UNIVERSITY of RWANDA

Laidlaw Scholars
Undergraduate Research and Leadership Programme

LAIDLAW RESEARCH AND LEADERSHIP PROGRAM

FINAL REPORT

PROJECT-NAME: Extraction of Humic acid from landfill compost, composted human solid waste, crop residues conditioned with cow dung, and its characterization.

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Declaration

I Antoinette ABIJURU with registration number of 218008331, student in University of Rwanda, NYARUGENGE Campus, College of Science and Technology, School of science, chemistry department, Bio-organic chemistry option and one of winner in Laidlaw Research and leadership scholarship program in University of Rwanda .I hereby declare that the work contained in this final report of Laidlaw scholarship is my original work and I have not previously in its entirely or in part submitted it at any Institution. Information recorded in this document was based on daily work done in two summers conducted at college of science and technology in Chemistry Laboratory.

Signature: 

Date: April 15, 2021

Approval

This project report has been submitted to Laidlaw scholarship with the approval of the following supervisor:

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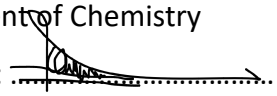
Signature: 

Date: April 15, 2021

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- Above all we would like to express our greatest gratitude to God Almighty for the strength and courage he gave us throughout this study.

List of Acronyms and abbreviations

A: 30:70 Coffee husks and cow dung ratio

B: 30:70 Cow dung and Rice husk

C: 70:30 Coffee husk and cow dung

LC: Landfill compost

HW: Composted human solid waste or human waste

E4: Absorbance measured at wavelength of 465nm

E6: Absorbance measured at wavelength of 665nm

HS: Humic Substances

HA: Humic Acid

FA: Fulvic acid

B.C: Before Christ

IHSS: International Humic Substance society

UV: Ultra violet

DOC: Dissolved organic carbon

NOM: Natural organic Matter

UK: united Kingdom

USA: united States of American

SOM: Soil organic matter

pH: Hydrogen potential

PVC: Polyvinyl chloride

COOH: Carboxylic acid

OH^- Hydroxide

NaOH: Sodium Hydroxide

HCL: Hydrochloric acid

µm: Micro-meter

CO_2: Carbone dioxide

HCO_3: Bicarbonate

Abstract

Rwanda has various sources for humic substances that can be used for large scale production of humic acid. Extracted humic acid is mostly used in agriculture to amend soil with low organic matter due to over-agriculture practice to enhance the plant growth. Humic substances is a dark colored fully decomposed remains of plant and animal organic matter through the process called humification. This project aims of finding the potential source of extracting humic acid for increasing the yield from agriculture in any weather seasons and reducing environment pollutants. The potential sources that we investigated on in this study include 30:70 coffee husk and cow dung named as A, 70:30 coffee husk and cow dung named as C, 30:70 cow dung and rice husk named as B, compost from landfill named as LC and composted human solid waste named as HW. All tested samples were treated with 0.1M sodium hydroxide NaOH for dissolving all humic substances. Humic acid was recovered in all sources after precipitating humic acid in humic substances through adding concentrated hydrochloric acid (38%).Analysis was done with UV-visible spectrophotometer through determining E4/E6 ratios and concentrations. Also weight were measured with electrical balance. The results revealed that in all tested sample, landfill compost contain the highest weight and concentration compared to other tested potential with 0.691g and 770ppm, respectively. Also result shows that landfill compost (LC) has low E4/E6 ratio of 4.31 compared to other potential source tested. Low E4/E6 ratio signify high degree of aromatic condensation, high molecular weight in the range of 5000 to 1000 Dalton. In all tested potential source after landfill compost 30:70 cow dung and rice husks (B) with weight of 0.5055g, concentration of 484.54ppm and low E4/E6 ratio of 4.641 follow. Finally, in all tested sample landfill compost (LC) found to be large production humic acid compared to other potential sources tested in Rwanda. This was confirmed after comparing the obtained weight, concentrations and E4/E6 ratios.

Key words: Humification, Humic acid, Agriculture residues, composted human solid wastes.

Table of Contents

List of Figures

List of Tables

List of Equations

CHAPTER 1: Introduction

1.1 Backgrounds

Humic acid is one of two known naturally acid. Humic acid is extracted from various sources only by precipitating extracted humic alkaline substance. It comprise a complex mixture of physically and chemically heterogeneous substances which show a relatively high molecular weight with a large number of oxygen containing functional groups –COOH and –OH in particular (Bellingham, 2012)

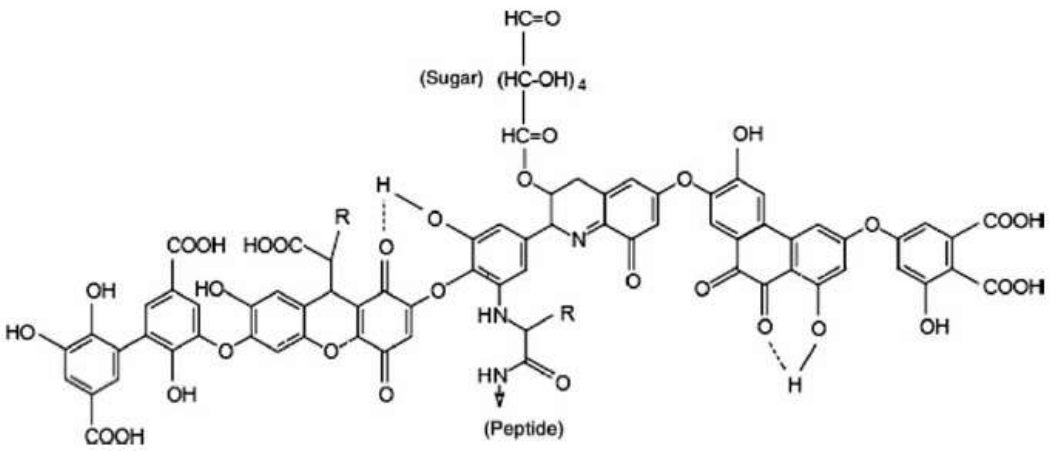

Figure 1.Model structure of humic acid according to Stevenson (1982)

Humic acids possess many functional group which improves physical and chemical properties of the soil. These functional groups include aromatic backbone and amines which helps humic acid to be biological active , the oxygen containing functional group (Carboxyl, phenol hydroxyl and ketone) which improve plant root development, water holding capacity and cation exchange capacity (Asing et al .,2009 ; Bellingham ,2012). Humic acids are thought to be complex aromatic macromolecules with amino acids, amino sugars, peptides, aliphatic compounds involved in linkages between the aromatic groups(Shamia et al., 2017).

The HS are formed by secondary synthesis reaction during the decay process and by transformation of biomolecules originating from dead organism and microbial activity(Rocha et al., 1998). These compounds are important in soil conservation especially for water holding

capacity, and in the complexation of metals in aquatic system. Humic substances (HS) are made up of humic acid (HA), fulvic acid (FA) and other organic waste residues called humins. Most of the data on HA , FA and humins refers to the average properties and structure of a large assembly of components of diverse structure and molecular weight (Bandichhor, 2014). HA is documented to interact with over 50 elements from periodic table, carcinogenic moieties, nutrient, radionuclides, toxic metals and anthropogenic compounds(Letters & Bhatt, 2017).

HS can be prepared from different sources especially from organic sources. During of our research we focused on landfill compost, composted human solid waste, agricultural residues and cow dung. Plant residues such as those obtained from agricultural activities such as coffee husk and rice husks are available in large quantities around the world (Yan et al., 2013)

Humic substances are highly found in natural soil-ecosystem, where they have persisted for hundreds of years (Vusie, 2006). They are the mostly widely occurring organic products for living things synthesis on the earth surface, exceeding the amount of organic substance containing carbon in living organisms by approximately one order of magnitude (Vusie, 2006). They give a concentrated and economical form of organic matter that can replace humus depletion caused by conventional fertilization methods in soil.

