

Comparison of Biomass Content for the Evaluation of Cellulosic Ethanol Fuel Production from Predominant Perennial Grasses in South-South, Nigeria

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Abstract

Spectrophotometric and gravimetric methods of analyses were used to quantitatively compare the biomass compositions of three predominant perennial grass species; *Eleusine indica* (Ei), *Pennisetum purpureum* (Pp), and *Panicum maximum* (Pm) that are found in the study area. The range of values for the percentage composition of the biomass components; cellulose, hemicellulose and lignin were 26.10 ± 0.85 – 34.04 ± 0.24 , 32.51 ± 1.85 – 42.57 ± 1.17 and 3.09 ± 0.09 – 5.62 ± 0.17 respectively. The sample with the highest amount of cellulose showed a promising raw material for the establishment of an ethanol fuel biorefinery in the region while that with the highest amount of hemicellulose which was different from the former showed that optimum production of the desired product could be obtained if advanced technologies are put in place to successfully ferment the pentoses fraction of the hemicellulose that may be present in the pretreatment hydrolysate. *Eleusine indica* with the highest amount of ash and metal (Mn) contents; $89,500 \pm 40.37$ ppm and 240.01 ± 3.04 ppm respectively was found to be likely that which will produce the least amount of the desired product as these substances may cause inhibitory effects to enzymatic activities in the reaction pathway and this specie was observed to have the lowest amount of the necessary biomass feedstock: cellulose and hemicelluloses, it also had the second highest amount of total extractable polyphenolics (TEPs); which is a potential inhibitor to enzymatic activities.

Keywords: Total Extractable Polyphenolics, Biofuel, Hemicelluloses, Cellulose, Lignin, Bioethanol

1. Introduction

The problem of global warming has been undeniably accepted worldwide. This is because global warming affects all nations of the earth, as the average temperature of the earth and its atmosphere is increasing. Certain gases, known as green house gases (GHG) have been identified as the major causes of global warming. Carbon dioxide from fossil- fuel, power stations and car exhaust are the main sources of GHG.

Biomass is the only alternative energy source that has been demonstrated to be able to supply liquid, solid, and gaseous renewable as well as sustainable (green) fuels [Ohimain *et. al.*, 2014]. Bioethanol can be produced from biomass such as starch and sugar from cassava, corn and sugarcane which are basically sources of food for human; the bioethanol produced from these sources are classified under the first (1st) generation biofuels while those produced from biomass such as agricultural and forest residues as well as non-food feedstock such as grasses, corncobs, wooden spills, bagasse, etc. are referred to as second (2nd) generation biofuels. Exploring the first generation biofuels may possibly lead to the problem of food crisis globally [Wongwatanaipaiboon *et. al.*, 2012], rather the choice of the second generation biofuels will be vital to manage the present challenges of climate change and still guaranty food security, though it is cost intensive considering the total production cost [Sims *et. al.*, 2008]. With thorough researches, optimum production could be achieved to save the cost effectiveness of the entire process.

The use of plant biomass as sources of energy give numerous advantages, as their sources of energy are green and renewable [Jank *et. al.*, 2013]. Also because of the lower content of sulphur in biomass, they are considered easier to gasify than fossil-fuels like coal and petroleum [Hall, 1997].

Generally, the average composition of grass biomass is 25-40% cellulose, 25-50% hemicellulose, 5-15% lignin; their structures are as shown in Figure 1, which emphasizes their precursors and possible effects in the reaction pathways. Grasses can as well be practically combusted in furnaces and boilers to produce steam for a Rankine cycle (steam cycle) for power generation [Wongwatanaipai boon *et. al.*, 2012].

