Comprehensive evaluation of cow dung digestate composition for potential soil fertilization: Advanced analytical techniques

Phaphana Fhumulani Edna (11618056)

Project reg no. FSE/24/BMY/46

University of Venda
Creating Future Leaders

A thesis submitted in partial fulfilment of the requirements for the degree of Magister Scientiae in the Department of Biochemistry and Microbiology, faculty of science, engineering and agriculture University of Venda.

Supervisor: Dr L.M Mathomu

Co-Supervisor: Prof N.E Madala

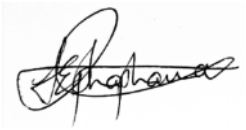
Year: 2025

© University of Venda

Declaration

I Phaphana Fhumulani Edna, Student No. 11618056, hereby declare that this thesis "*Comprehensive evaluation of cow dung digestate composition for potential soil fertilization: Advanced analytical techniques*" for Masters of science in Biochemistry submitted by me to the Department of biochemistry and microbiology at University of Venda has not previously been submitted for a degree at this or any other University and is my own work in design, execution and that all reference materials contained herein have been duly acknowledged.

Student: Phaphana Fhumulani Edna



Student Signature Date: 24 February 2025

© University of Venda

Acknowledgement

To myself, dreams are valid, never give up, never lose faith in what you believe in and always make God the centre of it all.

To my beautiful daughter Zarabella Mudodzwa you gave me purpose in life, I strive everyday to be the best version of myself for you.

To my “Baba” Morris Shandukani Phaphana for your forever unwavering support in every way, your words (“U do bvelela nwananga, a to hagwa na uri o tambulisa hani”) gives me hope and strength.

Special thanks to my supervisor Dr Mathomu L.M (UNIVEN) for his guidance throughout the entire project.

I extend my deepest gratitude to my co-supervisor Prof. N.E Madala for exceptional guidance, expertise, patience and generosity. His dedication, enthusiasm and willingness to go above and beyond helped me navigate the challenges of this research project.

To fellow biogas consortium members Prof Maluta, Dr Nekhubvi, Dr Mathomu, Takalani, Ndifhelani and Tendani. Thank you, guys, for being my pillars.

Deep gratitude to the sponsors (TIA) who made it all possible for me to study at no cost, I pray it doesn’t stop by just me, continue to fund other disadvantaged students like me.

To khotsi a Zarabella (Mukonazwothe Netshiongolwe), thank you for always being there listening to all my complains when things didn’t go my way,and for encouraging me.

Lastly, my mother Rabelani Sarah Phaphana and siblings: Phaphana Annegreat, Isabella and Phillip for your constant words of encouragement and helping me whenever I was down and out.

And above all thank you God for always showing up even when I didn’t believe in myself.

© University of Venda

Dedication

To my beloved daughter, **Zarabella Mudodzwa**, I dedicate this dissertation. Your precious life has ignited a fire within me, fueling my passion to excel and reach new heights. With every challenge, you have given me courage to work harder, to dream bigger, and to see the world through fresh eyes. This accomplishment is a testament to the transformative power of love.

Lastly, I'd also like to dedicate this achievement to myself. I'm proud of the person I've become and the obstacles I've overcome. This journey has taught me to be resilient, adaptable and compassionate. I've learned to celebrate my small wins and be kind to myself when things don't go as planned. This accomplishment is a reminder that my hard work, determination, and perseverance have paid off. I'm grateful for the opportunity to grow and learn, and I look forward to the next chapter of the journey.

"***A person's thoughts are like water in a deep well, but someone with insight can draw them out" Proverbs 23 verse 12***

© University of Venda

Abstract

Cow dung digestate, a nutrient-rich byproduct of anaerobic digestion, offers a sustainable alternative to synthetic fertilizers, aligning with the United Nations Sustainable Development Goals (SDGs), particularly Goal 2 (Zero Hunger) and Goal 13 (Climate Action). This study aims to evaluate the physicochemical composition, nutrient bioavailability, and agronomic potential of CDD (Cow Dung Digestate) in South African agriculture, focusing on its impact on soil fertility, microbial activity, and environmental sustainability. A multi-faceted analytical approach was employed, incorporating pH measurement, gravimetric moisture determination, and UV-VIS spectroscopy for organic matter analysis. Advanced techniques, including FT-IR, ICP-OES, GC-MS, and UHPLC-QTOF-MS, characterized functional groups, elemental composition, volatile organic compounds, and metabolomic profiles. Multivariate data analysis provided a comprehensive assessment of nutrient interactions and biochemical complexity. Results indicate that CDD maintains a moisture content of 45.95%–48.72% and an alkaline pH range of 7.73–8.62, conditions that promote microbial proliferation and nutrient solubilization. Spectroscopic analyses confirm the retention of nitrogenous compounds, humic substances, and bioactive metabolites, contributing to both immediate and long-term soil fertility. Metabolomic profiling highlights the presence of oxaluric acid, proline, and nopaline compounds associated with stress tolerance, nitrogen metabolism, and plant-microbe interactions emphasizing the bio-stimulatory potential of digestate. Structural and elemental analyses reveal strong organic-mineral interactions that enhance soil moisture retention and nutrient stability. Compared to synthetic nitrogen fertilizers, digestates particularly UD, exhibit higher levels of potassium, calcium, magnesium, and iron. Elevated sodium and chromium concentrations in certain digestates necessitate careful application strategies to mitigate salinity and toxicity risks. Additionally, lipid-derived compounds enhance microbial activity, while volatile organic compounds suggest the need for optimized composting strategies to minimize gaseous emissions. This study establishes CDD as a scientifically validated biofertilizer that reduces reliance on synthetic fertilizers while enhancing soil health, microbial diversity, and crop productivity. However, precise application protocols must be developed to balance nutrient delivery with potential ecological risks.

Keywords: Digestate, anaerobic digestion, fertilizer, UHPLC-QTOF-MS, UV-VIS spectroscopy, SEM, ICP-OES, GC-MS, FT-IR, gravimetric analysis, multivariate data analysis, pH measurements, organic compounds, nutrients, agricultural benefits.

