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EFFECT OF INCREASED HEAVY METAL TOXICITY ON THE QUALITY OF VERMICOMPOSTED BIOSOLIDS: (A CASE STUDY OF SLUDGE FROM BUGOLOBI AND LUBIGI WASTEWATER TREATMENT PLANTS).

BY

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A RESEARCH DISSERTATION SUBMITTED TO KYAMBOGO UNIVERSITY DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN WATER AND SANITATION ENGINEERING (GMEW) DEGREE OF KYAMBOGO UNIVERSITY

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DECLARATION

I, **Mafabi Grace**, declare that this submission is entirely original work of mine, and that, to the best of my knowledge and belief, it does not contain any text or writing that has been published or written by someone else, or that has been used to award a degree at a university or other higher education institution, unless proper attribution has been made in the text and reference list.

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APPROVAL

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DEDICATION

I dedicate this thesis to my wonderful family, particularly to my daughter Celine Tibyonza, who has had to put up with my absence while I worked on this project.

ACKNOWLEDGMENT

My deepest appreciation goes out to my research supervisors, Dr. Eng. Anne Nakagiri and Dr. Charles Onyutha for their support and counsel throughout this research. I would like to express my gratitude towards Mr. Aziz from MAURIK and Mr. Said Ntambi for the assistance rendered in attending to the various experimental setups for the research.

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I pray for the utmost blessings from the Almighty God.

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LIST OF ACRONYMS

COD	Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
TSS	Total Suspended Solids
MPL	Minimum Permissible Limits
NEMA	National Environment Management Authority
UNBS	Uganda National Bureau of Standards
mg/L	Milligrams per Liter
Mg/Kg	Milligrams per Kilogram
NWSC	National Water and Sewerage Cooperation
BSTP	Bugolobi Sewage Treatment Plant
WWTP	Wastewater Treatment Plant
TOC	Total Organic Carbon
MUARIK	Makerere University Agriculture Research Institute Kabanyolo
UIRI	Uganda Industrial Research Institute
TKN	Total Kjeldahl Nitrogen
C/N	Carbon to Nitrogen ratio
TN	Total Nitrogen
AAS	Atomic Absorption Spectrometry
SDGs	Sustainable Development Goals
NDPIII	National Development Plan III
IMO	Indigenous Micro-Organisms
WSPs	Waste Stabilisation Ponds
ANOVA	Analysis of Variance
NDIR	Non-Dispersive Infrared Absorption (NDIR).

ABSTRACT

Sewage sludge management is one of the current global environmental issues. In many developing countries like Uganda, the problem is amplified due to uncontrolled and untreated industrial effluents. This tends to increase heavy metal toxicity of wastewater and consequently that of sewage sludge thereby posing danger to human health and the environment. This research explored means of stabilising such sewage sludge of increased heavy metal toxicity through vermicomposting to allow for safe disposal. Sewage sludge samples were picked from Bugolobi and Lubigi sewage treatment plants and tested for quality and bio-safety. An optimal earthworm stocking density for sewage sludge vermicomposting was determined using 0.0, 1.0, 2.0, 2.5, and 3.5kgworms/square meter of exposed surface area of vermicompost bins. Using an optimal earthworm stocking density, vermicomposting of sewage sludge at higher heavy metal concentration was carried out and the resulting biosolids assessed with respect to their Total Nitrogen, Phosphorus, Organic Carbon, Carbon-Nitrogen ratio, faecal coliforms, and electro-conductivity. The results from the tests were compared with standards for organic fertilizers set by Uganda National Bureau of Standards. The results indicated the presence of faecal coliforms, Lead levels, and Electro-conductivity beyond maximum permissible limits. The characteristic of sewage sludge was observed to improve with age while sewage sludge from Lubigi treatment plant which predominantly receives domestic waste water exhibited relatively better characteristics in comparison to sludge from Bugolobi treatment plant. Earthworm stocking density had significant influence on the level of faecal coliforms and electro-conductivity of vermicompost. The research ascertained that a stocking density of 2.5kgworms/m^2 was optimal for sewage sludge vermicomposting. Heavy metal toxicity was observed to cause earthworm mortality beyond 0.0021 moles of Lead in sewage sludge. Increased bio-accumulation of heavy metals Lead and Zinc in earthworms was observed at higher heavy metal concentrations. Vermicomposting at higher heavy metal toxicity levels yielded better quality biosolids in terms of bio-safety and quality except for increment in concentration of the spiked metals and electro-conductivity. Further research, therefore, on speciation of spiked heavy metal concentrations after vermicomposting is recommended in order to conclusively ascertain the absence of any potential threat of such vermicompost to the environment.

Keywords: - Vermicomposting, Heavy metal, Toxicity, Biosolid, Sludge, Stocking density

CHAPTER 1: INTRODUCTION

1.1 Background

Waste water management has become a global concern which has impacted on the environment and aspects of human survival for instance access to safe water with continued disposal of untreated wastewater from urban centres directly into water bodies (UNEP. 2017). In a number of developing counties in Africa, and more especially Uganda, the case is not any different especially with the escalating level of industrialisation and urbanisation (Oyoo, 2010). The wastewater treatment capacity in Uganda has not grown in tandem with the growing population and industrialisation for instance only 10% of Kampala city's 1.5 million inhabitants are served by a sewerage system whereas the remaining 90% rely on on-site sanitation. As a result, more than 46% of generated waste water from settlements and industries is released untreated into the environment (IWA, 2017).

In line with the above, most industries in Kampala city directly dispose of their untreated effluents in Nakivubo channel. The channel eventually pours into Lake Victoria through the Inner Murchison Bay thereby polluting and affecting this ecosystem (Komakech et al., 2014, Mbabazi et al., 2010, Björnberg and Elenström, (2016). An assessment of the Nakivubo wastewater quality showed an increased level of pollution with chemical oxygen demand (COD), biochemical oxygen demand (BOD), and total suspended solids (TSS) levels exceeding maximum permissible limits (MPL) i.e., 336mg/L, 200.3mg/L and 266mg/l respectively (Kayima et al., 2010). The channel also exhibits an increased heavy metal content with Lead (Pb) and Mercury (Hg) levels surpassing MPL i.e. >0.1 mg/l and >0.01 mg/l (Nansubuga et al., 2015). The increased pollution of the channel significantly affects water supply in Kampala for instance more chemicals such as Aluminium Sulphate (Alum) are required to treat water to acceptable drinking standards. This implies that the required Alum may soon reach the maximum allowable limit for human consumption (Oyoo, 2010).

To curb pollution of Lake Victoria by wastewater from Nakivubo channel, Bugolobi Sewage Treatment Plant (BSTP) has been extended by National Water and Sewerage Cooperation (NWSC) to 45,000M^3. This will ensure pre-treatment of wastewater from the channel estimated at 0.63m^3/s flow (Kayima et al., 2010). This is expected to improve the quality as well as increase the quantity of sludge generated at BSTP beyond the current 285M^3/day (KFW, 2017). Additionally, wastewater treatment plants (WWTPs) mainly abstract nutrients and organics through both primary and secondary treatments leaving heavy metals, pesticides, and medications that are released into the environment as micro-pollutants (Kim and Farnazo, 2017).

The disposal of sludge with such toxic contaminants as pesticides, pharmaceuticals, acids, heavy metals, radioactive material, and pathogens is very challenging due to its potential negative effect on the environment (Zhang, Wang, and Wang, 2017) and humans health (Lowman et al., 2013). In Uganda, sewage sludge is normally disposed of onto agricultural fields and according to NWSC, this kind of application is limited to non-food crop production such as floriculture due to the possible existence of multiple heavy metals (NETWAS Uganda, 2011).

An analysis of the bio-solids at BSTP showed the presence of heavy metals for example Chromium (Cr) at 35.00±1.06 (mg/Kg) and Cu at 7.20±0.04 (mg/Kg) (Nakiguli et al., 2018). This plant's high heavy metal constitution of its sewage sludge was also ascertained by Sekabira et al., 2010. In their current state, therefore, the biosolids from Bugolobi STP have a lot of salts and heavy metals in them which could lead to several health effects such as cancers in humans and soil salinization with repeated application.

More than 50% of the sewage sludge generated at BSTP is sold to farmers as fertilizers (NETWAS Uganda, 2011). This therefore calls for a more effective sewage sludge stabilisation mechanism to warrant safe disposal especially in consideration of the increased heavy metal toxicity levels of the sludge with addition of wastewater from the Nakivubo channel. Anaerobic digestion alone which is the most common form of waste water treatment in Uganda in form of septic tanks, ponds and conventional WWTP has limitations in terms of the final effluent and sludge quality. According to McCord et al. (2020), anaerobic digester effluent does not meet waste water discharge quality standards for field application as it poses water quality issues if not properly handled due to its constituents such as TS - 26,091 mg L^{-1}, COD - 3471 mg L^{-1}, and BOD_5 = 246 mg L^{-1}.

According to Sinha et al. (2009), vermicomposting of sewage using a variety of earthworm species such as Red Tiger worm spp and African Night Crawler spp offers total stabilisation with significant reduction in the heavy metal content. This is possible due to the adaptability of the earthworms to live in varying conditions in which they feed on sludge converting it into a more stabilized and nutritious product called vermicompost which can then be safely applied to land.

In Uganda, the use of earthworms has been successfully tried in a pilot study of tiger worm-based toilets in northern Uganda (Nyende and Achire, 2018). However, little has been studied on the potential use of vermicomposting to improve heavy metal-laden sewage sludge from STPs in developing countries like Uganda to benefit all kinds of agricultural applications.

1.2: Problem Statement

Bugolobi STP has been expanded to 45,000m^3 to include pre-treatment of wastewater from Nakivubo channel which is highly polluted with heavy metals including Lead, Zinc and Cadmium (Sekabira et al., 2010). The channel has also been observed to contain high amounts of heavy metals including Chromium (Cr) – 35.00±1.06 (mg/Kg) and Copper (Cu) - 7.20±0.04 (mg/Kg) as a result of pollutant sources such as storm water and industries (Nakiguli et al., 2018, Kayima et al., 2010). The incorporation of waste water from Nakivubo channel characterised by increased heavy metal toxicity poses a disposal problem for resultant sewage sludge at the plant due to environmental threat from constituent pollutants.

Additionally, sewage sludge management in Uganda has been mainly achieved through land application for instance more than 50% of sewage sludge generated at Bugolobi STP is sold to farmers as manure (NETWAS Uganda, 2011). However, due to environmental concerns of land application such as pollution of underground and surface water sources, cancers, and genetic disorders in humans (Harrison and Oakes, 2003), several countries have backtracked on this practice. With growing emphasis on reuse and energy recovery options, the anticipated increase in heavy metal concentration of sewage sludge at Bugolobi STP with the additional of waste water from Nakivubo channel makes reuse of this sludge unattainable due to prevailing environmental and health constraints. Therefore, this research explores the use of vermicomposting in stabilizing such sewage sludge into an environmentally friendly and reusable compost.

1.3: Objectives / Research Questions

1.3.1: Main Objective

This study evaluated the impact of higher sewage sludge heavy metal concentrations on the quality of vermicomposted bio-solids.

1.3.2: Specific Objectives

I. Characterize the quality of sewage sludge at Bugolobi and Lubigi STPs.
II. Determine an optimal earthworm stocking density for sewage sludge vermicomposting.
III. Assess vermicompost quality for sewage sludge at spiked heavy metal concentrations.

1.3.3: Research Questions

I. Does sewage sludge from Bugolobi and Lubigi STPs meet disposal standards for organic fertilizers in Uganda?
II. Are the levels of Lead and Zinc within sewage sludge at the two plants within maximum permissible limits?
III. How can this sludge be vermicomposted to achieve maximum stabilization before disposal?
IV. Does heavy metal toxicity of sewage sludge hinder the performance of vermicomposting and if so, at what level does this happen?

1.4: Justification

The inclusion of pre-treatment of wastewater from the Nakivubo channel with all its toxic contaminants at Bugolobi STP results into elevation of these pollutants' levels in resultant sludge thereby making re-use as a disposal mechanism unattainable. Pollutants such as heavy metals and pharmaceuticals at such heightened levels in sludge do not warrant safe land application which is the main disposal mechanism in Uganda. To address this challenge, more environmentally friendly mechanisms such as vermicomposting have been used to stabilise sewage sludge. This has been attributed to the presence of Metallothionein (MTs) proteins in earthworms which play a big role for instance in the bio-remediation of heavy metals with the formation of insoluble transformative products which render the heavy metal pollutants insoluble, unavailable, and consequently stabilised.

The earthworms have been known to be highly adaptive to variation in their environments. However, with the unprecedented levels of toxicity from Nakivubo channel and consequently resultant sewage sludge, it's worth ascertaining the possibility of vermicomposting for such sewage sludge. This research, therefore, explored the potential of sewage sludge stabilisation with vermicomposting at increased heavy metal toxicity levels to warrant re-use within the environment with minimal impacts to both flora and fauna.

1.5: Significance

I. This research is aligned with Sustainable Development Goals (SDGs) 3, 6, 14, and 15 as well as Uganda's National Development Plan (NDP III) for the promotion of environmentally friendly water and resource management through strengthening pollution control and minimizing land degradation.

II. In line with SDG 13 – climate action, vermicomposting of sewage sludge ensures limited emission of greenhouse gases as compared to other sludge stabilisation mechanisms and thereby cutting the global warming effect from the release of such gases to the atmosphere.
III. Vermicomposting is a cost-effective process that offers a more nutrients enriched and homogeneous product that is of high quality and bio-safety for use as organic fertilizer in agricultural production of both food and non-food crops.
IV. The earthworms from the process can be reused as feed-in poultry and fish farming.

1.6: Research scope

The scope of this research is summarized in the sub-sections below;

1.6.1: Content Scope

A comprehensive literature review on wastewater treatment, stabilisation, and disposal of sewage sludge with emphasis on vermicomposting was performed. The research mainly focused on heavy metals i.e. Lead (Pb) and Zinc (Zn) with spiking of the heavy metal concentration in subsequent setups achieved with Lead. Qualification of bio-solids after vermicomposting was mainly based on the quality and bio-safety characteristics of the bio-solids i.e. level of nutrients (Total Nitrogen – TN & Total Phosphorous - TP), pathogens (faecal coliforms), Total Organic Carbon (TOC), Electro-Conductivity (EC and pH), and the Carbon to Nitrogen Ratio (C/N).

1.6.2: Location Scope

This research utilized samples from Bugolobi sewerage treatment plant located at Latitude 0.318483° and Longitude 32.606890°. The experimental setup for vermicomposting of sewage sludge was performed at Makerere University Agricultural Research Institute - Kabanyolo (MUARIK).

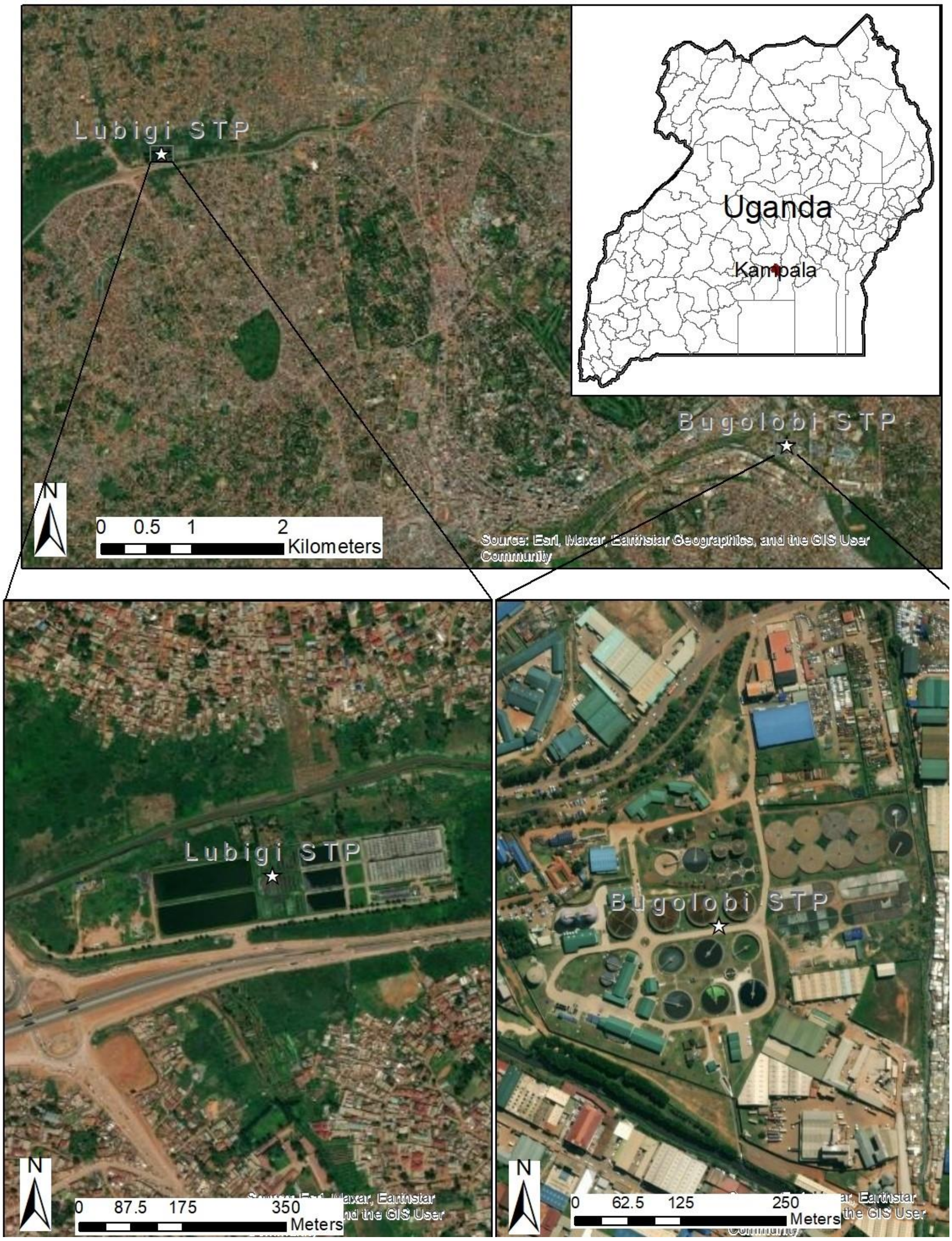

Figure 1. 1: Aerial view of Bugolobi and Lubigi Sewage Treatment Plants

1.6.3: Time scope

The experimental setups and laboratory testing were started in November 2021 and progressed through to April 2022 during which two separate setups each running for at least forty-two (42) days were performed followed by data analysis and reporting writing throughout May and June 2022.

