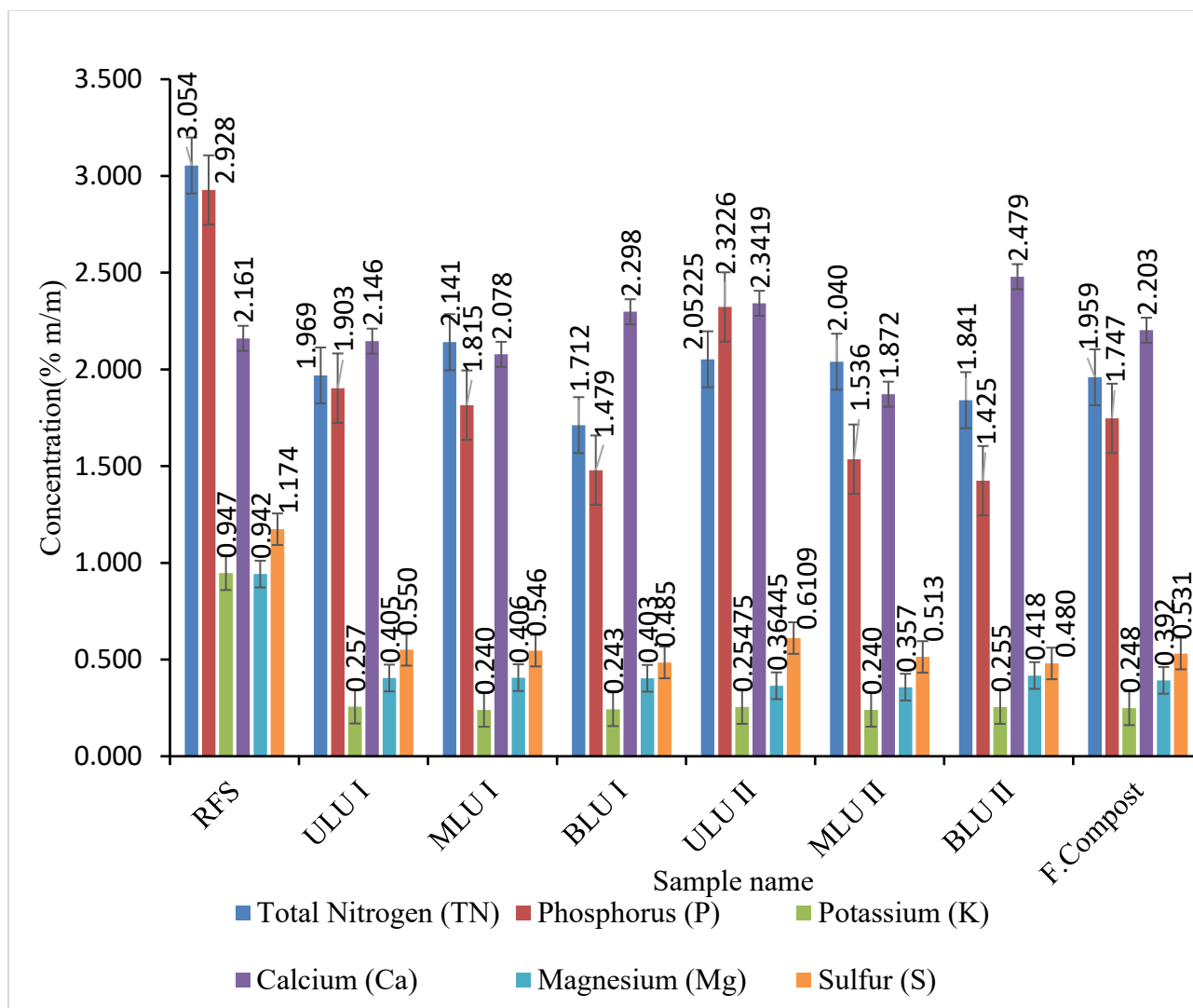


Figure 21: Macro-Nutrients obtained in raw faecal sludge and vermicompost produced at Rulindo FSTP

(RFS: Raw faecal sludge, ULU I: Upper layer unit I, MLU I: Middle layer unit I, BLU I: Bottom layer unit I, ULU II: Upper layer unit II, MLU II: Middle layer Unit II, BLU II: Bottom layer unit II, F. compost: Final compost)

Micro-Nutrients content of vermicompost produced at Rulindo FSTP

Except for zinc, which is slightly lower, some of the micronutrient concentrations obtained in our study at the Rulindo Faecal Sludge Treatment Plant (FSTP) are slightly higher compared to the research findings reported by [111]. In their study, the micronutrient concentrations in dewatered faecal sludge compost were found to be iron (18.72 mg/kg, or 0.001872%), zinc (699.74 mg/kg,

or 0.069974%), and manganese (321.88 mg/kg, or 0.032188%). From the results obtained, there is an increase in micronutrient concentrations in vermicompost compared to raw faecal sludge, except for sodium and zinc, as shown in the following data: 0.674%, 0.003%, 0.194%, 0.041%, 1.217%, and 0.088% for aluminum, lithium, sodium, manganese, iron, and zinc, respectively, in raw faecal sludge. In contrast, the concentrations in vermicompost are as follows: aluminum (Al = 0.734%), iron (Fe = 2.565%), manganese (Mn = 0.058%), zinc (Zn = 0.052%), lithium (Li = 0.008%), and sodium (Na = 0.089%).