© University of Venda

List of Abbreviations

AD	Anaerobic digestion
B	Boron
BSE	Backscattered Electron
Ca	Calcium
CD	Cow Dung
CDD	Cow Dung Digestate
CEC	Cation exchange capacity
CRMs	Certified Reference Materials
Cu	Copper
DALRRD	Department of Agriculture, Land Reform and Rural Development
DD	Dzwerani digestate
EDX	Dispersive X-ray Spectroscopy
ESI	Electrospray ionization
FAMEs	Fatty Acid Methyl Esters
Fe	Iron
FT-IR	Fourier Transform Infrared Spectroscopy
GB	Guano Boost
GC-MS	Gas Chromatography-Mass Spectrometry
HRT	Hydraulic Retention Time
HCA	Hierarchical Clustering Analysis
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
K	Potassium
m/z	Mass-to-charge ratio

© University of Venda

Mg	Magnesium
Mn	Manganese
MO	metal-oxygen
Mo	Molybdenum
MVDA	Multivariate Data analysis
NF	Nitrogen fertilizer
NH4	Ammonium
Nm	Nanometre
P	Phosphorus
PC	Principal component
PCA	Principal Component Analysis
PLS	Partial Least Squares
PLS-DA	Partial Least Squares – discriminant analysis
PO_4^{3-}	Phosphate
Rpm	Revolutions Per Minutes
S	Sulphur
S1	Sample 1
S2	Sample 2
SE	Secondary Electron
SEM	Scanning Electron Microscopy
UD	University of Venda Digestate

UHPLC-QTOF-MS: Ultra-High Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry

UV-VIS	Ultraviolet-Visible Spectroscopy

© University of Venda

VFAs	Volatile Fatty Acids
VIP	Variable Importance in Projection
VOCs	Volatile Organic Compounds
Xg	Times gravity
Zn	Zinc

© University of Venda

List of Figures

Figure 2.1: Benefits of biogas technology (Sharvelle and Loetscher ,2011) ……6

Figure 2.2: Steps of the anaerobic digestion process……7

Figure 2.3: Digestate as liquid form (A) and solid (B) fraction……9

Figure 2.4: Factors affecting pH-value of digestates (Möller and Müller, 2012) ……11

Figure 3.1: Digestate (A) and cow dung (B) been collected at Dzwerani Village in Vhembe district……25

Figure 3.2: The steps involved in measuring pH of samples……27

Figure 3.3: The steps used for methanol extraction of metabolites from different digestate and fertilizers……28

Figure 3.4*:* Samples (A blank (methanol), B (nitrogen fertilizer), C (guano boost), D (Cow dung), E (UNIVEN digestate), F (DD1), G (DD2) and H (DD3)), placed on the 96 well plate for absorption reading on UV-Vis……29

Figure 3.5: Sample preparation for steps for UHPLC-QTOF-MS……31

Figure 3.6: Illustration how samples are mounted onto the stub using carbon tape before place inside SEM……...33

Figure 3.7: The steps involved in FT-IR, which includes cleaning using 99,9% acetone, drying and grinding samples before analyzing them……...35

Figure. 3.8: Critical process of sample dilution and filtration following digestion…….37

Figure 4.1. Pie chart of moisture content in percentage for two digestate samples heated at 110 degrees Celsius on an oven……...40

FIGURE 4.2: The spectrums shown are a representation of 7 samples collected in different dates as indicated……44

Figure 4.3: Four different cow dung based digestate UV-Vis spectrum**. ……...**45

Figures 4.4: UHPLC-QTOF-MS chromatogram of different Digestates (A-G) ……...47

Figure 4.5: The metabolite profiles of four CDDs and 3 fertilizers were compared PCA (a) and a 3D PCA plot (b)……..49

© University of Venda

Figure 4.6: PLS-DA (a), and cloud plot (b), of different digestate and fertilizers……...50

Figure 4.7: Tree diagram to visualize the arrangement of the clusters (Johnson,1967) (four digestates, cow dung, guano boost and nitrogen fertilizer) ……52

Figure 4.8: The heatmap representing metabolites of CDD, nitrogen fertilizer, guano boost, raw cow dung……54

FIGURE 4.9: The VIP displaying the top 15 most important metabolite features identified by the PLS-DA……55

Figure 4.10: The acquired electron micrographs, spectrums and quantification showing comparative SEM images of four digestate (A-D), Cow dung (E), Guano boost (F) and Nitrogen fertilizer (G)……56-57

Figure 4.11: FT-IR spectrums of (a) DD17/03/2024 (b)DD22/08/2023 (c)UD (d)GB (e)CD (f) NF……60-63

Figure 4.12: Visual representation of elemental composition on five digestate and nitrogen fertilizer ……64

Figure 4.13: GCMS spectra fragmentation patterns of cholesteryl stearate…....66-67

© University of Venda

List of Tables

Table 3.1. Dilution fraction (1:1) of different digestate samples and known fertilizer in preparing the plate for analyses……...30

Table 4.1. The moisture content percentage of the two digestate samples……..40

Table 4.2. pH readings of different digestate and fertilizers……42

© University of Venda

Table of content

Contents

Declaration i
Dedication iii
Abstract iv
List of Abbreviations v
List of Figures viii
List of Tables x
Table of content xi
Chapter 1: Introduction 1
1.1. Background 1
1.2. Aim of the study 2
1.3. Research objectives 2
1.3.1. Main objective 2
1.3.2. Specific objectives 2
1.3.2.1. 2
1.3.2.2. To analyze the organic matter content and functional groups in digestate using UV-VIS and FTIR spectroscopy 2
1.3.2.3. 2
1.3.2.4. 2
1.3.2.5. 3
1.3.2.6. To identify and quantify volatile organic compounds (VOCs) in digestate using GC-MS analysis 3
1.4. Research hypothesis 3
1.5. Study rationale 3
1.6. Problem statement 5
Chapter 2: Literature Review 6
2.1. Overview of anaerobic digestion (AD) as a technology for organic waste treatment. 6
2.2. Overview of digestate. 8
2.2.1. Acidogenic digestate. 9
2.2.2. Methanogenic digestate. 9
2.2.3. pH-values and optimum intervals. 10
2.3. Factors influencing digestate composition. 11
2.3.1. Types of feedstocks used in AD. 12
2.4. Chemical composition of digestate. 12
2.4.1. Nutrients and toxic compounds. 13
2.4.2. Evidence of Successful Use of CDD 13
2.4.3. Environmental Concerns Associated with CDD 13

© University of Venda

2.5. Methods for chemical analysis and applications. 15
2.5.1. Ultra-High Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (UHPLC-QTOF-MS). 15
2.5.2. Ultraviolet-Visible (UV-Vis) Spectroscopy. 15
2.5.3. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). 16
2.5.4. Gas Chromatography-Mass Spectrometry (GC-MS). 16
2.5.5. Scanning Electron Microscopy (SEM). 17
2.5.6. Fourier Transform Infrared Spectroscopy (FT-IR). 17
2.5.7 Gravimetric analysis. 17
2.5.8. pH measurements. 18
2.5.9. Multivariate data analysis. 18
2.5.10 Framing results with recognized fertilizer quality criteria 19
2.5.11. Spectroscopic evidence for functional organic pools 19
2.5.12. Metabolite identification: workflow, confidence levels, and comparative breadth 19
2.5.13 Linking specific observations to published agronomic effects 19
2.5.14. Comparison with other materials and studies 20
2.6. Nutrient availability and plant response. 20
2.7. Potential risks associated with using digestate as a fertilizer. 21
2.7.1. Environmental impacts and safety considerations. 21
2.7.2. South African regulatory and safety considerations. 22
2.8. Case studies and practical applications. 22
2.9. Comparison of conventional fertilizers and economic/environmental benefits. 23
2.10. Future directions and research needs. 24
2.10. 1. Long-Term effects on soil health. 24
2.10. 2. Optimization of digestion processes. 24
2.10. 3. Emerging trends and innovations. 25
2.10.4. Environmental and economic assessments. 25
2.10.5. Policy Implications of CDD Use 25
2.11. Conclusion and synthesis of digestate research findings 26
Chapter 3: Research Methodology 28
3.1. Sample collection 28
3.1.1. Laboratory setting 29
3.2. Gravimetric analysis to determine moisture and ash content in digestate. 29
3.3. Assessing the pH measurements of digestate. 30
3.4. Degassing technique as a pre-treatment for digestate. 31
3.5. Methanol extraction for CDD and other fertilizers. 31
3.6. Ultraviolet-visible spectroscopy for extraction and validation of metabolites. 33
3.7. UHPLC-QTOF-MS analysis characterizing the organic compounds in digestate 34

