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Characterization of Fresh Cattle Wastes Using Proximate, Microbial and Spectroscopic Principles

[1]S. Katheem Kiyasudeen, [1]Mahamad Hakimi bin Ibrahim and [2]Sultan Ahmed Ismail

[1]Environmental Technology Division, School of Industrial Technology,
Universiti Sains Malaysia, Pulau Pinang, 11800, Malaysia
[2]Ecoscience Research Foundation (ERF), 98, Baaz Nagar,
3/621 East Coast Road, Chennai - 600041, India

Abstract: The main aim of this study is to perform a comparative study on freshly collected cow dung samples to guide farmers in Pulau Pinang, Malaysia. Hence, fresh cow dung samples (CD1, CD2 and CD3) from three different farms (F1, F2 and F3) provided with locally available resources such as grass, palm tree wastes and corn were subjected to proximate analysis, microbial and FTIR characterization principles. Results from proximate analysis showed that the CD2 had significantly high ($p<0.05$) nutrient characteristics such as total carbon (41.89±0.11%), total nitrogen (2.65±0.01%), crude protein (16.90±0.06%) and organic matter (75.40±0.2%) than the other two cow dung samples, CD1 (total carbon-38.84±0.06%, total nitrogen-1.40±0.15%, crude protein-8.9±0.09% and organic matter-69.92±0.1%) and CD3 (total carbon–39.16±0.04%, total nitrogen-2.05±0.2%, crude protein-13.07±0.17% and organic matter-70.48±0.07%). Total viable counts of bacteria also showed that the CD2 ($2.84\pm0.01 x10^5$ cfu/g) had substantially higher ($p<0.05$) total colony forming units than CD1 ($1.78\pm0.05 x10^5$ cfu/g) and CD3 ($2.47\pm0.01 x10^5$ cfu/g). FTIR studies showed similar pattern for both CD2 and CD3 whereas certain dissimilarity was observed in CD1. Based on this comparative study, it is recommended to use grass as major diet along with nutrient rich supplements for cows in the selected farms in Pulau Pinang, Malaysia to enhance cow dung's nutrient quality for the better agricultural practices such as manure production, composting and vermicomposting. Future studies can be focussed on nutrient content of the feed ration and changes occur inside cow's digestive system in order to bring more scientific evidences.

Key words: Cow dung · Characterization · FTIR · Proximate analysis · Nutrients

INTRODUCTION

In recent years, use of manure as fertilizers has been gradually declined due to separation of crop and livestock production, transportation issues and increased availability of synthetic fertilizers [1]. Usage of commercially available synthetic fertilizers pollutes the environment and degraded the soil quality [2]. Due to increasing interest about the harsh effects of synthetic chemicals and improperly treated animal wastes on the environment and human health, researches on alternative farming becomes inevitable [3]. Animal manures are well known for supplying many nutrients for crop production and organic matter which in turn helps in improving soil structure, water holding capacity, drainage, reduces wind and water erosion, provides a source of slow release nutrients, promotes growth of earthworms and beneficial microbes [1]. Dairy animals produce about 1.61 ft^3 (12.0 gal) of fresh manure (feces and urine) per 1,000 lb average live weight per day and it contains 14.4 lb of total solids per AU per day [4]. Cow manure has been considered as a potential fertilizer since ancient times as it contains essential macro and micronutrients for crop growth and is a low cost alternative to synthetic inorganic fertilizers. It has various uses such as fuel, disinfectant, purifier, mosquito repellent, a good source for plant growth [5] and a valuable feed for earthworms in vermicomposting process [6-8]. The relationship between

Corresponding Author: S. Katheem Kiyasudeen, Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Pulau Pinang, 11800, Malaysia.
Tel: +60164018271, E-mail: katheem.kiyas@gmail.com.

the feed and the excreta in cattles has been well established by many researchers [9-12]. They also suggested that the quantity and quality of the nutrients obtained from the excreta (e.g. dung in case of cows) is influenced by various impact factors such as feed input, climatic conditions, animal stocking density, etc. To be more precise, feed input is a critical nutrient input for estimating nutrient excretion [13] but these feed stuffs may vary in composition due to many reasons unlike chemicals that are chemically pure. A lot of work has been done on the conservation of nutrients in manure, but most of the times the attention has always been on Nitrogen, Phosphorous and Potassium while other nutrients are not given adequate attention [14].