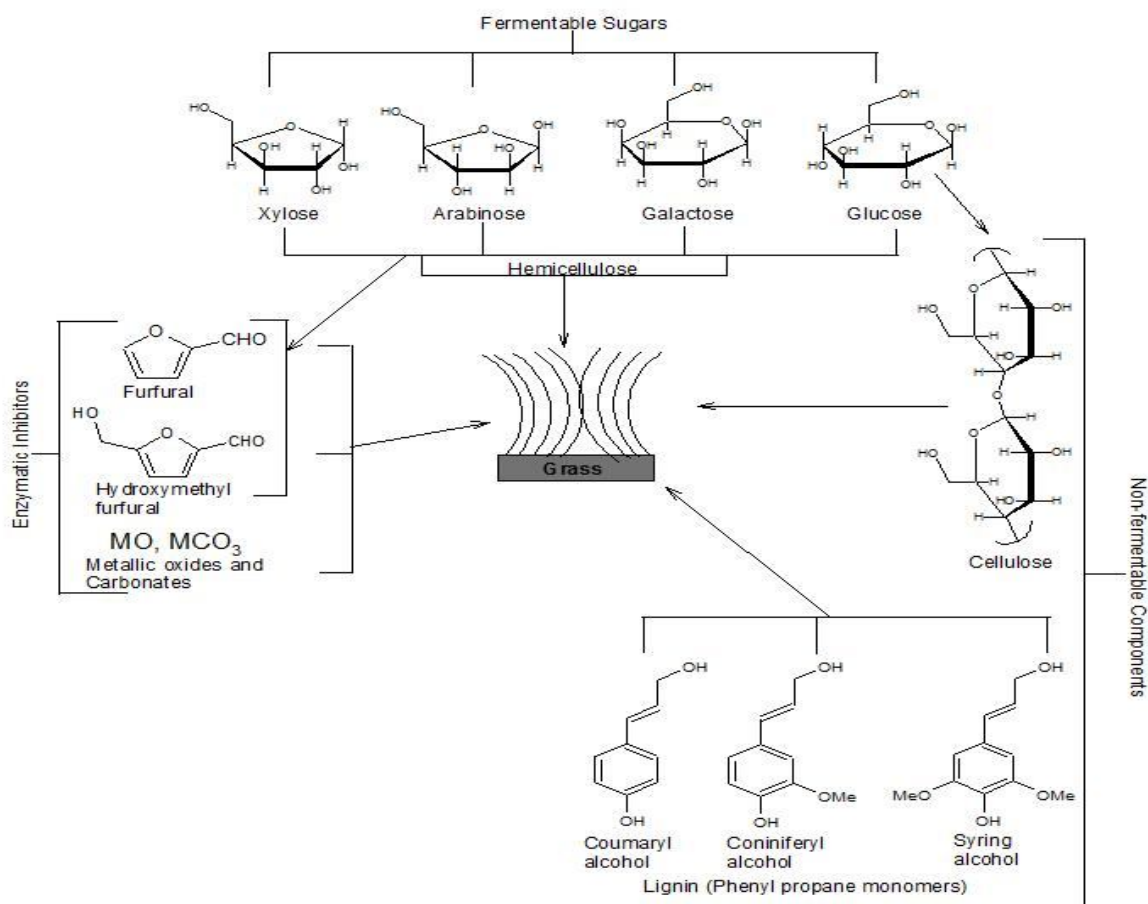


Figure 1: Components of grass biomass showing fermentable and non-fermentable components as well as components that can cause recalcitrant effect to hydrolysis and fermentation reactions in the reaction pathway.

2.0 Materials and Methods

2.1. Biomass Handling

Three predominant grass species; *Eleusine indica*(Ei), *Pennisetum purpureum* (Pp), and *Panicum maximum* (Pm) were identified and collected from the University of Port Harcourt, Nigeria.

The biomass were washed thoroughly and copiously with deionized water, chopped into size (1-2cm) and air-dried in a greenhouse for six days.

The dried biomass were ground into powdered form before they were sieved to 0.15mm, 0.20mm, 0.35mm, 0.45mm and 0.71mm particle sizes using a DH-300T test sieve machine. The dried and sieved biomass was preserved in a cool and dry place at an average temperature of 12⁰C, until used for further work.