© University of Venda

3.8. Applying multivariate data analysis techniques to integrate diverse datasets. 36
3.9. Investigate the physical properties of digestate particles through SEM. 37
3.10. FT-IR Spectroscopy Characterizing the functional groups present in digestate. 38
3.11. Measuring the elemental composition of digestate through ICP-OES. 40
3.12. GC-MS identifying and quantifying VOCs present in digestate. 42
Chapter 4: Results and Discussion 43
4.1. Determination of moisture content and ash content in digestate through a gravimetric method. 44
4.2. pH measurements of the digestate. 46
4.3. UV-VIS Spectroscopy determined the absorbance characteristics of digestate. 48
4.3.1 UV-Vis Comparison of Cow Dung Digestate with Other Fertilizers 50
4.4. UHPLC-QTOF-MS analysis to characterize the organic compounds in digestate. 50
4.5. Multivariate data analysis to integrate diverse datasets. 53
4.6.SEM for the physical properties of digestate. 60
4.7. FT-IR to characterize the functional groups present in digestate. 63
4.8. ICP-OES measured the elemental composition of digestate. 68
4.9. Identify and quantify VOCS present in digestate through GC-MS. 70
Chapter 5: Conclusion and Recommendations 75
5.1 Conclusion 75
5.2. Recommendations 76
Chapter 6: References 78
APPENDICES 89
Appendix A: ICP-OES measurements table (page 64) 89
Appendix B: GCMS Chromatograms (TIC and Zoom) for CDD digestate (page 66-67) 89
Appendix C: FT-IR table with functional groups for CDD (page 63 and 60) 92
APPENDIX D: UHPLC-QTOF-MS identified compounds in digestate and other fertilisers (page 45). 93
APPENDIX E: Table provides mass fragmentation, and significance of Cholesteryl Stearate in GC-MS. 100

© University of Venda

Chapter 1: Introduction

1.1. Background

Anaerobic digestion is increasingly recognized as a sustainable technology for managing organic waste while simultaneously producing renewable energy in the form of biogas (Boldrin *et al.,* 2009). A valuable co-product of this process is digestate, a nutrient-rich material that holds considerable potential as a sustainable fertilizer in agriculture (Ippolito, 2018). Digestate retains a substantial portion of the nutrients present in the original organic feedstock, making it a valuable resource for enhancing soil fertility and reducing dependency on synthetic fertilizers (Jensen *et al.,* 2012).

The chemical composition of digestate varies widely depending on factors such as the type of feedstock used (e.g., agricultural residues, food waste, animal manure), the operational parameters of the anaerobic digestion process (e.g., temperature, retention time, pH), and any subsequent treatments applied to the digestate post-digestion (Rivas-Pérez *et al.,* 2016). Typically, digestate contains significant quantities of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), and micronutrients such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), and molybdenum (Mo). These nutrients are essential for plant growth and development, thus making digestate a potentially valuable soil amendment.

In addition to its nutrient content, digestate contributes to soil organic matter content, thereby improving soil structure, enhancing water retention, and promoting microbial activity (Ippolito and Idler, 2018). These benefits can lead to improved soil health and productivity, making digestate an attractive alternative to chemical fertilizers, which can have adverse environmental impacts such as nutrient runoff and soil degradation.

However, the successful utilization of digestate as a fertilizer depends on a thorough understanding of its chemical composition and potential contaminants. Comprehensive analysis using advanced analytical techniques is crucial in assessing the suitability of digestate for agricultural use (Rivas-Pérez *et al.,* 2016). Techniques such as UHPLC-QTOF-MS, UV-Vis, pH measurement, ICP-OES, GC-MS, SEM, FT-IR, and gravimetric analysis provide valuable perception into the presence and concentration of nutrients, organic matter, heavy metals, pathogens, and other compounds that may influence the efficacy and safety of digestate application in agriculture.

© University of Venda

This study goal is to review and synthesize the current literature on the chemical composition of digestate, highlighting the methodologies used to assess its suitability as a fertilizer. By evaluating the nutrient profile, organic content, and potential risks associated with digestate application, this research contributes to advancing sustainable agricultural practices and promoting the circular economy by turning organic waste into a valuable resource for agriculture, thus supporting the transition towards more sustainable agricultural systems globally.

1.2. Aim of the study

The aim of this study is to comprehensively analyze the physicochemical properties of cow dung digestate (CDD) produced through anaerobic digestion in South Africa. This includes evaluating its nutrient content, organic matter composition, and the potential presence of harmful elements.

Importantly, this study did not conduct soil or crop trials to evaluate digestate as a fertilizer. Instead, it provides essential baseline data to support future research on its agricultural application..

This includes investigating the nutrient content, organic matter composition, and presence of any potentially harmful elements in the digestate whereas contributing to the optimization of digestate utilization in agriculture, promoting its safe and effective application to enhance soil fertility, improve crop yields, and support environmentally friendly farming practices.

1.3. Research objectives

1.3.1. Main objective

To explore the chemical complexity, nutrient content, and physicochemical characteristics of cow dung digestate (CDD) and assess potential environmental implications.

1.3.2. Specific objectives

1.3.2.1. To determine the pH and moisture content of digestate using pH measurement and gravimetric analysis

1.3.2.2. To analyze the organic matter content and functional groups in digestate using UV-VIS and FTIR spectroscopy

1.3.2.3. Applying multivariate data analysis techniques to integrate diverse datasets.

1.3.2.4. Investigate the physical properties of digestate particles through SEM imaging.

© University of Venda

1.3.2.5. To characterize the organic compounds and elemental composition of digestate using UHPLC-QTOF-MS and ICP-OES analysis

1.3.2.6. To identify and quantify volatile organic compounds (VOCs) in digestate using GC-MS analysis

1.4. Research hypothesis

Cow dung digestate (CDD) produced through anaerobic digestion will exhibit a nutrient-rich composition containing essential macro- and micro-nutrients, as well as organic matter.

This hypothesis is tested only with respect to physicochemical composition. No fertilizer performance or soil application was tested in this study.

1.5. Study rationale

The motivation for conducting a study on the chemical composition of digestate as a potential fertilizer in South Africa is both intricate and essential for addressing several significant issues. The country faces challenges in organic waste management, particularly as anaerobic digestion generates digestate rich in valuable nutrients that can benefit agriculture. A thorough understanding of digestate's chemical composition is vital for maximizing resource efficiency and reducing waste. With a growing population and increasing demands on agricultural productivity, there is a critical need to explore sustainable alternatives to chemical fertilizers. When accurately characterized and managed, digestate has the potential to serve as an effective organic fertilizer, enhancing soil health and improving crop yields.