The current study predominantly aims to evaluate proximate, microbiological and FTIR characteristics of fresh cowdung samples collected from three different farms in Pulau Pinang, Malaysia in which cows are fed with locally available resources such as palm tree wastes, corn wastes and grass. This research work also serves as a report on cow dung quality and also beneficial for the farmers, researchers and students to choose the appropriate combination of feed from the locally available resources to obtain cowdung of better quality and ultimately to be used for commercial and research purposes specifically recycling animal wastes, manure production and vermicomposting.

MATERIALS AND METHODS

Fresh cow dung samples CD1, CD2 and CD3 were collected from three different farms F1- Kampung selamat, F2-Bukit gambir and F2-Sungai dua around Pulau Pinang, Malaysia in which the cows were fed with locally available resources. Table 1 shows the feed details and their ratio.

Composite Sampling: Cow dung samples were collected according to recommended methods of manure analysis [15]. After obtaining seven sub-samples, they were thoroughly mixed in a container. From the container, single composite sample of approximately 1 kg was taken out and shifted to the polythene bag and sealed air tight. Then the samples were stored in deep freezer at -20°C for further analysis. All cow dung samples are subjected to statistical analysis in three replicates expressed in Mean ± Standard deviation on oven dry basis.

Proximate Analysis

pH and Electrical Conductivity (EC): The pH of manure suspension with cow dung and water ratio of 1:10 was determined using a pH meter (Model: Eco testr pH2) and electrical conductivity in the manure suspension was measured by an electrical conductivity meter expressed in dS/m (Model: Eco testr EC) by standard procedures [16, 17].

Moisture and Total Solids (TS): Cow dung samples were analyzed for moisture and dry matter content using oven drying method [18, 19]. 10g of each composite samples were weighed and dried in hot air oven for 24 hours at 105°C. Dry weight of the sample was taken till it showed its constant weight.

Organic Matter (OM) and Ash: Approximately 2g of dried and powdered samples were placed and burnt in a furnace for 4 hours at 550°C by Loss of Ignition method [20]. After ashing, the samples were allowed to cool in a dessicator. Final weights of the samples were measured with a precision balance.

Crude Protein: 1g cow dung samples (carried out in triplicates) were subjected to Kjeldahl acid digestion (combination of 25 mL H_2SO_4 and Kjeldahl catalysts) using Gerhardt Kjeldatherm digester and allowed to cool for 1 hour and subjected for distillation (32% NaOH and 2% H_3BO_3 combination) using Gerhardt Kjeldahl distillation system VAPODEST 45s in which auto titration using 0.1 N HCl was done [21].

Chemical Characteristics: Total Carbon (TC), Hydrogen (H), Total Nitrogen (TN) and Sulfur (S) values of the samples were determined using TOC-SSM 5000A analyser and Perkin Elmer 2400 Series II CHNS-O Analyzer [22].

Microbiological Analysis: The serial dilution and standard plate count method was used for culturing bacteria and fungi using Nutrient agar and Potato dextrose agar (PDA) [23, 24]. The plates were incubated at 28±2°C and the colony counts were recorded as total viable count.

FTIR Assay: The infrared spectra of each composting step were recorded between 4000 cm^{-1} and 400 cm^{-1} by using a Perkin-Elmer 1600 FTIR. Pellets were prepared by mixing 2 mg ground sample with 200 mg KBr, later compressing the mixture under vacuum for 10 min. In order to limit moisture interference, both composting samples and KBr were dried at 105°C for 72 hours before making the pellet [25-27].

Table 1: On field profile of cow dung samples

Contents	CD1*	CD2**	CD3***
Location	Kampung selamat (F1)	Bukit gambir (F2)	Sungai dua (F3)
Colour	Slight green	Thick green	Thick green
State	Watery	Semi solid	Semi solid
Chief ingredients and its ration	Corn (100%)	Grass, fruit peels, bread wastes (80:10:10)	Palm tree wastes, grass (50:50)

* CD1- Cow dung from Kampung selamat; **CD2-Cow dung from Bukit gambir; ***CD3- Cow dung from Sungai dua

Statistical Analysis: The analysis of variance was performed to compare means of three replicates of all samples. One-way ANOVA is carried out on all parameters using the IBM SPSS Statistics version 22 at $p<0.05$ as significant level.