1.7: Conceptual Framework

The conceptual organization presented in Figure 1-2 below provided the basis for planning this study. In this conceptual framework, factors such as the temperature, moisture content, and pH of sewage sludge were the independent variables which were closely monitored and controlled since they had significant bearing on the outcomes of the experimental setups. Optimisation of the vermicomposting process was achieved through inoculation of the setups with Indigenous Micro-Organisms (IMO). Given that the research is investigating the quality of vermicomposted bio-solids, the biosolids characteristics such as heavy metal content, nutrients content, microbial count, Total Organic Carbon (TOC), and Carbon to Nitrogen ratio (C/N) which depicts maturity of biosolids formed the dependent variables. Most of the parameters indicated were measurable and were reached after a series of laboratory testing.

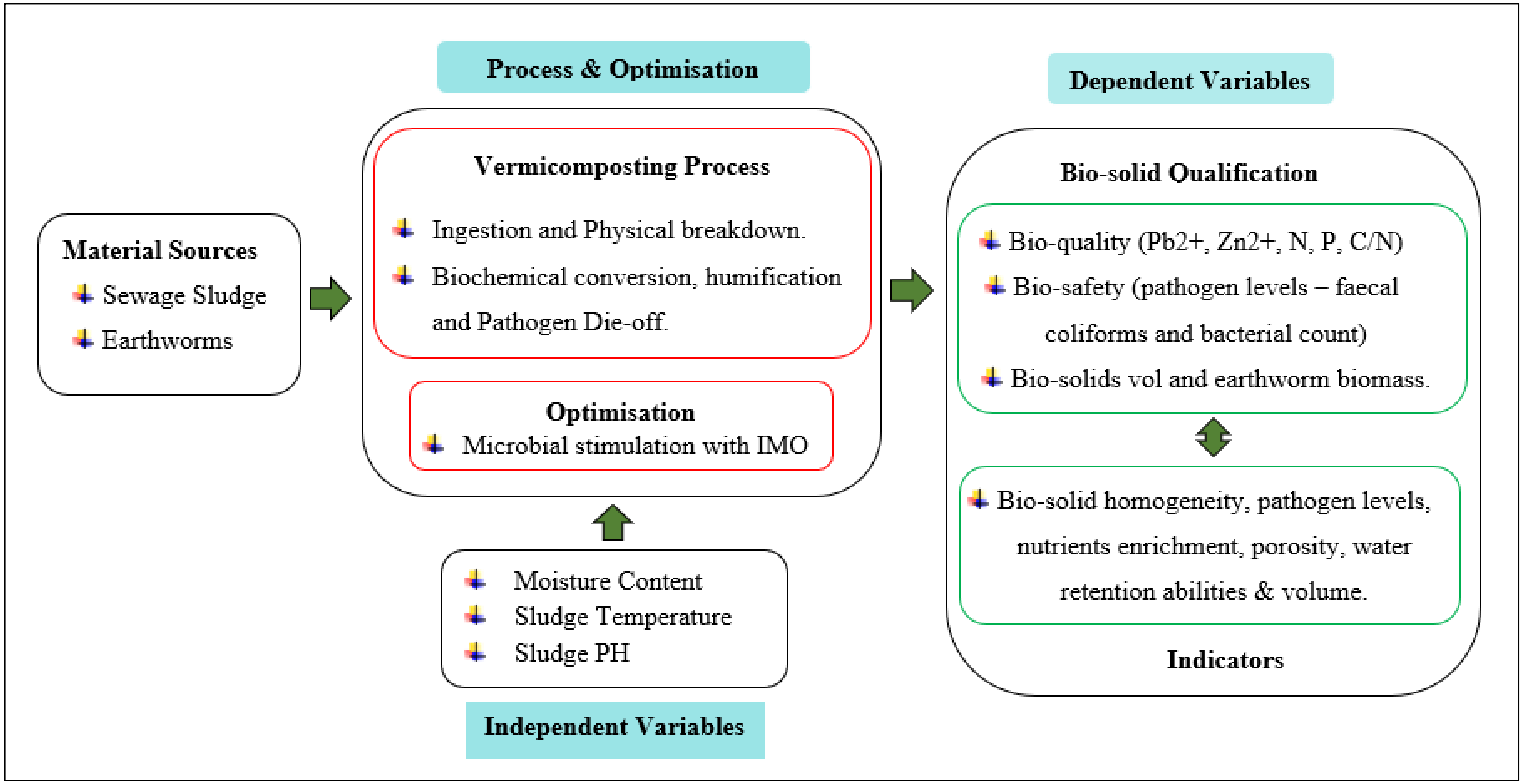

Figure 1. 2: Conceptual Framework for the Research

1.8: Outline of the dissertation

There are five chapters in this thesis. The backdrop of the study, problem description, aims, research questions, significance, and justification for undertaking the research are all provided in Chapter 1 of the introduction. In Chapter 2, the study's pertinent literature review, information that has already been published in journals and research papers is cited. The materials and procedures utilized in the experimental setting are discussed in Chapter 3, the laboratory results from the experimental setups are presented in Chapter 4, their analysis is discussed in Chapter 4, and the research study is concluded with recommendations in Chapter 5.

CHAPTER 2: LITERATURE REVIEW

2.1: Introduction

This chapter discusses research literature that has been published in books and periodicals in the past. An understanding of wastewater treatment, sewage sludge, sewage sludge stabilisation, and disposal mechanisms is provided by this review with an in-depth review of vermicomposting which was the focus of this research.

2.2: Wastewater Treatment

Wastewater treatment involves the removal of organics, pathogens and nutrients such as Nitrogen and Phosphorous from municipal sewage water before disposal. However, a number of micro-pollutants like pesticides, pharmaceuticals, and Nano-sized metals are not adequately removed during the process which then become a threat to the environment and the entire human and ecological system (Kim and Farnazo, 2017). The process separates the liquid and solid phases of waste water. The treated liquid part/effluent generated from wastewater treatment is usually disposed of directly into water bodies such as seas, lakes, and rivers where natural purification involving dilution and bacteriological action takes place (R Wood, Bell, and Wilkinson, 1993). The solid phase usually referred to as sludge, is a semi-solid product of waste water treatment that comprises of substantial amounts of organics and nutrients which require delicate handling but carry beneficial advantages (F Dilek Sanin, Clarkson and P Aarne Vesilind, 2011).

The two fundamental reasons for wastewater treatment are environmental protection through pollution control and public health safeguards in controlling the prevalence of waterborne diseases such as cholera and dysentery (Gray, 2004). In wastewater treatment, eliminating pollutants or altering the hazardous and complicated nature of constituents, requires a combination of physical, chemical, and biological processes to be applied to produce a more stable product with minimal impact on the environment. Wastewater treatment can be done through conventional wastewater treatment or use of waste stabilisation ponds. The section below highlights the processes involved in each of the wastewater treatment mechanism.

2.2.1: Conventional Wastewater Treatment

Traditional wastewater treatment was started in the late 19th and early 20th centuries mainly in the United Kingdom and United States to control pollution of water bodies by subjecting waste water through a combination of physical, biological, and chemical processes remove some or most of the pollutants (Nathanson and Archis Ambulkar, 2018). Stringent regulations over time have created a need for higher levels of wastewater treatment for instance pre-treatment of industrial wastewater to prevent negative effects of toxic chemicals onto the biological processes used at sewage treatment plants. The section below therefore presents a step by step process of wastewater treatment including preliminary, primary, secondary / bio-filtration, and tertiary wastewater treatment, as well as sludge treatment, which stabilizes the sludge produced during the process.

Preliminary Treatment

The goal of this initial stage of wastewater treatment is to remove oils, large solids, fats, and other substances from wastewater in order to safeguard facilities that treat waste water after this stage and therefore has limited effect on pathogens in the liquid waste stream. During this stage, screening is carried out with the help of bar racks (20 to 60 mm), bar screens (6 to 12 mm), and fine screens (0.25 to 3mm) to remove floatable or suspended coarse solids such as rubber, vegetable, plastic, and paper among other items. With the aid of grit chambers, grit removal is accomplished by gravity separation of heavy particles with a specific gravity greater than putrescible organic matter, such as sand and gravel. Flow equalization to achieve a constant flow rate is also ensured during this stage (Dodane et al, 2012).

Figure 2. 1: Niayes faecal sludge treatment plant screening in Dakar, Senegal

(Source: https://www.researchgate.net/figure/Bar-screen-at-Niayes-faecal-sludge-treatment-plant-Dakar-Senegal-photo-Linda-Strande_fig3_269037413. Accessed at 23/7/2023.)

Primary Treatment

Removal of settleable organic solids and floating organic material is the primary goal of primary treatment in order to lower the suspended solids burden for future treatment operations. Primary wastewater treatment is usually divided into two units i.e. primary sedimentation and complementary processes. Under primary sedimentation, the tanks are constructed with gently sloped bottoms and have sludge hoppers with relatively steep sides and when efficiently operated can 50 to 65 percent of the suspended solids and 25 to 40 percent of BOD (J. Paul Guyer, 2018).

According to Nathanson and Archis Ambulkar (2022), primary treatment of wastewater eliminates around 60% of the total suspended particles and roughly 35% of BOD. Complementary processes include coagulation which is mainly about chemical destabilisation of colloids allowing aggregation and flocculation. This results into the formation of large particles / flocs which thereafter settle to the bottom of the sedimentation tank. Primary treatment results in a highly organic-rich sludge that is pumped to sludge digesters where anaerobic digestion takes place for about eight (8) weeks before sludge is finally transferred to drying beds (Demirbas, Edris and Alalayah, 2017).

Secondary Treatment

This focuses on removing soluble / dissolved biodegradable organic constituents that escape primary treatment by biological means in which micro-organisms feed on these organic constituents for their growth and reproduction releasing carbon-dioxide, water, and energy. According to Frankel, (2022), secondary treatment of wastewater removes more than 90% of remaining suspended solids from the wastewater which is accomplished with aerobic, anaerobic or anoxic bacteria. Secondary treatment at wastewater treatment plants can either be carried out using trickling filters or through the activated sludge (AS) process.

According to Foladori et al. (2010), the AS process is the most commonly used technique and relies on biomass growth through oxidation of organic or inorganic compounds to form secondary sludge. In the trickling filter method, a bed of stones is constructed onto which sewage is sprayed from the top, and bacteria at the surface of these stones absorb pollutants from the sewage reducing its BOD as it descends the stones after which it is collected. The benefits and drawbacks of the two most widely applied techniques, biological filters and activated sludge processes, are summarised in Table 2.1 below.

Table 2. 1: Comparison between filters and AS processes

S/N	Mechanism	Advantages	Disadvantages
1	Biological Filters	A minimal energy footprint	In general, performance is worse than with an activated sludge approach.
		Simple operation that requires less upkeep and supervision than the activated sludge method	Large-scale constructions if nitrogen removal standards are mandated.
		Excellent settling qualities of the sludge	High capital costs (which may be 20% more than those associated with activated sludge)
		Greater resistance to contaminants and load fluctuations than activated sludge	Effective pre-treatment is necessary
		In most cases tailored to small communities	Responsiveness to clogging
2	Activated Sludge	Whatever size of community-appropriate (except very small ones)	Capital costs that are relatively high
		Good removal of all pollution-related factors (SS, COD, BOD5, N by nitrification and denitrification)	High level of energy use
		Optimized for the defense of delicate receiving regions	Regular monitoring and qualified workers are needed.
		Partial stabilisation of sludge	Ability to detect hydraulic overloads
		Simple to use concurrent de-phosphatation	Sludge's settling characteristics are not always simple to manage.
			High sludge production that needs to be thickened.

Source: (Marcos Von Sperling and International Water Association, 2007)

Tertiary Treatment

This is the final stage of wastewater treatment and removes inorganic compounds, bacteria, viruses, and parasites which makes the effluent safe for reuse and disposal in the environment. The pathogens are eliminated by means of procedures like coagulation, flocculation, sedimentation, filtering, and disinfection.

2.2.2: Waste Stabilisation Ponds

Waste stabilisation ponds (WSPs) remove sediments, nutrients, pathogens, and helminth eggs effectively by using a network of three or more man-made ponds i.e. anaerobic, facultative, and maturation ponds sunk into the earth. According to R. S. Suglo, (2018), WSPs are large, shallow basins commonly used for the treatment of domestic waste water and agro-industrial waste. They are an appropriate technology for treatment and removal of pathogenic microorganisms from wastewater in tropical and subtropical regions that are characteristic of warm climatic conditions. WSPs are mainly natural, efficient, cost-effective methods of treating wastewater in rural and remote communities as compared to other techniques because of their distinctive qualities that guarantee ease of use, low energy requirements, and low maintenance needs (Bolton et al., 2010).

In addition, Ansa et al. (2012) observed that WSPs achieved superior treatment efficiencies as compared to conventional techniques like activated sludge with bacteria reductions averaging 1 to 2 logs whereas almost 99% of protozoa and helminth eggs were removed. The benefits and drawbacks of WSPs as a wastewater treatment technology are outlined in Table 2.2 below.

Table 2. 2: Advantages and Disadvantages of WSPs

S/N	Advantages	Disadvantages
1	WSPs can withstand hydraulic and biological stress loads.	WSPs demand a considerable amount of land.
2	They successfully reduce pathogens, BOD, and solids.	High startup costs, including, if relevant, the cost of land
3	When used with aquaculture, WSPs remove a significant amount of nutrients.	Professional design and construction are necessary.
4	Due to their minimal operational costs, they are economical.	Sludge needs to be properly removed and treated.
5	WSPs don't need electricity to function.	De-sludging (normally every few years)
6	If designed and maintained properly, there won't be any fly or odor issues.	Mosquito management is necessary.
7	With locally accessible resources, WSPs can be constructed and repaired.	Monitoring the salinity is necessary if the effluent is reused.
8	Effluent can be recycled for irrigation in agriculture or aquaculture.	In some cases, colder climates may not be suitable

Source: https://sswm.info/factsheet/waste-stabilisation-ponds, Accessed 25/7/2023.

The effectiveness of WSPs in eliminating organic micro-pollutants depends on a number of variables, including pond type and configuration, operational treatment plant parameters, wastewater quality particularly the pollutant's characteristics, sunlight, temperature, redox conditions, and pH. (Gruchlik, Linge and Joll, 2018). Algal concentrations have a direct impact on the disinfection of wastewater, although with higher efficiency in warmer climatic circumstances and according to Liu, Hall and Champagne, (2015), elevated pH and dissolved oxygen (DO) which are linked to the presence of algae, are crucial elements for efficient disinfection of wastewater. Davies-Colley et al. (1999), observed that increasing sunlight exposure too enhanced WSPs' ability to disinfect.

Similar to traditional wastewater treatment, wastewater is first subjected to preliminary treatment, which includes screening and grit removal after which it is systematically channelled through a series of waste stabilization ponds in the order of anaerobic ponds, facultative ponds, and maturation ponds as detailed below.

Anaerobic Treatment Ponds (APs)

Anaerobic ponds are the smallest of the ponds and created for pre-treatment of wastewater to remove BOD, SS, and COD from wastewater. They are constructed with a depth of 3-5m in order to limit the penetration of oxygen to the inner layers and therefore maintain the life of anaerobic bacteria that breakdown organic matter contained in domestic and industrial wastewaters. According Marcos Von Sperling, (2007), the rate of conversion of organic matter in APs is a bit low owing to the slow growth rate of anaerobic bacteria but with BOD removal efficiencies averaging 50-70%. Accordingly, to ensure efficient operations of the APs, they should be designed for a detention time of 3-6 days, depth of 3.5-5m, and length to breadth ratio of 1:3 (Marcos Von Sperling, 2007).

The process tends to produce Hydrogen Sulphide (H_2S) gas whose rate of production increases with increasing organic loading from 2.76 to 5.77 kg COD/m3.d, the PH from 3 to 7, the hypoxic conditions, and the hydraulic retention duration (Polprasert and Chatsanguthai, 1988), (Chen et al., (2017). The production of H_2S gas typically results in odour nuisance at the WSPs. This, however, can be minimised by using impermeable or permeable coverings, controlling sulphate concentration in raw wastewater to below 500mg SO_4^{2-}/l, recirculating secondary effluent, and designing the pond properly (Paing, Picot, and Sambuco, 2003).

Facultative Treatment Ponds (FPs)

Facultative ponds (FPs) typically have a retention span of 5–30 days and are 1–2.5 m deep. According to Pearson et al., 2005, the efficiency of FPs increases in shallower ponds whereas the H2S emission starts to manifest at depths greater than 2.2m, and therefore 1.25m average depth is recommended for efficiency of the FPs (Tilley et al., 2008).

FPs comprise of aerobic zones close to the surface and anaerobic zone at deeper layers with capacity to treat wastewater with BOD range of 100 to 400 kg/ha/day corresponding to 10 to 40 g/m^2/day at temperatures above 20°C. The algal production of oxygen occurs in the aerobic zones up to a depth of approximately 500mm at which light can penetrate whereas within the deeper layers, there is limited oxygen and the pond is predominantly anaerobic. The FP therefore ensures further treatment of wastewater through sedimentation and aerobic oxidation of organic material, reduction of odour and pathogens, in addition to sedimentation of organics that settle as sludge at the bottom of the ponds (Tilley et al., 2008).

Maturation Treatment Ponds (MPs)

Pathogen elimination is the major purpose of maturation ponds. They receive effluent from facultative ponds and their size and number depends on the desired bacteriological quality of the final effluent. MPs have a retention duration of 15-20 days and are 0.5-1.5 m deep. The pond's shallowness guarantees that it is well oxygenated and provides enough light and temperature, both of which are essential to the pond's functioning mechanism. Under optimal conditions, MPs removal efficiencies for MPs in series can reach 6 log10 for faecal bacteria, 4 log10 for viruses, protozoan, cysts, and helminth eggs though this is highly variable with a removal efficiency of the pathogen in practice only averaging 3 log10 (Verbyla, 2017).

MPs only remove a small portion of BOD_5 and some nutrients and their performance are affected by the hydraulic retention time, water clarity, PH (high PH required), and pond depth. Pond depth is significant in way that it influences factors such as ultraviolet radiation, high DO, and temperature which highly aids the process, and predation by other organisms. The MPs must be adequately designed in terms of size and number as these factors have significant influence the quality of the effluent emitted (Ho, Van Echelpoel and Goethals, 2017).

At the end of wastewater treatment, the effluent from either conventional treatment plants or waste stabilisation ponds is discharged into the environment and the sewage sludge undergoes further stabilisation before final disposal. Stabilisation of sewage sludge is required because the efficiency of most STPs doesn't meet minimum standards (Vitez, Sevcikova, and Oppeltova, 2012) in addition to the formation of more or less toxic compounds for instance surfactant alkyl phenol ethoxylate (APE) group from detergents, motor oils, paints that gives rise to more toxic products, especially after anaerobic digestion (Petrović and Barceló, 2000). This calls for careful handling of the resultant sludge and stabilisation of the same where necessary before disposal.