The decrease in concentrations of sodium and zinc indicates that the composting process, facilitated by the biological activity of *Eisenia fetida*, can reduce the levels of these elements. In contrast, for elements that increased in concentration, this suggests that the process may not efficiently reduce such elements. The rise in their concentrations may be attributed to the degradation of organic matter in raw faecal sludge, which has a higher organic content compared to vermicompost. This behavior was also observed by [107] in their research. It is believed that the increase in nutrient and heavy metal concentrations is due to the substantial degradation of organic matter, which results in a net loss of dry weight during composting. For example, when sawdust was used as a bulking agent, the concentrations increased from the initial feedstock to mature compost as follows: sodium from 1 to 1.4 g/kg, iron from 2175.1 to 2955.7 mg/kg, manganese from 109.5 to 204.3 mg/kg, and zinc from 245.3 to 349.4 mg/kg [107]. Except for zinc, which is slightly lower, some of the micronutrient concentrations obtained in our study at Rulindo Faecal Sludge Treatment Plant (FSTP) are slightly higher compared to the research findings reported by [111]. In their study, the micronutrient concentrations in dewatered faecal sludge compost were found to be iron (18.72 mg/kg, or 0.001872%), zinc (699.74 mg/kg, or 0.069974%), and manganese (321.88 mg/kg, or 0.032188%).

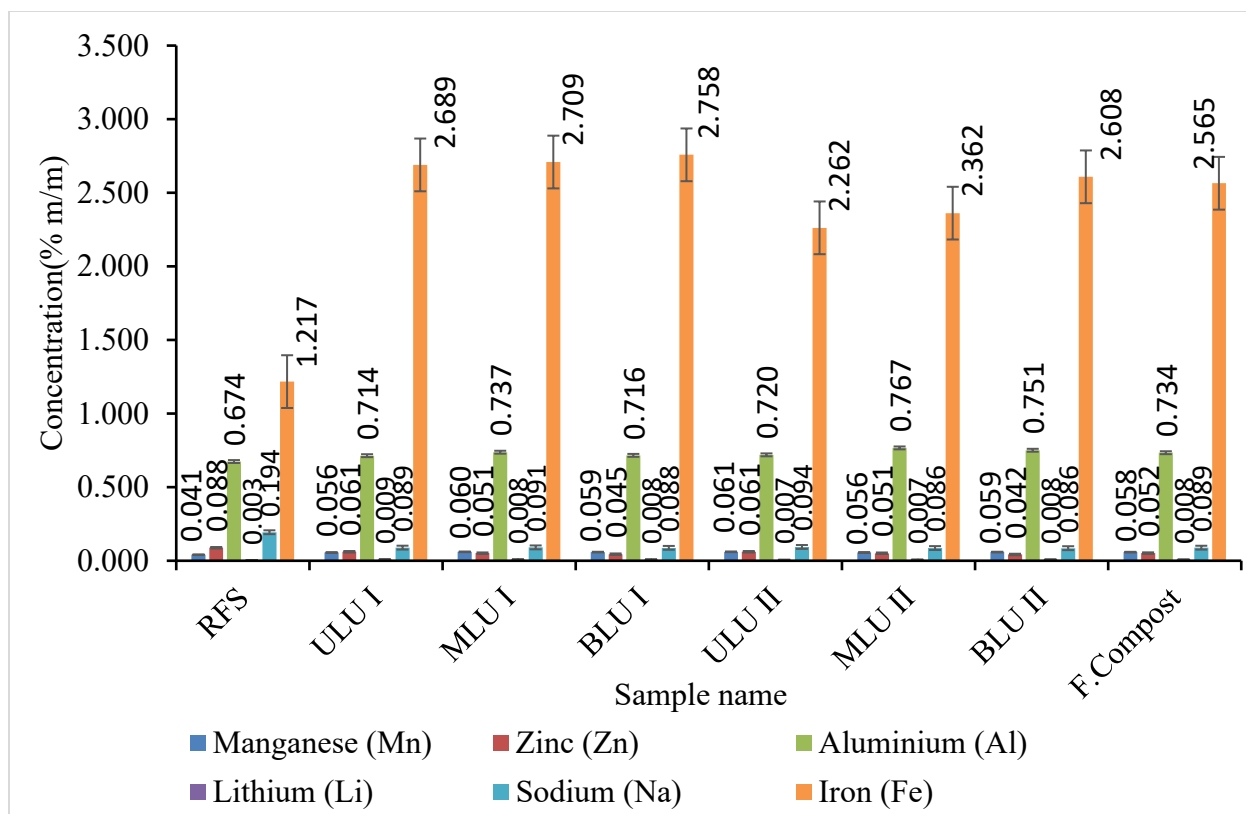


Figure 22: Micro-nutrients results detected in raw faecal sludge and vermicompost at RFSTP

(RFS: Raw faecal sludge, ULU I: Upper layer unit I, MLU I: Middle layer unit I, BLU I: Bottom layer unit I, ULU II: Upper layer unit II, MLU II: Middle layer Unit II, BLU II: Bottom layer unit II, F. compost: Final compost)