2.2. Analysis of Biomass composition: Cellulose, Hemicellulose and Lignin.

The biomass composition was determined using the method of fiber analysis as adopted by the University of Nebraska Ruminant Nutrition Laboratory, (2009).

The neutral detergent fiber (NDF), acid detergent fiber (ADF), permanganate lignin (PML) and ash (ASH) contents of the biomass were first determined before the evaluation of cellulose (CEL), hemicelluloses (HEM) and lignin(LIG) present in each sample. These were carried out with the biomass of 0.15mm particle size.

2.2.1. Determination of the Amount of Neutral Detergent Fiber (NDF).

1±0.004 g of the ground biomass sample with 0.15mm particle size was weighed using a FA/JA-B series analytical balance and 0.5g of Sodium Sulphite was added for the removal of protein. The sample was subsequently placed in a Berzelius 600 ml beaker and 100 ml of neutral detergent solution was added at room temperature.

The contents of the beaker were refluxed for one hour followed by the addition of Alpha-amylase.

At the end of the reflux period, the sample solution was filtered using a vacuum pump with a dry Whatman 541 filter paper.

The residue was washed copiously with boiling water, filtered and dried in an oven for a minimum of 6hours; then transferred into desiccators and the dry filter paper and residue reweighed (Van Soest *et. al.*,1991; Anderson and Ingram, 1989).

Calculation:

$$\% \text{NDF} = \frac{(\text{Dry filter paper} + \text{Residue}) - (\text{Dry filter paper})}{(\text{Sample Weight})} \times 100$$

2.2.2. Determination of the Amount of Acid detergent Fiber (ADF) and Hemicellulose (HEM)

Dried mass of samples after NDF analysis were weighed using a FA/JA-B series analytical balance and placed on a 600ml beaker with 100ml of acid detergent solution added at room temperature. The contents were refluxed for one hour with the addition of 0.5 Alpha-amylase repeatedly after reflux begun and ten minutes before filtering high starch samples.

At the end of the reflux period, the beaker was removed separately and condenser wiped with moist sponge to remove condensed detergent solution. The residue was washed with several volumes of boiling water and filtered with a vacuum pump using dry Whatman 541 filter paper then dried in laboratory oven at 100°C for 6 hours and transferred to desiccators before being reweighed to obtain the weight of dry filter and residue (Van Soest,1963).

Calculation:

$$\% \text{ ADF} = \frac{(\text{Dry filter paper} + \text{Residue}) - (\text{Dry filter paper})}{(\text{Initial Sample Wt})} \times 100$$

$$\% \text{ Hemicelluloses} = \% \text{NDF} - \% \text{ADF}$$

2.2.3 Determination of the Amount of Permanganate Lignin (PML) and Cellulose (CEL)

Dried mass of sample after ADF analysis was filtered into a Gooch crucible, the crucible was placed in shallow Pyrex dish with 2 to 3cm of distilled H₂O in it and 25ml of KMnO₄-Buffer solution was added into the crucible then left to stand for 90minutes with a short glass rod for stirring contents and breaking up lumps, adding more solution in some cases (solution was purple at all times); the crucible and the level of water in pan was adjusted to avoid excess flow of solution out of the crucible. After 90minutes, the crucible was removed and transferred to filtering apparatus and the residue sucked dry but not washed. The Crucible was placed in clean Pyrex dish and half filled with demineralising solution, the content of crucible was filtered after 15 minutes and refilled approximately half full with demineralising solution. The sides of crucible were rinsed with demineralising solution, then left to stand until the fiber was white (20-30 minutes). The Crucible and content was washed 3 times with 80% ethanol and the glass rod rinsed, then removed so as to cause no loss in dry matter, before filtration with Whatman 541 filter paper. The fiber was dried at 105°C over night then transferred into desiccators and weighed to obtain the cellulose residue.

