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# Extraction of Humic Acid from Agricultural Crop Residues Conditioned with Cow Dung, Compost from Landfill and Human Waste

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**Abstract:** Rwanda has various sources for humic substances that may be utilized for the large scale production of humic acid which can be used as soil amendment to enhance the plant growth. Humic substance is a dark colored fully decomposed remains of plant and animal organic matter through the process called humification. This work aims of finding the potential source of extracting humic acid for reducing environment pollution and increasing the yield from agriculture in any weather seasons The potential sources that were investigated 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 human waste named as HW. All tested samples were treated with 0.1M sodium hydroxide NaOH for dissolving all humic substances. Humic acid was recovered after all sources were allowed to be precipitated with concentrated hydrochloric acid (38%). Analysis was done with UV-visible spectrometer through determining E4/E6 ratio and concentration. Also masses were measured with electrical balance. The results shows that in all tested sample, landfill compost and 30:70 cow dung and rice husks have low E4/E4 which signify high degree of aromatic condensation, high molecular weight compared to the other sources these values are 4.31 and 4 respectively. The concentration also was found to be 770 ppm for landfill compost and 484.54ppm for 30:70 cow dung and rice husks. Finally, in all tested samples landfill compost (LC) and 30:70 cow dung and rice (B) husks found to be considered as potential source for production of humic acid compared to the tested potential source.

**Keywords:** Humic Acid, Landfill Compost, Coffee Husk, Rice Husk, Cow Dung

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## 1. Introduction

Humic acid is an organic polymer which is consisted of humic substances [1]. The Humic substances are formed by secondary synthesis reaction during the decay process and by transformation of biomolecules originating from dead organism and microbial activity [2]. Humic acid is extracted from various sources of humic substances or hilius (humic like substances in atmosphere) [3] only by precipitating extracted humic alkaline substance [4]. Humic substances is a generic name used to explain three fractions which are humic acid, fulvic acid and humins [5].

Humic acid is black or dark brown precipitate, which is

insoluble in acid condition [5]. Humic acid structure comprised up with major elements of carbon (C), oxygen (O), nitrogen (N), hydrogen (H) [6]. Also, its structure is dominated with aromatic ring, bridges, aliphatic chains, aliphatic ring and various functional group, but the most important functional groups which perform nearly all function are phenolic, carboxylic group, methoxy, carbonyl and hydroxyl [7].

Humic substances are extremely versatile and critical components of the natural soil-ecosystem, where they have persisted for hundreds of years [8]. Humic acid gives a concentrated and economical form of organic matter that can replace humus depletion caused by conventional fertilization

methods in soil. Humic substances are extensively used all over the world due to their benefits in agricultural soils, especially in soils with low organic matter [9]. Humic substances improve soil structure through increasing water holding capacity, availability of nutrient (avoid leaching of the nutrient), adhesion, compactness, and microorganism in soil. Also humic substances enhance plant growth through

improving enzymatic activity, root respiration and increase plant capacity to resist to abiotic stress such as high pH, and salinity [7]. Humic conditioning are most obtained from fossil sources such as leonardite, peats, manures, coals and other sources [10], but other sources such as, landfill compost, agricultural residues or livestock residue like cow dung and faces may be envisaged.