RESULTS AND DISCUSSIONS

CD1 collected from cows (L1) fed with Corn as major feed was slightly green in colour and watery in state. CD2 obtained from the cows which are fed with grass as major diet, fruit peels and bread wastes was thick green in colour and semi-solid in state. Similarly, CD3 had grass as a major diet along with Palm tree wastes had thick greenish colour and also semi-solid in state.

Proximate Characteristics: Table 2 shows the obtained proximate characteristics of the fresh cowdung samples, CD1, CD2 and CD3. pH is a very important assay to evaluate quality of any manure as it determines the availability of nutrients, microbial activity and physical conditions [28]. pH can be high or low depending upon feedstock and production conditions [3]. Comparison of pH of three samples after ANOVA test shows that the CD1 had almost neutral pH (7.5±0.05) which is significantly higher ($p<0.05$) than CD2 (6.5±0.05) and CD3 (6.5±0.05). Values of pH are in accordance with many works on fresh cow dung [19, 29] and is suitable for plant applications. Electrical conductivity (EC) expresses ion contents of any solution [30]. It provides better information about the soluble salts in cow manure. Manure can have high levels of EC due to the larger amounts of salt based minerals commonly added to the feed [16]. EC value of CD1 (1.92±0.01 dS/m) is significantly lower ($p<0.05$) than other two samples, CD2 (2.9±0.011 dS/m) and CD3 (2.7±0.011 dS/m).

Moisture is one of the major parameter in estimating the quality of any manure or substrate for vermicomposting. Low moisture level can significantly affect the earthworm survivability and reproduction. Earlier studies showed that more water content in the manure will cause less cohesion in soil as it separates the soil particles and causes softening of soil which leads to nutrient loss [2]. Similarly, total solids or dry matter content is an important factor in determining the handling characteristics and relative nutrient content of manure. Significantly high ($p<0.05$) moisture level was observed in CD1 (90.21±0.2%) than the other two samples, CD2 (80.73±1.1%) and CD3 (81±1%) whereas the levels of total solids are in contrast with the levels of moisture i.e. CD2 (19.26±1.1%) and CD3 (19±1%) had significantly high ($p<0.05$) total solids level than CD1 (9.7±0.20%).

In vermicomposting process, substrates that are poor in organic matter do not usually support large numbers of earthworms. Since cow dung has been used as a successful substrate for rearing earthworms, presence of organic matter in higher amount is mandatory. Range of 80-85% of organic matter has been reported to suit the better growth of earthworms [2, 7]. From the ANOVA tests performed, it is obtained that the organic matter (%) of CD2 (75.40±0.2) is significantly higher ($p<0.05$) than the CD3 (70.48±0.07) and CD1 (69.92±0.1) whereas the levels of ash content (%) are in contrast with the levels of organic matter (%) i.e. CD1 (30.08±0.11) had significantly high ($p<0.05$) ash (%) levels than CD2 (24.59±0.2) and CD3 (29.52±0.07). Among the analysed cowdung samples, CD2 is assessed to possess organic matter (%) level more suitable for rearing earthworms.

It has been proposed that a proper balance of C and N nutrient levels contributes to proper development of earthworms, plants and microorganisms, as well as their synergistic interactions. Cow manure has prominent economic importance as a fertilizer, feed supplement due to high carbon and nitrogen source. It is well established that the carbon and nitrogen are two primary nutrients required for any cell growth and serves as a major quality parameter for manures, substrates for earthworms etc. High carbon, nitrogen and C:N ratio are determined from the fresh cow dung samples. Total carbon (%) of CD2 (41.89±0.11) is slightly higher ($p<0.05$) than the other two samples, CD3 (39.16±0.04) and CD1 (38.84±0.06) whereas total nitrogen (%) of CD2 (2.65±0.01) is substantially higher ($p<0.05$) than CD3 (2.05±0.2) and CD1 (1.40±0.15). All the samples showed increased value of carbon and nitrogen. From that, the C:N ratio was determined. C:N ratio of CD1 (27.67±0.34) is significantly higher ($p<0.05$)

Table 2: Proximate characteristics of the fresh cow dung samples CD1, CD2 and CD3. Each value is the Mean±SD of three replicates. Significant P<0.05 via Duncan test. [abc] represents that the values are significantly different