Humic substances in the soils stimulates plant growth beyond the effects of mineral nutrients alone. Humic substances are extensively used all over the world due to their benefits in agricultural soils, especially in soils with low organic matter. They forms an integral part of the ecosystem as they play an important role in global cycling of nutrients and carbon. Humic products provides economically and ecologically friendly solutions by increasing plant nutrients and reducing the use of conventional fertilizers (Rupiasih & Vidyasagar, 2009) at the commercial scale.

It is generally acknowledged scientifically that soil fertility depends on a combination of factors like concentration of micro and macro nutrients in the soil, the amount of applied fertilizers, the type of fertilizers (liquid or solids) and the availability of water. These concepts are also relevant for effective use of humic acids as compounds for enhancing plant growth. Agriculture, as one of South Africa's most important industries, is likely to benefit from humic acids products because of their contribution to improved micronutrients release efficiency in plants. A year-to-year consistency in production rates may be possible with humic-amended soils (Vusie, 2006).

Humic acid are most obtained from fossil sources such as leonardite, peat ,coal , manures (Vusie ,2006) ,but other sources such as composted human solid waste ,landfill compost, compost from agricultural residues or livestock residues such as cow dung may be envisaged.

It is acknowledged in the literature that humic acids have been shown to stimulate both the number and length of roots in young, growing plants (International et al., 2015). In this study, we focused on assessing the presence and characterization of humic acid in landfill compost, composted human solid waste and rice husk, coffee husk conditioned with cow dung.

1.2. Research Questions

How environmental pollutants come from solid and liquid waste (human solid waste) dumped at landfills, cow dung and agriculture residue such as coffee husks and rice husks can be reduced through extracting humic acid in it and be used as soil amendment?

1.3 Problem Statement

In Rwanda there is a pressure to increase productivity of agricultural system to meet domestic and international demands. Most of the agricultural system practices generates crops and its residues can be obtained. This combined with the high economic cost associated with crop failure, has encouraged wide spread of the fertilizer usage and other agrochemicals. In some parts of our country, especially in eastern province, demand for agricultural productivity has led to marginalization of farmers, forcing them to cultivate unsustainable soils, in risky climatic areas, in order to produce food. These pressures, especially when combined with poor cultivation techniques such as removal of plant materials after harvesting and poor ways of use of residues obtained (here for our case we mentioned coffee husk and Rice husk) leads to increase runoff and soil erosion.

Use of fertilizer is indeed important to achieve optimum yield. However it necessary to understand fully the implication of various type of the fertilizer applied in the soil. Excessive use of reduced (energy rich) sources of nitrogen in fertilizer here in Rwanda mostly in eastern province has one of the causes of soil acidification following its biological oxidation to nitric acid.

In Rwanda coffee cherries are mostly processed using the dry method, there by leading to an annual release of more than 240,000 tons of coffee husk into the environment. This represents a major environmental problem mainly due to the high content of tannin and phenolic compounds contained in this product (Gómez-brandón, et al., 2013).Also rice husks contaminate environment due to contains high amount of tannins and phenolic compounds.

Dumping wastes (Solid and liquids) at landfill come as solution for reducing hygienic disease in urban households. Even though this practice was achieved successful, but the way in which wastes are dumped in different landfill in Rwanda is also a challenge which result to environmental pollution. These waste pollute environment in two ways the first is the formation of leachate for solid waste and undefined pits which are designed for keeping liquids waste, where both contaminate ground water mostly during rainy season. The second way is the emission of global warming gases formed during decomposition of wastes and results to cause global warming.

In Rwanda development is increasing up in all sectors such as agriculture, farming, and medicine and in other field. The injection of antibiotic in cow was also developed to cure some disease .Even though these antibiotic cure disease, the remaining are excreted in cow dung. During rainy seasons, rainy water flow these remaining antibiotics in cow dung into river and contaminate it, and it we end up drinking treated water with antibiotics.

We as research we carried this project for finding all possible way in which these above mentioned negative effect can be reduced through using all these source of humic acid, which will be used to enhance plant growth mostly in soil with low organic matter. We were motivated to conduct this research because there no any publication paper which dealt with extraction of humic acid from landfill compost, composted human solid waste and agriculture residues such as rice husks and coffee husks conditioned with cow dung.

1.4 Objective of the study

1.4.1 General Objectives

This project was implemented with aim to extract and analyze humic acid from agricultural residues (coffee husk and Rice husk) conditioned with cow dung, landfill compost and separated human waste.

I.4.2 Specific Objective of this study

The specific objective of this project are the following;

- To test the hypothesis that landfill compost is a potential source for extracting humic acid compared to the sources collected randomly, these include three different ratios of coffee husks and Rice husks conditioned with cow dung and human waste. The comparison will be done after treating with humic acid precipitation methods.
- To measure the weight of all extracted humic acid powder.
- To calculate the correspondence concentration for each sample with UV-Visible spectroscopy.
- To determine the molecular weight or size, degree of condensation of aromatic carbon content of humic acid though calculating E4/E6 with UV-Visible spectroscopy.

This methods was formulated according to the standard precipitation method recommended by the International Humic Substances Standard for the production of humic acids (Rupiasih & Vidyasagar, 2009)

1.5. Scope of the project

This research aims of extracting humic acid from landfill compost, composted human solid waste, and coffee and rice husks conditioned with cow dung. Normally extraction of humic acid was achieved for all sample. Also we did some of its characterization .The potential source of humic acid from among tested sample was done after analysis of extracted humic acid powder. This is what this report contain.

This project didn't cover the investigation of humic acid effect on plant growth as it is well known to enhance plant growth in all circumstance and in any weather. Also we didn't do the required amount of humic acid powder in all different type of soil finds in Rwanda.

1.6. Significance of this study

The project has various role to the environment and economy of Rwanda. Extraction of humic acid in these sample will reduce the global warming, high emission of polluting gases from landfill rice husks and coffee husks containing high level of tannins and phenolic which increase greenhouse effect and reduce quantity of antibiotics flows in the river from cow dung.

It is well know that most of the field nowadays are extensively cultivated. After long time results to the soil with low organic matter. This activity affect strongly the yield harvested compare to the previous years and as the yield is very low the more people suffer from hunger and affect their living standard (wealth).

This project will improve the wealth of farmers through increasing the yield of their crops .Humic acid increase the soil organic matter which will in turn to increase the yield as plant obtained the nutrient required to grow has high water holding capacity and , also it reduce leaching of micro and macro nutrient as result of over use of field.

CHAPTER 2: Literature Review

2.1 Introduction about Humic Acid

Humic acids is a complex organic macromolecules which can be extracted from different sources of humic substances or hilius (humic like substances in atmosphere) (Graber & Rudich, 2006). Humic substances is a generic name used to explain three fractions (Vusie, 2006) . These fractions include: humic acids (HA, HAs), fulvic acids (FA, FAs) and humin where they differ in colors and solubility. Humic acid is a black or dark brown precipitate which is obtained when an acid is added into humic alkaline solution. Whereas ,the remaining yellow liquids was coined as fulvic acid and it is soluble in acid (Jana et al., 2016).The insoluble black solid residue after treatment of humic substance with alkalis is called humin . Humic acid was coined in the 1800's (Billingham, 2012)

Humic substances furnish necessary high amount organic matter which might substitute humus reduction raised by intensive agronomic activity or new adoptive fertilization methods .Increase of humic substances in soil believe to induce plant growth and production rather than the existence soil mineral only. All over the world use of humic substances has increase for agriculture purpose because of its benefit in the soil with less organic matter (Vusie,2006) .Also, it was revealed that humic products are environmental friendly in terms of economic and ecology, through increasing nutrient uptake of plant and reduction use of conventional fertilizer (Wright & Lenssen, 2013).