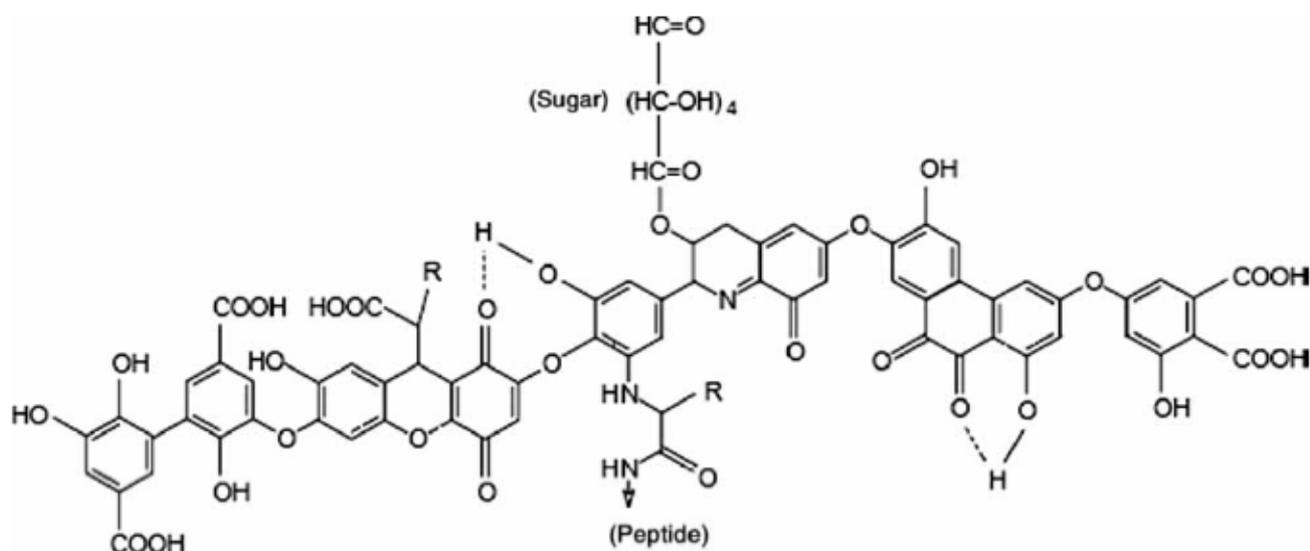


Figure 1. Proposed structure of humic acid.

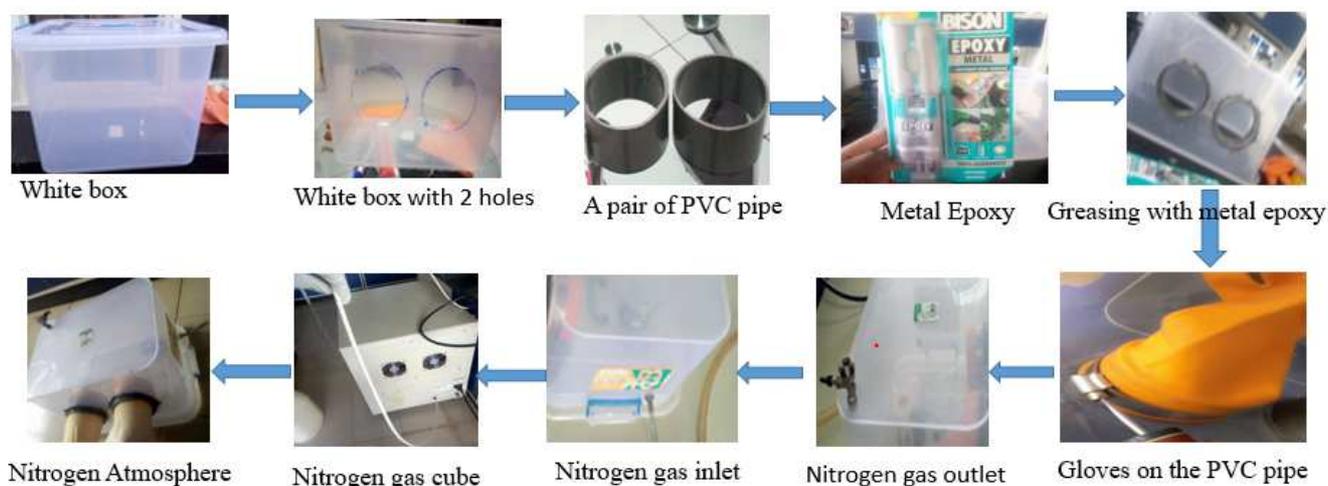


Figure 2. Step used to create nitrogen atmosphere.

Humic acid heterogeneity is favoured by method used to extract, origin, types of humic substances source and nature of the chemical extractive agent [11]. Humic acid or humic substances form an integral part of the ecosystem as they play an important role in global cycling and availability of nutrients, carbon content, water holding capacity [12], and soil detoxication [6]. Humic products provide economically and ecologically friendly solutions by increasing plant nutrients and reducing the use of conventional fertilizers [13] at the commercial scale.