Parameters	CD1	CD2	CD3
pH	7.5±0.05[b]	6.5±0.05[a]	6.6±0.05[a]
EC (dS/m)	1.91±0.01[a]	2.9±0.011[c]	2.7±0.011[b]
Ash (%)	30.08±0.11[c]	24.59±0.2[a]	29.52±0.07[b]
Crude protein (%)	8.9±0.09[a]	16.90±0.06[c]	13.07±0.17[b]
Total solids (%)	9.7±0.20[a]	19.26±1.1[b]	19±1[b]
Moisture (%)	90.21±0.2[b]	80.73±1.1[a]	81±1[a]
Organic matter (%)	69.92±0.1[a]	75.40±0.2[c]	70.48±0.07[b]
TC (%)	38.84±0.06[a]	41.89±0.11[c]	39.16±0.04[b]
Total N (mg/L)	1.40±0.15[a]	2.65±0.01[c]	2.05±0.2[b]
Sulfur (%)	1.26±0.005[c]	1.24±0.005[b]	1.17±0.005[a]
Hydrogen (%)	7.77±0.11[c]	7.64±0.005[a]	7.66±0.01[b]

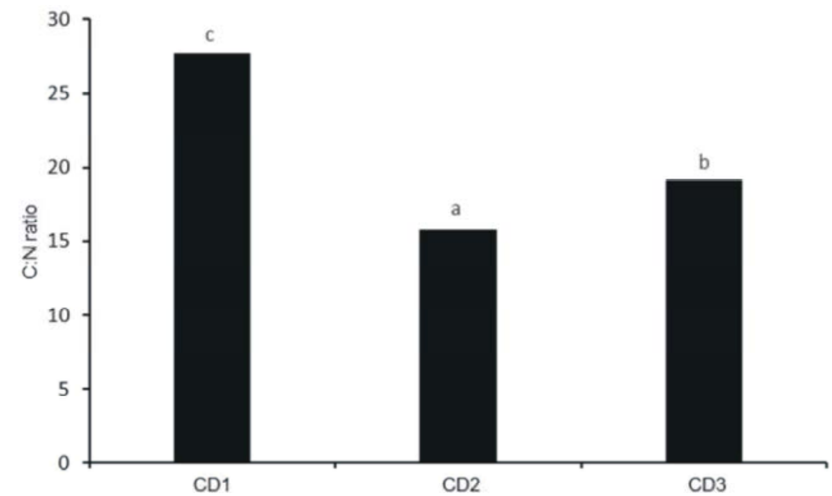

Fig. 1: Total colony forming units (CFU/g) of bacteria of the fresh cow dung samples CD1, CD2 and CD3. Values are means ± standard deviation of three replicates. Different letters indicate significant difference at P<0.05 by Duncan's test

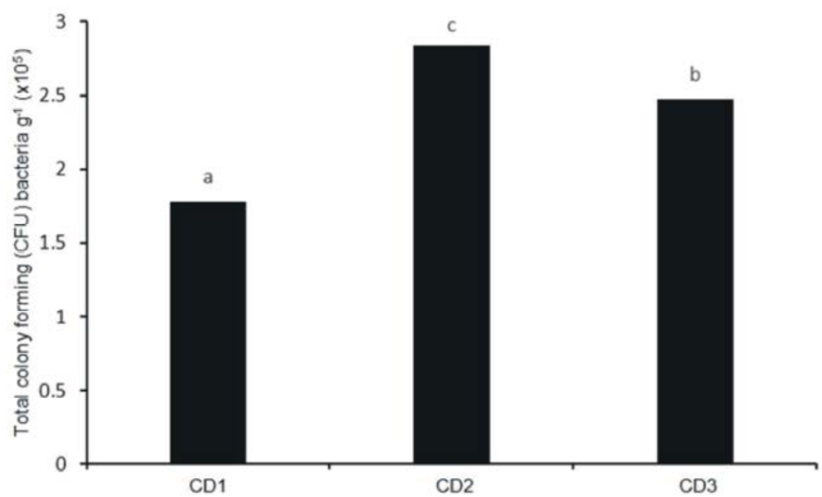

Fig. 2: Total colony forming units (CFU/g) of fungi of the fresh cow dung samples CD1, CD2 and CD3. Values are means ± standard deviation of three replicates. Different letters indicate significant difference at P<0.05 by Duncan's test