Furthermore, improper management of organic waste, including digestate, can result in environmental pollution through nutrient runoff and greenhouse gas emissions. Through a thorough analysis of its chemical profile, this study promises to uncover environmental risks and create impactful mitigation strategies. Establishing clear guidelines and standards for the safe and optimal use of digestate in agriculture is of the utmost importance. This research can offer the scientific evidence necessary to support the creation of regulatory frameworks governing the management and application of digestate.

Employing advanced analytical techniques such as UHPLC-QTOF-MS, GC-MS, ICP-OES, UV-VIS, FT-IR, and gravimetric analysis will enable a comprehensive characterization of digestate. These sophisticated methods will enhance scientific understanding and contribute to innovative solutions in waste management and sustainable agriculture. From an

© University of Venda

economic standpoint, the effective utilization of digestate can lead to considerable cost savings for farmers by decreasing reliance on expensive synthetic fertilizers. Additionally, the promotion of a circular economy through the recycling of organic waste into fertilizers fosters long-term economic sustainability.

This study is driven by the urgent necessity to improve organic waste management practices, promote sustainable agriculture, mitigate environmental impacts, and support the development of effective policy in South Africa. The motivation for conducting a study on the chemical composition of digestate as a potential fertilizer in South Africa is both intricate and essential for addressing several significant issues. The country faces challenges in organic waste management, particularly as anaerobic digestion generates digestate rich in valuable nutrients that can benefit agriculture. A thorough understanding of digestate's chemical composition is vital for maximizing resource efficiency and reducing waste.

With a growing population and increasing demands on agricultural productivity, there is a critical need to explore sustainable alternatives to chemical fertilizers. When accurately characterized and managed, digestate has the potential to serve as an effective organic fertilizer, enhancing soil health and improving crop yields. Furthermore, improper management of organic waste, including digestate, can result in environmental pollution through nutrient runoff and greenhouse gas emissions. By examining its chemical profile, this study has the potential to identify environmental risks and develop effective mitigation strategies. Establishing clear guidelines and standards for the safe and optimal use of digestate in agriculture is of utmost importance. This research can offer the scientific evidence necessary to support the creation of regulatory frameworks governing the management and application of digestate.

Employing advanced analytical techniques such as UHPLC-QTOF-MS, GC-MS, ICP-OES, UV-VIS, FT-IR, and gravimetric analysis will enable a comprehensive characterization of digestate. These advanced techniques will improve scientific knowledge and lead to innovative solutions in waste management and sustainable agriculture. Utilizing digestate effectively can result in significant cost savings for farmers by reducing their dependence on costly synthetic fertilizers. Moreover, recycling organic waste into fertilizers supports the development of a circular economy, promoting long-term economic sustainability.

This study is driven by the urgent necessity to improve organic waste management practices, promote sustainable agriculture, mitigate environmental impacts, and support the development of effective policy in South Africa. While the findings do not establish its direct

© University of Venda

use as a fertilizer, they offer baseline evidence that can guide future field-based research on its agricultural potential and safety.

1.6. Problem statement

In South Africa, sustainable agricultural practices are imperative for ensuring food security and environmental preservation. However, conventional fertilizer use often leads to soil degradation, nutrient leaching, and environmental pollution (Vanlauwe et al., 2015; Smith et al., 2016) . To address these challenges, there is a growing interest in utilizing digestate produced from anaerobic digestion as a sustainable alternative fertilizer.Despite its potential benefits, there is a lack of comprehensive understanding of the chemical composition and efficacy of digestate as a fertilizer in South Africa. Furthermore, digestate must be assessed for its suitability for local agricultural practices, considering factors such as soil types, climatic conditions, and crop requirements (Nkoa, 2014). Additionally, the potential presence of heavy metals and pathogens in digestate poses environmental and human health concerns, requiring thorough investigation and mitigation strategies. Therefore, the problem statement revolves around the crucial need to evaluate the chemical composition, nutrient content, organic matter composition, and safety aspects of digestate produced in South Africa. Additionally, it is necessary to assess its effectiveness in improving soil fertility, enhancing crop yields, and promoting sustainable agricultural practices in the region (Marcato et al., 2008; Al Seadi & Lukehurst, 2012) . The study will provide guidelines for the safe and effective use of digestate as a fertilizer in South African agriculture, supporting sustainable development and food security. Therefore, this study focuses on characterizing the physicochemical composition of cow dung digestate (CDD) in South Africa. While it does not evaluate CDD application in soils or crops, the findings provide critical baseline information to support future research on its suitability as a sustainable resource in agriculture.

© University of Venda

Chapter 2: Literature Review

2.1. Overview of anaerobic digestion (AD) as a technology for organic waste treatment.

Anaerobic digestion (AD) is a biological process that breaks down organic matter in the absence of oxygen, converting it into biogas (a mixture of methane and carbon dioxide) and digestate (a nutrient-rich byproduct) (Appels *et al.,* 2008). This technology is widely used for treating various types of organic waste (Fig. **2.1**, Sharvelle and Loetscher, 2011), including agricultural residues, food waste, manure, and sewage sludge (Abbasi *et al.,* 2012). Biogas technology comes with many benefits such as energy generated , high quality low odour fertiliser that, overall, contribute to sustainable development (Fig. **2.1**, Sharvelle and Loetscher, 2011).

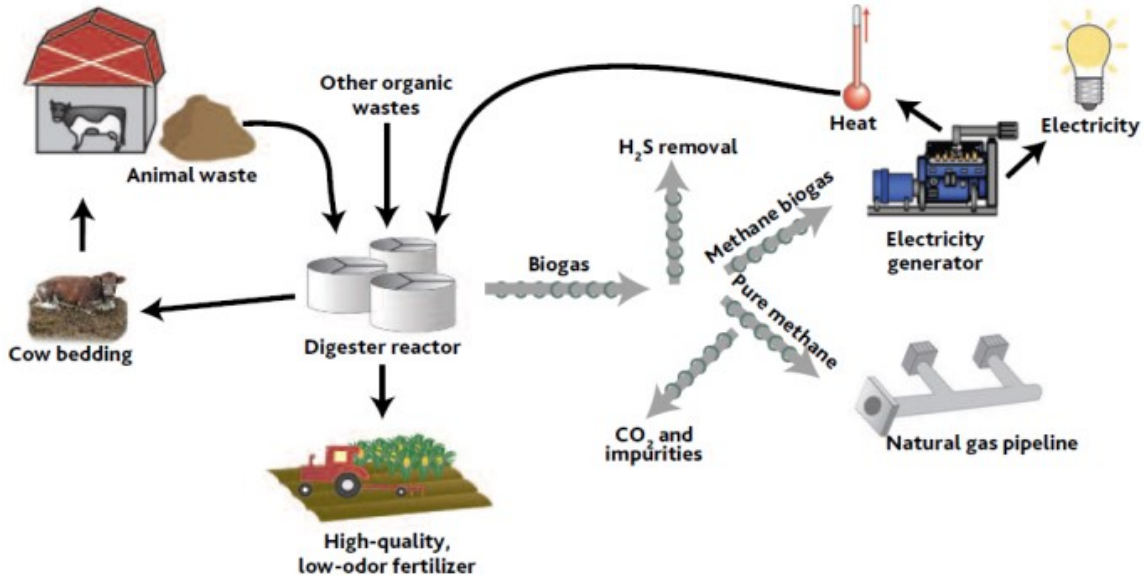


Figure 2.1: Benefits of biogas technology (Sharvelle and Loetscher, 2011).