2.3: Sewage sludge

A semi-solid and composite by-product of wastewater treatment comprising a mixture of solids, organics, and chemical pollutants (Lowman et al., 2013). About 50% of organic matter contained in sewage is converted into sludge during the wastewater treatment processes (Kacprzak et al., 2017) which sludge accounts for about 1% of the total wastewater treated (Turovskiĭ and Mathai, 2006). The limited efficiency of WWTPs across the world however has a significant bearing on the final composition of chemicals within sewage sludge in that several pollutants remain concentrated in the sludge after the treatment process which makes its disposal challenging (Smith, 2009).

According to Usman et al. (2012), sewage sludge contains significant amounts of organic matter and nutrients like nitrogen (N) and phosphorous (P), as well as chemical irritants, toxic heavy metals like zinc (Zn), lead (Pb), and cadmium (Cd), as well as pathogens like bacteria, viruses, and protozoa that could be harmful to humans, animals, and plants. Since sewage sludge contains both organic matter and nutrients, it is widely used as a soil supplement. For example, more than 60% of the 6.2 million dry metric tons (MT) of sewage sludge produced annually in the United States of America (USA) are dumped on land (Harrison et al., 2006). A few underdeveloped nations, including South Africa, have had success using sewage sludge on agricultural land (Snyman, 2007) while in others like Oman, the practice has not yet picked up due to the association of sludge with human waste (Jaffar Abdul Khaliq et al., 2016).

The sewage sludge produced by Bugolobi STP and other wastewater treatment facilities is disposed of in landfills in Uganda as a soil amendment after composting for six to eight weeks (Lowman et al., 2013). Regulations established by international and local environmental protection organizations, such as the Environmental Protection Agency (EPA) in the United States and the Uganda Bureau of Standards (UNBS) in Uganda, serve as guidelines for the management of sewage sludge. However, in Uganda, adherence to set guidelines is still low which poses a great threat to the environment. More emphasis should be directed on reuse and energy recovery options such as biogas production as sustainable disposal options for sewage sludge generated across the country.

2.3.1: Characterisation of sewage sludge

Sewage sludge is usually categorized depending on its degree of treatment and constituents to guide its disposal mechanism as a way of minimizing adverse effects to the environment and public health. In order to control the availability of heavy metals like arsenic, cadmium, chromium, copper, lead, mercury, molybdenum, nickel, selenium, and zinc in sewage sludge as well as pathogens and general environmental pollution, the EPA and the European Union (EU) have issued several guidelines and standards for the safe disposal of sewage sludge, such as 40 CFR Part 503 and European Council Directive 86/278/EEC (Commission of the European Communities (CEC), 1986) (EPA Biosolids Biennial Review Report, 2021).

According to EPA, (1993), biosolids are either categorized as class A, class B or Exceptional Quality (EQ). Class A biosolids are those in which pathogens have been reduced to below detectable levels, whereas class B biosolids have pathogens reduced to levels at which they are unlikely to pose a threat to public health and the environment and therefore should not be applied to land or exposed to the public under specific conditions such as after considerable reduction of pathogens by environmental factors to below detectable levels i.e. Salmonella sp. bacteria should have a density of less than 3 MPN per 4 grams of total solids or fewer than 1,000 Most Probable Number (MPN) per gram of total solids (dry weight) for faecal coliforms (EPA Biosolids Biennial Review Report, 2021).

In light of the potential dangers to public health from their usage, the guidelines state that Class B biosolids cannot be sold or donated for use in agriculture or any other type of public use. Strict restriction in the use of such biosolids is desired to limit risks involved for instance 30 days minimum restriction for public exposure, crop harvesting, and grazing. Class B biosolids must have a density of faecal coliforms less than 2 million MPN or CFU per gram of total solids (dry weight) at the time of disposal (Boczek, 2019). On the other hand, due to their high quality, EQ biosolids are permitted for use in any type of land application because they meet Class A criteria and adhere to tight heavy metal pollutant restrictions (States., 1995; Spinosa and Vesilind, 2015).

Similar regulations have been partially enacted in Uganda by UNBS, (2017), but enforcement has been difficult due to significant expenses associated with ensuring sewage sludge is stabilized to the necessary standards. According to some research carried out, the biosolids from Bugolobi STP have faecal coliforms beyond 3 million CFU g-1 and are therefore not safe for agricultural use or any other disposal into the environment (Lubuulwa and Olupot, 2019). While there is a potential risk from anthropogenic chemicals like pharmaceuticals, hormones, and pesticides in addition to heavy metals like arsenic, selenium, and antimony, existing directives and guidelines in Uganda and around the world have failed to set limit values for organic and emerging micro-pollutants in sewage sludge. Table 2-3 below highlights some of the current UNBS organic fertilizers disposal guidelines especially for sewage sludge intended for land application.

Table 2. 3: Contaminant limits for Organic fertilizers in Uganda (UNBS, 2017)

Heavy Metals	**Limit (mg/kg dry weight)**	**Test method**
Arsenic (As)	10	ISO 17318
Lead (Pb)	100	ISO 17318
Mercury (Hg)	2	ISO 17318
Cadmium (Cd)	5	ISO 17318
Copper (Cu)	300	ISO 11047
Chromium (Cr)	50	ISO 17318
Other Properties		
Salinity (Conductivity) mhos/cm, max.	5	ISO 11265
Total Nitrogen, %, m/m, min	1	ISO 11261
Organic carbon, %, m/m, min.	12	ISO 10694
Moisture Content (Solid Organic fertilizer) (%), m/m	30-35	ISO 11465
P^H	6-10	ISO 10390
E. Coli	Absent	
Salmonella	Absent	

To ensure sewage sludge meets set guidelines and standards, several stabilisation mechanisms are in use and the section below highlights such mechanisms in Uganda and across the world.

2.3.2: Stabilisation of Sewage sludge

Sewage sludge's beneficial constituents for instance nutrients such as Nitrogen and phosphorus favour its use as a fertilizer in agricultural production. However, toxic contaminants such as heavy metals, pharmaceuticals among others necessitate having adequate conditioning and stabilisation of sludge before final disposal to prevent adverse environmental and human health impacts.

Pre-treatment of sewage sludge through methods such as thermal treatment, ultra-sonification, ozonation, acidification, alkalinisation, or enzyme addition among others increases sewage sludge stabilisation and biogas production potential (Braguglia, Mininni, and Gianico, 2008). According to Levantesi et al. (2010), pre-treatment improves sludge microbiological quality making it safer for disposal. In addition, it facilitates oxidation of refractory compounds such as pesticides, pharmaceuticals, antibiotics which are normally difficult to remove, and reduction in sludge volumes due to the solubilisation effect on particulate organic matter. (Rivas Ibáñez et al., 2015).

However, sewage sludge pre-treatment is not famously practiced on large-scale WWTPs due to underlying costs required to reach recommendable stabilisation levels (class A biosolids). These costs for instance may be in the form of higher energy requirements for effective ultra-sonification or higher doses of ozone among others. Pre-treatment has detrimental effects on the quality of biosolids as well since it lowers their organic matter content, solubilizes nutrients, and increases their heavy metal level (Braguglia, Mininni, and Gianico, 2008, Zhang, Wang and Wang, 2017).

Typically, sewage sludge stabilisation with anaerobic digestion takes place in reactors where biogas that is composed of 60–65% methane (CH4), 30–35% carbon dioxide (CO2), and a minor amount of other gases is tapped off and utilised to generate heat and power. The toxicity of sewage sludge is reduced by 2–5 times through anaerobic digestion, in accordance with Coarita Fernandez et al. (2020) in addition to limiting odour problems and reduction in sludge volume. Sewage sludge digestion however isn't the ultimate stabilisation mechanism as it needs to be followed up with more effective processes such as dewatering using filter presses or centrifuges which reduces the amount of sludge produced, makes transportation easier, raises calorific value, and even lessens leachate output in landfills (Wu, Dai, and Chai, 2020), thermal drying, open sun drying as at Bugolobi STP, lime addition to alter the pH to > 11, eliminate microbiological risk, immobilise heavy metals in sewage sludge, and increase the benefits to agriculture (Wong and Selvam, 2006).

The relative concentration of oxidised and reduced substances in a solution ultimately determines the Redox potential (Eh). The Eh has a significant impact on environmental toxicity of heavy metals in terms of their bioavailability and migration and consequently the advantage of immobilisation on heavy metals. This is increased with lime addition, however, the excessive elevation of pH with lime addition may affect plant growth and it is therefore recommended that more natural passivating agents such as bentonite be used since they pose a limited threat to the environment even after application for long periods (Zhang et al., 2017). The availability and cost of lime may also provide a barrier to its use in stabilising sewage sludge.

Vermicomposting on the other hand, is an economically and environmentally sound sewage sludge stabilisation mechanism. In light of the rising socio-economic and environmental costs of dealing with current and future waste disposal as well as the risk of emission of greenhouse gases (GHG), such as Methane (CH4) and Nitrous Oxides from landfills or their management by composting, vermicomposting offers a tangible alternative to sewage sludge management. The process is odourless, rapid as compared to normal composting with process timing almost halved, and offers an end product that is environmentally safe and highly nutritious for plant growth (Sinha et al., 2009; Norbu, Visvanathan, and Basnayake (2005)).

Vermicomposting of sludge sewage

As a stabilising technique, vermicomposting uses earthworms to break down organic debris and create nutrient-rich pellets known as worm castings, which are deposited from the worms' back ends (Chaoui, 2010). Vermicomposting reduces the level of pathogens and bio-available heavy metals in sewage sludge by transforming unstable heavy metal fractions into stable ones and significantly prevents the leaching of these harmful metals and persistent contaminants to the ground thereby protecting groundwater from pollution (Azgin, 2021; Ludibeth, Marina and Vicenta, 2012). Vermicomposting also reduces sludge volume thereby easing disposal, eliminating foul odour, and increases plant metabolites for instance higher levels of nitrates, microbial biomass, and soil enzymes such as Azotobacterial spp. which are important for plant growth hence causing growth stimulation

(5-7 times). All these factors make vermicomposting an essential stabilizing mechanism for sewage sludge destined for land application

In addition, vermicomposting has reduced methane emission because it is majorly an aerated process thereby ensuring environmental preservation (Munroe, 2007). Nevertheless, a number of studies have demonstrated that the concentration of heavy metals after the composting process may tend to rise due to weight loss of organic matter (OM) as a result of the volatilization of gases like H_2O and CO_2, which results in an overall rise in the concentration of heavy metals like Pb and Cd. The risk of the spike in the heavy metals is controlled because vermicomposting reduces the bio-available heavy metal fraction while increasing the residual fraction thereby ensuring limited pollution of the environment (He et al., 2016; Wang et al., 2017; Gupta and Garg, 2008; Song et al., 2014).

Earthworms used in the vermicomposting process possess a number of desirable qualities, including the ability to display high biomass consumption, high efficiency of biomass conversion to body proteins, which is a physiological trait required to achieve high growth rate and reproduction, high degree of adaptability and tolerance to varying environmental conditions, and the capacity to consume large amounts of biomass (Sinha et al., 2009). Many earthworm species, including the Tiger worm species (spp), Red Tiger worm species (spp), Indian Blue worm species (spp), and African Night Crawler species (spp), which are typically employed in the vermicomposting process, have these characteristics (Sinha et al., 2009).

Earthworms

Earthworms are segmented-bodied, long, narrow, cylindrical, bilaterally symmetrical organisms that are classified according to the depth at which they live in the soil. For example, epigeic earthworms, which are primarily surface dwellers, endogeic earthworms, which can burrow up to 15 cm deep, or anecic earthworms, which can burrow up to 1 m deep, are those that live in the soil. Due to the fact that they are voracious garbage eaters and bio-degraders, epigeic earthworms like Eudrilus eugeniae, Eisenia foetida, and Perionyx excavatus are suitable for vermicomposting. The best organism for degrading organic wastes is Eisenia foetida (red wriggler, red worm, tiger worm, etc.), which can function in a variety of temperature and moisture conditions and consume up to 75% of their body weight in organic matter each day (Sinha et al., 2009, Chaoui, 2010).

Figure 2. 2: Earthworms for vermicomposting of organic matter

Source: Research Pictures by Mafabi Grace, 16/01/2022

During the vermicomposting process, earthworms work in tandem with microorganisms (bacteria, fungus, and actinomycetes) to stabilise sewage sludge. According to Dominguez, Edwards, and Webster (2000), earthworms play a key role in the process by consuming pathogens in their stomach and altering the substrate with their digestive enzymes to make it more microbially active, for example by expanding the surface area. The worms achieve nutrient enrichment especially phosphorous through mineralization, phosphorous mobilization, and the action of alkaline phosphatase excreted by the composting worms (Yadav and Garg, 2011; 2009). Earthworms have been used in the bioremediation of heavy metals, and results have included a considerable drop in the overall content of heavy metals like Zn (15.10-39.60%), Fe (5.20-29.80%), Mn (2.60-36.50%), and Cu (8.60-39.60%) (Nakiguli et al., 2018, Suthar, 2010, Urdaneta et al., 2008, Sosnecka, Kacprzak and Rorat, 2016) with a build-up of 7600 mg/g dry weight of Pb and over 100 mg/kg Cd in earthworm tissue. The presence of metallothionein (MTs) proteins in earthworms, which have a very high capacity to bind metals and subsequently their attraction and removal from compost, is said to aid in the bio-remediation of heavy metals, according to Sinha et al. (2009).

Mechanism of vermicomposting

Vermicomposting is an intricate process that involves both physical operations such substrate aeration, mixing, and grinding as well as biochemical activity controlled by microbial decomposition of the substrate in earthworms' intestines (Venkatesh, 2007). Through burrowing, earthworms establish aerobic conditions in the waste products, which prevents anaerobic microorganisms from releasing hydrogen sulphide (H2S) and other unpleasant odours. The grume secreted in the earthworms' mouth softens the sludge, which is then transported to the oesophagus where it is neutralised by calcium excreted by the oesophageal inner walls before moving on to the gizzard and intestine for further processing.

In the intestines and gizzard, earthworms work as a bio-reactor breaking waste material into minute fragments through their grinding and crushing effect with the help of gizzard stones and gut muscle movement which increases the surface area hence optimizing enzymatic action. Casts are created as a result, which are microscopic particles between 2-4 microns in size that are then transported to the intestine for enzymatic reactions (Chaoui, 2010). Low oxygen levels in the gut also encourage pathogen die-off, which when combined with coelomic fluids, which are secreted by worms and have anti-bacterial qualities, completely disinfects the final product.

Endogenous enzymes secreted by earthworms in the gut, such as proteases, lipases, amylases, cellulases, and chitinases, or partially exogenous enzymes from associated flora and fauna after their excretions, achieve biochemical degradation of the ingested material in the gut. These enzymes speed up the biochemical conversion of the cellulosic and proteinaceous materials in the waste organics (Suthar, 2010). By housing millions of decomposing microorganisms and promoting them up to 1000-fold build-up in the gut, earthworms operate as a biological stimulant. When organic matter enters the earthworm's intestines, it is mineralized into ammonium, which the microorganisms then nitrify into plant nutrients. The result is a compost that is more homogeneous, less polluted, nutrient-rich, soft, and highly porous and has a higher water retention capacity (Chaoui, 2010, Sinha et al., 2009).

The process of humification, in which the huge organic particles are transformed into a complex amorphous colloid including phenolic compounds, is the last step in the vermi-processing and breakdown of organic waste. As a result, humus is created from more than 25% of the organic materials. In addition to stabilising heavy metals, the creation of humic compounds also reduces their mobility and phytotoxicity in biosolids by offering a large number of non-specific and specific sites for metal adsorption that result in the formation of insoluble organometallic complexes (Zhang et al., 2017).

Vermicomposting system

The vermicomposting system comprises the composting structure, habitat, and ecosystem (worms and microbes) which need to be optimally conditioned to allow for the effective vermicomposting process (Christie, 2011). The vermicomposting structure should be well aerated and with a relatively high surface area to depth ratio to maintain aerobic conditions within the setup. The structure can be in the form of plastic worm bins, wooden bins or flow-through containers, vermicomposting trenches, pits, heaps, and windrows.

Materials with a high carbon to nitrogen ratio, or C/N, which are also bulky, absorbent, and resistant to deterioration should make up the bedding material. The bedding can be categorized as primary bedding materials which can work in isolation for example shredded paper, coarse compost, and manure mixes, or secondary bedding material which are normally used in the mixtures, for example, sawdust, wood chips, or bulky plant waste.

Figure 2. 3: A sample of a plastic composting bin

(Source: *Research pictures by Mafabi Grace, 16/01/2022*)

However, to promote optimal worm activity, a temperature range of 20-25°C and moisture content of 60-75% should be maintained inside the system to facilitate the earthworm respiration. Earthworms are remarkably adaptive to severe and variable environmental circumstances. A balance between the moisture content and aeration needs to be ensured as the two are inversely proportional i.e., an excess of moisture cuts off the oxygen supply creating anaerobic conditions in the system which eventually kills off the earthworms (Sinha et al., 2009).

In addition, the composting system environment should be one with minimal sunlight, and quiescence, with proper bedding and feeding rates to facilitate earthworm activity (Christie, 2011). The earthworm stocking density in the composting system has an impact on the efficacy of vermicomposting as well. According to Wang et al. (2017), a stocking density of 2.5-3.5kg/m^2 is effective in the stabilisation of sewage sludge whereas Ndegwa, Thompson, and Das (2000) suggest that the ideal worm stocking density for vermicomposting sewage sludge mixed with cow dung and paper chips is 1.60 kg/m2 and the optimal feeding rate is 0.75 kg/kg/day. The stocking density has to be optimized to avoid starvation of the worms and discourage dormancy in the setup.

It is important to guarantee that the carbon to nitrogen ratio (C/N) is at least 20 within the system as a ratio below this limit results in the production of ammonia gas (NH_3) which kills off the worms as well affects the aeration within the system whereas elevated levels of C/N also affect microbial activity. A product with the best fertiliser value and the lowest risk of environmental pollution has a C/N of 25 (Ndegwa and Thompson, 2000). However, as the vermicomposting process advances, the C/N lowers as a result of the ongoing decomposition of organic matter, the mineralization of nitrogen, and the microorganisms' metabolism of carbon as a source of energy (Song et al., 2014).

To lower the amount of heavy metals and subsequently improve substrate qualities including air space, moisture content, C/N ratio, and pH, sewage sludge composting is typically done with bulking agents. Some of the bulking materials utilised include sawdust and other municipal garbage. This has a good impact on the rate of composting and also dilutes the concentration of heavy metals in the final biosolid (Zhang et al., 2017). The process should be set to run between six (6) to ten (10) weeks and no longer than that since it has been noted that earthworms start releasing heavy metals into their environment between ten and fifteen weeks. Additionally, later phases of the process are characterized by limited productivity which is mainly attributed to the exhaustion of the feed substrate at that stage (Yadav and Garg, 2011). A correctly constructed vermicast system will convert organic waste into vermicast in 22–30 days, according to Chaoui (2010).