In this study, we have investigated extraction of humic acid from agricultural crop residues conditioned with cow

dung, compost from landfill and Human waste. The nitrogen atmosphere has been created by our team and the action plan for faecal waste treatment has been designed by our group. In this work different parameters have been studied. Degree of humification, aromatization, oxygen content, carbon content were done by calculating E4/E6 ratio and concentration of humic acid solids has been determined using UV- instrument.

## 2. Materials and Methods

In this study chemicals/reagents and equipment used were

found in University of Rwanda, College of Science and Technology in Chemistry/Biology laboratory. Sodium hydroxide (NaOH) with 4.5N and concentrated hydrochloric acid (HCl) with 38% were from chemistry laboratory. Biofuge manufactured by haraeus used was from applied biology laboratory.

### 2.1. Creation of Nitrogen Atmosphere

In this research, we changed normal atmosphere to nitrogen atmosphere. This atmosphere was designed for purging inorganic carbon during precipitation. It has been done through designing a glove box. Also some of guidelines of making glove box from other research have been referenced [14].

For the first, a clear storage box tub was required because an operator needs to observe some of changes that might take place inside of box during chemical reaction. With a marker two holes on white box which may fit for two plastic pvc adapter have been drawn. After, two holes following a circle drew with a marker on the box with a razor blade or a sharp knife has been made. 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 used has diameter of 90 cm.

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. A pair of gloves (elbow strength waterproof gloves) has been taken and 2 waterproof gloves have been fixed on two PVC pipe inside of white storage box with two hose clamp. This glove box that has been 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 at the bottom of glove box we added a brass hose bard which serves 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. 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. After making a glove box, nitrogen atmosphere was created in this way. A small tube from nitrogen gas cube was connected to the gas inlet on glove box. When nitrogen gas is concentrated inside of white glove box, other gases which was inside of box start to be escape through gas outlet tube. Then, after 2-3 minutes depend on size of white storage box with a flame of match an operator can confirm if glove box is fulfilled with inert gas of nitrogen.

### 2.2. Sample Collection

All samples were collected in different places; landfill waste such as landfill compost and faecal sludge were collected from GICUMBI district, Northern province, cow dungs were from NGOMA district, Eastern province, coffee husks were from KIREHE District, Eastern province, and rice husks were collected from KAYONZA District in Eastern province. After collection, all samples were dried in oven operated a 60°C, also except landfill compost other samples were homogenized through crushing in mortal and pestles for reducing period of time a reagent will dissolve humic substances. After all samples were sieved for removing large particles such as stones and sands. A siever with diameter size of 18  $\mu\text{m}$  was used.

In this research, also we focused on how faecal sludge are separated from other wastes. We did this because we have used faces as one of the sample. The chart below shows how faces 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 faecal sludge collected by a vehicle. The faecal sludge path through from chamber 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 faces 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 serves for killing all anaerobic microorganisms. When all these three anaerobic tank fulfil with separated liquids, these liquids waste path through filter channel (4). This channel is packed with sands which serve for further separating of liquids waste. Separated liquids throws 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 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 faces and other waste with high density than water and urine. Those wastes pass through van pipe transporter (9). Settled wastes from chamber named as 2 pass through van pipe to the separation house (10). This house is well constructed with concrete and above is packed with sand which allow movement of waste water, urine that have settled with faces. As waste water cease from faces, faces started to be dried with sunlight that pass through the white aluminium sheet of separating house. When faces are dried above sands are stored in storage house (11) for ready being used as organic fertilizer or being used in extraction of humic acid.

Constructed trash dryer house (12) serve for air and sunlight drying of some of separated trash which separated at reservoir channel (1). Finally, trashes dried in trash dryer

house are burned in incinerator (13). The figure 3 below has been drawn with an authorization from Manager of GICUMBI landfill.



Figure 3. Action plan faecal sludge treatment.