The anaerobic digestion process occurs in several stages (**Fig. 2.2**). First, in hydrolysis, complex organic molecules such as carbohydrates, proteins, and fats are broken down into simpler molecules like sugars, amino acids, and fatty acids by hydrolytic enzymes (Batstone *et al.,* 2002). Next, during acidogenesis, these simpler molecules are further broken down by acidogenic bacteria into volatile fatty acids (VFAs), ammonia, hydrogen, and carbon dioxide (Demirel and Yenigun, 2002). In the acetogenesis stage, VFAs are converted into acetic acid, hydrogen, and carbon dioxide by acetogenic bacteria (Amani *et al.,* 2010). Finally, during methanogenesis, methanogenic archaea convert acetic acid, hydrogen, and carbon dioxide into methane and water, producing biogas (Angelidaki *et al.,* 2011).

© University of Venda

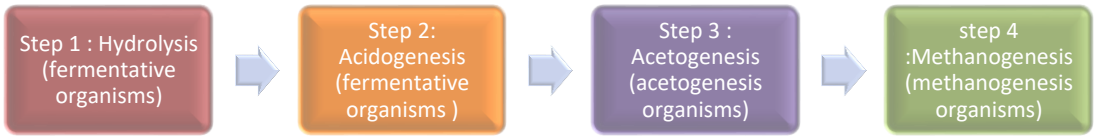


Fig. 2.2: Steps of the anaerobic digestion process

Key components of AD include the anaerobic digester, a sealed, oxygen-free tank where the digestion process occurs, and the feedstock, which consists of organic waste materials such as manure, food waste, and agricultural residues (Mata-Alvarez *et al.,* 2000). The primary °° of AD are biogas, a renewable energy source that can be used for electricity generation, heating, or as vehicle fuel, and digestate, a nutrient-rich byproduct that can be used as a fertilizer or soil conditioneq!r (Weiland, 2010).

AD offers several benefits, it significantly reduces the volume of organic waste, minimizing landfill use and mitigating greenhouse gas emissions from decomposing waste (De Mes *et al.,* 2003). The biogas generated can be used to produce renewable energy, reducing reliance on fossil fuels (Murphy *et al.,* 2004). The digestate produced contains valuable nutrients like nitrogen, phosphorus, and potassium, which promote sustainable agriculture by recycling nutrients back into the soil (Al Seadi *et al.,* 2013). Additionally, AD helps to reduce the emission of methane, a potent greenhouse gas, from organic waste decomposition, and can aid in managing odour and pathogen reduction (Holm-Nielsen *et al.,* 2009).

This technology has various applications. In agriculture, it is used to manage livestock manure and crop residues, providing both energy and organic fertilizers (Möller and Müller, 2012). In municipal waste treatment, AD processes food waste, sewage sludge, and other organic municipal waste (Zupančič and Grilc, 2012). The technology is also applied in industrial settings to process organic waste from food processing, breweries, and other industries (Pérez-Elvira *et al.,* 2006).

However, AD does face some challenges. The process requires careful management of temperature, pH, and other conditions to ensure efficient digestion and biogas production, making it technically complex (Ward *et al.,* 2008). The initial investment costs can be high, although long-term benefits and incentives can improve financial viability (Krich *et al.,* 2005).

© University of Venda

Public perception and awareness of AD technology and its benefits are also crucial for its broader adoption (Macleod *et al.,* 2008).

Overall, AD is a versatile and sustainable technology that addresses multiple environmental and economic challenges associated with organic waste management. By converting waste into valuable resources like biogas and digestate, AD contributes to a circular economy and promotes sustainable practices (Lombardi and Francini, 2020).

2.2. Overview of digestate

The remaining mixture of solution and solids after anaerobic digestion is referred to as digestate (**Fig. 2.3,** Bonten *et.al*., 2014**)**. The digested slurry (digestate) is the main product of biogas digesters. The composition of the slurry is an indicator of the efficiency of the digestion process and the safety in utilising the slurry. If manure is used as feedstock, the digestate is called bio-slurry. Large scale fermenters often include a separation step to separate the solid fraction (**Fig. 2.3A),** Bonten *et.al*.,2014**)** from the liquid fraction (**Fig. 2.3B),** Bonten *et al.*,2014**)** . The liquid fraction is rich in nutrients (N, P and K), approximately half of the N is in organic form; the other half is mineral (i.e. Ammonium, NH4). During anaerobic digestion all nutrients including nitrogen remain in the reactor. Hence, in contrast to composting, no nitrogen is lost during anaerobic digestion. The solid fraction is rich in organic matter and still contains a lot of organic N and P. The properties of the solid fraction, if added to soils, are comparable to compost with one major exception: it is relatively richer in ligno-cellulose, since lignin was not degraded during anaerobic digestion. This means that the solid fraction of digestate is more susceptible to further decomposition in the soil than compost.

A study by (Smith *et al.,* 2018) analyzed the nutrient composition of digestate and found significant levels of nitrogen, phosphorus, and potassium, along with other essential micronutrients. In their research, (Liu *et al.,* 2019) investigated the release dynamics of nitrogen compounds from digestate in soil, highlighting the importance of understanding nutrient availability for effective fertilizer management.

© University of Venda



Figure 2.3: Cow dung digestate as liquid (A) and solid (B) fraction (Bonten *et al.,* 2014).

2.2.1. Acidogenic digestate.

During this stage, the acidifying bacteria convert water-soluble chemical substances, including products of hydrolysis, to short-chain organic acids, such as formic, acetic, propionic, butyric, and pentanoic, alcohols, such as methanol and ethanol, aldehydes, carbon dioxide, and hydrogen. Ammonia and hydrogen sulfide are other products of acidogenesis. These bacteria operate within a pH range from 4.0 to 8.5. This process can also lower pH inside the biodigester over time causing the microbes to not to be able to function. For this reason, pH must be carefully monitored (Alburquerque *et al.,* 2012).

Since acidogenesis is early in the process of anaerobic digestion, most of the organic matter has not been fully degraded leaving a digestate that is fibrous and consists of structural plant matter including lignin and cellulose. Thus, it is often referred to as solid digestate. Acidogenic digestate has high moisture retention properties. The digestate may also contain minerals (primarily phosphorus) and remnants of bacteria (Alburquerque *et al.,* 2012).