Heavy metals content in vermicompost from Rulindo FSTP

Other than the toxic heavy metals like Cd, As, Hg, Ni, Cr, and Cu, the only heavy metals detected in the produced vermicompost are vanadium (V = 0.004%), titanium (Ti = 0.047 %), and lead (Pb = 0.004%). Among these identified elements, lead (Pb) is the only one considered a toxic element. However, its concentration is very low (0.004 %) to pose any harmful impact based on the maximum levels indicated by the UNBS standard for organic fertilizers and soil conditioners (Pb < 100 mg/kg, or 0.01%) [99], as well as the Cambodia national standard for organic fertilizers (Pb < 100 mg/kg) [9]. Additionally, research conducted in Kenya indicated that Pb should not exceed 840 mg/kg, based on the permissible level in sludge set by the USEPA (1993) [112]. Some studies, including those by [9], [107], and [105], have investigated heavy metal contamination in faecal sludge for agricultural production. These studies classify zinc as an essential heavy metal found in

organic fertilizers. However, the concentration of zinc in the vermicompost produced at Rulindo Faecal Sludge Treatment Plant (FSTP) ($Zn = 0.052\%$) remains low compared to the maximum limits indicated by various standards: the Cambodia standard ($Zn = 1000 \text{ mg/kg}$, or 0.1%) [9], the EU range, which specifies that zinc should fall between $210\text{--}4000 \text{ mg/kg}$ [107], and the French norms ($Zn < 600 \text{ mg/kg}$, or 0.06%) [105]. This confirms that the produced vermicompost is free from toxic heavy metals.

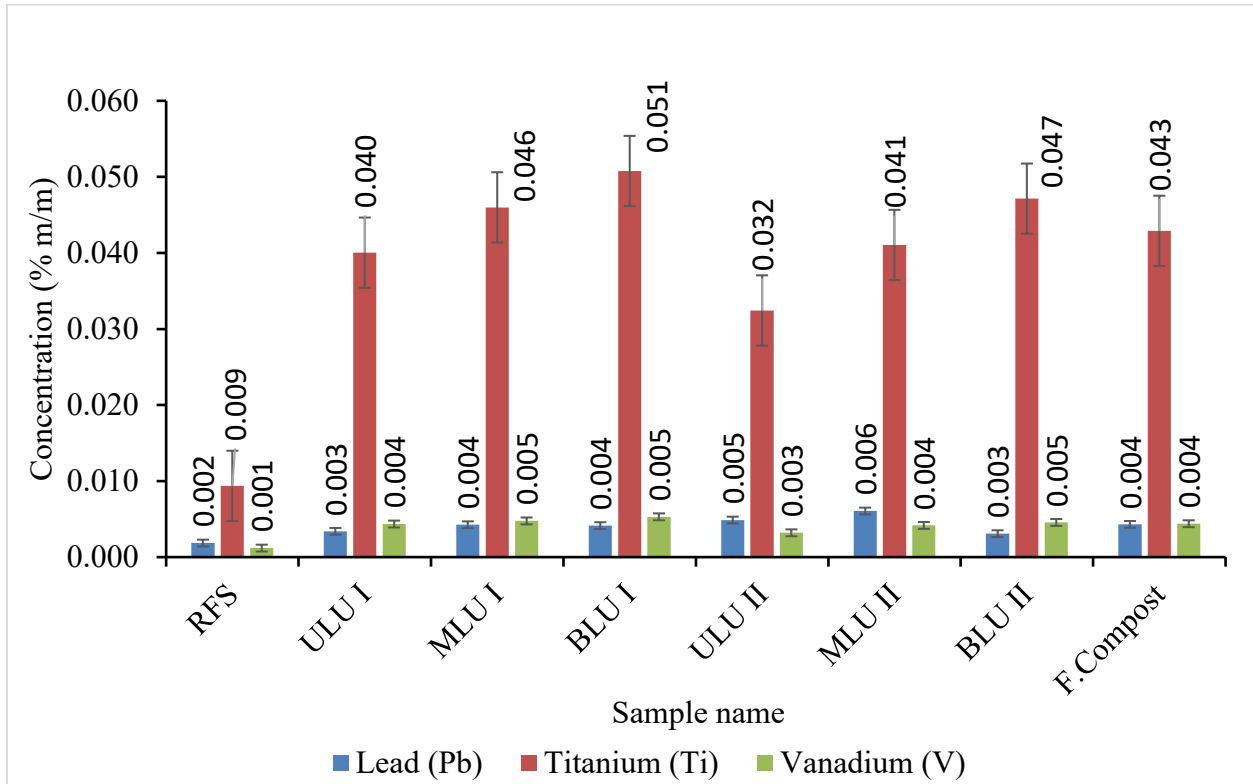


Figure 23: Heavy metals detected in raw faecal sludge and vermicompost produced at Rulindo FSTP

(RFS: Raw faecal sludge, ULU I: Upper layer unit I, MLU I: Middle layer unit I, BLU I: Bottom layer unit I, ULU II: Upper layer unit II, MLU II: Middle layer Unit II, BLU II: Bottom layer unit II, F. compost: Final compost)