### 2.3. Extraction Process

In this research all collected samples were treated with acid-base precipitation method for extracting humic acids, where sodium hydroxide was used to extract the acid. Before extraction, except dried rice husks and coffee husks, other samples were stored in refrigerator at less than 4°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°C to remove moisture content. After removing moisture content samples were dried into powder for easy extraction. Extraction process was begun by weighing 10g of each samples and mixture of coffee husk were as follow: 30: coffee husk and cow dung, 70:30 coffee husk and cow dung and 30:70 cow dung and Rice husk. Five samples were firstly treated with 0.1M in beaker where agitation were applied for dissolving completely a solution of samples. There after each sample were carried in centrifuge for separation of humic acid, humins and fulvic acid the centrifuge tube used each was measuring 50ml In centrifuge a speed of 4000 rpm in 45 minutes was applied to separate humic substance this was done twice. After this humic acid sludge were separated with other humic substances then humic acid sludge were dissolved with 0.1M and carried into Nitrogen atmosphere in order to remove unwanted gases by dissolving concentrated hydrochloric acid (HCl) with 35-38%. Obtaining humic acid sludge were collected into beaker and we put in into oven for 13 hours. After drying all obtained powder were weighted

with electrical balance and stored in vial prior to further analysis.

### 2.4. Chemical Determination of Humic Acid

The determination of degree of humification, aromatization, oxygen content, carbon content were done by calculating E4/E6 ratio. This was done through determining wavelength at 465nm and 655 nm, respectively using UV visible spectrophotometer. The humic acid precipitate obtained was dissolved with 0.01M sodium hydroxide. Then we allowed it to be shaken on shakers in 6 hours. After we filtrated humic acid 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 solution [9]. The Cary 1E spectrophotometer with 1cm quartz cell was used during analysis.

### 2.5. Determination of Concentration of Humic Acid Solids Using UV - Instrument

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 states that absorbance is directly proportional to the concentration was based on for making the standard calibration curve [15]. Standard calibration curve is used to determine the concentration of unknown substance [16]. Finally, the concentration of the components can be found from the absorbance spectrum of the sample [17]. The absorbance band used were 540nm.