During the vermicomposting process, the quality of the substrate is checked from time to time through the monitoring of parameters that affect the process such as temperature, moisture, pH, organic matter (OM), N, and C/N. The capacity of the cationic interchange (CCI), which reveals the humic fraction of the substrate and rises over time, is one measure in addition to others that reflect the vermicompost's level of maturity and stability. Similarly, throughout the process, the pH, TOC, and C: N are expected to reduce whereas the EC, TKN, TK, TP, and heavy metals content is expected to increase as compared to the initial mixtures (Gupta and Garg, 2008).

Influence of toxicity on earthworm activity

Sewage sludge has been noted to be phytotoxic with growth inhibition ranging from 70.45-100% (Adamcová and Vaverková, 2016). The presence of heavy metals, high salinity levels, and developing contaminants like detergents and pharmaceuticals are the key contributors to the toxic nature of sewage sludge. Earthworms are repelled by salinity levels exceeding 5 mg/g, or 0.5% salinity in which case ammonium is usually the primary cause of salinity (Chaoui, 2010). Increased pollution from industries whose wastewater is discharged into the environment untreated results in a metal-laden sludge product after wastewater treatment, which contributes to the heavy metal toxicity of sewage sludge (Kim and Farnazo, 2017; Barakat, 2011).

Utilizing earthworms in the improvement of such sewage sludge implies that the worms have to get in contact with such levels of metal pollution and start the degradation process of the substrate. With their delicate nature having to solely depend on the interface between their bodies and the surrounding for survival especially respiration through their moist skin, the toxic surrounding, therefore, inhibits earthworm activity or kills off the worms themselves and consequently affecting the effectiveness of the vermicomposting process, for instance, the time required to achieve full stabilisation of sewage sludge (Strande and Brdjanovic, 2014).

Earthworms may not remove toxic substances but cause variation in their chemical structure rendering them harmless to the environment for instance reduction of the mobility of heavy metals by converting the bio-available portion into residual fractions which have minimal effect on the environment. The increasing overall heavy metal concentrations in earthworm tissues, which are well explained by the phenomena, or the development of stable metal-humus complexes as a result of the humification of organic materials are two possible explanations (Singh and Kalamdhad, 2016, He et al., 2016, Song et al., 2014).

Increased toxicity's impact on earthworm activity has been assessed in several phytotoxicity tests or earthworm avoidance tests using single concentrations to determine the quality of field soils or multi-concentrations to determine the toxicity of a spiked chemical in which case earthworms tended to avoid spiked soils / heavy metal-laden soils. However, when bulking material and microorganisms were added to the setup, earthworms tended to outcompete the toxicity of the soils. This was because the microorganisms produced volatile compounds that changed the chemical structure of the concentrates and made them more palatable to the earthworms (Syed et al., 2016).

The results of the earthworm avoidance test whose duration lasts between 24 to 48 hours can be correlated to longer-lasting tests, including the 56-day earthworm reproduction test though the latter is more labour-intensive and requires longer incubation periods (Garcia et al., 2008; Frankenbach et al., 2014). It is wise to ascertain the impact of heavy metal concentration on earthworm activity over such a process because the reproductive tests' duration is comparable to that of vermicomposting.

2.3.3: Sewage sludge disposal and re-use

Any waste management operation that is not a recovery operation is referred to as a disposal operation, even if it has a by-product like the extraction of materials or energy. This has become one of the most challenging and growing environmental concerns in wastewater treatment (Adamcová and Vaverková, 2016). This is due to its associated environmental effects as well as costs that may reach 50% of an STP’s operating costs resulting from the ever-increasing amount of sludge generated (Turovskiĭ and Mathai, 2006). STPs are less efficient in removing pollutants and therefore the resultant sludge from the treatment process usually contains varying amounts of organics, nutrients, pathogens, and heavy metals which makes its disposal difficult (Oleszczuk et al., 2012). STPs have also been linked to the development of multi-drug resistant bacteria and antibiotic resistance genes, both of which pose a risk to human health (Czekalski, Gascon Diez and Burgmann, 2014; Czekalski et al., 2012).

The main methods used to dispose of sewage sludge from STPs are landfilling and incineration. Sludge stability, reduced volume, and moisture content are all goals of pyrolysis, gasification, or incineration, which makes it easier to store and transport. Sewage sludge ash (SSA), which is created by incinerating sewage sludge, is known to include greater concentrations of Ni, Cu, Zn, Cd, Sn, and

Pb and may bio-concentrate through eco-systems when applied to the ground, having detrimental effects on human health (Wang et al., 2013).

However, according to Chen and Poon (2017), SSA may be used for other beneficial uses such as mixed in mortar where it was found to accelerate the rate of heat evolution from cement hydration and could even replace cement up to 10% in mortars without significant effect. This is possible because SSA is comprised of compounds such as SiO_2, CaO, Al_2O_3, Fe_2O_3, MgO, and P_2O_5 which make it a good pozzolanic material that is cementitious when finely ground (Yusuf et al., 2012). SSA which approximates to 1.7million tones worldwide can also be reused as a clay substitute in the production of sintered bricks, tiles, and pavers, as well as high-density ceramics, Portland cement, and other products (Donatello and Cheeseman, 2013).

Practices for disposing of sewage sludge, such as landfilling, are becoming increasingly expensive and impractical and coupled with the increasing desire to reuse sewage sludge for different benefits, land application of increasingly becoming the preferred sewage sludge disposal practice (States, 1995). Land application is a sewage re-use mechanism in which the bio-solids are spread onto land especially agricultural fields. This is usually done to fertilize soil as sludge contains beneficial constituents such as nutrients including Nitrogen, Phosphorus, and Potassium (K) as well as micronutrients for instance Iron (Fe), Boron (B), Copper (Cu), and Nickel (Ni). Contained organic matter in sewage sludge plays a vital role in conditioning of poor soils for instance improvement of soil porosity thereby facilitating root growth and aeration. Organic matter also enhances soil water retention, binding of contaminants and carbon sequestration important for limiting greenhouse gas emissions, pathogen reduction among others which is beneficial to agriculture.

The microbial population is also increased with sewage sludge addition thereby ensuring an improved balance of biochemical processes required for plant growth (Usman et al., 2012; Turovskiĭ and Mathai, 2006; Oleszczuk et al., 2012). Sewage sludge application to the land is governed by rigorous regulations and supervision to avoid impacts on the environment. Below are some of the guidelines developed by EPA, (1995) for sewage sludge destined for the land applicants.

Table 2. 4: Limits for pollutants in sewage sludge for land applications (States, 1995).

Pollutant	**Maximum allowed concentrations for all bio-solids (milligrams per kilogram)**	**Limits of Pollution for EQ and PC Bio-solids (milligrams per kilogram)**
Arsenic	75	41
Cadmium	85	39
Chromium	3,000	1,200
Copper	4,300	1,500
Lead	840	300
Mercury	57	17
Molybdenum	75	—d
Nickel	420	420
Selenium	100	36d
Zinc	7,500	2,800
Applies to:	All sewage sludge that is land applied	Bulk sewage sludge and bagged sewage sludge
From Part 503	Table 1, Section 503.13	Table 3, Section 503.13

In the US and the majority of EU countries, the application of biosolids generated from the STPs onto agricultural fields has been rampant (Gattie and Lewis, 2004). According to Hudcová, Vymazal, and Rozkosny (2019), the application of biosolids as fertilizer to agricultural fields accounted for about 60% between 2014 and 2015. In other countries like Belgium, Switzerland, and Romania, stringent measures have been put in place which restricts the application of sewage sludge onto land. The practice has been embraced in several African countries including Uganda where the increasing use of biosolids from NWSC STPs such as Bugolobi and Lubigi has been reported (NETWAS Uganda, 2011).

Land application of sewage sludge has its negative side as it has been associated with several issues such as the existence of ongoing and new contaminants, as well as the danger of contamination of soil and water due to the ease with which heavy metals can be transported thanks to its organic components, and the preferential flow paths created by the wormholes (Graber et al., 2001, Graber and Gerstl, 2011), toxic heavy metal pollution, presence of pharmaceuticals and the danger of pathogen-containing bioaerosols being transported and released. Events involving the land application of sewage sludge have been linked to serious health impacts and physical symptoms include mucous membrane irritation, respiratory and gastrointestinal distress, headaches, skin rashes, and occasionally death (Harrison and Oakes, 2003, Lowman et al., 2013, Khuder et al., 2007).

Additionally, sludge application has been associated with modifications to the soil's microbial community and diversity thereby causing irreversible damage to the soil that may last decades or even centuries. This is mainly attributed to pathogenic microorganisms like bacteria, fungus, and viruses present in sewage sludge which usually survive the wastewater treatment process and get disposed of together with the sewage sludge. This implies that adequate care should be taken in the reuse of sewage sludge for agricultural purposes to prevent such impacts on the environment and human health. In line with this, NWSC has precautionary limited the application of biosolids to non-food crops for instance flower beds because there may be a health risk to people (NETWAS Uganda, 2011). Biosolids are sold to farmers at a fee of about UGX. 10,000 per tonne from the STPs.

According to Zhang et al. (2017), the concentration and speciation of heavy metals is a very important aspect in determining the toxicological effects of heavy metals contained in sewage sludge. Four different kinds of heavy metal speciation exist including acid-soluble/exchangeable fractions, reducible fractions, oxidizable fractions, and residual fractions. The acid-soluble fraction is bound to carbonates and is thought to be readily bioavailable. The reducible fraction is bound to iron and manganese oxides and is potentially bioavailable.

The stability of sewage sludge through mechanisms such as vermicomposting that improve the availability of nutrients and immobilize harmful heavy metals that would otherwise have adverse effects on both flora and fauna is highly sought especially for metal-laden wastewater that is characteristic of the expanded Bugolobi STP in Uganda and similar STPs in developing countries to warrant safe disposal and re-use.

2.4: Key findings and knowledge gaps

From the literature review, management of sewage sludge generated from wastewater treatment remains an enormous challenge due to several factors including the inefficiency of wastewater treatment plants, emerging pollutants like detergents and pharmaceuticals (Petrović and Barceló, 2000), increasing cost of wastewater treatment, inadequate regulation locally and the world over among others yet this has to be effectively done to ensure environmental sustainability.

The success of this requires total harmonization of all key processes aimed at ensuring the safety of sewage sludge disposal concerning both flora and fauna for instance pre-treatment processes such as thermal treatment, ultra-sonification, ozonation, acidification, alkalinisation or enzyme addition have been reported to have numerous advantages (Braguglia, Mininni, and Gianico, 2008; Rivas Ibáñez et al., 2015) though there is still room to have them embraced globally, anaerobic digestion which reduces sewage sludge odour, volume and toxicity by 2-5 folds (Coarita Fernandez et al.,2020), and a wide range of sewage sludge stabilisation mechanisms such as dewatering, thermal drying, lime addition, composting or vermicomposting.

As mentioned above, sewage sludge stabilisation has been the focus of a lot of research. For instance, vermicomposting, the most popular method of sewage disposal at the moment, is an environmentally friendly and economically viable method for sewage sludge destined for land application. Aspects of the ideal conditions for the process, such as temperature, PH, moisture content, and carbon-to-nitrogen ratio (C/N), have been well studied, but less emphasis has been placed on emerging constraints like increasing toxicity of both wastewater and sewage sludge globally which defeats the use of sludge for agricultural purposes.

The presence of metallothionein (MTs) proteins in earthworms has been found to play a significant role in the bioremediation of heavy metals during the vermicomposting of sewage sludge. These promote the transformation of bio-available heavy metal fractions into residual fractions in addition to the formation of humic substances which offer a large number of both general and specialised sites for the adsorption of metals. This decreases the mobility of heavy metals and phytotoxicity in biosolids by causing the creation of insoluble organometallic complexes. The details of these transformational heavy metal products from vermicomposting have been scarcely studied.

Additionally, very little research has been done on the problem of phytotoxicity of sewage sludge caused by the presence of such heavy metals and their effects on vermicomposting. This research has therefore contributed towards bridging the gap of vermicomposting concerning increasing toxicity by studying the effect of increased heavy metal toxicity on the quality and safety aspects of vermicomposted bio-solids which will add to the world of knowledge and ensure safe land application of such sewage sludge.

CHAPTER 3: METHODOLOGY

3.1: Introduction

This chapter describes the procedure used to carry out the study. The major goal of this study was to ascertain how elevated heavy metal toxicity will affect the vermicomposted biosolids' quality.

3.1.1: Research Steps

The research steps that were followed in this study are presented below in figure 3-1;

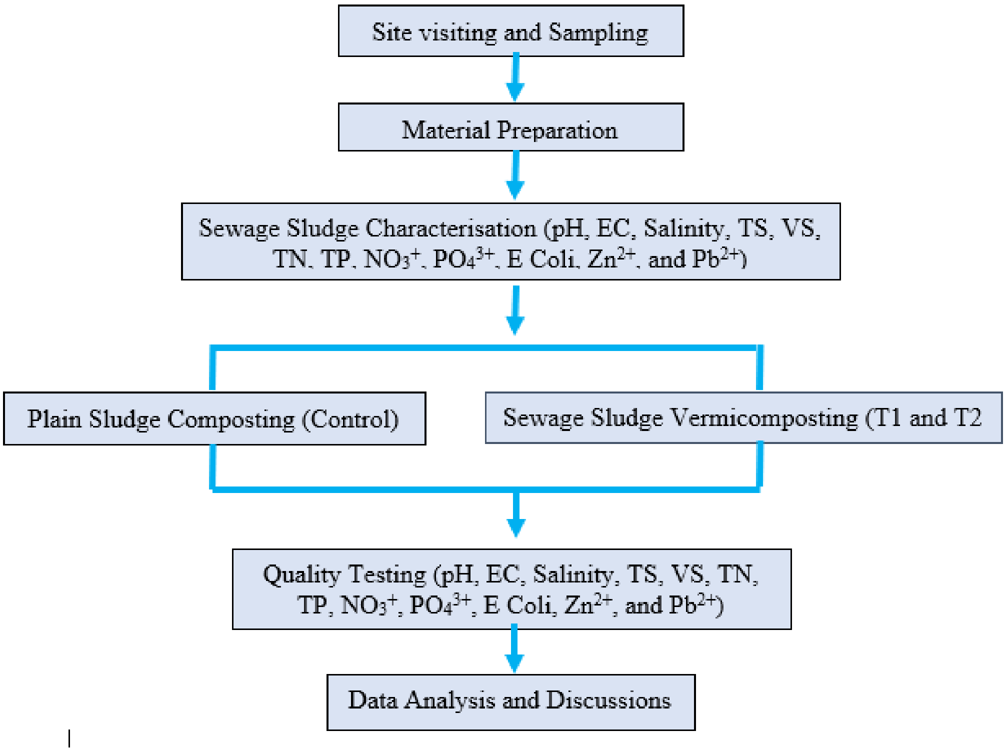

Figure 3. 1: Methodological flow chart for the research study

3.2: Materials and Experimental setups

Materials used for this research included sewage sludge from Bugolobi and Lubigi Sewage Treatment Plants (STPs). The two plants were considered because they are the largest waste water treatment plants in Uganda in addition to the fact that they incorporate treatment of various wastewater streams including storm water and faecal sludge that was key for the research. Indigenous Micro-organisms (IMO) in liquid form were used in optimising vermicomposting process whereas earthworms of the African Night Crawler Species (Eudrilus Eugeniae) obtained from Makerere

University Agricultural Research Institute Kabanyolo (MUARIK) were used in the experimental vermicomposting process.

3.2.1: Sewage sludge and Characterization

Bugolobi STP (0°19'6.54"N, 32°36'24.80"E) and Lubigi STP (0°20'49.43"N, 32°32'42.03") provided the sewage sludge samples for this study. Sludge sampling followed both selective and random sampling criteria during which sewage sludge samples at two (2) weeks (fresh) and six (6) weeks (matured sludge) were picked. The fresh sludge considered was the 2 weeks old sludge to avoid the initial thermophilic phase of sludge digestion during vermicomposting which is dangerous to earthworms. Random grab samples were picked from a given composting windrow and mixed to form composite samples in sealed polythene bags.

Characterization of sewage sludge was done for sewage sludge samples from both Bugolobi and Lubigi STPs and was meant to ascertain the quality of sewage sludge from these plants and their rated compatibility with disposal guidelines set by the Uganda National Bureau of Standards (UNBS, 2017) for solid organic fertilizers. The samples were transported in ice-cooling boxes at 4^0C to Makerere Department of Plant Sciences, Microbiology and Biotechnology (0°20'7.62"N, 32°33'59.26"E) and the Uganda Industrial Research Institute (UIRI) Laboratories (0°20'10.57"N, 32°37'30.98"E) for testing and analysis of the various parameters.

Figure 3. 2: Two (2) weeks old sewage sludge sample picking at Bugolobi STP.

The samples were tested for parameters such as Heavy metals Lead (Pb) and Zinc (Zn), total organic carbon (TOC), total phosphorus (TP), and electro-conductivity (EC), the potential for Hydrogen (pH) and the level of faecal coliforms to confirm conformity with the established standards for disposal of sewage sludge as an organic fertilizer.

3.2.2: Earthworms and Optimal Stocking density for Vermicomposting

Earthworms used for the vermicomposting process were mature African Night Crawlers (Eudrilus Eugeniae) of at least 3 weeks of age obtained from MAURIK (0°28'5.73"N, 32°36'26.78"E) vermiculture center at a fee of UGX. 20,000 per kilogram. The worms were hand-picked and weighed to reach the required stocking densities per square meter (m^2) of the exposed surface of the composting bin but with allowances of 0.2kg worms per 1kg of cultured worm mixture to cater for the compost materials within the mixture.

Figure 3. 3: Inoculating of sludge samples with earthworm densities during setup

To obtain an optimal earthworm stocking density for vermicomposting of sewerage sludge, various worm densities were used including 0.00 (control), 1.00, 2.00, 2.50, and 3.50kg-earthworms/m^2 of the exposed surface area of the vermicomposting bin. This was in line with findings by Wang et al. (2017) and Ndegwa, Thompson, and Das (2000) who established stocking densities 2.5-3.5kg/m2 and 1.60 kg/m2 respectively as effective during vermicomposting.

The vermicomposting system for this experimental setup comprised of black worm bins in the form of stackable crates obtained from Crest Tanks (U) Ltd, weighing 4.26kgs and measuring 0.65m x 0.37m x 0.33m (L x W x D), approximately 0.240405 m^2 of surface area which was used in calculation of the required worm weighting for each of the above stocking densities to give the treatments in the table 3-1 below.