2.2 History of humic substances (humus)

Humus has been known since ancient time .Humus come from roman's word which means soil or earth .After 1,800 years this word " humus "named as "terra" in the 1^{ST} century B.C ,because of its misuse in the literature, but in the 1700's this term of humus was reused again in Europeans literature which means" loam "or mould (Feller, 1997). Soil organic matter is also known as humus .This term of humus has been known as a basic unit of soil ecosystem in term of physical, chemical and biological properties. Humic products which have capacity to resist on microbial degradation consist of 70%to 80% of organic matter in soil and this is associated with many functions in the soil (Piccolo, 2001)

Actually, in soil high percentage of humic substances (which includes humic acids) are comprised up large amount of carbon. About 6.0×10^{12} tonnes of humic acid in term of carbon detected in

soil (Billingham, 2012) .And this raised from 2.0 to 4.5 times amount of carbon which exist naturally in terrestrial biomass (Piccolo, 2001).Whereas , in atmosphere the amount of hilius dissolved organic carbon (DOC) flux in rainwater was about 430×10^6 t c/year.(Billingham ,2012)

Although ,from the roman's periods to the 20th century there has been a steady problem of extracting large quantity of humic acids in the composts and organic manures. Various research shows that humic acids to be the main component of compost and organic manures .This was revealed by researcher through extraction process of humic acids from those source (Jana et al., 2016) .These humic substances can be used to improve structure and quality of soils.

2.3 Source of Humic Substances

Humic substances are natural organic matter (NOM) which have persisted and detected many years in soil, water (rivers, lakes ,oceans) and sediments , but also they occupy large proportional in different organic matter such as brown coals , composts , peats and carbonaceous shales. Excluding the formation of hilius (humic like substances) in atmosphere other sources of humic substances are formed from natural process called humification (Mayhew, 2004)

After this process, Humic substances are characterized black color or dark brown of the topsoil, while in leaf litters and composts humic substances have a yellow or brown color (Bellingham, 2012) .In addition, humic substances in high amount contribute to the brown stain in lakes and freshwater creeks , also existence of humic substances in Antarctic continent was determined (Peña-Méndez et al., 2005).

Although, there are many source of humic acid .Humic substances have versatile concentration of Humic or Fulvic acid (Shamia et al., 2017). In previous time, soil and water serve as source of humic acid and these humic acid has been used to amend soil, but they are many other source of humic acids .Leonardite (oxidized sub-bituminous coal) are formed after 70million year (Mayhew, 2004) serve as major source of humic acid compared to the quantity of humic acid extracted from other source of organic matter.

Presently, use of humic base product from leonardite has become popular not only in outdoor horticulture, greenhouse cropping, but also for organic growers.

Table 1.Different sources of humic substances in different concentration (Vusie, 2006)

Natural source	% Humic /Fulvic acid
Leonardite	24/90
Compost	5 to 25
Peat	5 to 20
Peat moss	5 to 20
Lignite	5 to 15
Manure	1 to 3
Soft coal	2 to 5
Hard coal	0 to 1

Humic product are sold in different areas from various traders. Where the largest market of humic acids are in Europe(Germany , Biomechanical in UK, Spain ,Italy), North America (USA, America) ,India , Dayal fertilizers , Malaysia , Humic Tech in Australia, and in other countries (Anon ,2004;Asing et al.,2009) . Malaysia is known to import all of its humic acids from foreign countries in form of solid or liquids (Asing et al., 2009).

2.4 Humification Process

Where does humification start? Humification begins through when there is physical degradation, reorganization, and enzymatic modification of SOM by soil fauna. Which is also followed by direct biodegradation of soluble fractions .These soluble fraction known to be characterized by highly solubility and low molecular weight such as, sugars, proteins and organic acids, which are more likely to be assimilated by micro-organisms. These assimilation give nitrogen and carbon as origin of energy, reproduction, growth of the biomass frequently go with ingestion of nutrient from the soil (Billingham,2012).The recalcitrant molecules are formed as a result of carbon source which have been used.

HUMIFICATION PROCESS

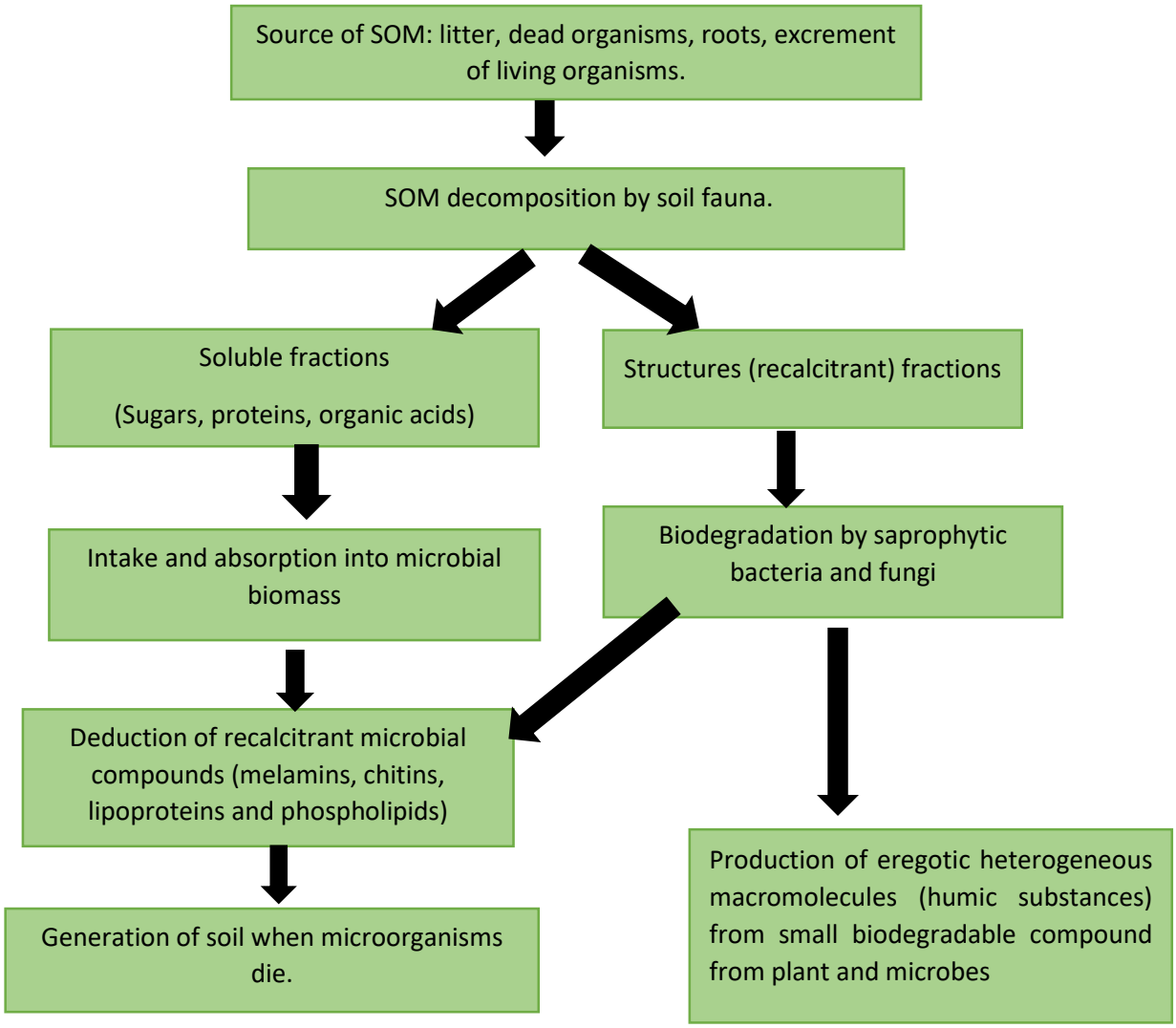

Figure 2. Humification process in the soil

2.5 Bio-organic study of humic acid

Bio-organic chemistry deals with organic molecules. Study organic structure and mechanisms involve to explain relationship between organic structures and its biological properties. Humic acid is an organic macromolecule with large amount of molecular weight (Sarlaki et al., 2019)

Humic acids are reversible heterogeneous macromolecules with molecular weight approximately arranged from 300 to 10,000 units (Kurniati et al., 2018) or from 5,000 to 100,000 Dalton (Asing et al., 2009). These molecular weights are highly dominated by carboxylic (COOH) and phenolic(OH) functional group .The hydrophilic acidity of humic acid improve hydration the result to rise water uptake capacity in soil .Humic acid contain both hydrophilic and hydrophobic properties (Gayathri et al., 2020)