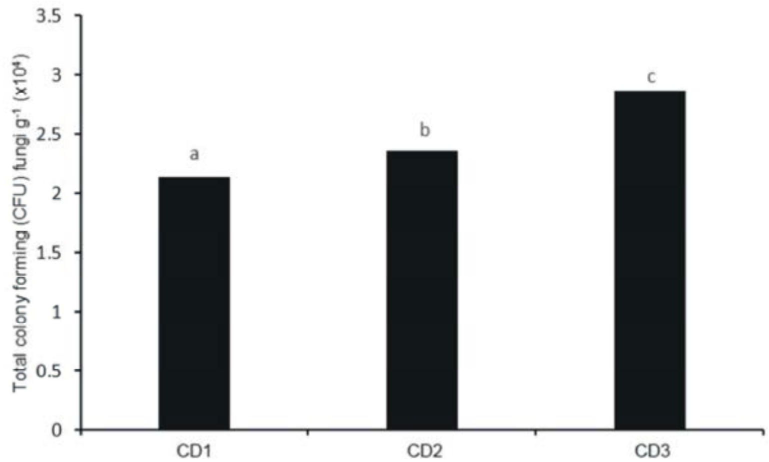

Fig. 3: C:N ratio of fresh cow dung samples CD1, CD2 and CD3. Values are means ± standard deviation of three replicates. Different letters indicate significant difference at P<0.05 by Duncan's test

than the CD3 (19.1±0.26) and eventually CD2 (15.80±0.09) (Fig. 1). Obtained values of C:N ratio of all samples are in accordance with Lazcano *et al.*, Otero et al and Ezekoye & Ezekoye [19, 31, 32], on their works on fresh cow manure. Crude protein is the most important dietary nutrient in the case of manure. Proper evaluation of crude protein reflects the quality of protein intake or of the feed. CD1 (16.90±0.06) had significantly higher ($P<0.05$) crude protein (%) than the CD3 (13.07±0.17) and CD1 (8.9±0.09). These results (carbon, nitrogen, C:N ratio and crude protein) serve as a critical point in differentiating the quality of cow dung samples. Increased carbon, nitrogen and crude protein levels as reported in CD2 indicates the availability of nutrient richness than the other two fresh cow dung samples, CD1 and CD3. Sulfur and hydrogen are the secondary macronutrients that determines the quality of soil, manure etc. [2]. Percentage of Sulfur (%) in CD1 (1.26±0.005) is higher than ($P<0.05$) CD2 (1.24±0.005) and CD3 (1.17±0.005). Similarly, the percentage of Hydrogen in CD1 (7.77±0.11) is slightly higher than CD3 (7.66±0.01) and CD2 (7.64±0.005). Presence of high Sulfur and Hydrogen levels also strongly indicates that these fresh cow dung samples could be attributed to be a better choice as a manure for plants and also a suitable substrate for earthworms in vermicomposting when pre-treatment is employed rather than direct application. Pre-treatment or aerobic composting process can reduce the nutrient levels by volatilization (ammonia is proved to toxic for earthworms) and microbial processes.

Total Viable Count of Bacteria and Fungi: Microbial loads were expressed as colony forming units (cfu/g) of the three cow dung samples. Total viable count is an important factor that determines the quality of any manure [32]. Figure 2 and 3 shows the bacterial and fungal colony forming units of CD1, CD2 and CD3 respectively. Through the results, it is clear that the subjected fresh cow dung samples are rich in microbial colonies. Total CFU/g of bacteria in CD2 ($2.84±0.01x10^5$cfu/g) is significantly higher ($p<0.05$) than CD3 ($2.47±0.01x10^5$ cfu/g) and CD1 ($1.78±0.05x10^5$cfu/g) whereas total CFU/g of fungi in CD3 ($2.78±0.01x10^4$ cfu/g) is substantially higher than CD2 ($2.36±0.04$ $x10^4$cfu/g) and CD1 ($2.14±0.01$ $x10^4$ cfu/g). As manure quality depends on microbial presence, these cow dung could serve as a potential fertilizer.