CONCLUSION

In Rwanda and in other developing nations, insufficient faecal sludge treatment, inadequate policies and regulations, and weak institutional frameworks are key challenges that hinder sustainable sanitation, where a central sewer network is lacking, on-site treatment and reuse of faecal sludge have emerged as critical strategies to address sanitation challenges while promoting resource recovery. However, the chemical and biological safety of end products (e.g., vermicompost and treated wastewater) resulting from faecal sludge treatment remains a significant concern, particularly regarding potential contamination by pathogens, heavy metals, organic pollutants, excess nutrients, or other harmful substances. The physicochemical and biological safety of vermicompost and wastewater from the Rulindo Faecal Sludge Treatment Plant in Rwanda was comprehensively analyzed using analytical methods, including UV spectrophotometry and ICP-OES. Although some physical and chemical parameters of the wastewater comply with the permissible limits set by the Rwanda Utilities Regulatory Agency (RURA) and the Food and Agriculture Organization (FAO) for wastewater discharge and reuse in irrigation, high concentrations of nutrients (e.g., nitrate and phosphate) and microbial loads (total coliforms, faecal coliforms, *Salmonella*, and *Shigella*) raise concerns regarding its suitability for irrigation. Furthermore, MAR, EC, and salinity values observed in the effluent were high, indicating its unsuitability for irrigation. Notably, the vermicompost produced at the Rulindo FSTP is of high quality for agricultural use based on nutrients contents and is chemically safe, as toxic heavy metals were undetectable in the samples, however, other researches on biological safety to investigate its biological contaminants including *Ascaris* eggs are highly recommended. Taken together, the findings of this study indicate that although certain physical and chemical parameters of wastewater meet permissible limits established by local and international standards for wastewater discharge and irrigation, the Rulindo FSTP may still pose environmental pollution risks and threaten human health due to elevated levels of nutrients, salinity, moisture absorption rate, and bacterial contamination. Therefore, the use of effluent from Rulindo FSTP for irrigation should be restricted until further technical adjustments are implemented to ensure safety. Further research is needed to build on these findings, while regular monitoring of untreated wastewater discharge at Rulindo FSTP is essential to assess water quality. Additionally, enhanced disinfection protocols are required to address pathogenic contamination and meet acceptable safety standards.

RECOMMENDATIONS

Rulindo FSTP effluents may pose threats to the environment, agriculture, and public health. To mitigate these concerns and assure safe, sustainable reuse, a multifaceted approach is suggested:

1. Upgrade Treatment Protocols for Biological and Chemical Safety

Implement advanced disinfection measures (e.g., UV treatment, chlorination) to improve pathogen removal (Salmonella, Shigella, coliforms) and align effluent quality with safety standards. Concurrently, adopt nutrient and salinity mitigation strategies, such as vegetative buffers (e.g., canna plants) or dilution of treated water, to reduce eutrophication risks and soil degradation.

2. Strengthening Monitoring and Regulatory Alignment

Institutionalize routine water quality monitoring at Rulindo FSTP to track critical parameters (e.g., pathogens, nutrients, conductivity) and ensure compliance with local and international reuse guidelines. Collaborate with regulatory bodies to establish clear, enforceable standards for permissible limits of contaminants in treated water and vermicompost.

3. Scale Innovations Through Pilot Testing

Launch pilot projects to evaluate the efficacy of treated vermicompost and water on diverse crops under local agricultural conditions. These studies will refine application guidelines, optimize resource recovery, and support scalable, context-specific solutions for sustainable waste management.

By integrating technical upgrades, robust monitoring, community engagement, and policy alignment, Rwanda can transform faecal sludge treatment into a model for safe resource recovery. This approach balances agricultural productivity with environmental protection and public health, advancing national sanitation goals while contributing to global sustainability targets.

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APPENDICES

Appendix 1: Results table of Physical-chemical characterization of water sample at Rulindo FSTP (see figure: 11, 12 and 13)

Sampling points	RFS	LST		TBF II		TRWT		Limit	FAO
Parameters		Mean	SD	Mean	SD	Mean	SD	RURA Guidelines	Irrigation water guidelines
PH	8.19	7.42	0.177	7.595	0.152	7.83	0.099	5 - 9	6.5-8.4
Conductivity(mS/cm)	8.68	6.21	1.704	5.64	0.354	5.17	0.608	4000 μS/cm	700 μS/cm
Temperature (C)	28.9	22.90	2.828	21	0.636	20.85	1.202	3 (variation)	
D.O	0.13	0.60	0.141	3.11	0.346	3.74	0.226	60%sat.	
Turbidity (NTU)		148.50	157.685	24.5	10.960	13.00	2.828	30	
TSS (mg/L)		75.00	80.610	13.5	3.182	7.50	2.121	50	≤10
COD (mg/L)	3514	392.33	228.636	214	28.284	199.00	16.497	250	
BOD5(mg/L)	659	141.25	129.754	39	3.889	38.50	2.828	50	

RFS: Raw faecal sludge, LST: liquid storage tank, TBF II: tiger biofilter II outlet, TRWT: treated water tank, SD: standard deviation.

Appendix 2: Results table of Chemical characteristics of water produced from Rulindo FSTP (see figure 14, 15 and 17)

Sampling points	LST		TBF II Outlet	TRWT		LIMIT		
	Mean	SD	Mean	SD	Mean	SD	RURA guidelines (2020)	FAO guidelines (2015)
Total Nitrogen (TN) (mg/l)	383.000	72.832	386.250	114.198	305.750	81.671	30	
Nitrates (mg/l)	349.311	42.866	371.850	127.067	225.530	70.046	20	20-30
Total Phosphorus (mg/L)	28.400	18.554	9.580	6.788	8.380	0.651	5	
Potassium (K) (mg/L)	366.500	40.305	416.500	21.920	397.500	3.536	100	2
Sodium (Na)(mg/L)	7.540	0.820	8.910	0.127	8.360	0.028	400	
Calcium (Ca)(mg/L)	16.925	1.391	19.385	8.350	12.834	2.855	500	
Magnesium (Mg)(mg/L)	79.050	20.662	116.740	64.997	103.030	43.628		
Sulfur(S)(mg/L)	74.380	5.148	67.460	2.178	58.950	1.485		
Boron (B)(mg/L)	0.148	0.129	0.139	0.091	0.315	0.243	0.5	
Strontium (Sr)(mg/L)	0.162	0.032	0.193	0.105	0.128	0.039		
Silcon (Si)(mg/L)	4.106	0.104	3.754	0.147	2.512	0.122		
Antimony (Sb)(mg/L)	3.433	0.087	3.139	0.122	2.100	0.102		
Iron (Fe)(mg/L)	0.496	0.628	0.077	0.015	0.027	0.026	3.5	5
Manganese (Mn)(mg/L)	0.408	0.188	0.252	0.109	0.540	0.064	0.1	0.2
Vanadium (V)(mg/L)	0.113	0.018	0.134	0.043	0.133	0.031		0.1
Sodium adsorption ratio (SAR) (%)	1.088		1.08		1.098			<6
Magnesium adsorption ratio (MAR) (%)	82.37		85.37		88.92			<50