Chemically, biologically, physical function of humic acids depend on its structure. This part of bio-organic chemistry is going to discuss deeply the structure model of humic acids and relate its structure function on reactivity of humic acid, but also explain relationship between structure of humic acid and its function in agriculture (Billingham, 2012)

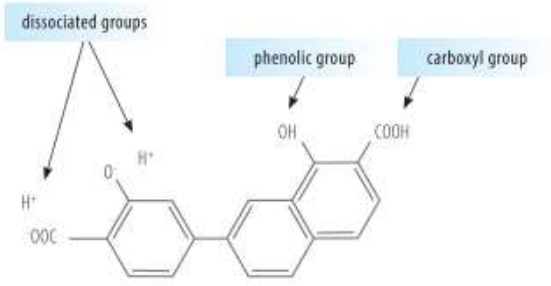

Figure 3.Carboxyl and phenolic group of humic acid which are involved in reactions

CHAPTER 3: Materials and Methods

3.1 Introduction

This research activities were conducted in three steps as follows: **Step I**: Testing the hypothesis that landfill compost is a potential source of extracting humic acid from other sources such as separated human waste and various ratios of rice husks and coffee husks conditioned with cow dung, which were selected randomly. **Steps II:** Comparing the obtained masses, determined concentration and calculated E4/E6 ratios of extracted humic acid products from all selected samples. **Steps III**: Confirming the hypothesis that landfill compost is the potential source of humic acid from other sources selected randomly. This chapter is going to describe all materials and methods used to do all activities mentioned in steps.

3.2 Materials and Reagents/Chemicals used during extraction

Table 2. Materials, and Chemicals used in extraction of humic acid and their roles

Materials/Reagents	**Function**
Magnetic stove	Moving the stirrer bar
Ultraviolet visible spectra	To measure E4/E6 ratios for degree of aromaticity and molecular size of humic substance
Nitrogen gas cylinder	Providing nitrogen gas in created glovebox
Florence flask with a holed stopper	Reaction container
0.1 M of NaOH	Extraction and dissolution of humic acids
Concentrated HCl (35-38%)	For precipitation of humic acids
pH meter	To measure the pH during reaction
Buckner funnel	For filtration purpose.
Vacuum oven	For drying at a given temperature under vacuum
MSE centrifuge	To remove suspended solids from solution
Distilled water	To prepare solutions and solvents
Filter paper	To filter small size suspended solids from the solution.
Labeling paper	To label each and every materials containing sample
Beaker of 250 ml	Facilitate in stirring of solution
Nitrogen Atmosphere	This was used for purging inorganic gases (CO_2,HCO_3)

3.3 Creation of Nitrogen Atmosphere

Nitrogen atmosphere is an isolation environment which excludes all other kinds of reactive gases like oxygen, carbon monoxide, methane gas, carbon dioxides, nitrous gas and its derivatives and other form of gases. An isolation atmosphere can be created by making a glovebox. The glovebox is a piece of equipment which can be designed in different form to serve different tasks , which includes to minimize the user's risks from hazardous contents within the glovebox ,but also it can be used to protect air and moisture sensitive substances away from other pollutants in the earth atmosphere. (Wu, 2014)

3.3.1. Procedure of Creating Glovebox

In our project we faced with a challenge of changing atmosphere from normal atmosphere into nitrogen atmosphere. This atmosphere were required for precipitating the extracted humic substance while purging the inorganic gases such as carbon dioxide and bicarbonate. We did this gloved helped with glovebox making guidelines (Wu, 2014)(Laaziri, n.d.). To do this glovebox we have used various materials such as, a clear plastic storage tub , a pair of glove, a razor blade or knife or rotary tools ,zip ties, hose clamps, water tap , epoxy metal , and golden silicon .

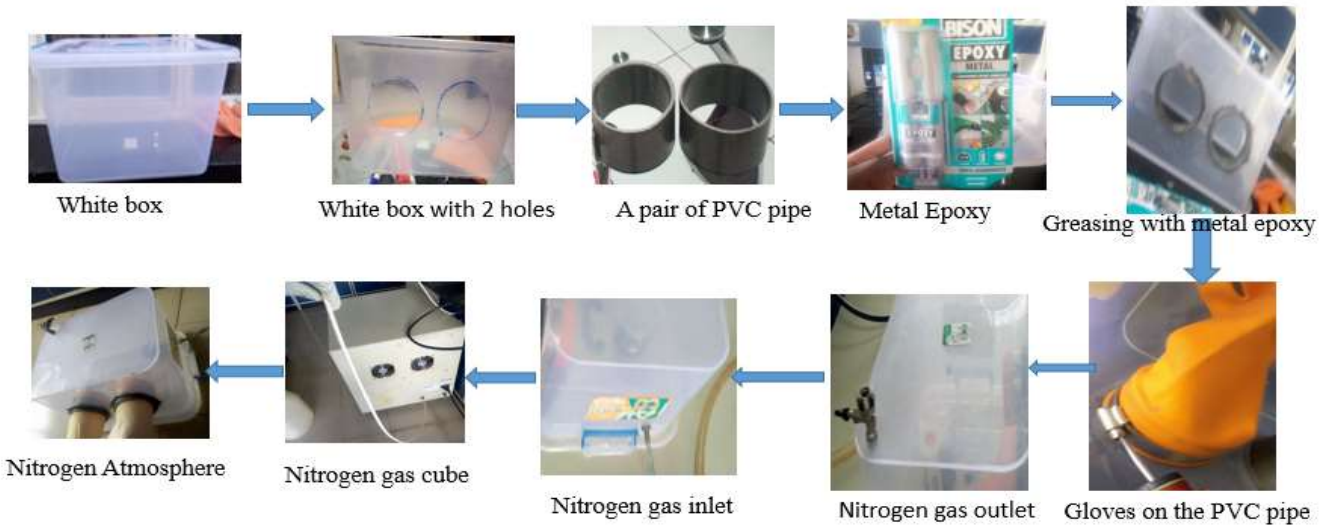

Figure 4. Steps used to make glovebox (Nitrogen atmosphere)

For the first, a clear storage box tub is required because an operator needs to observe some of changes which might takes place inside of box. With a marker we drew two holes on white box which may fit for two plastic PVC adapter. After, we made two holes following a circle drew with a marker on the box with a razor blade or a sharp knife. After cutting the holes on a clear plastic storage tub, two PVC pipe was inserted in the holes as well as increasing box size for more fitness of PVC pipe in the white storage box. The Polyvinyl Chloride must have 90 cm of Diameter.

After inserting both of two PVC in the hole of white box, we sealed the arm port for minimizing the entrance of dust or other unwanted gases .A sealant called epoxy metal was used to cover all gaps between PVC pipe and clear storage box. After removing all gaps, we waited 12 hours for drying. We took a pair of gloves (Elbow strength waterproof gloves) and we tried to fix those 2 waterproof gloves on 2 PVC pipe inside of white storage box with a hose clamps. This glovebox that we made was for creation of Nitrogen atmosphere (Inert atmosphere), so we put a tap at the top of white storage box for gas outlet, but also at the right side of glovebox we added a brass hose bard which serve as a gas inlet from a cube of nitrogen.

A complete glove box must be well closed during process, but in case the cover of box does not fit with the lid of box different sealant can be added. Either at the base or in the cover of box to make it more fit. According to the kind of box purchased addition of the sealant should be used. In this section a sealant called golden silicon was added inside of cover box for increasing fitness of the lid of a box and the cover, but using golden silicon as a sealant took time to be dried, but the quickest and easiest way of sealing a box is to cover with a weather stripping at the lip of box also it depends on the type of box. In case we wanted to make a glovebox which minimize the quantity of outside gas that might enter in the created Nitrogen atmosphere.