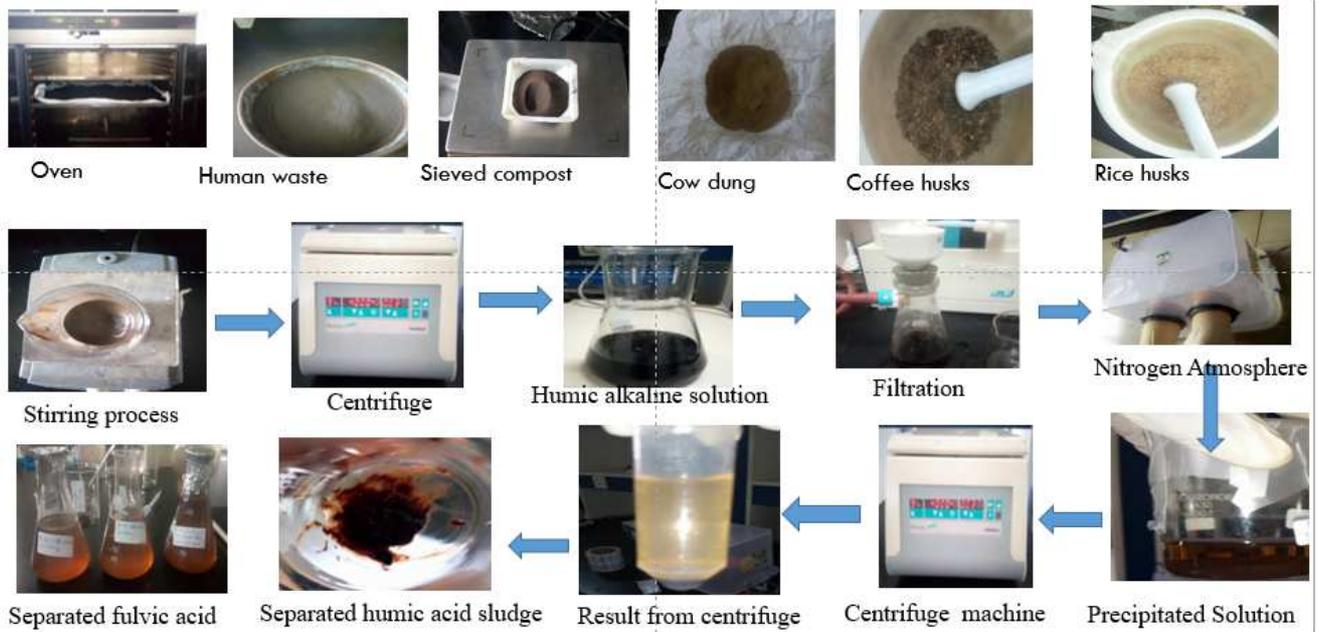


Figure 4. Full procedures used to extract humic acid.

### 3. Results and Discussion

Results recorded for all tested samples were carried out after extraction of humic acid powder in all sample. Amount of grams samples and procedure used were the same for all tested samples as figure 4 demonstrate.

#### 3.1. 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 acid.

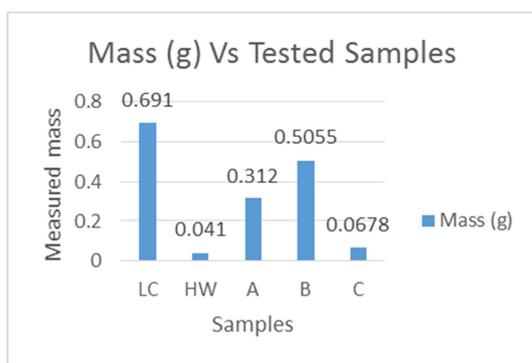


Figure 5. Weight of all extracted humic acid powder.

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 (30:70 cow dung and rice husk ratio) which has weight of 0.5055g, this is also followed by sample A (30:70 coffee husk and cow dung

ratio) which weight of 0.312g, followed by sample C (70:30coffee husk and cow dung ratio) which has weight of 0.0678g and the last one in all tested potential source is HW which has weight of 0.041g. As the weight of humic acid is high, more will be selected to be used.

#### 3.2. 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 at 60°C.

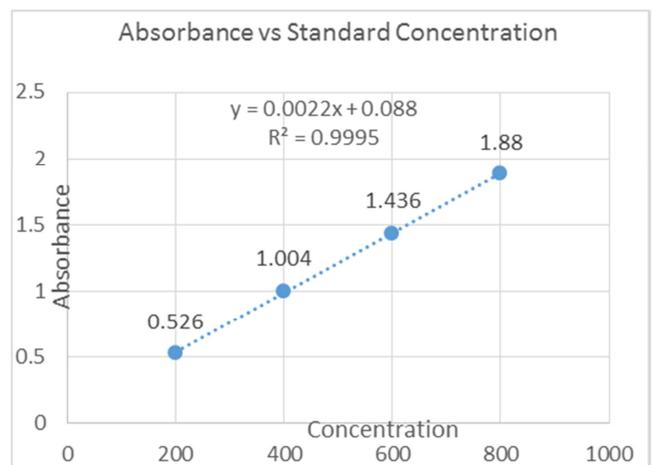


Figure 6. Moisture content of tested sample.

#### 3.3. Results Recorded for Concentrations

The results were recorded after drawing calibration curve of standard solution. Standard calibration curve is use to determine the unknown concentration in all tested sample.