The processes above were repeated for samples that were found to be yellow after filtration. This was required on samples where lignin/ADF was greater than 35% (Van Soest, and Wine, 1968).

Calculation:

$$\% \text{Lignin} = \frac{(\text{ADF Residue} - \text{Cellulose Residue}) \times 100}{(\text{Initial Sample Weight.})}$$

The ash content was obtained by heating the cellulose residue above in a laboratory furnace at 500°C for 1hour, then allowed to cool in a desiccators before it was weighed to obtain the % Ash (ASH) content.

$$\% \text{Cellulose} = \% \text{Cellulose Residue} - \% \text{ASH}$$

2.3.1. Determination of Total Extractable Polyphenolics (TEPs)

A solution of 100ppm tannic acid was made by dissolving 0.05g of tannic acid in a 500ml volumetric flask then made up to the mark and three other standard solutions of 50, 25 and 12.50ppm were prepared.

0.75±0.001g of dried and 0.71mm sieved particle sized biomass was weighed (W) into a 50ml beaker, 20ml of 50% Methanol added and covered with parafilm then place in a water bath at 77.50°C for 1hour. The extract was filtered with Whatman filter paper 1 into a 50ml volumetric flask using 50% aqueous methanol to rinse and make up with water then the solution mixed properly by stirring.

1ml of the unknown or standard solutions was put differently into a 50ml volumetric flask using a pipette followed by the addition of 20ml water, 2.5ml Folin-Denis reagent and 10ml sodium carbonate (17%) ; the solutions were stirred to mix well then make to the mark with water and left to stand for 20minutes. Then the absorbance was read at 760nm (using UV 1801 Series UV-Vis Spectrophotometer) for the unknown, standards and blank solutions.

Calculation:

A graph of absorbance against concentration of the standards was plotted and the concentration of the unknown and blank were found.

The mean blank value was subtracted from the mean unknown value to give a value for the corrected concentration, C (ppm)

$$\text{Total Extractable Polyphenolics (\%)} = \frac{0.005 \times (\text{ppm})}{\text{Weight of Sample (W)}}$$

2.3.2 Analysis of Metals (Mn, Co, Pb, and Cd)

1.0±0.5g of the biomass was weighed into a beaker using FA/JA-B series analytical balance. One milliliter (1ml) perchloric acid (HClO₄) and 3ml of conc. HNO₃ were added to the beaker, stirred and allowed to heat for 15 minutes until gases disappeared in a fume cupboard then filtered. The sample was allowed to cool and made up to 100 ml with distilled water then an aliquot of 50 ml was taken for analysis using a Buck Scientific Model 210VGP Atomic Absorption Spectrometer (AAS) with standard solutions of the required metals as well as their hollow cathode lamps. Graphs of absorbance against concentration (mg/l) of standard solutions were plotted and concentration of the unknown determined from values of their absorbance, the actual concentration of the unknown in milligram per kilogram (mg/kg) was calculated from the equation below (Ekpo, 2005 and Ekpo *et. al.* 2014).

$$\text{Conc. (mg/ kg)} = \frac{\text{absorbance of solute} \times \text{vol. of solvent}}{\text{absorbance of standard} \times \text{weight of dry biomass}}$$

3.0. Results and Discussion

Table 1: % Composition of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) in Grass Samples.

Grasses	NDF	ADF
<u>Eleusine indica</u> (Ei)	78.08±0.09	45.55±1.05
<u>Pennisetum purpureum</u> (Pp)	76.23±0.98	36.92±1.51
<u>Panicum maximum</u> (Pm)	77.18±0.43	34.61±1.70

As shown in Table 1 and Figure 2, the amounts of Neutral detergent Fiber (NDF) for the three grasses are not excessively different from each other, rather the amount of ADF for Eleusine indica showed remarkable increase from others and it is the shortest of the three grass species.

Table 2: % Composition of Biomass in Grass Samples.