After making a glovebox, Nitrogen Atmosphere can be created .A small tube from Nitrogen gas cube was connected to the gas inlet to the inside of the white storage box . When nitrogen gas is concentrated inside of white glovebox, other gases which was inside of box start to be removed through gas outlet tube .Then, after 2-3 minutes depend on size of white storage box with a flame of match an operator can be used to confirm if a white box if full of inert gas of nitrogen.

3.4. Sample Collection

GIHEMBE refugee camp is located on a small hill in GICUMBI District, Northern Province. The camp currently is a home to about 20,000 refugees from the Democratic Republic of Congo who fled political turmoil in their country in 1997. Waste types from refugee camp. both solid and liquids waste are dumped at GICUMBI Landfill which is located in GICUMBI District, RUKOMO sector, KINYAME cell and in GASHARARA village. We took two samples which are landfill compost and separated human waste.

- Landfill compost composted after 10 month were collected with a shovel before being placed in cleaned plastic bucket. We collected 1kg of landfill compost.

Figure 5.Landfill compost

- Separated human waste were collected after being treated in the following way. We drew this chart with using Revit software under authorized by GICUMBI landfill managements.

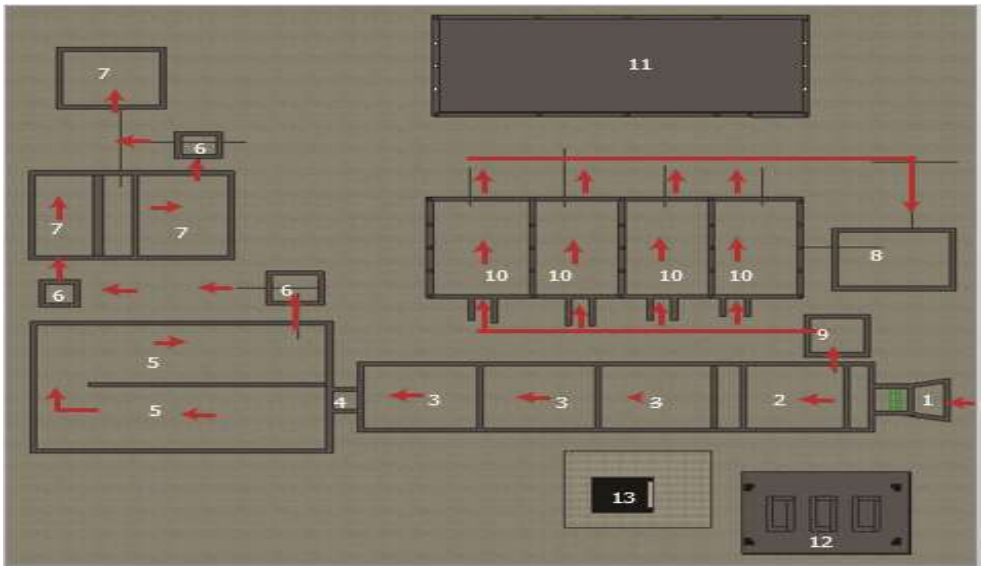

Figure 6.Action Building of Human Waste Treatment.

In this research we focused also on how fecal sludge is separated due to we have feces as one of the sample. This chart above shows how feces are separated from water, urine, toilet paper and other wastes. This chart has 13 parts and each parts has its importance. Reservoir channel (1). This part serve for receiving fecal sludge collected by a vehicle. The fecal sludge path through from chamber 1 to the chamber called settling chamber (2). This room has 2m in length and 1.2 m in width and serve for partial separation of feces and liquids wastes according to their density. At the top of this chamber there is a big PVC pipe which allow the movement of separated liquid waste to the next tank called anaerobic chamber (3).

These tanks are 3 consecutive tanks which has 3m in depth and 1.80cm width. This tank serve for killing all anaerobic microorganisms. When all these three anaerobic thank fulfill with separated liquids, these liquids waste path through filter channel (4). This channel is packed with sands which serve for separated liquids waste. Separated liquids throw to the open area called aerobic chamber (5).This chamber serve to kill all aerobic micro-organisms. The liquid waste from aerobic chamber pass through transporter pipe (6).The liquid waste from aerobic chamber and pass through transportation pipe pass move to the Reed zone (7) where large quantity of Nitrogen is consumed by reed plants. Denitrogenated liquid waste are stored in storage tank of remained liquids (8).

Back at the bottom of settling tank (2) there is a pipe which transport partial settled waste which include feces and other waste with high density than water and they pass through Van pipe transporter (9). Settled waste from chamber coined as 2 that pass through van pipe move to the separation house (10) .This house is well constructed with concrete and above is packed with sand which allow the movement of waste water ,urine that have settled with feces. As waste water cease from feces, feces started to be dried with sunlight that pass through the white aluminum sheet of separating house. When feces are dried are stored in storage house (11) for ready being used as organic fertilizer or being used in extraction of humic acid. Constructed trash dyer house (12) serve for air drying of some of separated trash which separated at reservoir channel (1). Finally one trash are dried in trash dryer house are burned in incinerator (13) .The figure (3) below has been designed with the Authorization of GICUMBI Landfill management.

- We collected one kg of 2 month composted human solid waste. These wastes were kept in storage house**.** Composted Human waste was collected shovel.

Figure 7.Human waste

- Rice husks were collected from KAYONZA District, MUKARANGE Sector, NYAGATOVU Sector, and KABEZA VILLAGE. A shovel also was used to collect dried rice husks and were stored in well cleaned bucket.

Figure 8. Rice husks

- Cow dung were collected from Eastern province, NGOMA District, RUKIRA Sector, NYINYA cell, KABIMBA Village .A shovel was used to collect cow dung, after was directly stored in well cleaned small bucket for being easily transported in the laboratory without contaminating the sample.

- 1kg of cow dung were collected.

Figure 9.Cow dung

- Coffee husks were collected from Eastern province, KIREHE District, MUSHIKIRI sector, RUGARUMA Cell, RYOGIRE Village .1kg of coffee husks were collected.

Figure 10.Coffee husks

Before extraction, except rice husks, other samples were stored in refrigerator at less than 4^{o}C to reduce microbial activity prior to extraction. After one day, all samples such as Landfill compost, human waste, coffee husks, cow dung samples, and were dried in an oven during 14 hours at 60^{o}C to remove moisture content. The elimination of moisture content was confirmed after measuring the weight loss. All dried sample in oven were weighted after allowing to be cooled in desiccator containing silica gel at 25^{o}C, this was done to reduce the re-absorption of moisture content.

Except landfill compost, other sample were homogenized prior to sieving. After drying, composted human solid waste, coffee husks, rice husks, cow dung were grinded in mortar with pestle before being sieved in 0.18µm sieve. We grinded samples for increasing the surface area of samples all sample were labeled with labeling paper prior to storage.

Sample and materials used were chosen according to their availability and suitability .The hypothesis that landfill compost can be a large production of humic acid was done after comparing all obtained results for landfill compost and other sources selected randomly as a major source.

3.5 Extraction of humic acid

In this research all collected samples were treated with acid-base precipitation method for extracting humic acids. We have used standard procedure for humic acid extraction as was. Recommended by IHSS (Rocha et al., 1998 ; Lamar et al ., 2014) .NaOH (0.1M) was used to extract all humic substance while 35-38% concentrated Hydrochloric acid was used to precipitate all humic acid present in the extracted solution extraction used for (Vusie, 2006).This techniques was applied for all collected sample.

3.5.1 Procedures used to extract humic acid from all collected samples

- After drying in oven, grinding in mortal with pestles and sieving with 0.18 µm sieve. We weighted 10 g of each sample on electrical balance. Sieving and weighing was done for each collected samples.

- We measured 10g of sieved landfill compost , 10g of sieved mixture of 30:70 cow dung and Rice husks which were symbolized as B , 10g of sieved mixture of 70: 30 coffee husks and cow dung which were symbolized as C, 30:70 coffee husks and cow dung which were symbolized by A and Composted human solid waste symbolized with HW.