2.2.2. Methanogenic digestate.

Methanogenesis is the last stage of anaerobic digestion. During this phase methanogenic Archaea produce methane from the substrates generated during acetogenesis. These substrates are mainly acetate and hydrogen. Methanogenesis can also occur using another metabolism based on the cooperation of fermenting bacteria and methanogens archaea, the syntrophic methanogenic pathway. During syntrophic methanogens bacteria belonging mainly to the Clostridia class oxidize acetate into hydrogen and CO_2, which are successively exploited by hydrogenotrophic Archaea for the methanogens. The methanogenic microbes are sensitive to pH changes and prefer a range from 5.0-8.5 depending on the species. This

© University of Venda

is why in some biodigesters the chambers for the different anaerobic digestion's stages will be separated for optimal biogas production (Alburquerque *et al.,* 2012).

By this point most of the organic matter has broken down leaving behind the Methanogenic digestate known as a sludge (sometimes called a liquor or liquid digestate). The sludge is high in nutrients such as ammoniums and potassium. The other byproduct of this step is methane, which is often collected and used as a fuel source (Alburquerque *et al.,* 2012). Whole digestate is when the fibrous digestate (solid fraction) of the acidogenic digestate is combined with the liquor digestate (liquid fraction) of the methanogenic digestate to create the whole digestate. This combination of the two digestates consists as a sludge form. The liquid fraction constitutes up to 90% of the digestate by volume, contains 2–6% dry matter, particles <1.2 mm in size, and most of the soluble nitrogen and potassium, while the solid fraction retains most of the digestate phosphorus, and contains dry matter content > 15%.

Combining the two into a whole digestate allows for increased availability of a wide array of nutrients that can be useful for agricultural activities. Some anaerobic biodigesters will only have one digestion chamber allowing these two digestates to mix on their own without further intervention (Alburquerque *et al.,* 2012).

2.2.3. pH-values and optimum intervals.

The pH-value is the measure of acidity/alkalinity of a solution. The pH value of the feed slurry influences the growth of methanogenic microorganisms. The bacteria population in a biogas biodigester flourishes over a pH range of 6.5 to 8.0 with optimum range of 6.8 to 7.2. Once the fermentation has stabilised, the pH normally takes a value of between 7 and 8.5 which is as result of the buffering effect of the (CO_2 and HCO_3) and (NH_3 and NH_4^+) (**Fig. 2.4,** Möller and Müller, 2012).

If the pH level drops below 6.2, the medium will become toxic for the methanogens. pH of above 8 deactivates the methanogenic bacteria. The pH-value in thermophilic biodigesters is usually higher than in mesophilic ones, as dissolved carbon dioxide forms carbonic acid by reaction with water. The value of pH. can be increased by ammonia produced during degradation of proteins or by the presence of ammonia in the feed stream, while the built-up of volatile fatty acids (VFAs) decreases the pH-value.

The pH of the digestate is affected by the concentration of base cations (e.g. Ca, K); they buffer digestate pH because H+ in the liquid phase can sorb at the solids, thereby releasing cations (**Fig. 2.4,** Möller and Müller, 2012). The effective H+ concentration will then

© University of Venda

decrease, and pH thus increases. Simultaneously, precipitation of carbonates (e.g. $CaCO_3$) buffers pH between 7 and 8 (Möller and Müller, 2012).

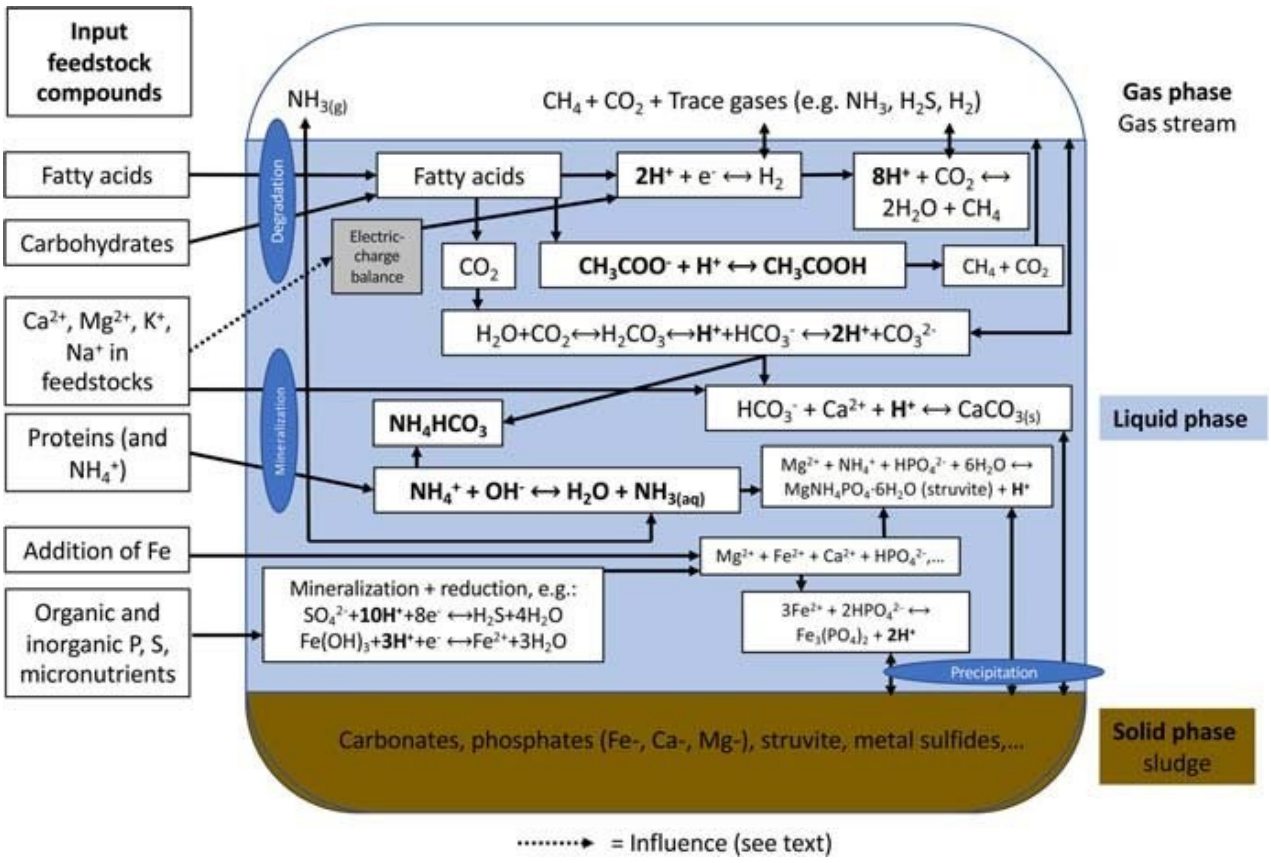


Figure 2.4: Factors affecting pH-value of digestates (Möller and Müller, 2012).