Grasses	HEM	CEL	LIG
<u>Eleusine indica</u> (Ei)	32.51±1.85	26.10±0.85	4.14±0.52
<u>Pennisetum purpureum</u> (Pp)	39.31±0.72	34.04±0.24	5.62±0.09
<u>Panicum maximum</u> (Pm)	42.57±1.17	32.71±1.02	3.09±0.17

Biomass composition is the most vital aspect of this research as fermentation to cellulosic ethanol fuel requires pretreatment of lignocelluloses to obtain desirable amount of polysaccharides, followed by saccharification reaction to obtain easily fermentable monosaccharide and subsequent fermentation of monosaccharide to cellulosic ethanol, which could be used directly as; a source of fuel, combined with other conventional fuels or used in the synthesis of fuels as in the transesterification reaction to obtain biodiesel.

In Table 2 and Figure 3, the biomass compositions indicated that the amount of hemicellulose in the samples increase in the order Ei<Pp<Pm and cellulose increases in the order Ei<Pm<Pp while lignin content followed an order of Pm<Ei<Pp.

Lignin is the biomass component that holds the other components closely tight to the secondary cell walls of plants; it is the only polymer found in plant cell walls that is not composed of carbohydrate, it is composed of three different phenyl propane monomers which are Coniferyl alcohol (found in all plant tissues), syringyl alcohol (common to hardwoods) and coumaryl alcohol (dominant in grasses) units. Lignin is a large group of aromatic polymers resulting from the oxidative combinatorial coupling of 4-hydroxyphenylpropanoids (Boerjan et al., 2003; Ralph et al., 2004).

Hemicellulose is made up of combination of hexoses and pentoses with the latter being predominant. Hexoses are easily fermentable while pentoses are difficult to undergo fermentation, except where special engineered enzymes are used. However, the yield of easily fermentable sugar could be obtained from a pretreated hydrolysate by carrying out a reaction to ascend the carbohydrate series (Finar, 2008); where pentoses are converted to hexoses before fermentation.

According to Zhu *et. al.*, 2009, pretreatment processes like the acid pretreatment process and sulphide/sulphate pretreatment to overcome recalcitrance of lignocelluloses (SPORL) produce fermentation inhibitory substance like furfurals which are mostly generated from pentoses. So, the more the amount of pentose sugars present in the lignocellulosic biomass the higher the amount of these inhibitory substances that will be formed.

This goes to show that utilizing a lignocellulosic biomass with high amount of hemicelluloses for the purpose of producing cellulosic ethanol fuel depends on the available technology to carry out fermentation of pentoses and the possibility to extract the fermentation inhibitory substances produced after the pretreatment stage of production. Among the considered grasses, Panicum maximum with the highest amount of hemicellulose of $42.57 \pm 1.17\%$ will have the advantage of its pretreated hydrolysate being utilized for fermentation.

Cellulose is the most required biopolymer for the purpose of this research. In Table 2 and Figure 3; the grass species have their cellulose composition in the increasing order of $Ei < Pm < Pp$. The range of the values is 26.10%-34.04%, which could be comparable to the work of Xue *et. al.*, 2011, where the range for the amount of cellulose was 33.94% - 39.46% from a set of ten grass species that were considered. Pernnisetum purpureum with the highest amount of cellulose of $34.04 \pm 0.24\%$ is likely to produce the highest yield of bioethanol. Eleusine indica, although having the least amount of cellulose composition among the considered grass species for this research, cannot be overlooked completely for the purpose of this production, because the ease of breaking down the fermentable sugar from cellulose during hydrolysis is also dependent on the crystal nature of the cellulose biomass, as most plant biomass form stronger crystal matrix within the bonds linking the disaccharides and monosaccharides together in the polysaccharide (Mosier *et. al.*, 2005).