- After, we placed 10 g for each sample in separated beaker of 250ml. After we treated it with 50ml of 0.1M sodium hydroxide (NaOH) for extracting all possible humic substances with some mean of agitation during of 2 hours. Agitation was done with magnetic stirrer.

- After 2 hours, we poured the stirred solution in centrifuge tubes, then we placed these centrifuge tubes in centrifuge machines operated at 4000rmp in 45 minute for separating the extracted humic substances from solid residue.
- These solid residue was treated 2 times with 0.1M of NaOH for completely recover of humic substances solution from treated solid residue. This steps was done for each samples

- After separating the humic alkaline substances solution solid residue were remained. We filtrated the obtained solution of humic substances through 55mm of filter paper by using buckener funnel and suction flask for removing suspended solids. After filtration we measured the pH values for each filtrated sample with glass electrode pH meter.

- After measuring the pH values for each sample, the filtrated solution was poured in a Florence flask, then we place in nitrogen atmosphere we created as we explained in (3.3).

- Then after concentrated hydrochloric acid (HCl 35- 38%) was added drop by drop by using pipette to adjust the pH of the solution which is less than 2, the solution was stirred under nitrogen atmosphere to precipitate the humic acid while purging inorganic carbons like CO_2 and HCO_3.
- After precipitating the solution was allowed to stand for about 4hrs to allow total precipitation of humic acid. After 4 hours we transferred the obtained solution into centrifuge tubes, then we placed into centrifuge machine operated at 3000 in 45 minute, for separating the formed precipitate called humic acid sludge from yellow liquids known as fulvic acid.
- The remaining humic acids sludge was recovered through re-dissolved in 0.1M of NaOH solution to the pH of 12 and re—precipitated with concentrated HCl until to the pH of < 2 and was re-centrifuged for purpose of removing impurities. This step was repeated twice in nitrogen atmosphere.

- Finally the humic acid sludge in centrifuge tube was placed in oven operated at 55^oC overnight for drying, then obtained powder product of humic acid were pulverized, weighted and stored in vials prior to analysis. Also the remaining yellow liquids was stored.

❖ The figure (11) below shows the full procedure of extracting humic acid from all tested sample. Each steps are the same for all samples and figure (12) shows extracted powder for all samples.

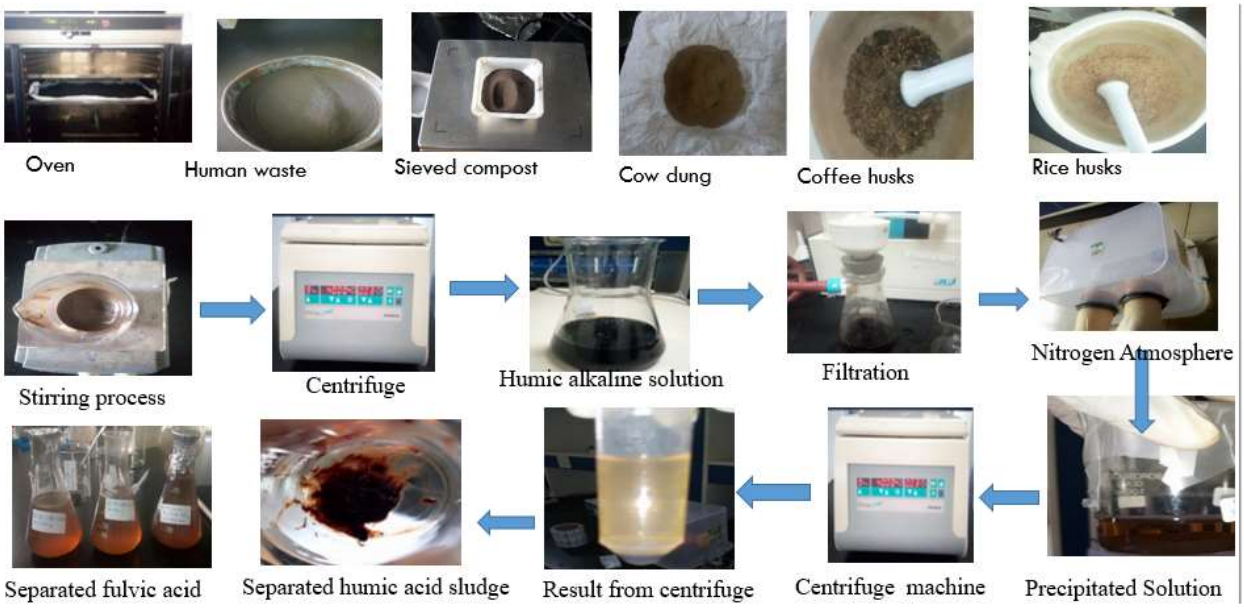

Figure 11. Procedures used to extract humic acid from all Samples

Figure 12. Extracted powder in all samples

3.6 Characterization of extracted humic acid

After obtaining powder product (Humic acid) for all collected samples qualitative and quantitative analysis was done using UV-Visible spectroscopy methods. These methods was used because of its availability and suitability for analysis of humic acid. Procedure used to characterize extracted humic acid are explained here below.

3.6.1 Determination E4/E6 ratio with UV-Visible Spectroscopic methods.

UV-visible spectrophotometer is well known instrument which is used in identification of humic acid. It provides information about degree of aromatization, degree of humification, concentration and molecular size.

A solution for all extracted humic acid samples was prepared through dissolving small amount of each extract of humic acid into 0.01M NaOH. A small amount of humic acid measured on the edge of spatula was placed in 250ml of conical flasks, then after 50ml of 0.01M of NaOH was added in flasks. The solution was placed on the shaker during 6 hours for allowing humic acid powder to be dissolved in the solution. After 6 hours we filtrated the solution with filter paper of 55mm for removing undissolved solid that may be interfere during analysis. Solution of 0.01M NaOH was used as a blank (Vusie, 2006)

The cary 1E spectrophotometer was used with 1cm quartz cell.E4/E6 ratio predict the degree of humification, aromatization and carbon content , oxygen content and molecular weight was determined after taking the ratio between absorbance measured at 465 and 665nm (Eshwar et al., 2017; Enev et al ., 2014)

3.6.2 Determination of concentration with UV-Visible spectroscopy

In nature humic substances have three absorbance band, in this research we have used one of its absorbance band to prepare standard solution. The beer-lambert law which state that absorbance is directly proportional to the concentration was based on for making the standard calibration curve. Standard calibration curve is used to determine the concentration of unknown substance(Manual, 2016).

Finally, the concentration of the components can be found from the absorbance spectrum of the sample (Chau, 2019)

$$A=\varepsilon bC$$

Equation 1.Beer-Lambert Law

Where ε stands for molar absorptivity ($M^{-}cm^{-}$), b is the pathlength, cuvette (cm) or 1 quart (cm) and C is the concentration of the absorber.

Standard solution was purchased from Branch of Dynapharm in Rwanda. A commercial foliar fertilizer which contain 0.68% of humic acid was purchased. After preparation of standard solution we poured small amount of each concentration into the cuvette with 1cm for recording its absorbance in UV-Visible spectrophotometer operated at wavelength of 450nm.

CHAPER 4: Results and Discussion

4.1 Introduction

This chapter of results and discussions is going to detail all results we have obtained based on the project objectives. The project objective include testing that landfill compost is potential source of humic acid extraction compare to other source selected randomly.

4.2 Moisture content determination for all samples collected

As previously stated, before extracting humic acid in all sample collected, we determined the moisture content for each. We measured masses of collected samples and masses of all samples after drying in the oven operated at 60^{o} C to 12 hours.