Appendix 3: Results table of biological characterization of water produced from Rulindo FSTP (see figure 18 and 19)

Sampling points	RFS		LST		BF II Outlet		TRWT		R.E
parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Total coliforms/ (cfu/100 mL)	2.015E+07	2.30E+07	3.130E+06	4.12E+06	2.092E+06	2.59E+06	1.302E+06	1.62E+06	93.54
Faecal coliforms/ (cfu/100 mL)	5.100E+05	1.56E+05	8.025E+06	1.13E+07	8.965E+05	1.22E+06	2.295E+05	3.17E+05	55.00
E. coli/ (cfu/100 mL)	2.510E+05	2.76E+05	1.900E+04	1.13E+04	5.501E+03	7.78E+03	<1.000E+00	0.00E+00	100.00
Salmonella/ (cfu/100 mL)	1.030E+05	6.65E+04	9.325E+04	1.00E+05	3.100E+04	2.26E+04	8.500E+03	6.36E+03	91.75
Shigella/ (cfu/100 mL)	1.565E+08	9.69E+07	3.101E+07	4.04E+07	2.780E+06	1.16E+06	2.992E+06	3.41E+06	98.09

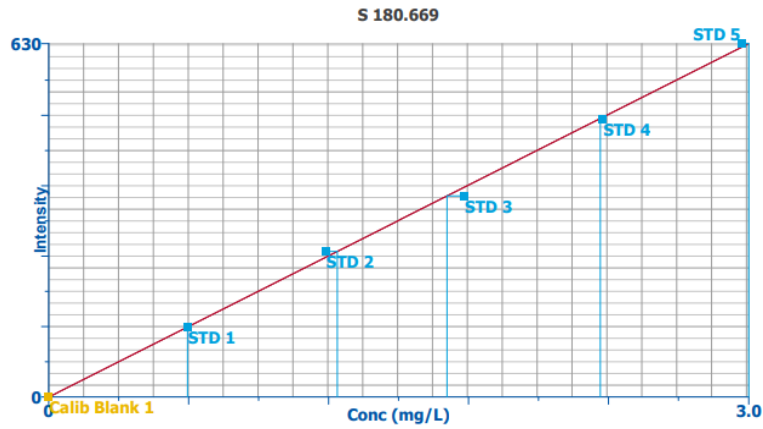
Appendix 4: Proximate analysis results of solid vermicompost produced from Rulindo FSTP (see figureb20)

PARAMETERS	RFS		ULU I		MLU I		BLU I		ULU II		MLU II		BLU II		t. compost
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	T.Mean
Moisture content (%)	94.18	2.04	51.15	7.90	61.81	1.38	60.67	0.48	54.08	0.37	60.98	1.48	60.57	1.16	58.21
Total solid (%)	5.83	2.04	48.86	7.90	38.19	1.38	39.33	0.48	45.93	0.37	39.02	1.48	39.43	1.16	41.79
Volatile Organic matter (VOS)	61.09	0.98	39.38	6.84	42.82	2.67	34.24	2.22	41.05	7.59	40.80	2.90	36.82	3.86	39.18
Total Organic carbon (TOC)	35.43	0.57	22.84	3.97	24.84	1.55	19.86	1.29	23.81	4.41	23.66	1.68	21.36	2.24	22.73

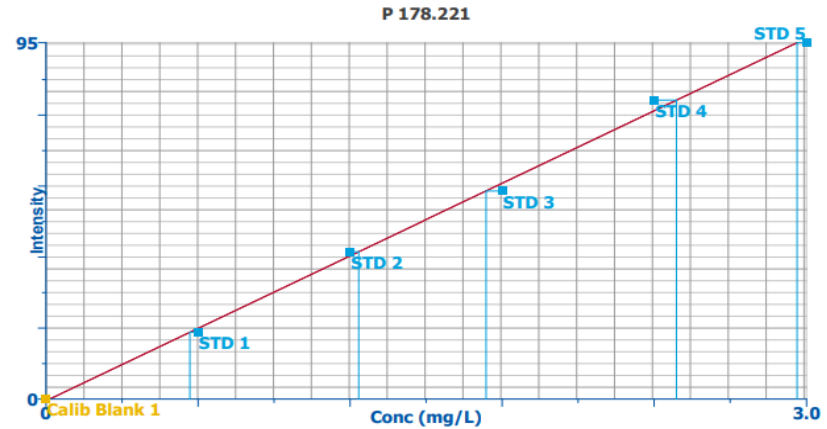
Appendix 5: Results table of Elemental composition of vermicompost produced at Rulindo FSTP (see figure 21, 22 and 23)