Table 3. 1: Stocking densities for sewage sludge vermicomposting

S/N	Description of Treatments and Setup, T1	Stocking Density (Kilogram Worms/m2)	Exposed surface area (m2)	Required weight of worms (Kg)
1	T_1, Plain Sewage Sludge + IMO (Control 1).	0.0	0.2405	0.0000
2	T_2, Sewage Sludge + IMO + 1.00kg-earthworms/sq.m.	1.0	0.2405	0.2405
3	T_3, Sewage Sludge + IMO + 2.00kg-earthworms/sq.m.	2.0	0.2405	0.4810
4	T_4, Sewage Sludge + IMO + 2.50kg-earthworms/sq.m.	2.5	0.2405	0.6013
5	T_5, Sewage Sludge + IMO + 3.50kg-earthworms/sq.m.	3.5	0.2405	0.8418

In setting up the vermicomposting system, worm bins were perforated to aid the aeration and flow of effluent from the system (Nyende and Achire, 2018). Filling of the plastic worm bins was limited to 0.3m height from the bottom of the bins to further improve aeration within the setup and avoid compaction of the bottom of the pile. The bins were raised 1m from the ground with help of wooden stands to prevent the entry of insects into the system and to create a proper working platform. Coarse compost was initially filled to about 0.03m as bedding at the bottom of bins to provide cover for the earthworms from unfavourable conditions within the composting system.

The obtained worm weights were added to 38kgs of sewage sludge in each stacked compost bin for the setup of the five stocking densities i.e. 1.0, 2.0, 2.5, 3.5kgworms/m^2, and the control setup which had no earthworms. Indigenous Micro-Organisms - IMO bacteria especially the dominant Stenotrophomonas maltophilia, Bacillus sp, and Chryseobacterium ureilyticum in liquid form were inoculated into the sewage sludge at the point of introduction of the earthworms to improve the conditions for optimal worm activity. Half litre ($\frac{1}{2}$ litre) of the IMO mixture was applied to each of the vermicomposting systems in particular to remove ammonia gas (NH3) that is created during the anaerobic digestion of sewage sludge and could harm the earthworms. IMO microorganisms were also needed to make the sewage sludge less offensive and more pleasant to the neighbourhood (Nyende and Achire, 2018, Nakagiri et al., 2017).

Figure 3. 4: Fabrication and weighting of compost bins for the setup

The experimental setups were all performed under shade to limit the effects of environmental factors such as rain and extreme heating from too much sunshine experienced within the tropics so as to maintain the temperatures within 25^{0}C. The pH of the samples was periodically monitored with the help of pH meter ensuring the ranges did not exceed palatable levels for earthworms. Moisture content was maintained within 60-75% of the samples.

A batched process of vermicomposting was used in which the setups were left to stand in the dark for six (6) weeks i.e., forty-two (42) days while monitoring temperature and moisture content to allow optimal conditions for worm activity. Earthworms were physically removed in the following weeks and at the conclusion of the vermicomposting process, and samples were taken in sampling bags that were labelled with waterproof ink with information on stocking density, date, age and time of samppling. These samples were tested and results compared with UNBS, 2017 disposal limits for organic fertilizers. This formed the selection basis for the most appropriate stocking density for sewage sludge vermicomposting.

Figure 3. 5: Setting up the different vermicomposting system designs

3.2.3: Heavy metal toxicity and effect on vermicomposting.

The heavy metals considered for this research experiment were Lead (Pb) and Zinc (Zn) which are some of the most common heavy metals in waste water. Variation in heavy metal toxicity of sewage sludge was achieved through spiking Pb concentration in sewage sludge using Lead Nitrate solution ($Pb(NO_3)_2$). The heavy metal toxicity of sewage sludge was first tested through an acute toxicity test during which the nominal concentration of Pb in sewage sludge was varied in increments of 1.00, 2.00, 3.00, 4.00 and 5.00 times. Earthworms (20No.) were introduced in the setups for 48 hours after which they were observed and analysed for survival rates with increasing lead toxicity of the sewage sludge.

The Pb used for spiking the concentration was obtained in solution as Lead Nitrate i.e. ($Pb(NO_3)_2$) and the various concentrations were achieved through molarity calculations and comparison with a standard solution of One Molar Lead Nitrate i.e. 1M ($Pb(NO_3)_2$) as detailed below.

$$\boldsymbol{Nominal\ Lead\ Concentration\ in\ Sewage\ Sludge\ at\ 2\ weeks = \frac{234.1307mg}{Kg}} \text{............(1)}$$

$$One\ Molar\ Pb(NO_3)_2\ solution = 331.2g\ of\ Pb^{2+}$$

Where, g; gram, mg; milligram, Kg; Kilogram, Pb; Lead, Pb(NO3); Lead Nitrate

Considering volumes of reagents, 1 Mole of $Pb(NO_3)_2$ is contained in 1000ml, therefore the nominal concentration of Pb^{2+} in moles and in volume can be calculated as;

$$\boldsymbol{Nominal\ moles\ of\ Lead\ at\ 2\ weeks = (\frac{\left(\frac{234.1307}{1000}\right)\left(\frac{g}{Kg}\right)}{331.2\ (\frac{g}{mol})}}\ \textbf{(mol/Kg)........................(2)}$$

$$Nominal\ moles\ of\ Lead\ at\ 2\ weeks = 0.0007069164 \text{ moles}$$

Where, g; gram, mol; mole, Kg; Kilogram

Spiking of the concentration was therefore achieved by multiplying the nominal concentration of lead in sewage sludge through the required spiked concentrations i.e. 1.00, 2.00, 3.00, 4.00 and 5.00 – times increments using the 1M $Pb(NO_3)_2$ solution.

$$\text{i.e., } Moles\ at\ x2.00\ Concentration = \frac{2X(234.1307)mg}{Kg} = 2\ X\ 0.0007069164$$

$$Moles\ of\ Lead\ at\ x2.00\ concentration\ =\ 0.0014138327\ moles$$

$$Moles\ of\ Lead\ at\ x3.00\ concentration\ =\ 0.0021207492\ moles$$

$$Moles\ of\ Lead\ at\ x4.00\ concentration\ =\ 0.0028276656\ moles$$

$$Moles\ of\ Lead\ at\ x5.00\ concentration\ =\ 0.0035345820\ moles$$

The volume of 1M $Pb(NO_3)_2$ solution required to spike sewage sludge to the required concentrations was calculated using the dilution formula below;

$$C_1V_1 = C_2V_2 \ldots\ldots (3)$$

$$\text{Required volume for double concentration} = 0.0007069164 * 1000 \text{ (mls)}$$

Where, C_1; starting concentration, V_1; starting volume, C_2; final concentration, and V_2; final volume.

Therefore, required volume of 1M $Pb(NO_3)_2$ Solution for doubling concentration = 0.7069164mls whereas to triple the concentration, $0.0014138327 * 1000$ (mls) were added and this was calculated for all concentrations. These volumes were added to ½ liter solution of IMO2 and then added to sewage sludge with thorough manual turning to ensure proper mixing. The treated sewage sludge mixture was then subjected to vermicomposting first for an acute toxicity test with 20 worms and thereafter using an optimal earth worm stocking density.

The physio-chemical characteristics of vermicomposted sewage sludge at different concentrations was established together with the corresponding bioaccumulation in the earthworms. Considering results of the acute toxicity test, the experimental design for objective three (3) only considered concentrations for levels of x1, x2 and x3 based on the following table 3-2;

Table 3. 2: Experimental design for Vermicomposting at increased lead concentration

S/N	Description of Treatments and Setup, T2
1.	T_6, Sewage Sludge + IMO x1.0 Heavy Metal Conc. + Optimal earthworms.
2.	T_7, Sewage Sludge + IMO x2.0 Heavy Metal Conc. + Optimal earthworms.
3.	T_8, Sewage Sludge + IMO x3.0 Heavy Metal Conc. + Optimal earthworms.

Figure 3. 6: Setup of the acute toxicity test for sewage sludge vermicomposting

Figure 3. 7: Experimental setups for vermicomposting of sewage sludge at MAURIK

Samples from each of the experimental setups were picked after intervals of one (1) week for six (6) weeks to monitor the progress of vermicomposting. The section below highlights some of the test methods used in the determination of the parameters of the samples.

3.3: Laboratory Tests

The Department of Plant Sciences, Micro-Biology, and Bio-Technology at Makerere University's College of Natural Sciences, as well as the UIRI Laboratories, conducted the laboratory testing. Table 3-3 below provides a summary of the tests that were run;

Table 3. 3: Standard tests for the analysis of quality parameters

S/N	Laboratory Test	Standard Method
1	Temperature	LCD Digital Thermometer (McGee, 1988).
2	Moisture Content	Drying at 105^0C for 24 Hrs (Nielsen, 2017).
3	PH	pH meter (Bates, 1973).
4	Electrical Conductivity (EC)	Electrical resistance method (Rayment and Higginson, 1992)
5	Total Nitrogen (TN)	Semi-Micro Kjeldahl (Bremmer and Mulvaney, 1982).
6	Total Phosphorous (TP)	Ammonium Molybdate – Ascorbate acid method (Knudsen & Beegle, (1988)
7	E. Coli and Salmonella	Most Probable Number (MPN) (Bolton *et al.*, 1982)
8	Heavy metals - Zinc (Zn) and Lead (Pb)	Atomic Absorption Spectroscopy (Hill & Fisher, 1999).
9	Total Organic Carbon	Oxidation method (Yoon *et al.*, 2018)

The temperature of the sample was monitored utilising a -10 to 50°C smart LCD pocket thermometer and using three (3) readings averages. In the determination of moisture content (MC) of sewage sludge, the samples were air dried after which the sludge was ground to remove crumps and increase surface area. The crushed sludge was then sieved using a 2mm sieve to obtain uniform particle sizing of the sample and thereafter the MC determined with 24 hours of 105°C oven drying and calculation for the parameters completed using the formulae;

$$\boldsymbol{MC = \frac{(M_0 - M_1)x100}{M_0}} \quad \textbf{(4)}$$

Where; ***MC*** stands for the sample's moisture content, ***M_0 and M_1*** are the weights of the sample before and after oven drying respectively.

Figure 3. 8: Refrigeration of samples and determination of MC and TS

Potentiometric electro-conductivity (EC) and pH measurements were made in a 1/10 suspension of the sample in deionized water using a calibrated pH-HANNA metre with 0.2 accuracy. 10g of the sample's sludge was dissolved in 25ml of deionized water, mechanically mixed for ten (10) minutes at 230 rpm, let stand for thirty (30) minutes, and then stirred once more for two (2) minutes before measurement during which the calibrated meter electrode was left until the reading was stable.

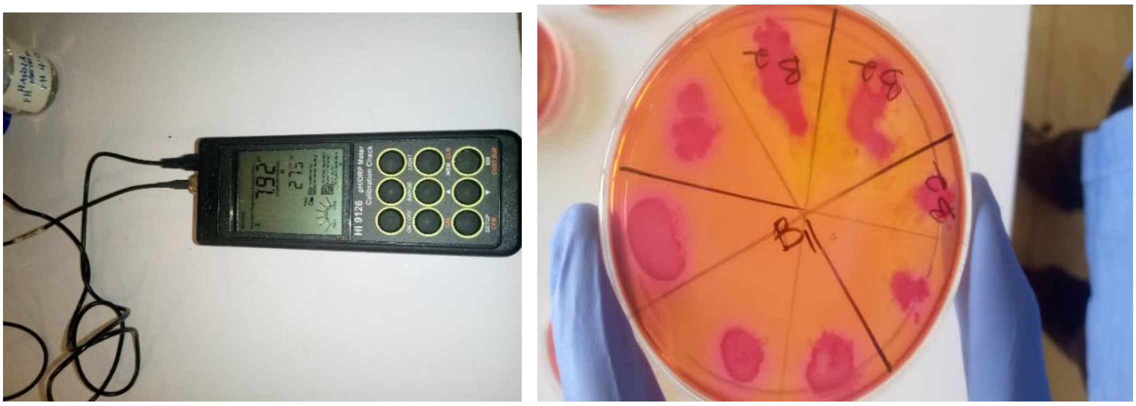

Figure 3. 9: pH - HANNAH instrument and a Completed Coliform Test (CCT)

The samples' total nitrogen and total phosphorous were measured using the semi-micro Kjeldahl method (Bremmer & Mulvaney, 1982) and the ammonium molybdate-ascorbate acid method as reported by Knudsen & Beegle, (1988), respectively, with phosphorous extracted using Mehlich 1 extractant. To detect TOC, organic compounds in quantifiable forms were first oxidised in a combustion furnace at a high temperature to produce carbon dioxide, which was then passed through scrubber tubes to eliminate interferences before being measured by non-dispersive infrared absorption (NDIR).

Heavy metals Lead (Pb) and Zinc (Zn) were tested using atomic absorption spectrometry (AAS). Collected samples were placed in an oven and the temperature brought to 60°C for a period 48hrs. They were then ground to a particle size of 1 mm and chilled in a desiccator. In a 75-ml digestion tube, 1g of the processed material was digested for 3 hours at 145°C with 5 ml of concentrated nitric acid. The solution was filtered through glass fibre into a 25-ml volumetric flask using a funnel after cooling for 48 hours. After that, the solution was divided across 50-ml Falcon tubes and centrifuged at 3000 rpm. Beer's Law was used to quantify the heavy metals produced by the operation;

$$\boldsymbol{A = a * b * c} \quad \textbf{(5)}$$

Where **A** – absorbance measured by the instrument, **a -** absorption coefficient (constant for a given element and instrumental conditions), **b** - path length (constant for a given element and instrumental conditions), **k** – machine constant, and **c** - concentration of substrate being analysed thus simplifying the equation to;

$$\boldsymbol{A = k * c} \quad \textbf{(6)}$$

3.4: Analysis of results

Laboratory test results were tabulated and averages obtained for all parameters under investigation. Graphical plots of data were obtained from the average results to ease comparison of the different experimental setups with respect to their physical-chemical and microbial characteristics and in reference to specifications of UNBS, 2017 guidelines on requirements for organic fertilizers destined for land application.

The results were analysed using R statistical package (version 3.4.3) developed by R development Core Team and graphs presented with the aid of GraphPad Prism (version 8.0.2.263). In order to determine the impact of different stocking densities and heavy metal concentrations on the quality of the vermicompost, one-way analysis of variance (ANOVA) was utilised at (95% confidence interval), p=0.05.

3.5: Ethical Consideration

The Makerere University College of Natural Sciences, Department of Plant Sciences, Micro-Biology, and Biotechnology, and Uganda Industrial Research Institute (UIRI) provided the necessary clearances, which are attached in the appendices of this report. This research study adhered to the guidelines established by the university research committee. The authors of all the informational sources included in this study, including reviews, manuscripts, textbooks, and reports, have been correctly mentioned and given due credit.

CHAPTER 4: RESULTS AND DISCUSSION

4.1: Introduction

This chapter presents results from various experimental setups on sewage sludge vermicomposting, including characterising sewage sludge from Bugolobi and Lubigi STPs, figuring out the ideal earthworm stocking density for vermicomposting, and analysing the effect of heavy metal toxicity on vermicomposting in comparison to UNBS standards (UNBS, 2017), and EPA 40 CFR Part 503 standards for use and disposal of sewage sludge.

4.2: Characterisation of sewage sludge

Sludge's features as a whole at Bugolobi and Lubigi STPs at two (2) weeks – (fresh sludge) and six (6) weeks – (matured compost) were determined for parameters such as total nitrogen (TN), faecal coliforms, electro-conductivity (EC), Lead (Pb), and total organic carbon (TOC) among others. This section presents findings from the laboratory tests conducted on the sewage sludge samples as Table 4.1 provides a summary.

Table 4. 1: Physiochemical characteristics of fresh and ready sludge at Bugolobi STP

Parameters	Sludge Stage		
	Sludge at Two weeks	**Sludge ready for uptake**	**Recommended minimum/maximum**
Total Nitrogen (%)	2.54±0.05b	2.54±0.04b	1.00a
Total Phosphorus (%)	3.64±0.05c	2.95±0.03b	1.00a
Lead (mg/kg)	133.48±3.42b	168.05±4.32c	100a
Zinc (mg/kg)	1179.3±5.16a	1287.64±15.49b	2800c
Electroconductivity (mS/cm)	12.88±0.00b	16.90±0.00c	5.00a
MPN	280±0.00c	220±0.00b	0.00a
Total Organic Carbon (%)	32.48±2.27b	52.02±2.45c	12.00a
C: N - Ratio	13±0.74a	21.96±0.72b	25.00c

Values are Means±SEM (n=3). Turkey's test (P<0.05) for every given parameter in the specified sludge stages indicates significant difference between the values when means with different letter of the alphabet inside a certain row. [SEM, standard error of the mean]

The research observed that Total Nitrogen (TN) was 2.54% and 2.59% for fresh and matured sludge samples from Bugolobi STP respectively indicating a slight increment in the TN of matured sludge with all the two samples scoring above recommended 1% minimum standard for organic fertilizers (UNBS, 2017). The Total Phosphorous (TP) content recorded for the two samples was higher as compared to their TN and stood at 2.93% and 3.69% for fresh and matured sludge samples respectively. The high level of these nutrients in sewage sludge at Bugolobi STP points to its nutrient-enriching potential to support plant growth.

The level of these nutrients increased between fresh and matured sludge which phenomenon was also observed by Pattnaik and Reddy, 2010. This can be explained from the net loss of mass from mineralization and oxidation of organic matter during which carbon-dioxide gas (CO_2) is lost to the atmosphere thereby concentrating the contained nutrients (Nayak, Varma and S. Kalamdh, 2013; Huang et al., 2004). The increment in Phosphorous is particularly attributed to phosphorous mobilization and alkaline phosphatase activity secreted by earthworms during vermicomposting (Zhu et al., 2016; Yadav and Garg, 2009). However, though nutrients are important for plant growth, high levels of nutrients could bring about eutrophication with repetitive and uncontrolled application to agriculture fields.

The recorded Lead (Pb) contents for fresh and matured sludge at Bugolobi STP were 133.48mg/Kg and 168.05mg/Kg respectively above 100mg/Kg permissible limit for land application (UNBS, 2017). The observed Zn contents i.e. 1185.245mg/Kg and 1290.206mg/Kg for the two samples respectively conformed to set limits by EPA 40 CFR Part 503 even for EQ biosolids i.e. 2800mg/Kg implying that Zn for the two samples was within acceptable limits for disposal. There was an observed general increment in the levels of both Pb and Zn for the two samples which is similar to observations made by Sosnecka, Kacprzak and Rorat, (2016), explained as a result of weight and biomass reduction during composting due to solubilization of organic matter which progressively increases solids content and consequently sewage sludge's content of these heavy metals.