Table 3. Measured moisture contents in all sample collected

Sample Names	Mass of collected samples (g)	Mass obtained after drying in oven (g)	Moisture content (%)
Landfill compost (LC)	1000	250	63
Human waste (HW)	1000	550	45
Cow dung	1000	387	61.3
Coffee husks	1000	642	35.8
Rice husks	1000	935	6.5

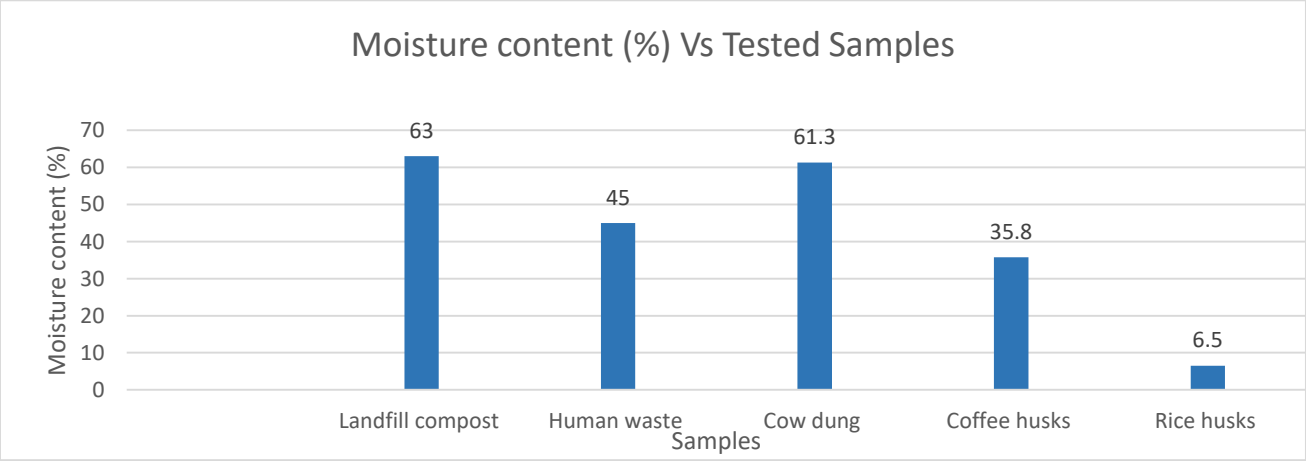

Figure 13. Illustration of measured moisture content in all sample.

4.3 Determination of measured mass (weight)

After extraction, with electrical balance we measured the obtained mass of humic acid .This was done for all collected sample. The value of mass obtained after extraction was also used for confirming the hypothesis that Landfill compost is potential source for extracting humic compared to the other selected potential source.

Table 4. Weight of extracted humic acid powder in all samples

Sample names	Measured mass (g)
LC	0.691
HW	0.041
A	0.312
B	0.5055
C	0.0678

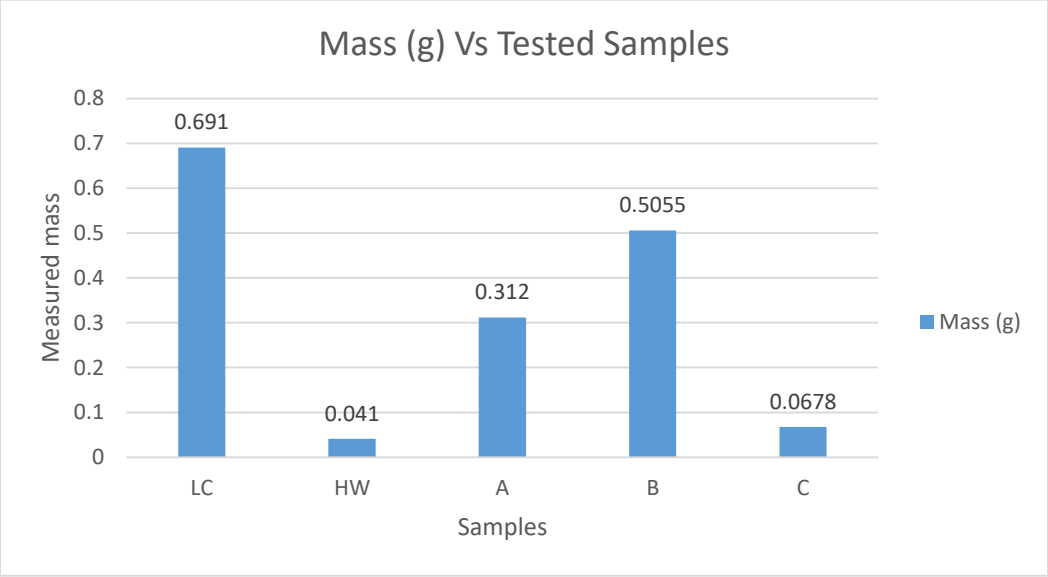

Figure 14. Weight of extracted humic acid in all samples

In all sample collected we get its correspondence mass. In all extracted samples, LC found to have high amount of 0.691 g of humic acid powder than other investigated potential source. This is followed by sample B which has weight of 0.5055g , this is also followed by sample A which

weight of 0.312g , followed by sample C which has weight of 0.0678g and the last one in all tested potential source is HW which has weight of 0.041g. In research done by vusie ,he found that the weight of humic acid extracted from Black Liquor was 10g while other sample were lower than 10g , this was one of many reasons that led vusie to confirm that Black liquor to be a large production of humic acid in South Africa (Vusie, 2006). This research shows that all tested samples only landfill compost (LC) is the one with high weight (g) compared to other mentioned potentials source. As the weight of humic acid is high, more will be selected to be used.

4.4. Results recorded for concentrations

The result recorded after preparing standard solution were calibrated for being used to determine the unknown concentration in all tested sample

Table 5. Absorbance and concentration of prepared standard solution

Solution (ppm)	Absorbance
200	0.526
400	1.004
600	1.436
800	1.880

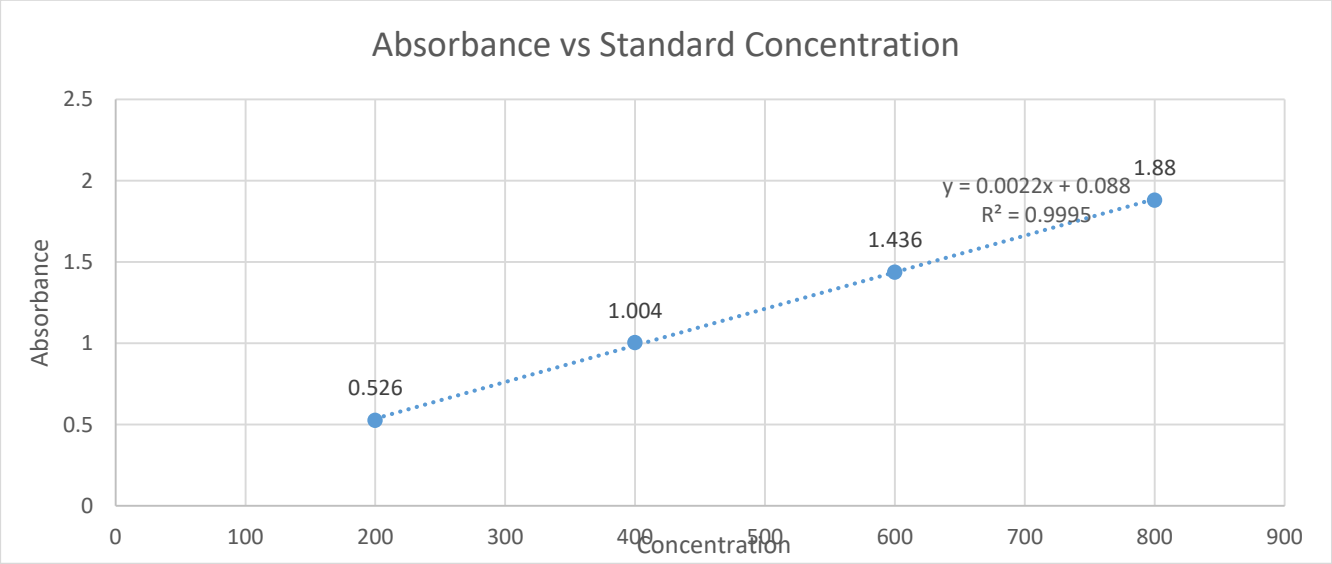

Figure 15.Calibraion curve of standard solution prepared at 450nm

After drawing the calibration curve, each humic acid extracted powder was prepared and after we recorded the absorbance for each sample. The linear regression obtained from standard calibration curve was used to calculate the concentration of humic acid in all sample. Then after all concentration obtained was compared.