	RFS		ULU I		MLU I		BLU I		ULU II		MLU II		BLU II		compost
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	T.mean
Al (%)	0.674	0.0688	0.714	0.0446	0.737	0.032	0.716	0.0019	0.720	0.0174	0.767	0.0526	0.751	0.0339	0.734
Ca (%)	2.161	0.1071	2.146	0.1281	2.078	0.153	2.298	0.1779	2.342	0.3148	1.872	0.0485	2.479	0.5199	2.203
K (%)	0.947	0.1730	0.257	0.0007	0.240	0.007	0.243	0.0052	0.255	0.0066	0.240	0.0117	0.255	0.0113	0.248
Li (%)	0.003	0.0001	0.009	0.0006	0.008	0.001	0.008	0.0004	0.007	0.0007	0.007	0.0002	0.008	0.0000	0.008
Mg (%)	0.942	0.1817	0.405	0.0614	0.406	0.012	0.403	0.0128	0.364	0.0660	0.357	0.0351	0.418	0.0571	0.392
Na (%)	0.194	0.0091	0.089	0.0008	0.091	0.004	0.088	0.0030	0.094	0.0051	0.086	0.0045	0.086	0.0018	0.089
P (%)	2.928	0.0467	1.903	0.0917	1.815	0.055	1.479	0.1673	2.323	0.2406	1.536	0.1037	1.425	0.1986	1.747
S (%)	1.174	0.0280	0.550	0.0631	0.546	0.077	0.485	0.0148	0.611	0.0694	0.513	0.0277	0.480	0.0054	0.531
Mn (%)	0.041	0.0031	0.056	0.0074	0.060	0.001	0.059	0.0016	0.061	0.0082	0.056	0.0011	0.059	0.0014	0.058
Fe (%)	1.217	0.0266	2.689	0.2092	2.709	0.320	2.758	0.2357	2.262	0.1834	2.362	0.1798	2.608	0.0920	2.565
Pb (%)	0.002	0.0002	0.003	0.0003	0.004	0.001	0.004	0.0010	0.005	0.0033	0.006	0.0026	0.003	0.0000	0.004
Ti (%)	0.009	0.0005	0.040	0.0043	0.046	0.005	0.051	0.0012	0.032	0.0087	0.041	0.0020	0.047	0.0039	0.043
V (%)	0.001	0.0001	0.004	0.0006	0.005	0.001	0.005	0.0001	0.003	0.0008	0.004	0.0001	0.005	0.0004	0.004
Zn (%)	0.088	0.0240	0.061	0.0019	0.051	0.006	0.045	0.0019	0.061	0.0004	0.051	0.0050	0.042	0.0023	0.052
TN (%)	3.05		1.969		2.141		1.712		2.052		2.04		1.841		1.959
C:N	12:1														12:1

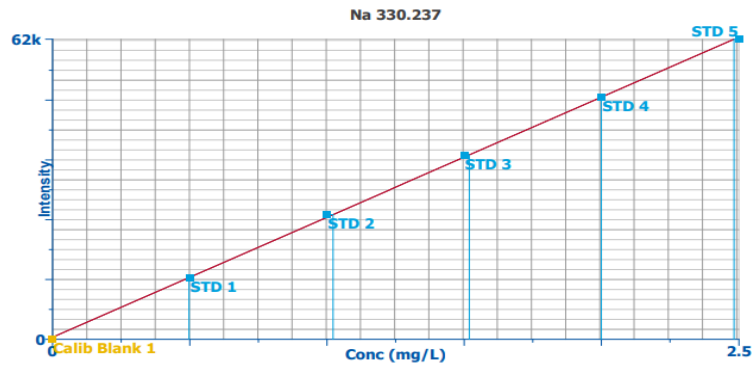
Appendix 6: Calibration curves of some elements analyzed on ICP OES



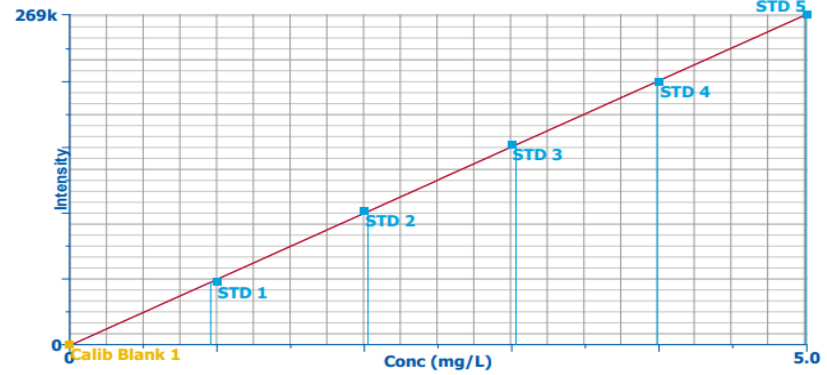
Slope 207.78329
 Intercept -0.26479
 Correlation coefficient 0.999270



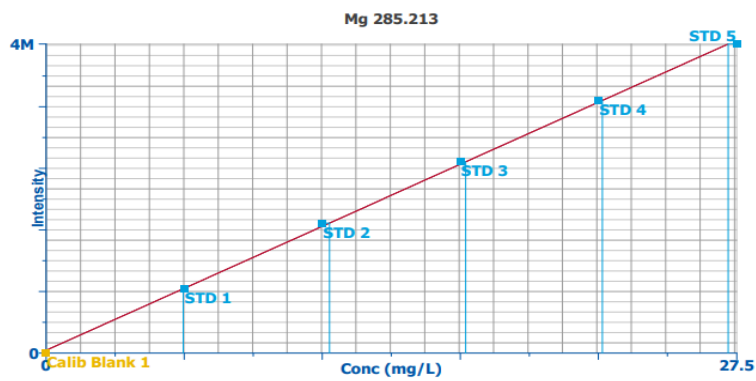
Slope 32.28176
 Intercept -0.46769
 Correlation coefficient 0.998770
 Mn 403.075