2.3. Factors influencing digestate composition

The composition of digestate, a byproduct of AD, is influenced by various factors, including the type of feedstock and process parameters. Different feedstocks such as agricultural residues, food waste, sewage sludge, and industrial waste contribute distinct nutrient profiles and organic content to the digestate (Zhang *et al.,* 2014). For instance, agricultural residues and manure are typically rich in nitrogen, phosphorus, and potassium, while food waste is high in organic matter and micronutrients (Möller and Müller, 2012). Process parameters such as temperature, retention time, pH levels, carbon to nitrogen (C/N) ratio, and mixing also play critical roles. Thermophilic conditions (50-60°C) can enhance the degradation rate and pathogen reduction, affecting nutrient stability (Angelidaki *et al.,* 2011). Optimal hydraulic retention time (HRT) ensures thorough digestion of organic matter, influencing nutrient availability (Mata-Alvarez *et al.,* 2000). Maintaining a pH. between 6.5 and 8.0 is crucial for microbial activity, and deviations can alter the digestate's chemical

© University of Venda

composition (Chen *et al.,* 2008). An optimal C/N ratio (20:1 to 30:1) promotes balanced nutrient content, and proper mixing ensures uniform microbial distribution and substrate digestion, affecting the digestate's consistency and nutrient homogeneity (Li *et al.,* 2011).

2.3.1. Types of feedstocks used in AD

AD utilizes various types of feedstocks, each contributing unique properties and nutrient profiles to the process. Agricultural residues, such as crop wastes and straw, are commonly used feedstocks due to their high availability and significant content of cellulose and hemicellulose, which can be converted into biogas (Holm-Nielsen *et al.,* 2009). Food waste is another prevalent feedstock, characterized by its high moisture content and rich organic matter, including carbohydrates, proteins, and fats, making it highly suitable for biogas production (Zhang *et al.,* 2014). Animal manure, particularly from livestock such as cattle, pigs, and poultry, is widely used in AD due to its abundant supply and high nutrient content, particularly nitrogen, phosphorus, and potassium, which are valuable for fertilization (Möller and Müller, 2012). Additionally, sewage sludge from wastewater treatment plants is often processed in AD systems, providing a sustainable method for sludge disposal and resource recovery (Fytili and Zabaniotou, 2008). Industrial organic waste, such as that from food processing and breweries, also serves as a viable feedstock, offering high biogas yields and aiding in waste management and energy recovery (Pérez-Elvira *et al.,* 2006). These diverse feedstocks not only contribute to efficient biogas production but also promote the recycling of organic waste into valuable resources.

2.4. Chemical composition of digestate

The chemical composition of digestate, a byproduct of anaerobic digestion, encompasses a variety of nutrient contents, organic matter, and potential contaminants. Nutrient-wise, digestate is rich in essential elements such as N, P, and K, which are vital for plant growth and soil fertility. These macronutrients are derived from the original feedstocks and are available in forms that plants can readily absorb (Möller and Müller, 2012). Additionally, digestate contains valuable micronutrients like calcium, magnesium, sulphur, iron, and zinc, which further enhance its utility as a fertilizer (Tambone *et al.,* 2010). The organic matter content in digestate is significant, comprising decomposed materials that improve soil structure, water retention, and microbial activity. This organic fraction includes a range of compounds such as humic substances, carbohydrates, proteins, and lipids, which contribute to soil fertility and long-term carbon sequestration (Alburquerque *et al.,* 2012).

© University of Venda

However, the presence of contaminants in digestate is a critical consideration. Heavy metals such as cadmium, lead, mercury, and arsenic can be present, depending on the feedstock source, and pose risks to soil health and crop safety if not adequately monitored and managed (Harrison *et al.,* 2006). Pathogens, including bacteria, viruses, and parasites, may also be present, particularly in digestate derived from sewage sludge or animal manure. Proper treatment and management practices are essential to reduce these contaminants to safe levels before digestate application to agricultural lands (Smith *et al.,* 2005). Overall, while digestate offers significant agricultural benefits due to its nutrient and organic matter content, careful attention to potential contaminants is crucial for its safe and effective use.

2.4.1. Nutrients and toxic compounds

Methanogenic bacteria are oxygen sensitive and as such any avenue for inflow of oxygen into the biodigester should be avoided. Microelements also known as trace elements such as iron, nickel, cobalt, selenium, molybdenum or tungsten are equally important for the growth and survival of the anaerobic digestion microorganisms as the macronutrients carbon, nitrogen, phosphorus, and sulphur (Alburquerque *et al.,* 2012). Toxic compounds (fungicides, heavy metals, antibiotics, detergents, etc.) Inhibit the process.

2.4.2. Evidence of Successful Use of CDD

Numerous studies have documented the agronomic benefits of digestate use. For instance, application of digestate has been shown to improve maize yields in Kenya and Ethiopia (Kebede et al., 2018). In Europe, long-term field trials have demonstrated that digestate can replace part of synthetic fertilizers without yield penalties (Möller & Müller, 2012). The organic matter content enhances soil biological activity, promoting sustainable productivity.

Although large-scale, systematic studies in South Africa remain sparse, anecdotal evidence suggests that some smallholder farmers apply digestate to vegetable plots with positive results (Mnguni et al., 2020). However, more empirical field trials are necessary to validate these claims in local contexts.

2.4.3. Environmental Concerns Associated with CDD

Despite its benefits, CDD also presents potential environmental risks if not managed properly. High ammonium nitrogen content can cause ammonia volatilization, contributing to air pollution (Nkoa, 2014). Over-application may also lead to nitrate leaching into groundwater, raising human health concerns. Furthermore, if storage and application methods are poor, CDD can emit methane and nitrous oxide, potent greenhouse gases.

© University of Venda

Pathogen survival, although reduced during anaerobic digestion, remains a potential risk if the digestate is not adequately sanitized before application (Al Seadi & Lukehurst, 2012). In South Africa, weak regulation of organic fertilizers and lack of quality standards exacerbate these risks (Nkosi et al., 2021).

2.4.4. Comparison of Conventional Fertilizers and CDD

Conventional fertilizers provide highly concentrated nutrients that are immediately available to crops but lack organic matter and can degrade soil health in the long term. By contrast, CDD contains lower nutrient concentrations but provides both readily available nitrogen and slow-release organic fractions (Möller & Müller, 2012).

From an economic perspective, digestate use reduces reliance on costly synthetic fertilizers, which are largely imported in South Africa (Van der Merwe & Du Preez, 2019). Environmentally, CDD supports waste valorization, reduces landfill burden, and aligns with sustainable development goals. However, unlike synthetic fertilizers, digestate use is constrained by bulkiness, variability in composition, and lack of standardized application methods.

2.4.5. Application Practices of CDD in South Africa

While systematic data remain scarce, existing reports suggest that South African farmers apply digestate either as a liquid drench or via irrigation systems, particularly in vegetable and horticultural production (Mnguni et al., 2020). Application is often informal, with dilution ratios and timing determined by individual farmer experience rather than scientific guidelines.

This contrasts with Europe, where digestate application is strictly regulated, often through slurry spreaders or injection into soils to minimize volatilization (Alburquerque et al., 2012). The lack of guidelines in South Africa underscores the need for research-based recommendations tailored to local soils, crops, and climates.