Electro-Conductivity (EC) is indicative of salinity levels and concentration of metal ions in the sample and this was observed as 12.88mS/cm and 16.90mS/cm for fresh and matured sludge samples respectively which were above the recommended maximum limit of 5mS/cm (UNBS, 2017). The observed increment in EC can be attributed to similar increase in the amount of heavy metals with time. High levels of EC for the samples may be indicative of salinity levels that may exceed bearable salt tolerance limit for plant growth which may not warrant agricultural application. It was noted that fresh sludge (2 weeks) at Bugolobi STP offered better characteristics with respect to recommended limits for Pb, Zn and EC as compared to the matured sludge.

The sample's carbon-nitrogen ratio (C/N) and total organic carbon (TOC) are indicative of maturity of the sample. These all exhibited a similar decreasing trend between fresh and matured sludge for instance TOC from 52.02% to 32.48% whereas C/N from 21.96 to 13.0 respectively similar to a study by Ludibeth, Marina and Vicenta, (2012) in which the C/N of 12.46 was obtained. These results are consistent with experiments done by Baoyi et al. (2019) and Sharma and Garg, (2018), which explained the decrease in TOC and C/N phenomenon as a result of the ongoing mineralization and oxidation of organics that causes an increase in the samples' TN over time while the carbon content is decreased due to the release of CO2 during the process.

Due to the fact that plants cannot assimilate nitrogen unless this ratio is brought to the range of 20 or below, C/N is employed as an index for the maturity of organic/biological wastes as well as a crucial metric for fertilisers (Senesi, 1989). According to Rostami et al. (2010), elevated C/N affects microbial activity whereas reduced C/N brings about the release of Ammonia gas (NH3) to the atmosphere. The reduction in TOC and C/N observed for the samples signifies continued stabilisation and therefore implying that matured sewage sludge was more stabilised and readier for land application as compared to fresh sludge. Regarding the suggested parameters for mature compost, i.e., 12.00% minimum and 25% maximum, both TOC and C/N of the two samples were satisfactory (UNBS, 2017).

In terms of bio-safety, both sewage sludge samples from Bugolobi STP tested positive for faecal coliforms i.e., 280MPN and 220MPN for fresh and matured sewage sludge respectively and above recommended limit of 0MPN (UNBS, 2017). This is indicative of Escherichia Coli and presence of disease-causing organisms such as Salmonella. The results are comparable with the observation made by Lubuulwa and Olupot, (2019) who identified high levels of faecal coliforms in mature sludge at 3946±86”000” CFU/g.

The outcomes of the laboratory experiments thus revealed significant differences in the physicochemical properties of both fresh and matured sludge at P=0.05 (ANOVA, P0.001) with the exception of TN (ANOVA, P=0.997). The two sewage sludge samples did not satisfy all requirements for organic fertilizers as specified by UNBS, (2017) and EPA 40 CFR Part 503 however, it is critical to note that matured sludge at six (6) weeks performed better than fresh sludge at two (2) weeks (Tukey's test result: $P>0.05$). In this event the null hypothesis which states that "Neither Sludge at six weeks nor sludge at two weeks are in agreement with the recommended minimums" is accepted.

Therefore, sewage sludge from Bugolobi STP should categorically be handled as Class B sewage sludge requiring strict restriction in its use and application to agricultural fields so as to limit risks to both humans and the environment for instance 30 days minimum restriction for public exposure, crop harvesting, and grazing. In comparison with sludge at Lubigi STP, relatively better physio-chemical characteristics were observed for all parameters except for Zn which was still within the recommended limits. The results for EC, Pb, and faecal coliforms however much they were better than those of matured sludge from Bugolobi STP, they did not satisfy set limits for organic fertilizers. Table 4.2 below compares results of physico-chemical tests conducted on sewage sludge samples from both Bugolobi and Lubigi STPs.

Table 4. 2: Characteristics of ready sewage sludge at Bugolobi and Lubigi STPs

Physicochemical properties of sludge	**Sewage treatment plant (STP)**		**P – values and levels of significance**
	Bugolobi	**Lubigi**	
Electroconductivity (mS/cm)	16.9±0.00	5.9±0.00	<2e-16 ***
Lead (mg/kg)	161.02±3.17	156.34±2.26	0.106
Total Nitrogen (%)	2.52±0.02	2.58±0.01	0.012 *
Total Phosphorus (%)	2.91±0.02	4.33±0.02	0.0000000663 ***
Zinc (mg/kg)	1275.20±17.61	1547.19±6.81	0.0000153 ***
MPN/100g	280±0.00	160±0.00	<2e-16 ***

Values are Means±SEM, Inferential statistic is stated in the last column ((Only three of the most frequently used levels' significance levels are intended to be indicated by stars. It is marked with one star (*) if the p-value is less than 0.05. The p-value is marked with two stars (**) since it is less than 0.001. Three stars (***) are indicated and the p-value is less than 0.0001. Standard error of the mean (SEM)

The low Pb and EC could be explained by the fact that Lubigi STP predominantly receives domestic waste water as opposed to Bugolobi STP that operates mainly as a combined waste water treatment plant receiving waste water from all sources such as domestic, storm water, and industrial waste water. This in addition to the lower levels of faecal coliforms makes sewage sludge from Lubigi better suited for land application as opposed to Bugolobi STP but with further stabilisation.

4.3: Optimum earthworm stocking density for vermicomposting

The research observed that using different earthworm stocking densities did not significantly affect the TN, TOC, Carbon Nitrogen ratio (C/N), and Pb of the vermicomposted sewage sludge at the P=0.05 level (ANOVA, P=0.404, P= 0.425, P=0.938, and P= 0.0578, respectively) as these followed similar trend as the negative control sample of 0kgworms/m^2. However, electroconductivity and MPN were significantly different between stocking densities at P=0.05 (ANOVA, $P=9.7*10^{-6}$, $P=1.48*10^{-9}$ respectively). A summary of these parameters per earthworm stocking density is provided below in table 4-3.

Table 4. 3: Physiochemical characteristics of sludge with various stocking densities

Parameters	Earthworm Stocking Density (KgWorms/m^2) used in Vermicomposting					
	2.5	**1.0**	**0.0**	**3.5**	**2.0**	**Recommended min/max**
Total Nitrogen (%)	3.20±0.43b	3.39±0.43b	3.51±0.68b	3.54±0.68b	3.36±0.53b	1.00a
Total Phosphorus (%)	6.51±1.59a	3.91±0.41a	4.53±0.30a	4.54±0.30a	4.50±0.41a	1.00a
Lead (mg/kg)	251.33±39.23ab	254.29±58.58ab	228.21±38.42ab	277.85±73.67b	278.66±69.34b	100a
Zinc (mg/kg)	1246.47±272.74a	1201.34±203.37a	1283.88±182.22a	1185.20±135.82a	1196.20±114.42a	2800a
Electroconductivity (mS/cm)	8.76±0.85ab	10.50±1.98c	9.81±0.72bc	10.39±1.25c	8.59±1.36ab	5.00a
MPN	241.67±43.01a	263.33±32.36a	430.00±229.71b	193.333±60.49a	165.00±52.05a	0.00a
Total Organic Carbon (%)	46.25±15.54a	46.03±14.88a	45.59±14.97a	46.04±15.17a	46.06±15.34a	12.00a
C: N – Ratio	14.90±5.33b	15.28±6.13b	16.51±5.85b	15.00±6.40b	15.58±6.71b	25.00a

Values are Means±SEM (n=3); Turkey's test (P<0.05) for any given parameter in the stocking density denotes a significant difference between means with various letters of the alphabet in the same row. [Standard error of the mean, SEM]

Total Nitrogen

Total nitrogen of the samples for all stocking densities generally followed an increasing trend from the start of the vermicomposting process up to week 6. A graphical representation of the results is as shown in figure 4.1 below;

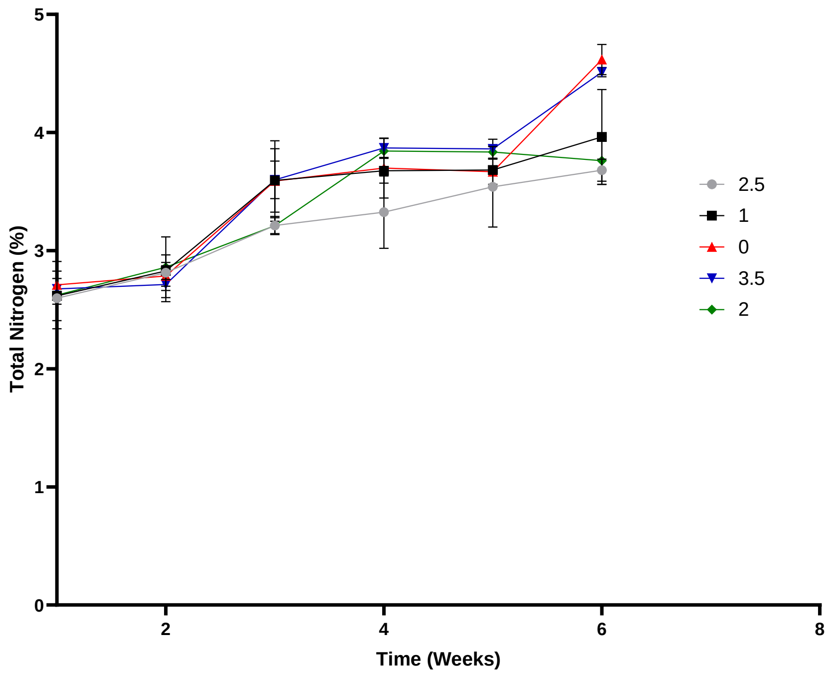

Figure 4. 1: Graph of change in Total Nitrogen over time for different stocking densities

From the results, earthworm amendments had no significant effect on the total nitrogen of the sludge over time (ANOVA, P=0.404). Setup with stocking density 3.5 earthworms/m^2 had the highest percentage of nitrogen of approximately 3.54% while stocking density 2.5 earthworms/m^2 had the lowest of all the five setups i.e., 3.20%. The analysis of results for all the setups further indicated an average 2-fold deviation in total nitrogen in the amendments as compared to the recommended minimum of 1.00 (UNBS, 2017).

There was no initial reduction in TN as a result of loss of NH_3 from ammonification as reported by Zeng et al. (2018) but rather progressive increment in TN which can be ascribed to the sewage sludge's initial two-weeks composting phase, action of IMO bacteria and earthworms in creating aerobic conditions in sewage sludge through their burrowing and shuttling activities thereby promoting increasing levels of Nitrates and nitrogenous excreta secreted by earthworms (NO3-N) (Fu et al., 2015; Yang et al., 2014).

Lead

Waste water especially from combined sewerage systems usually contains lead due to a number of sources such as industrial waste and leaded fuels. An encounter with lead has potential adverse effects to the environment and is toxic to humans at about 800mg (Acharya et al., 2009). Lead content in vermicomposted sewage sludge was assessed for the different stocking densities. The results from the laboratory tests are presented in figure 4.2 below.

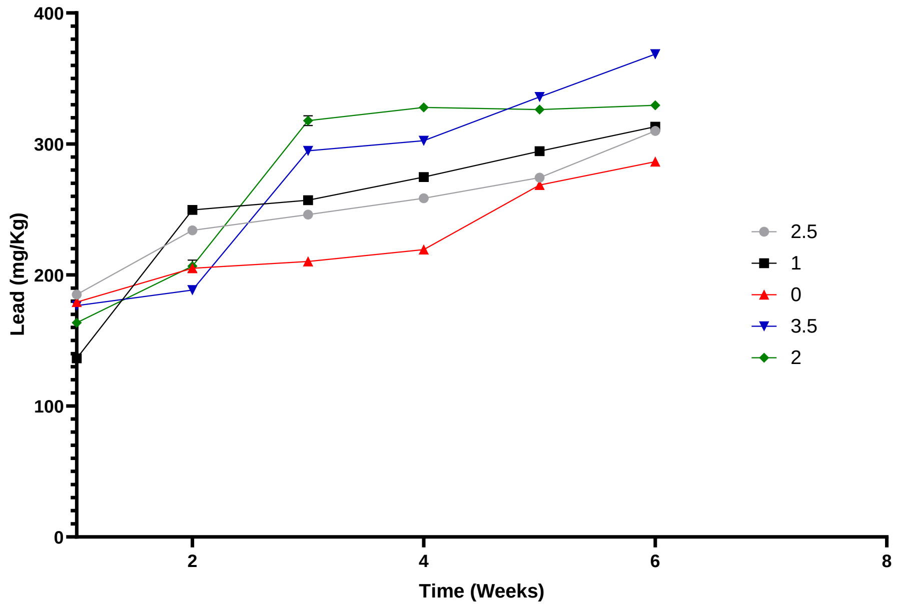

Figure 4. 2: Lead Content Changes over Time for different stocking densities

There was no observed significant difference in the trends of the samples in relation to their Lead content over the six-week composting period at P=0.05 (ANOVA, P=0.0578). The Lead amount in all samples increased with time and this can be attributed to the general reduction in mass with the loss of organic matter through decomposition similar to observations by Sosnecka, Kacprzak and Rorat, (2016). Vermicomposted samples were observed to have higher Lead concentrations as compared with the control sample due to enhanced microbial activity with earthworms which facilitates rapid organic matter (OM) decomposition. The Lead content in all samples was beyond recommended maximum 100mg/kg. However, it was observed that the Lead content in the samples at the end of the experiment was lowest for the control sample (0kgworms/m^2) i.e. 228.21±38.42mg/kg and increased with increase in earthworm stocking density of the samples with 3.5kgworms/m^2 stocking density having the highest Lead concentration at 277.85±73.67mg/kg.

According to Zhang et al., 2017, the increased Lead content in the vermicomposted samples may not pose great danger to the environment as earthworms stabilise the Lead in the sludge into residual forms through the creation of humic compounds and formation of insoluble organometallic complexes which reduces its mobility and phytotoxicity in biosolids.

Electro-Conductivity

A crucial factor in determining the quality of sewage sludge is the electro-conductivity of a sample, which is indicative of its salinity and heavy metal content especially for biosolids destined for land disposal. The results from vermicomposting of sewage sludge using different earthworm stocking densities are presented in Figure 4.3 below.

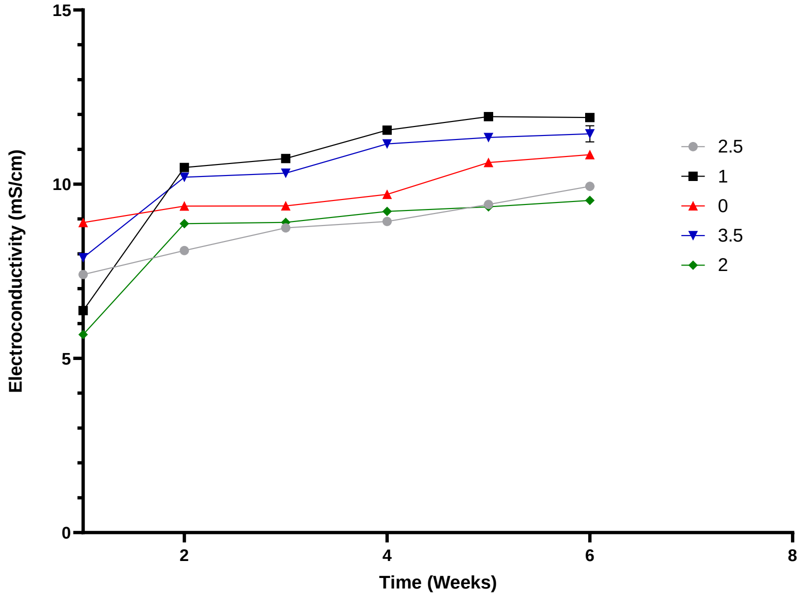

Figure 4. 3: Graphs of change in Electroconductivity with time

Electroconductivity of the sludge over time indicated significant differences at P=0.05 (ANOVA, $P=9.7X10^{-6}$) amongst the various worm stocking density treatments. Stocking density, $1.0kgworms/m^2$ recorded the highest value of electroconductivity in the range 10.50±1.98mS/cm. It was noted that on average, stocking density $1.0kgworms/m^2$ had twice as much the limit recommended for organic fertilizers, 5mS/cm (UNBS, 2017). Post hoc comparisons indicated that stocking densities, 2.5 and 2, 1 and 3.5 had similar effects on the electroconductivity (Tukey's test, P>0.05) with the values increasing throughout to week six.

Stocking densities 2.5kgworms/m^2 and 2kgworms/m^2 had comparably better EC results compared to stocking densities 0, 1 and 3.5kgworms/m^2. However, all stocking densities experienced a general increment in their EC overtime attributed to weight loss from decay of organic materials and rising salt and heavy metal concentrations (Garg et al., 2006; Suthar, 2007). According to Garrido et al. (2005), EC beyond 4000 µScm-1 restrains plant growth by inducing high osmotic pressures in the roots and therefore where sewage sludge exhibits such high characteristics of EC, continued use of resultant bio-solids on land leads to soil salinization which is a form of toxic soil pollution.

Faecal Coliforms

Faecal coliforms depict the presence of disease-causing organisms in the sample and these were tested for to confirm the neutralizing effect of the various stocking densities during vermicomposting process. Figure 4.4 below highlights the results from the laboratory tests on the samples.

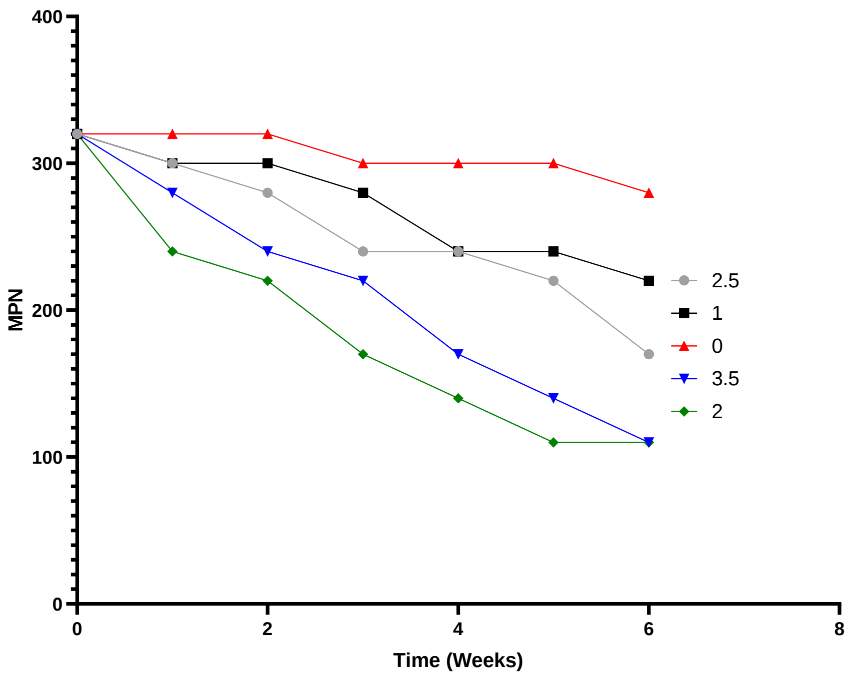

Figure 4. 4: Variation of Faecal Coliform against time for various stocking densities

Significant differences were observed in faecal coliforms at P=0.05 (ANOVA, P=2.87x10^{-10}). Stocking density, 0kgworms/m^2 had the highest number of faecal coliforms in the range 305.71±14.34MPN and showed no significant reduction over the course of the six weeks whereas

2kgworms/m^2 stocking density had the lowest level of faecal coliforms in the range 187.14±73.43MPN which was the only stocking density statistically similar with the recommended limit of 0MPN as per UNBS guidelines (UNBS, 2017).