Table 6. Calculated concentration of humic acid in all sample.

Samples	Absorbance (nm)	Concentration(ppm)
LC	1.782	770
HW	0.726	290
A	0.906	371.8
B	1.154	484.54
C	0.771	310.45

After calculating all concentration for all collected samples from standard calibration curve, we found that LC to have high concentration compared to the other selected potential source with concentration of 770ppm. LC is followed by sample B which has concentration 484.54 ppm, followed be A with 371.8 ppm , A followed by C which has Concentration of 310 ppm and the last was HW with concentration of 290ppm .As concentration increase more source is chosen as potential source due to small amount of humic acid will be required .

4.5. Qualitative analysis of extracted humic acid (E4/E6)

This value of E4/E6 is the ratio calculated between absorbance measured at 465 nm and 665nm. This value calculated for all samples is independent on the concentration (Vusie, 2006)

E4/E6 value is also called index of humification. This index relates molecular weight and size , degree condensation of the aromatic carbon network ,carbon content (Chen et al., 1976) and oxygen content of humic materials (Enev et al., 2014). The degree of aromatization is directly proportional to the degree of humification and this is characterized by low value of E4/E6 ratio (Machado et al., 2020).The high value of E4/E6 shows low aromatic condensation, large proportional of aliphatic structure (Chen et al., 1976) and presence of oxygen-containing functional group such as carboxyl ,carbonyl, ester , hydroxyl group (Enev et al., 2014) and high aliphatic group.

The high and low value ratio of E4/E6 was done after comparing with this range of 3.5 to 5.9 as Kononova proposed (Kononova, 1966). Above 5.9 ratio is considered as high E4/E6 ratio.

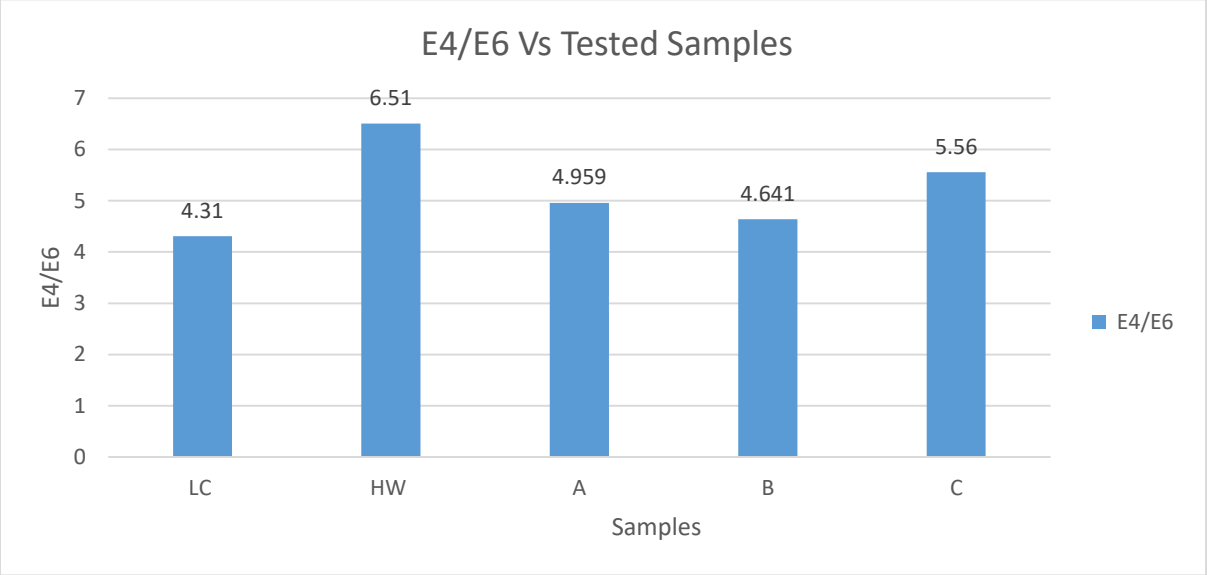

Figure 16. Calculated E4/E6 ratios for all tested samples

The Fig (16), shows the E4/E6 value for all tested samples , except composted human solid waste which has high value of E4/E6 equal to 6.51, other tested sample were in the accepted range from 3.5-5.9 as Kononova (1966) has proposed, but the others which are in the range also have difference degree of aromatization and molecular weight. LC which has low E4/E6 of 4.31 compared to other tested potential source. This refer to the highest degree of aromatic condensation, humification and molecular weight.

This is followed by sample B which has E4/E6 ratio of 4.641 ,this is also followed by sample A which has 4.959 value of E4/E6 ,A also followed by C which has E4/E6 value of 5.56 and the last is the Composted human solid waste (HW) with E4/E6 ratio of 6.51.HW with high E4/E6 ratio of 6.51 is due to its value is out range of E4/E6 ratio of 3.5-5.9 as Kononova (1966) has proposed.

The low value of E4/E6 obtained for LC may be due to the microbial activity which takes place in long period, result to high degree of humification, aromatic condensation and molecular weight or size in the range of 5,000 to 100,000 Dalton (Gayathri et al., 2020). While the high value ratio of E4/E6 obtained for HW shows high rate of aliphatic structure and low degree of aromatic condensation (Chen et al., 1976; Ywih Ch'ng et., 2018).

Chapter 5: Conclusion and Recommendations

5.1 Conclusion

This research aims of determining the potential source for extracting humic acid. This was done after extracting humic acid in all samples which were treated with precipitation method. These samples include Landfill compost, composted human solid waste, 30:70 coffee husks and cow dung (A), 70:30 coffee husks and cow dung(C) and 30:70 Cow dung and rice husks (B) . All collected sample were compared in terms of concentration, weight, and E4/E6 which shows molecular size and degree aromatic condensation. In all results Landfill compost was found to be large scale production of humic acid and can be used as soil amendment and plant growth enhancer because of the following reasons. Landfill compost (LC) found to have high amount of weight of 0.691g and was the highest weight compared to the other, highest concentration of 770ppm compared to other sources, and has low E4/E6 ratio of 4.31 and this refer to the high condensation of aromatic carbon content, high level of humification and molecular size.

5.2 Recommendations

This research recommend the following to any researcher want to do further research about humic acid and to Manager of Liquid and solid waste in Kigali city (Rwanda).

- Evaluation of –OCH3 and –OH groups of humic acids obtained in all tested sample should be done to evaluate the effect of extraction method on the degradation of humic acid.
- Determination of sugar content of humic acid. When sugar react with nitrogen tends to give pyridine which may be poisonous to plants. Once it appears that humic acids isolated from mentioned samples contain a small amount of nitrogen this is called nitrogenation of these substances. This poisonous substances formed may in turn to inhibit plant growth
- Determination of salt content. Accumulation of salt in the soils may lead to inhibitory effect on plant growth. By all means humic acid with high salt content must be purified as their use may have long term effect on the soil and therefore on plant growth on which they are applied. Chromatographic purification (Ion exchange) or dialysis may be ideal procedures to remove salt from humic acids in solution.
- This study suggest that after extraction of humic acid further application of humic acid should be done for heavy metal removal in water, to plant growth enhancer for various types of soil.
- This study suggested that Manager of solid and liquids waste of Kigali city should apply the techniques of separating liquid waste rather dumping in various pits constructed at NDUBA Landfill. This will reduce hygienic disease caused to the people who fetch contaminated ground. Also will reduce the high emission of greenhouse gases.
- Also Manager of solid and liquid waste in Kigali city should increase the rate in which segregation solid waste must be done at NDUBA Landfill for reducing the formation of leachate and greenhouse gases which pollute ground water for people living near NDUBA landfill. This will reduce hygienic disease but also will increase the rate in which humic acid will be extracted from landfill compost and reduce air pollutants.
- This program should find the way in which time of doing research part can be increased to 4 month for doing all analysis required for product to be sold to the farmers.

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