Table 3: Composition(ppm) of ash, total extractable polyphenolics and metals in the grass samples

Grasses	ASH	TEP	Mn	Co	Cd	Si	Pb
<u>Eleusine indica</u>	89,500±40.37	7,900.00±22.72	240.00±1.45	1.20±0.00	<0.01	<0.01	<0.01
<u>Pernnisetum purpureum</u>	68,600±17.15	6,300.00±11.79	41.70±0.65	1.83±0.05	<0.01	<0.01	<0.01
<u>Panicum maximum</u>	82,300±38.94	10,599.99±23.74	36.60±4.20	1.20±0.08	<0.01	<0.01	<0.01

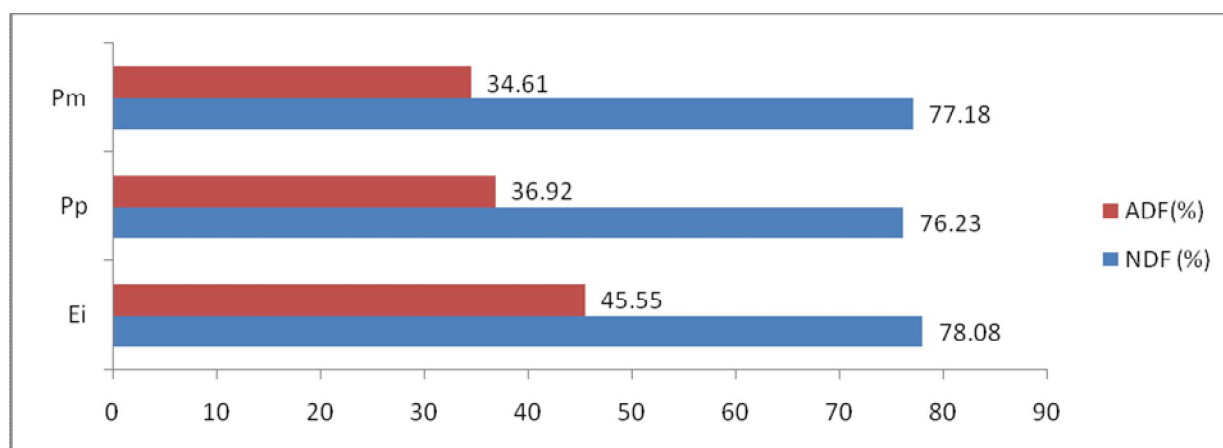


Figure 2: Composition (%) of Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) in Grass Samples

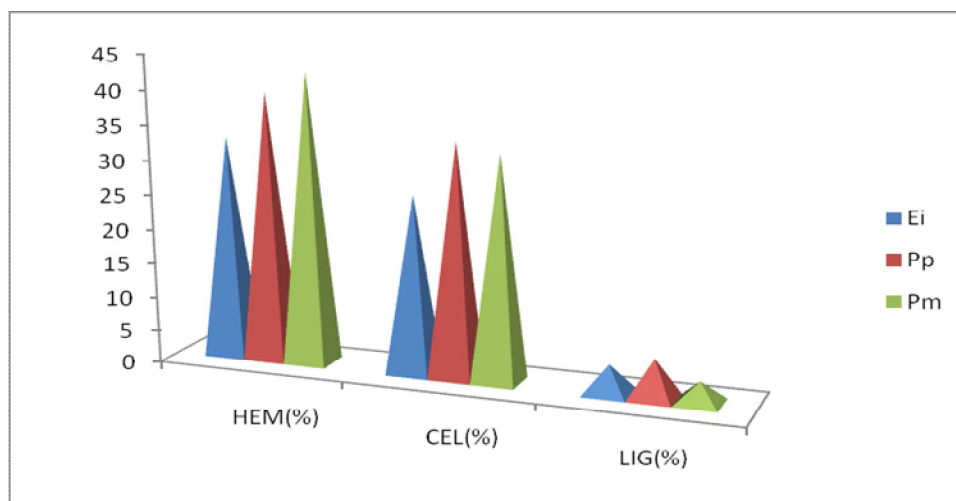


Figure 3: Composition (%) of Biomass in Grass Samples

Apart from substances generated in the pathway of the production processes which have inhibitory effects on enzymatic activities at the saccharification and fermentation stages of this production, there are other inhibitive substances that are components of the plant cell wall and are capable of causing similar effects: Some of these substances are shown in Figure 1 and they include; tannins, terpenes, resins, heavy metals (in the form of oxides and carbonate), Hibbert ketones, weak acids, phenolics etc (Palmqvist and Hahn-Hägerdal 2000). Two of these substances [metals (Mn, Co, Cd, and Pd) and total extractable polyphenolics (TEP)] were analysed quantitatively and others determined as the ash (ASH) content.