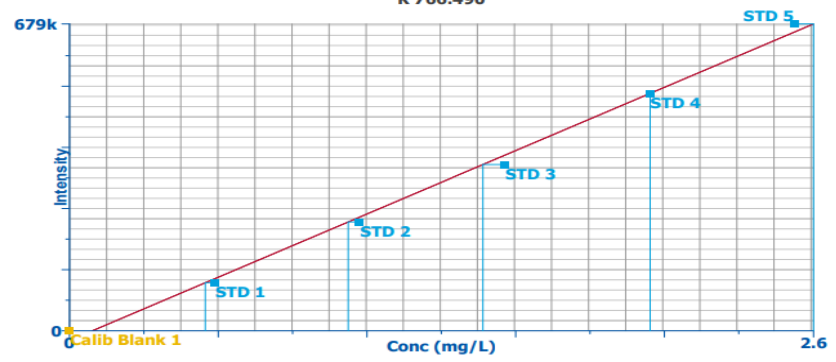
Slope 24651.2060
 Intercept 429.27116
 Correlation coefficient 0.999839



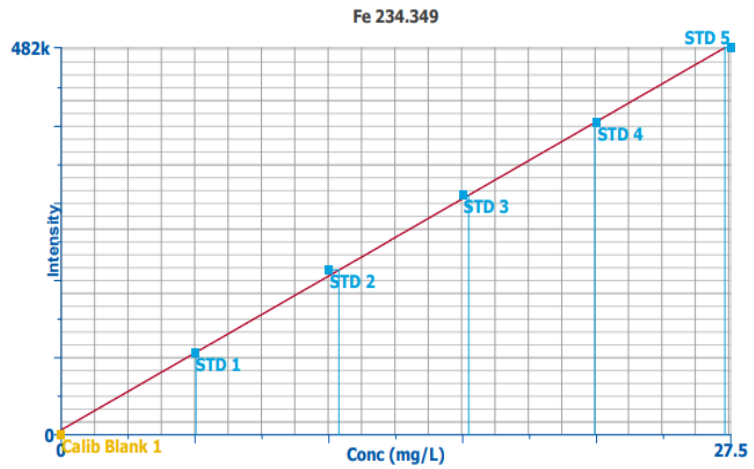
Slope 53982.7926
 Intercept -677.07469
 Correlation coefficient 0.999898
 K 766.490



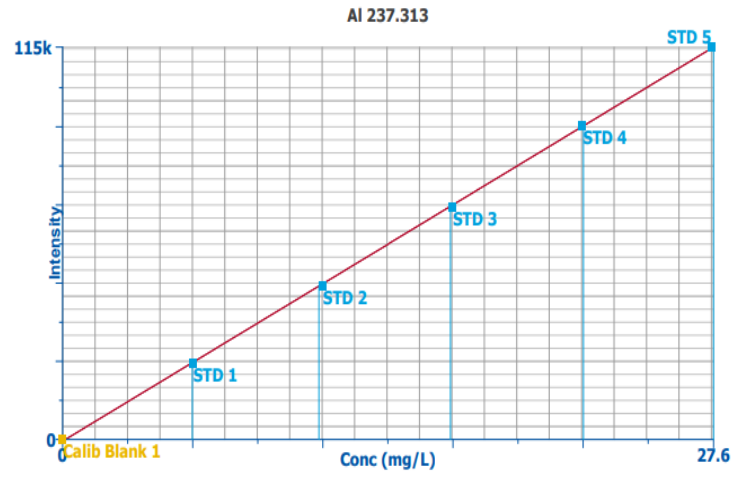
Slope 131912.100
 Intercept 32459.3669
 Correlation coefficient 0.999693



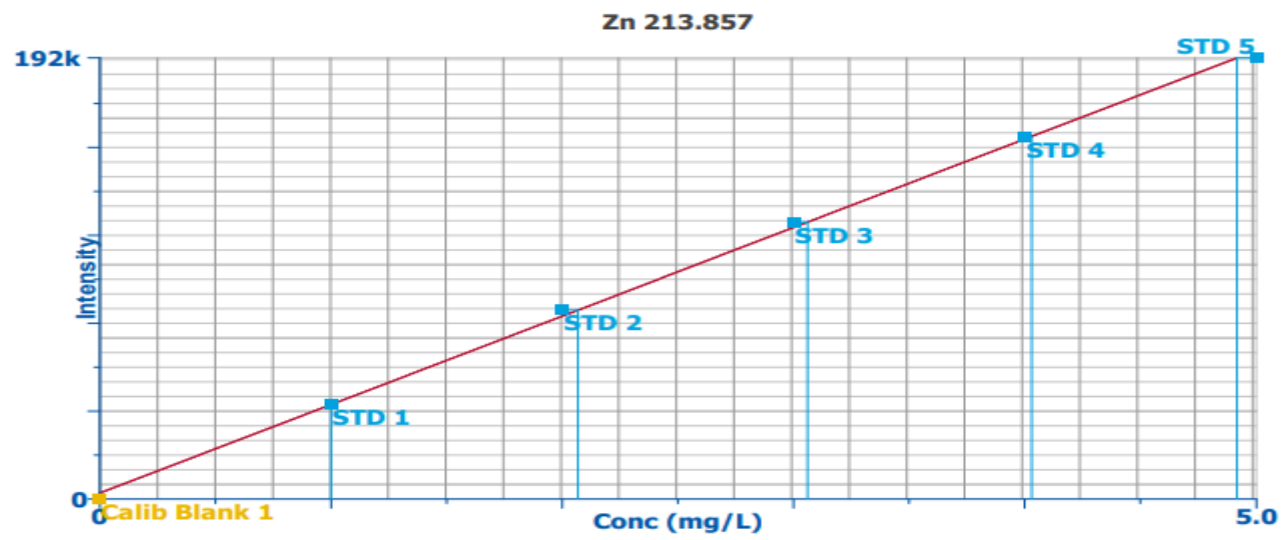
Slope 272940.586
 Intercept -21419.812
 Correlation coefficient 0.997891