With the expectation that these may have been lowered to undetectable levels after 8 to 10 weeks of the settings, the reduced levels of faecal coliforms in the various vermicomposting setups demonstrate that earthworms were significantly involved in the reduction of the faecal coliforms. The phenomenon of decreased faecal coliforms during vermicomposting is explained in a study by Monroy, Aira and Domínguez, (2009). It indicates that the earthworms' digestive processes were the primary reason for the 98% drop in coliform population after passing through them during vermicomposting.

Total Organic Carbon

The sewage sludge's overall organic carbon is a good indicator of the maturity of sewage sludge in terms of its readiness for disposal onto agricultural lands and was determined for samples with different stocking densities over a period of six weeks. Figure 4.5 below presents a trend of the results over the course of the vermicomposting process.

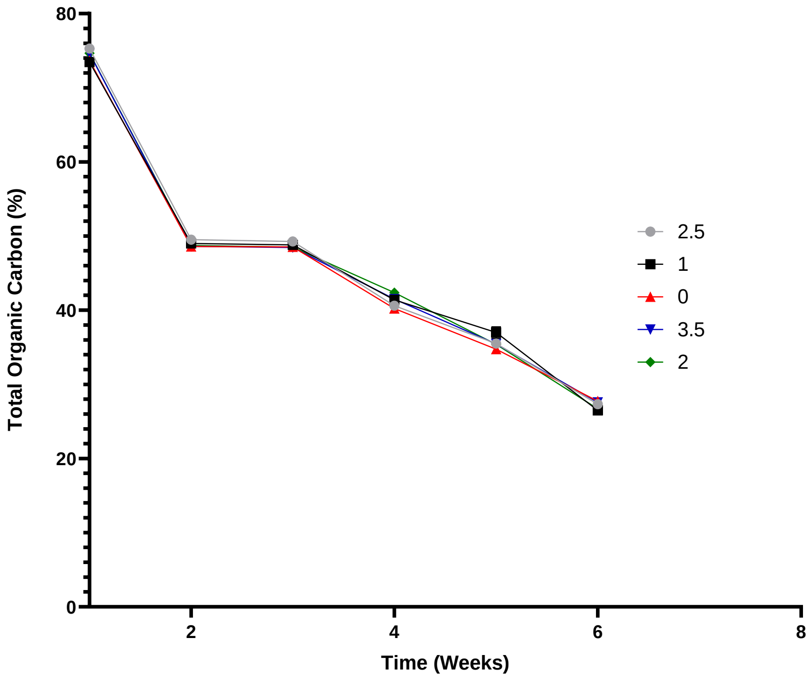

Figure 4. 5: Graph of Total Organic Carbon against time for various stocking densities

Different stocking densities resulted into varying levels of Total Organic Carbon (TOC) but with all stocking densities generally leading to a decrease in the amount of TOC over the six-week period. The negative control, 0kgworms/m^2 caused the most significant lowering of TOC achieving 45.59±14.97. This was 33.59% closest to the recommended 12% minimum implying that the stocking densities of 1.0, 2.0, 2.5, and 3.5kgworms/m^2 achieved better TOC results i.e., 46.03±14.88, 46.06±15.34, 46.25±15.54, and 46.04±15.17 since they achieved higher values in relation to the minimum. However, all stocking densities had similar reducing influence on TOC over the vermicomposting period (P>0.05 on the Tukey's test).

Carbon to Nitrogen Ratio

Similar to TOC, the Carbon-Nitrogen ratio (C/N) is an effective indicator of maturity of compost. For organic fertilisers, UNBS has set a maximum C/N restriction of 25, with the lower the C/N value, the better (UNBS, 2017). The study attempted to calculate C/N values for biosolids composited with different stocking densities and figure 4.6 below presents the results of laboratory analysis of the samples.

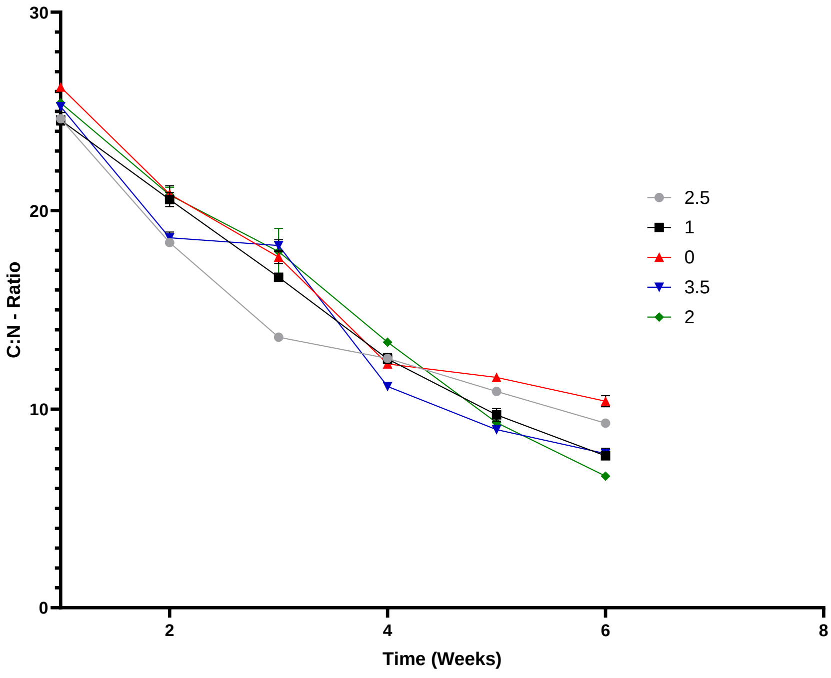

Figure 4. 6: Graph showing variation of C/N against time for various stocking densities

It was observed that the C/N for all stocking densities generally constantly reduced over the six-week period. Averagely, the stocking density of 2.5kgworms/m^2 initially had the best return with respect

C/N up to the fourth week whereas no significant differences were identified except that the 0kgworms/m^2 stocking density had the highest rise in the range of 16.51±5.85.

The relative performance of each stocking density with respect to the parameters' during vermicomposting was established from the Tukey test (comparison between means). The closeness of the parameters of the vermicompost from the different stocking densities compared to recommended minimum/maximum showed that stocking density 2.5kgworms/m^2 had the greatest resemblance of 50% to the recommended minimum/maximum, followed by 1.0kgworms/m^2 and, 2.0kgworms/m^2 at 41.67%, 3.5kgworms/m^2 at 33.33% and lastly stocking density 0.0kgworms/m^2 with the least influence on the parameters with respect to the permissible limits at 25%. This implies that stocking density 2.5kgworms/m^2 is optimal for vermicomposting of sewage sludge which is line with recommendations by Wang et al., 2017.

4.4: Heavy metal toxicity on the physio-chemical properties of vermicomposted sludge

Toxicological effects of heavy metals on earthworms

Investigation on the toxicological effects of heavy metals in earthworms using Lead concentration (Pb^{2+}) showed that it was toxic to earthworms above specific concentrations and exposure times and can have an impact on their variety, distribution, and abundance. Table 4.4 below shows results from acute toxicological test after 48hours.

Table 4. 4: Heavy metal toxicological effect on earthworms

Parameter	**Toxicity levels and Survival Rates of Earthworms**				
Lead Conc. (Moles)	0.0007	0.0014	0.0021	0.0028	0.0035
Number of worms	20.00±0.00c	20.00±.00c	20.00±0.00c	16.00±1.00b	13.67±0.58a

It was observed that Lead concentration (Pb^{2+}) in the ranges of 0.0007, 0.0014 and 0.0021 moles were palatable for earthworms and the number of earthworms before and after the setups didn't alter significantly. i.e., P=0.05 (ANOVA, P=1). At increased levels of Pb^{2+} i.e., 0.0028 and 0.0035moles, earthworm mortality was recorded with results of earthworm numbers at the end of the setup statistically different from the rest P=0.05 (ANOVA, P=0.001) and from each other P=0.05 (ANOVA, P=0.00178). Therefore, earthworm survival reduced with increase in Pb^{2+} above nominal 0.0007moles concentration of Lead in sewage sludge at Bugolobi STP.

The mortality of earthworms beyond 0.0021 i.e., 0.0028 and 0.0035 can be explained from a study by Mostafaii et al. (2016) in which earthworm toxicological symptoms were induced by heavy metal toxicity such as oozing of coelomic fluid from the worm's body, swelling of the clitellar region, posterior segments, and morphological changes involving curling and excessive mucus secretion with bloody lesions. This affects growth and metabolism of earthworms while causing cell membrane integrity to be destroyed or protein denaturation, which finally causes death.

Gudeta et al. (2023) explained the survival of earthworms with increasing heavy metal toxicity as due to special surface-active metabolites in the guts of earthworms called drilodefensins that defend earthworms against the effects of toxics elevating the antioxidant activities of their enzymes and converting them into harmless compounds or useful nutrients. The ability of earthworms to act as biofilters, bioindicators, bio accumulators, and transformers of oxidative polyphenols, microplastics, toxic heavy metals, and other pollutant hydrocarbons in addition to the presence of microorganisms in their gut which assist in fixation, accumulation, and transformation of these toxicants contributes to the survival and adaptability of the earthworms.

The expected median lethal concentration (LC50) for the setups with 20 earthworms over a period of 7-14days was 10 earthworms and considering fatalities in the setups with Pb^{2+} at 0.0028 and 0.0035 moles i.e., 4 and 6 mortalities respectively after 48 hours, Pb^{2+} in these ranges equivalent to four times the nominal lead concentration was therefore proved to be unpalatable for earthworm activity.

Earthworm Bioaccumulation of heavy metals

Bioaccumulation is a direct biological quantifier of heavy metal bioavailability as it measures the actual amount of heavy metals taken up by the earthworm and incorporates all of the effects of biotic (e.g., earthworm behaviour) and abiotic (e.g., soil pH) modifying factors over the duration of exposure (Shuo Yu, 2009). At the conclusion of the vermicomposting process, earthworms were tested for the concentration of these heavy metals to determine the role that they played in the vermicomposting of sewage sludge at higher heavy metal concentrations and table 4.5 below highlights the observations made.

Table 4. 5: Bioaccumulation of Lead and Zinc during vermicomposting

Earthworm Sample	1. Earthworm Initial Conc.	2. Nominal Conc. 0.0007	3. Double Conc. 0.0014	4. Triple Conc. 0.0021
Lead (mg/l)	<0.02±0.00a	6.71±0.07b	15.83±0.10c	20.15±0.82d
Zinc (mg/l)	66.11±0.11a	172.86±0.76b	222.60±0.34c	230.84±0.88d

Values are Means±SEM (n = 3); Tukey's test (P<0.05) indicates a significant difference between the values for any given parameter in the worm treatments when the means in a given row begin with a different letter of the alphabet. According to an ANOVA, there were significant differences in the lead concentrations of the worms across the four groups (P = 2.91e-11). Zinc concentrations in the worms were likewise significantly different in all four groups (P 2e-16) according to an ANOVA.

The bioaccumulation of worms was significantly affected by increasing the concentration of heavy metals (Pb2+) in samples 2, 3, and 4 at the P=0.05 level (ANOVA, P=2.91e-11 and P2e-16, respectively). Earthworms from the setup 4 with 0.0021 moles of Pb^{2+} had the highest mean lead and zinc concentrations i.e., 20.15±0.82mg/l and 230.84±0.88mg/l respectively, while the negative control – fresh earthworms indicated lowest levels of both lead and zinc i.e. <0.02±0.00mg/l and 66.11±0.11mg/l respectively.

According to the findings, earthworms bioaccumulated more heavy metals in their bodies when sewage sludge had greater heavy metal concentrations. Wang et al. (2018) also observed this tendency. In accordance with Veltman et al. (2007), the internal heavy metal concentration in the body of earthworms can be predicted and calculated, for example using the OMEGA model from Optimal Modelling for Ecotoxicological Applications.

Heavy metal toxicity and physicochemical properties of vermicomposted sludge

The laboratory test results on samples of sewage sludge vermicomposted at spiked concentrations of Lead are as listed in Table 4.6;

Table 4. 6: Characteristics of Vermicomposted Sludge at Different Lead Toxicity Levels.

Physiochemical properties	Setups with increased Lead Concentrations			Inferential statistic
	X1 = 0.0007	X2=0.0014	X3=0.0021	P-Value
TN (%)	3.99±0.99a	4.90±1.26b	4.97±1.38b	0.0194 *
TP (%)	4.54±0.31b	4.13±0.79a	4.67±0.33b	0.00378 **
Lead (mg/kg)	254.06±22.13a	435.04±78.17b	487.47±88.02c	7.34e-16 ***
Zinc (mg/kg)	1646.01±75.65a	1702.09±95.96a	1668.15±98.05a	0.138
EE (mS/cm)	7.90±0.35a	8.59±0.82b	8.77±0.75b	0.00022 ***
MPN	177.14±69.00b	144.29±66.83ab	115.71±71.17a	0.0202 *
TOC (%)	37.36±10.14a	38.20±8.73a	36.64±10.08a	0.872
C: N – Ratio	10.82±5.58a	8.94±4.32a	8.64±4.47a	0.293
MC (%)	65.88±4.77a	64.12±6.08a	63.60±7.65a	0.473
pH	6.29±0.30a	6.66±0.44b	7.00±0.47c	0.00000255 ***
Total Solids (%)	23.85±1.79a	24.14±2.25a	24.28±1.97a	0.784
Volatile Solids (%)	56.66±18.95a	57.09±19.12a	56.27±19.35a	0.991

A value is Means±SEM, means with a different letter of the alphabet inside a specific row, and the Tukey's test (P<0.05) for any given parameter in the lead amendments indicate a significant difference between the values Similar to this, the inferential statistic is listed in the final column. Three of the most frequent levels are indicated by stars. One star (*) is displayed when the p-value is less than 0.05. The p-value is less than 0. 01, is flagged with 2 stars (**). The p-value is less than 0.001, and is flagged with three stars (***)). [SEM, standard error of the mean]

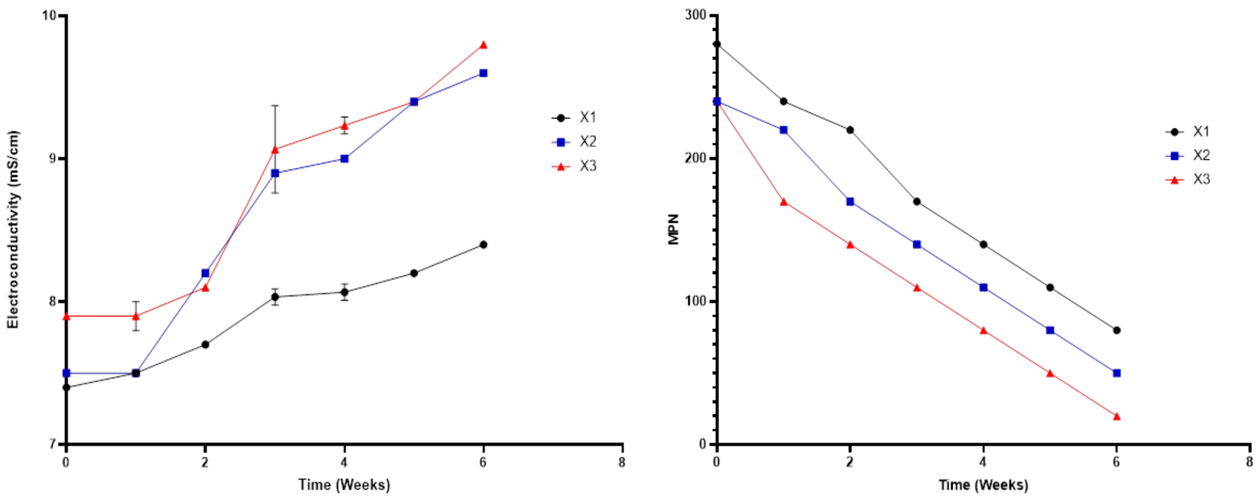

Figure 4. 7: Plots of A) Electroconductivity and B) Faecal coliform.

The electroconductivity (EC) significantly differed from one another after spiking with Lead Nitrate at P=0.05 (ANOVA, P=0.00022) with both spiked treatments X2 and X3 depicting higher electroconductivity and a generally increasing trend with time. All the recorded ECs of the samples were above the recommended maximum by UNBS (UNBS, 2017). The spiked sewage sludge samples X2 and X3 had increasingly better results with respect to faecal coliforms with lower levels of MPN as compared to the non-spiked sample X1 i.e., 144.29 and 115.71 as compared to 177.1 respectively. All samples experienced gradual decrease of the faecal coliforms however with significant difference at P=0.05(ANOVA, P=0.0202). The rapid fall of MPN in the spiked samples can be explained from the fact that heavy metal toxicity exerted its effect on the faecal coliforms by binding with cellular membranes of the organisms thus impairing their physiological and biochemical parameters (Sigel, 1974; Tridech et al., 1981).

There was no significant effect on moisture content by the treatments, at P=0.05 level (ANOVA, P=0.473]. Treatments X1 and X3 maintained the highest and lowest moisture contents respectively throughout the vermicomposting process which were all favourable for worm composting. However, the study established that Lead concentration spiking of sewage sludge had significant effect on pH at P=0.05 (ANOVA, P=0.00000255) whereby there was an observed increment of sludge pH for setup X3—the highest spiked sample—maintained the highest pH at the end of the vermicomposting process while the other boosted samples generally declined with time as shown in figure 4.8 below.

The lead nitrate utilised in the experimental setup, whose pH ranges from 8.5 to 9.0, can be used to explain the elevated pH of the spiked samples.