In Table 3, Figure 4 and Figure 5, Eleusine indica has the highest amount of the most accumulated metal (manganese) as well as ash content, while Pennisetum purpureum contains the least amount of ash and TEPs. In the other hand, Panicum maximum has the highest amount of TEPs. Plant ash is known to be a rich source of metallic oxides and carbonates and the removal of these metallic compounds will require severe reaction conditions such as the use of concentrated solution of acids, which may lead to the degradation of the required biomass feedstock (cellulose and hemicellulose) to form other organic inhibitive substances such as; organic acids, furfurals and 5-hydroxymethyl furfural (HMF) (Palmqvist and Hahn-Hägerdal 2000, Chandel *et. al.*, 2007a) and the extraction of polyphenols or any other organic inhibitor from the biomass feedstock will require the use of liquid-solute extraction, using an organic solvent without degrading the useful biomass component nor excessively generating other inhibitive substances.

According to the work of Ladisch *et. al.*, 2009, polyphenolic compounds are most often generated in the cause of pretreatment process. The effect of these polyphenolic compounds depend on the type of polyphenol present in the biomass (Mandel and Reese, 1965). Characterization of specific phenolic compound could be done with the used of Gas chromatography-Mass spectrometry (GC-MS) as well as High Performance Liquid chromatography (HPLC) methods of analysis (Popa *et. al.*, 2011).

So, from the values of TEPs in Table 3 and Figure 4, Panicum maximum may be less economically viable to be used as a raw material in this production compared to other grasses studied in this research work.

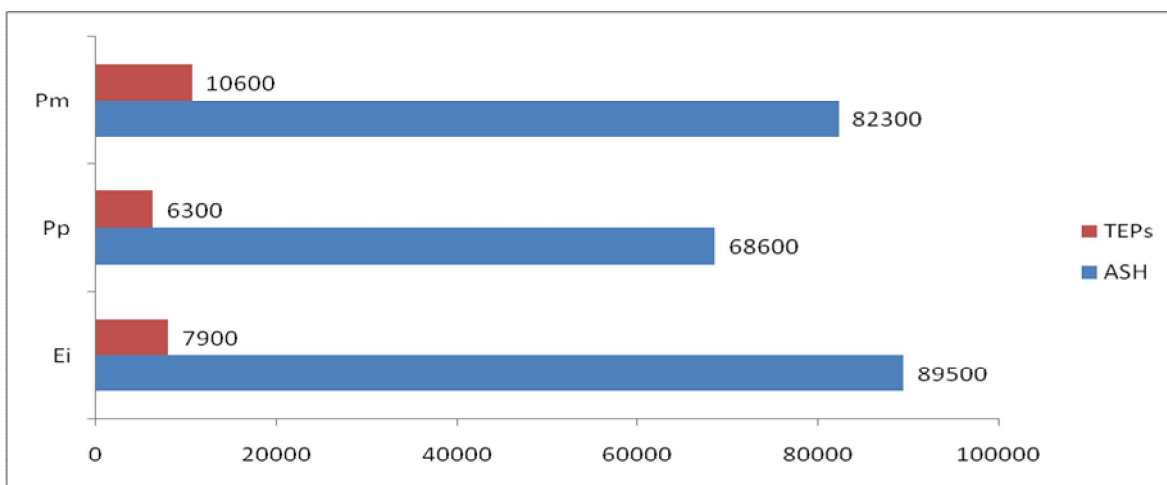


Figure 4: Amounts (ppm) of Ash and Total Extractable Polyphenolics (TEPs) Present in Biomass.