Slope 17473.3961
 Intercept 6087.14913
 Correlation coefficient 0.999614

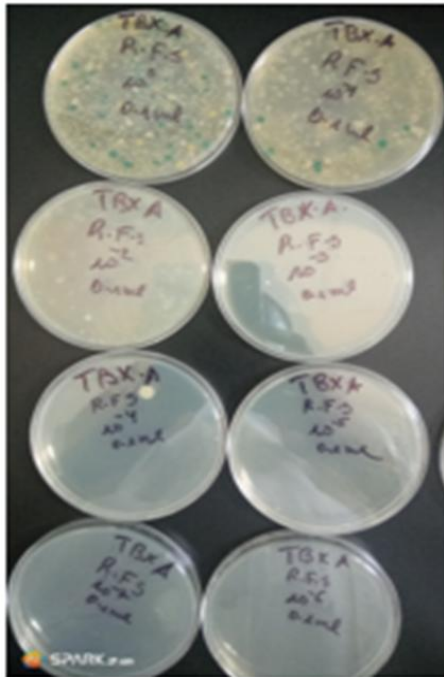


Slope 4202.28644
 Intercept -479.80781
 Correlation coefficient 0.999954

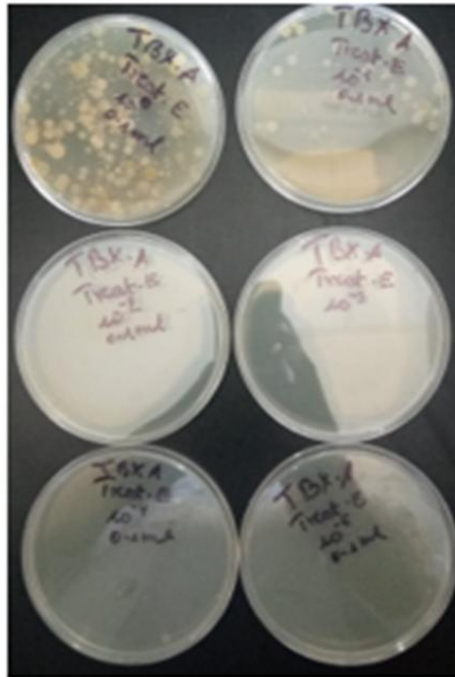


Slope 38522.5596
 Intercept 2632.32079
 Correlation coefficient 0.999421

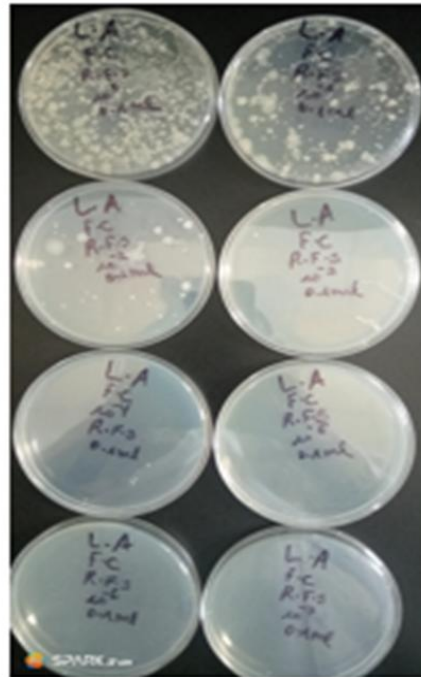
Appendix 7: Colonies in Petri-dishes after incubation period



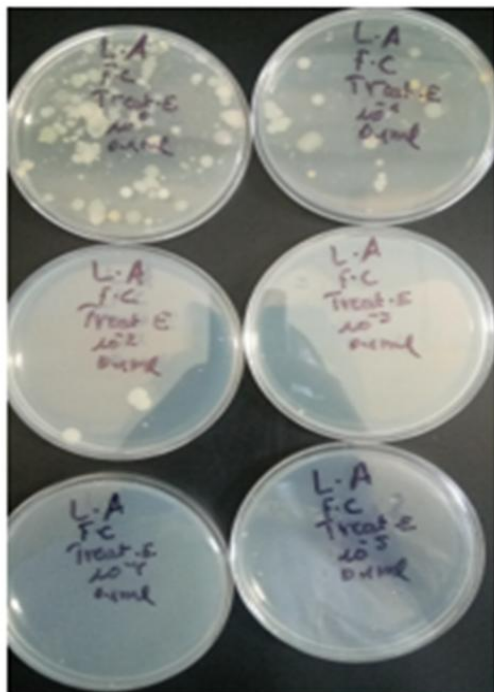
E.coli (RFS)



E.coli (TRWT)



Feecal coliforms (RFS)



Feecal coliforms (TRWT)



Salmonella & Shigella (RFS)



Salmonella & Shigella (TRWT)