FTIR Interpretation: FTIR spectroscopy has been applied in waste characterization and process control in waste research for several years. The infrared spectra of the samples CD1, CD2 and CD3 shows similar areas of absorbance and the differences lying in the intensity of certain bands (Table 3a, 3b and 3c & Fig. 3,4 and 5). The interpretation of the spectra is based on various works [26, 27, 33-35]. The main absorbance bands are considered. There is a large band centered at 3421-3406cm^{-1} is due to hydrogen vibrations of the OH groups of alcohols, phenols or carboxylic acid. The peak at 2918-2850cm^{-1} is due to CH vibrations of aliphatic groups. The band centered between 2360 and 2339cm^{-1} is due to C=C of terminal alkynes. Band between 1735 and 1721cm^{-1} is due to C=0 of aldehydes, ketone, carboxylic acids and esters. The bands around 1655 and 1639cm^{-1} is characteristics of aromatic amides and ketones (C-C, C=O). The bands between 1561 to 1544cm^{-1} is caused by the absorbance of N-H Amides. The bands centered around 1462 to 1425cm^{-1} is due to COO^- aliphatic

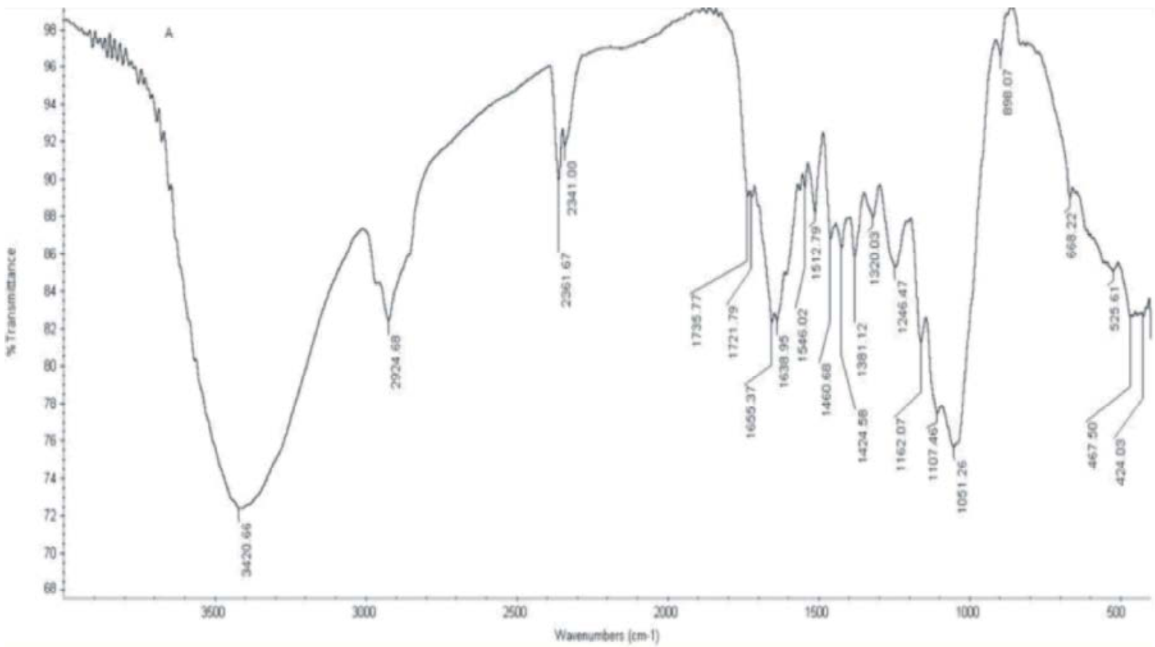

Fig. 4: FTIR spectra of CD1

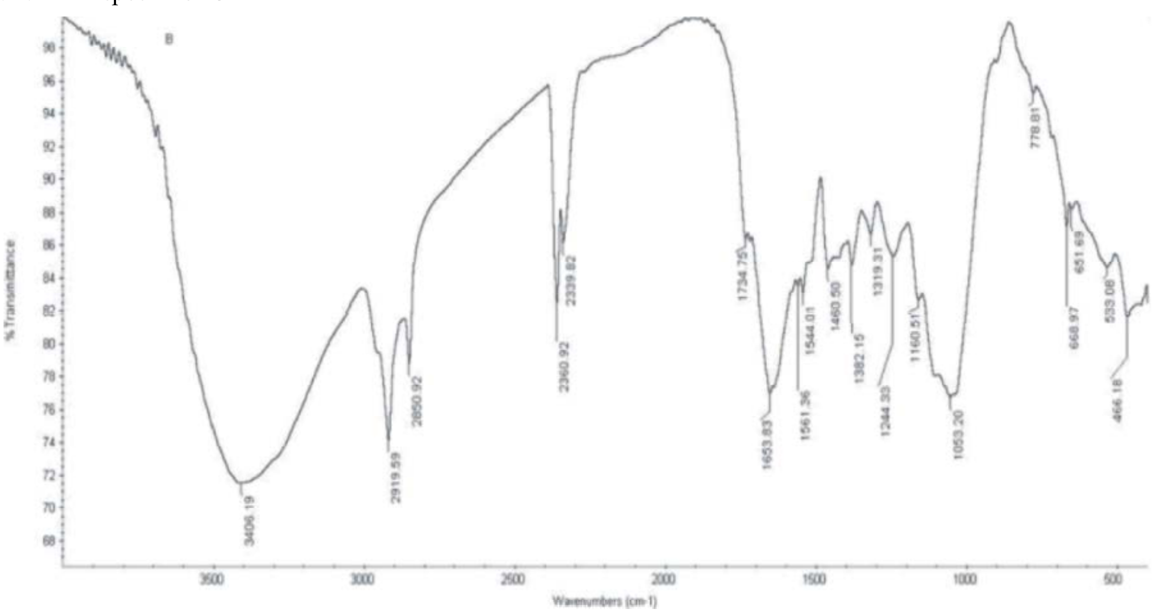

Fig. 5: FTIR spectra of CD2

groups, asymmetric, CH bending of CH_2 and CH_3 groups. The band at 1380-1382cm^{-1} is generally linked to the absorbance of N-O stretch of Nitrates, 1318-1320cm^{-1} is linked to Aromatic primary and secondary amines (C-N stretch), 1244- 1246cm^{-1} is due to carboxylic acids (C-O) and amides II (C-N), 1162-1053cm $^{-1}$ is due to polysaccharides (C-O-C & C-O) and <1000cm^{-1} is because of Aromatic ethers and polysachharides (C-H). The infrared spectra obtained from CD1, CD2 and CD3 had similar pattern with notable bands as exceptional. CD1 had peak at 1512cm^{-1} which is because of the absorbance by Lignin groups (Aromatic skeletal) and presence of S-O stretch at 1051 cm^{-1} is also a factor which sets a point of difference between CD1 and the others. These particular bands are absent in case of CD2 and CD3. This may be attributed to the nature of feed proportion as CD2 and CD3 shared grass as a major ingredient.