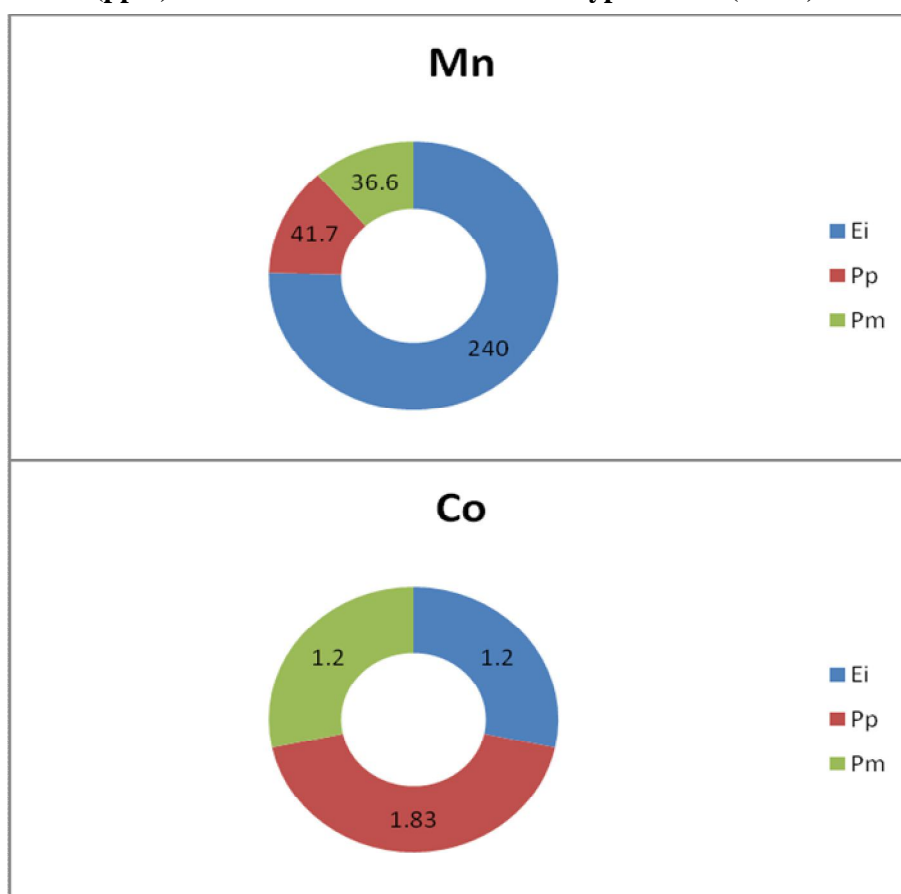


Figure 5: Amounts (ppm) of Manganese (Mn) and Cobalt (Co) present in Biomass.

4.0. Conclusion

The establishment of a biorefinery in the considered region for the production of cellulosic ethanol fuel may be viable, because of the availability of raw materials with required quantity of feedstock. Pennisetum purpureum is likely to produce the highest yield of bioethanol fuel followed by Panicum maximum, these are deduced from the increasing order of the amount of cellulose contained in their biomass; the latter may be more successful where improved technologies are applied to ferment the hemicellulose fractions present in the pretreatment hydrolysate as well as extracting the extreme high amount of total extractable polyphenolics (TEPs) so as to prevent the effects of these substances in the production pathway.

Eleusine indica may likely produce the least yield of the desired product as its carbohydrates contents are low compared to the other two samples. Moreover, the high amounts of ash and metal components (Mn) present in it prove that its pretreated feedstock may contain numerous metallic substances that may pose recalcitrance effects to enzymatic activities in the production pathway, such that the entire production processes may be difficult to manage.

5.0 Acknowledgement

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