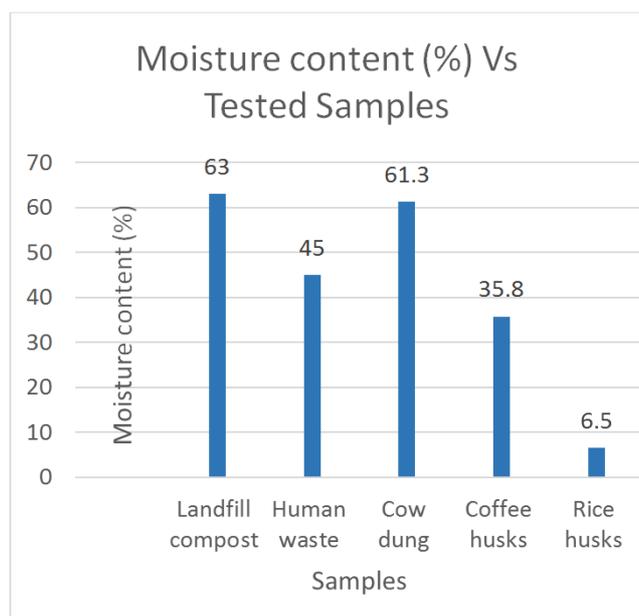


Figure 7. Calibration curve of humic acid standard solution.

After drawing the calibration curve, each humic acid extracted solution 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 1. Absorbance Vs concentration for tested samples.

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 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 770 ppm. LC is followed by sample B which has concentration 484.54 ppm, followed by A with the concentration of 371.8 ppm and the by C which has lowest concentration of 290 ppm. As concentration increase more source is chosen as potential source due to small amount is required to be used per meter square of field.

### 3.4. 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 [18] and oxygen content of humic materials [19]. The degree of aromatization is directly proportional to the degree of humification and this is characterized by low value of E4/E6

ratio [20]. The high value of E4/E6 shows low aromatic condensation, large proportional of aliphatic structure [18] and presence of oxygen-containing functional group such as carboxyl, carbonyl, ester, hydroxyl group [19] 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 was proposed by Kononova [21]. Above 5.9 ratio is considered as high E4/E6 ratio.

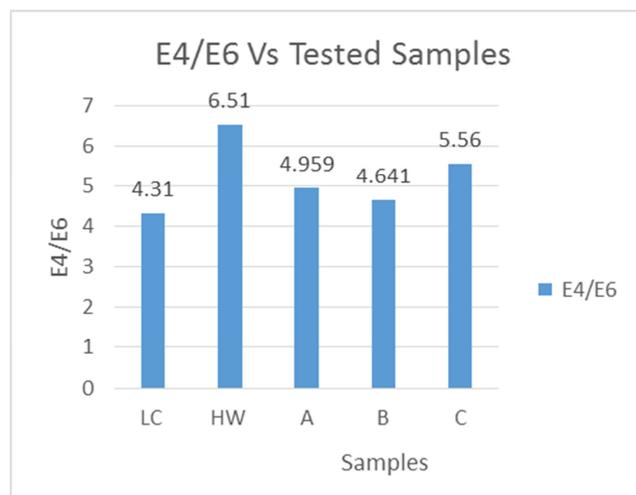


Figure 8. Measured E4/E6 ratio for all tested samples.

Figure 8, shows the E4/E6 value for all tested samples, except human 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 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 has high degree of aromatic condensation, humification and molecular weight.

This is followed by B sample 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 human waste (HW) with E4/E6 ratio of 6.51. HW with high E4/E6 ratio of 6.51 is due to this value is out range of E4/E6 ratio of 3.5-5.9.

The low value of E4/E6 obtained for LC is due to the microbial activity which takes place in long period, this result to high degree of humification, aromatic condensation and molecular weight or size in the range of 5,000 to 100,000 Dalton [11]. While the high value ratio of E4/E6 obtained for HW shows high rate of aliphatic structure and low degree of aromatic [18, 22].

## 4. Conclusion

This research aims of determining the potential source for extracting humic acid was done after extracting humic acid in all samples treated with acid-base titration. Landfill compost, human 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 of aromatic condensation. All results confirm landfill compost 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 found to have high weight of 0.691g and is the highest weight compared to the other. Also it has highest concentration of 770ppm compare to other sources, and low E4/E6 ratio of 4.31 is in the range of 3.5 to 5.9 and this refer to the high condensation of aromatic carbon content, high level of humification and molecular size. This research found that only human waste (HW) E4/E6 has value which is high due to is out of the proposed range of 3.5 - 5.9) of 6.51.

As results of this investigation landfill compost is the best source for large scale production of humic acid in Rwanda according to the parameter analysed such as weight, concentrations and E4/E6 ratios.

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