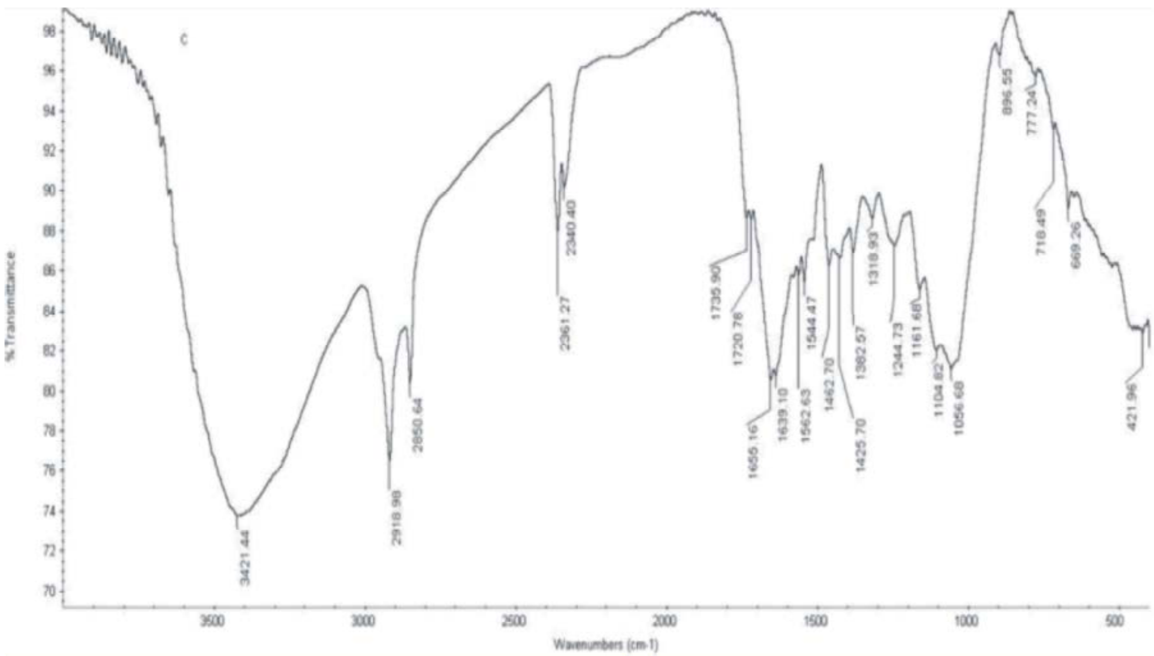

Fig. 6: FTIR spectra of CD3

Table 3a: Major absorption bands and assignments in FTIR spectra observed in CD1

Location Wavenumber (cm^{-1})	Vibration	Functional group or component
3420	O-H stretch	Bonded and non-bonded hydroxyl groups and water
2924	C-H stretch	Aliphatic methylene
2361-2341	C=C	Terminal alkynes
1735 - 1721	C=0	Aldehyde, ketone, carboxylic acids, esters
1655-1638	C=C, C=O	Aromatic, Amides and ketones
1546	N-H in plane	Amides II
1512	Aromatic skeletal	Lignin
1460-1424	C-H stretch	Aliphatic
1381	N-O stretch	Nitrate
1320	C-N stretch	Aromatic prim and sec. amines
1246	C-O, C-N	Carboxylic acids and Amide III
1162-1107	C-O-C,C-O	Polysaccharides
1051	S-O stretch	Inorganic sulfates in freeze-dried leachates
<1000	C-H	Aromatic ethers, Polysaccharides

Table 3b: Major absorption bands and assignments in FTIR spectra observed in CD2

Location Wavenumber (cm^{-1})	Vibration	Functional group or component
3406	O-H stretch	Bonded and non-bonded hydroxyl groups and water
2919-2850	C-H stretch	Aliphatic methylene
2360-2339	C=C	Terminal alkynes
1734	C=0	Aldehyde, ketone, carboxylic acids, esters
1653	C-O, C-C	Amide I, Carboxylates, Aromatic ring modes, alkenes
1561-1544	N-H in plane	Amides II
1460	C-H stretch	Aliphatic
1382	N-O stretch	Nitrate
1319	C-N stretch	Aromatic primary and secondary amines
1244	C-O	Carboxylic acids
1160-1053	C-O-C,C-O	Polysaccharides
<1000	C-H	Aromatic ethers, Polysaccharides

Table 3c: Major absorption bands and assignments in FTIR spectra observed in CD3

Location Wavenumber (cm^{-1})	Vibration	Functional group or component
3421	O-H stretch	Bonded and non-bonded hydroxyl groups and water
2918-2850	C-H stretch	Aliphatic methylene
2361-2340	C=C	Terminal alkynes
1735-1720	C=0	Aldehyde, ketone, carboxylic acids, esters
1655-1639	C=C, C=O	Aromatic Amides, ketone
1562-1544	N-H in plane	Amides II
1462	C-H stretch	Aliphates
1425	COO stretch	Asymmetric, CH bending of CH_2 and CH_3 groups
1382	N-O stretch	Nitrate
1318	C-N stretch	Aromatic primary and secondary amines
1244	C-Ostretch,O-H deformation C-O stretch, C-OH stretch	Carboxylic acids, Aryl ethers and phenols, Aliphatic OH
1161-1056	C-O-C,C-O	Polysaccharides
<1000	C-H	Aromatic ethers, Polysaccharides

CONCLUSIONS

Knowledge of the amount of manure and plant nutrients produced on a dairy farm is the first step in the proper operation of a manure handling and utilization system. Results obtained from this study proved that the cow dung samples CD1, CD2 and CD3 are suitable to serve as potential cow manure which can be applied to soil or composted using conventional methods for better agricultural practices. Significantly higher values notably crude protein, total carbon and bacterial load makes CD2 preferable than the rest to serve as potential fertilizer or as suitable feed for earthworms in vermicomposting. This may be due to the provision of food ration and additional ingredients as mentioned in Table 1. Whereas, CD2 and CD3 had recognizably closer in range in some parameters because of the similarity in major diet especially grass as feed. As an increase or decrease in nutrient values reflects on the dietary ingredients, it is recommended to add nutrient rich supplements to the cows along with grass as major diet to obtain better output i.e. cow dung rich in nutrients. Future studies may focus of quality of the ingredients to the cows and the analysis of nutrient changes that occur in cow's digestive system in order to furnish additional evidences.

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