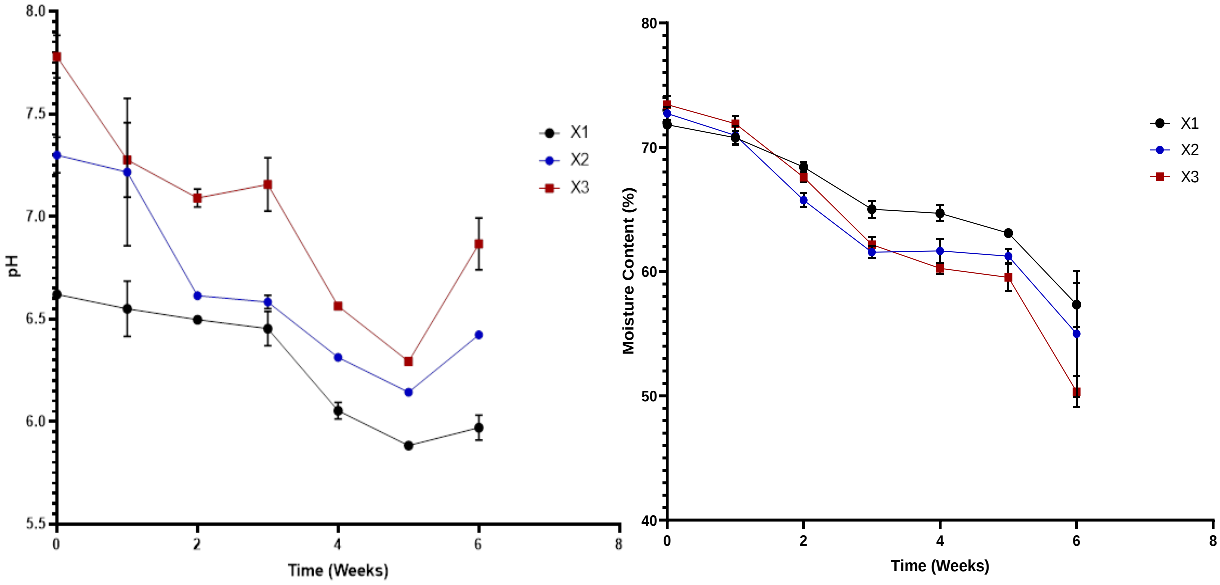

Figure 4. 8: pH and Moisture Content Variation with time

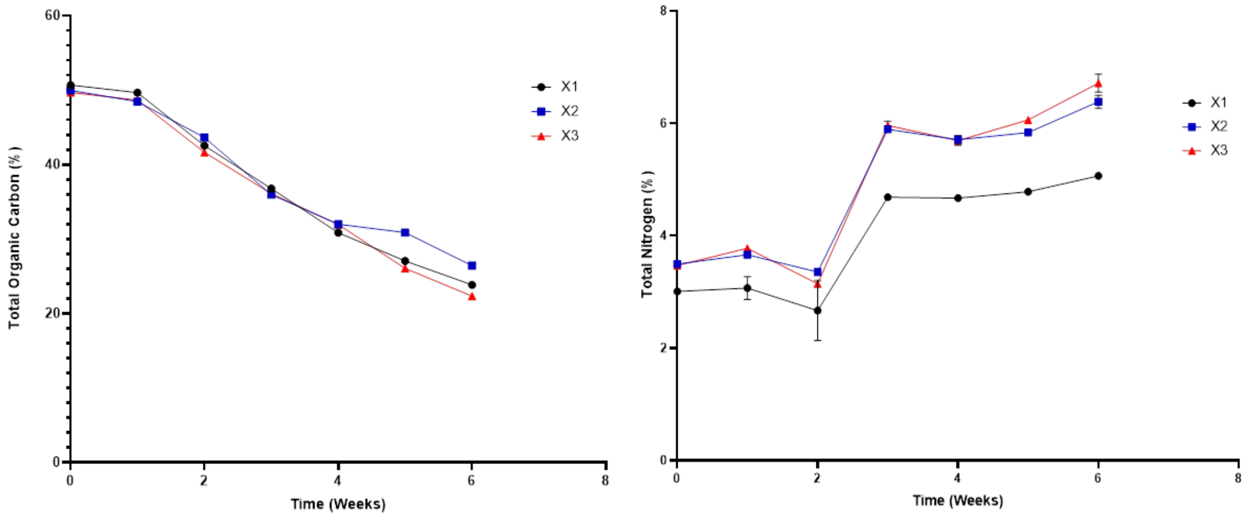

Figure 4. 9: Total Nitrogen and Total Organic Carbon Change over Time

Total Organic Carbon (TOC) results for the three treatments gave no significant difference in the readings at P=0.05 (ANOVA, P=0.872) as highlighted in figure 4.9 above. All the treatments followed a generally constant decrease pattern with time and had values beyond the recommended minimum, 12% at the end of the process. Similarly, the post hoc analysis of the results suggested no difference between the different means (Tukey's test, P>0.05). Considering Total Nitrogen (TN), significant differences were observed for the three treatments at P=0.05 (ANOVA, P=0.0194).

Treatments X2 and X3 had relatively similar effects on TN i.e., 4.90±1.26 and 4.97±1.38 respectively (Tukey's test, $P>0.05$) but appeared slightly higher as compared to treatment X1 (3.99±0.99).

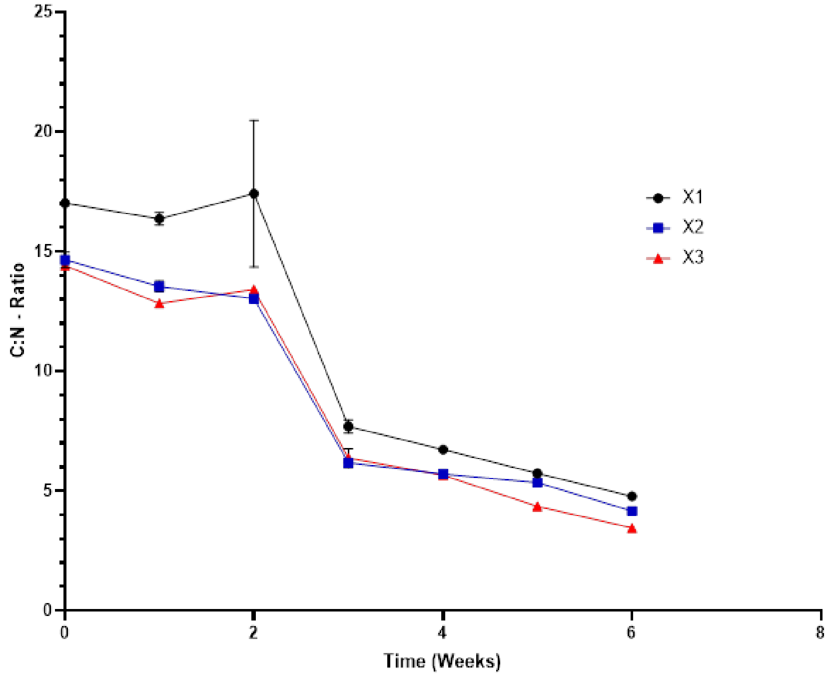

Figure 4. 10: Variation of the carbon-nitrogen ratio over time

The results of the study showed that there was still no significant difference in the Carbon-Nitrogen ratio (C/N) at $P=0.05$ (ANOVA, $P=0.293$) for the treatments since both total organic carbon and total nitrogen that are the key parameters were not significantly affected. Considering the heavy metals of Lead (Pb) and Zinc (Zn) in the treatments, significant difference for Lead Content was observed for all spiked samples with Lead Nitrate at $P=0.05$ (ANOVA, $P=7.34e-16$). Higher Pb concentrations were therefore observed with treatment X3 recording the highest Pb content after six weeks at 487.47±88.02, followed by treatment X2 with 435.04±78.17 and lastly the non-spiked sample - treatment X1 at 254.06±22.13.

The Post hoc analysis of the results indicated that all three treatments of sewage sludge had different effects (Tukey's test, $P>0.05$). In relation to the concentration of Zn, spiking of sewage sludge with Lead Nitrate had no significant effect on zinc concentration of the vermicomposted sewage sludge at $P=0.05$ (ANOVA, $P=0.138$). However, spiked treatments X2 and X3 showed higher means for Zn concentration after six weeks as compared to the non-spiked treatment X1. The increased Pb and

Zn contents of the spiked sewage sludge samples may not be of great concern as vermicomposting reduces these bio-available heavy metal forms to more stable, less soluble and unavailable metal complexes thereby minimizing their effect on the environment.

Therefore, with the exception of Lead content of the spiked samples and increased EC, the results from all the other quality and bio-safety parameters point to the fact that vermicomposting of sewage sludge yielded better quality compost at higher heavy metal concentrations up to such levels palatable to earthworm activity.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1: Conclusions

The following conclusions were reached in light of the outcomes of this research investigation;

I. The sewage sludge from Lubigi STP offered better prospects with respect to land application as compared Bugolobi STP with matured sludge at all the plants exhibiting better characteristics as compared to fresh sludge. Nitrogen, Phosphorous, Total Organic Carbon and Carbon-to-Nitrogen ratio of the sludge samples were within allowable limits. However, the sludge also contained a lot of heavy metals especially lead, electro-conductivity, and feacal coliforms above permissible limits. This implies that any potential use of such sewage sludge should follow strict restrictions as have been formulated for Class B biosolids.

II. An earthworm stocking density of 2.5kgworms/m^2 was optimal for sewage sludge vermicomposting.

III. Earthworms exhibited heavy metal tolerance limits of approximately 0.0021moles of Lead below which they showed increased bio-accumulation at higher heavy metal concentrations. Within palatable heavy metal concentrations, vermicomposting significantly improved the quality and bio-safety of resultant vermi-compost.

5.2: Recommendations

I. A vermi-composting facility based on a 2.5kgworms/m^2 earthworm stocking density should be established at Bugolobi sewage treatment plant to further improve sewage sludge to acceptable quality before disposal.

II. There is need to carry out further investigation on the speciation of heavy metals in the resultant biosolids after vermicomposting of such heavy metal spiked sewage sludge so as to conclusively ascertain environmental threat from the use of the biosolids.

III. Vermicomposting setups were run for six weeks, it would be crucial to determine what effect it would have to run the experimental setups for more than six weeks say 8-10 weeks. This would help ascertain at what point, total sterilization of sewage sludge with respect to feacal coliforms is achieved.

IV. There should be strict follow-up by accountable organizations like Kampala Capital City Authority (KCCA) and NEMA on waste water treatment especially from industries and factories upstream. This will minimize the concentration of heavy metals in sewage and consequently sewage sludge at the plant.

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APPENDICES

APPENDIX 1: UIRI Clearance

UGANDA INDUSTRIAL RESEARCH INSTITUTE

"A Lead Agency in Industrialisation of Uganda"

24 January, 2022

To

Mr Mafabi Grace
17/U/14685/PE
Kyambogo University
Banda – Kyambogo Rd
P.O.BOX 1 (UG)

Dear Sir

RE: Authorization to have your lab work conducted at Uganda Industrial Research Institute

Following the letter, you wrote to me requesting to have your Physicochemical properties of sludge analyzed at the chemistry laboratory of Uganda Industrial Research Institute, and in the matter of the above reference, I take great pleasure in granting you permission to have your work analyzed. Congratulations!

Looking forward to working with you

Yours sincerely

Muhereza Ivan

Head Chemistry Laboratory

This Certificate of Analysis is only valid if it bears an authorized signature and official stamp. This certificate has been issued without any alternation and may not be reproduced other than in full except with written approval from the Executive Director, Uganda Industrial Research Institute.

Office Address: Plot 424, Makubuya Road, Nakawa Industrial Area

Postal Address: P.O. BOX 7086, KAMPALA (U)

Telephone: +256-414-286 124/245, +256-414-286 695

E-mail:info@uiri.org
Website: www.uiri.org
Fax:+256-414-286 695

APPENDIX 2: Makerere University Laboratory Clearance

MAKERERE UNIVERSITY

P.O. Box 7062 Kampala, Uganda
http://www.sci.mak.ac.ug
http://www.science.ac.ug

Tel: +256-414-540765 URL:
Fax: +256-414-532780
Email: pmb@cns.mak.ac.ug

COLLEGE OF NATURAL SCIENCES
DEPARTMENT OF PLANT SCIENCES, MICROBIOLOGY AND BIOTECHNOLOGY

November 1, 2021,

The in-Charge Research Laboratory
Dear Sir,

RE: INTRODUCTION OF MR MAFABI GRACE – 17/U/14685/GMEW/PE

I take great pleasure in introducing to you the above-mentioned gentleman. He is a student pursuing a master's degree in water and sanitation engineering at Kyambogo University.
He seeks to have his research project work done in the Research laboratory in the fields of spectroscopic, chemical, conductivity, and thermogravimetric analysis anticipated to run for five months starting November 2021.
I therefore kindly request you to guide him and provide him with all the necessary help needed to accomplish his laboratory work.

Yours sincerely,

Abubakar Sadik Mustafa
Ass. Head of Labs & stores
P. O. Box 7062 Kampala - UGANDA
Tel: +256-779-764915

APPENDIX 3: NWSC Clearance

NATIONAL WATER AND SEWERAGE CORPORATION

TELEGRAMS WATERS KAMPALA
Telephone: +256-414-315 000
+256-312-280414/5
Fax:0414 – 256 288/345531/348447
Email: info@nwsc.co.ug

HEAD OFFICE

P. O. BOX 7053
PLOT 3, Nakasero,
KAMPALA

Ref.: BSS/R&D/22-05 **Date**: Jan 2, 2022

The Sen. Manager, Sewerage Services-KW

Re: Permission to collect sewage and sludge samples from the Nakivubo WWT Plant

This is to introduce to you Mr. MAFABI Grace, a student of Kyambogo University, pursuing an MSc. In Water and Sanitation Engineering, who has been granted permission to collect samples from the Bugolobi WWT Plant. The title of his research study is ***"Effect of Increased Sewage Sludge Toxicity on the Quality of Vermicomposted Biosolids- A Case Study of Bugolobi Sewage Treatment Plant"***

The student would like to have access to the wastewater treatment plant and take samples of sewage and sludge for analysis at a laboratory elsewhere. In this regard, you are kindly requested to provide him with the necessary access and assistance to enable him successfully accomplish his study. This permission will be valid until 30th July 2022.

Anticipating your usual cooperation.

Christopher Kanyesigye
Manager, Research and Development

APPENDIX 4: Data and Results

MAKERERE UNIVERSITY

P.O. Box 7062 Kampala, Uganda
http://www.sci.mak.ac.ug
http://www.science.ac.ug

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Fax: +256-414-532780
Email: pmb@cns.mak.ac.ug

COLLEGE OF NATURAL SCIENCES
DEPARTMENT OF PLANT SCIENCES, MICROBIOLOGY AND BIOTECHNOLOGY

Jan, 31st, 2022

RESEARCH TEST RESULT.

Student Name: MAFABI GRACE

Reg. No: 17/U/14685/GMEW/PE

WEEK 0

	Test parameter	Final sludge	Two-week sludge
1	Total Phosphorus, %	2.93	3.69
2	Total Nitrogen, %	2.53	2.59
3	Lead, mg/Kg	164.35	136.84
4	Zinc, mg/Kg	1290.21	1185.24
5	Electroconductivity, mS/cm	16.90	12.88
6	Volatile Solids, %	97.50	62.00
9	MPN/100g	220	280
10	Moisture content, %	64.23	70.18
11	Total Solids, %	35.77	29.82
12	PH	6.22	7.09
13	Total Organic Carbon, %	54.17	34.44
14	C/N Ratio	21.37	13.28

UGANDA INDUSTRIAL RESEARCH INSTITUTE

"A Lead Agency in Industrialisation of Uganda"

Research Test Results

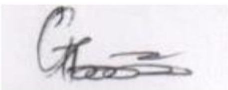
Student Name: MAFABI GRACE

REG. NO: 17/U/14685/GMEW/PE

Date of Analysis: 25th – 28th April 2022

TEST PARAMETERS	Sample ID	Result
Total Phosphorus (%)	X1	4.209461
	X2	3.089001
	X3	9.999453
Total Nitrogen (%)	X1	5.01842
	X2	6.38852
	X3	6.60323
Lead (mg/Kg)	X1	211.2900
	X2	450.9573
	X3	515.8743
Zinc (mg/Kg)	X1	1523.094
	X2	1609.782
	X3	1569.352
Electroconductivity (mS/cm)	X1	8.4
	X2	9.6
	X3	9.8
Volatile Solids (%)	X1	32.02
	X2	32.75
	X3	29.83
Total Organic Carbon (%)	X1	23.86452
	X2	26.45224
	X3	22.34624
C/N Ratio	X1	4.75539
	X2	4.14059
	X3	3.38414

Analyzed by



Waibale Wilber,

Scientist Chemistry Laboratory

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Tel: +256-414-286 124/245, Fax: +256-414-286 695 Email: info@uiri.org Website: www.uiri.org

UIRI

UGANDA INDUSTRIAL RESEARCH INSTITUTE

"A Lead Agency in Industrialisation of Uganda"

Research Test Results

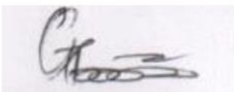
Student Name: MAFABI GRACE

REG. NO: 17/U/14685/GMEW/PE

Date of Analysis: 14th – 18th March 2022

TEST PARAMETERS	Sample ID	Result
Total Phosphorus	X1	5.086887%
	X2	4.854576%
	X3	4.957895%
Total Nitrogen	X1	2.976742%
	X2	3.487929%
	X3	3.456662 %
Lead	X1	221.4533 mg/Kg
	X2	273.8972 mg/Kg
	X3	289.9874 mg/Kg
Zinc	X1	1698.387 mg/Kg
	X2	1687.787 mg/Kg
	X3	1672.673 mg/Kg
Electroconductivity	X1	7.4 mS/cm
	X2	7.5 mS/cm
	X3	7.9 mS/cm
Volatile Solids	X1	88.00 %
	X2	88.97 %
	X3	87.98 %
Total Organic Carbon	X1	50.68736 %
	X2	49.97343 %
	X3	49.67453 %
C/N Ratio	X1	17.02780
	X2	14.32754
	X3	14.37066

Analyzed by



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UGANDA INDUSTRIAL RESEARCH INSTITUTE

"A Lead Agency in Industrialisation of Uganda"

Research Test Results

Student Name: MAFABI GRACE

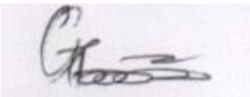
REG. NO: 17/U/14685/GMEW/PE

Date of Analysis: 25th – 28th April 2022

TEST PARAMETERS	Sample ID	Result
Lead (mg/l)	X0	<0.02
	X1	6.6988
	X2	15.7157
	X3	19.6802
Zinc (mg/l)	X0	66.017
	X1	172.959
	X2	222.226
	X3	230.080

Note: <0.02mg/l means below detection limit for the parameter

Analyzed by



Waibale Wilber,

Scientist Chemistry Laboratory