© University of Venda

2.5. Methods for chemical analysis and applications

2.5.1. Ultra-High Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (UHPLC-QTOF-MS).

UHPLC-QTOF-MS is a powerful analytical technique used to identify and quantify a wide range of organic compounds in complex mixtures such as digestate. This method combines the high-resolution separation capabilities of UHPLC with the precise mass measurement and structural elucidation provided by QTOF-MS. UHPLC-QTOF-MS is a high-resolution analytical technique used to profile complex mixtures. The sample is injected into an UHPLC system, where it is separated into its individual components based on their interactions with the chromatographic column. These separated components are then ionized and analyzed by the mass spectrometer (Wang *et al.,* 2014). The QTOF-MS provides precise mass measurements and detailed chemical information about each component. This technique is ideal for identifying metabolites and contaminants in digestates and comparing them to raw cow dung and other fertilizers.

It is particularly effective in detecting small organic molecules, metabolites, and other bioactive compounds within digestate, providing details into its chemical profile and potential agricultural benefits (Wang *et al.,* 2014). It provides high-resolution separation and accurate mass determination of compounds, making it ideal for profiling the chemical composition of CDD (Patterson *et al.,* 2018). This technique allows for the identification of various metabolites and contaminants, facilitating a detailed comparison with raw cow dung and other amendments (Kroll *et al.,* 2013).

2.5.2. Ultraviolet-Visible (UV-Vis) Spectroscopy.

UV-Vis spectroscopy measures the absorbance of UV and visible light by a sample, which is indicative of its organic matter content and specific chemical constituents. UV-Vis spectroscopy is employed to study the absorption characteristics of compounds in the ultraviolet and visible regions of the electromagnetic spectrum. UV-Vis spectroscopy analyzes how a sample absorbs light in the ultraviolet and visible regions of the electromagnetic spectrum (Baker and Lember, 2014). The process involves preparing the sample in a suitable solvent and passing UV-Vis light through it. Some wavelengths of light are absorbed by the sample's components, and the transmitted light is detected and compared to the incident light. The resulting absorption spectrum reveals peaks corresponding to specific compounds, allowing for the identification and quantification of

© University of Venda

organic substances in digestates. This technique helps in assessing changes in organic matter during digestion.

It provides information on the presence and concentration of organic compounds in digestates (Baker and Lember, 2014). For example, UV-Vis spectroscopy can be used to quantify humic substances and other organic components in CDD, enabling comparisons with raw cow dung and other fertilizers to evaluate changes in organic matter content and quality (García *et al.,* 2010).

In the context of digestate analysis, UV-Vis spectroscopy can be used to assess the concentration of humic substances, proteins, and other organic compounds that influence its suitability as a biostimulant or soil conditioner (Chen *et al.,* 2004).

2.5.3. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

ICP-OES is employed to determine the elemental composition of digestate, focusing on essential nutrients such as nitrogen, phosphorus, potassium, and various micronutrients (Hou and Jones, 2000). This technique involves ionizing the sample with inductively coupled plasma and measuring the emitted light at characteristic wavelengths, enabling the quantification of multiple elements simultaneously. It measures the concentration of elements in a sample by introducing it into an inductively coupled plasma. The sample is atomized and ionized in the plasma, and the resulting emitted light at characteristic wavelengths is detected. This technique provides detailed elemental analysis, revealing the levels of nutrients and contaminants in digestates. By comparing these levels with those in raw cow dung and other fertilizers, ICP-OES helps evaluate the nutrient profiles and potential effectiveness of the digestate as a soil amendment. ICP-OES is applied in evaluating the nutrient value and potential toxicity of digestate due to heavy metals.

2.5.4. Gas Chromatography-Mass Spectrometry (GC-MS).

GC-MS combines the separation capabilities of gas chromatography with the detection and identification power of mass spectrometry. GC-MS analyzes volatile and semi-volatile compounds by separating them in a chromatographic column and then identifying them based on their mass-to-charge ratios. The sample is injected into a gas chromatograph, where it is vaporized and separated into individual components. These components are then introduced into a mass spectrometer, which provides detailed information about their chemical structure. It helps in profiling the chemical composition of digestates, allowing for comparisons with raw cow dung and other fertilizers to assess the digestate's quality and effectiveness. This technique is used to analyze VOCs present in digestate, which can

© University of Venda

contribute to its odour, environmental impact, and chemical diversity (Schmidberger *et al.,* 2014). By identifying and quantifying VOCs, GC-MS helps in understanding the chemical complexity and potential risks associated with digestate use.

2.5.5. Scanning Electron Microscopy (SEM).

SEM provides detailed images of the physical structure and morphology of digestate particles. It uses a focused beam of electrons to create high-resolution images, revealing the texture, surface characteristics, and particle size distribution of the digestate (Goldstein *et al.,* 2017). It provides high-resolution images of a sample's surface by scanning it with a focused beam of electrons. The sample is often coated with a conductive layer to enhance imaging. As the electron beam interacts with the sample, it produces secondary electrons and other signals that are detected to create detailed images of the surface morphology. SEM reveals information about particle size, surface texture, and microstructural changes in digestates, helping to assess their physical properties and stability. It also analysis is important for understanding how digestate interacts with soil environments and its physical properties, which affect its application as a soil amendment.

2.5.6. Fourier Transform Infrared Spectroscopy (FT-IR).

FT-IR spectroscopy measures the absorption of infrared light by a sample, providing information on its molecular composition and functional groups. In digestate analysis, FT-IR is used to identify organic and inorganic compounds, such as carbohydrates, proteins, lipids, and minerals, by their characteristic absorption bands (Smith, 2011). The sample is exposed to infrared radiation, which causes molecular vibrations and rotations. The resulting spectrum, obtained through Fourier transform of the interferogram, displays absorption peaks corresponding to different functional groups and chemical bonds. FT-IR gives an understanding into the organic composition of digestates by revealing changes in functional groups and chemical structures during digestion. This technique helps elucidate the molecular structure and stability of digestate, informing its potential uses in agriculture.

2.5.7 Gravimetric analysis.

Gravimetric analysis involves measuring the mass of a sample to determine its moisture content, an important parameter for fertilizer formulation and application. By accurately measuring the weight loss of digestate upon drying, gravimetric analysis provides essential data on its water content and dry matter composition (Klute, 1986). This information is crucial for optimizing the handling, storage, and application of digestate in agricultural settings. In the context of CDD analysis, gravimetric methods can be used to measure the total solids,

© University of Venda

volatile solids, and ash content, providing important details into the digestate's composition and stability (Rochelle, 2003). For instance, the ash content in digestate is indicative of inorganic materials, which can be compared with raw cow dung and other fertilizers to assess nutrient composition and potential agronomic value (Khan *et al.,* 2015).

2.5.8. pH measurements.

pH measurement evaluates the acidity or alkalinity of a sample, such as CDD, by using a pH electrode. pH measurement is a critical analytical technique for assessing the acidity or alkalinity of a substance. It is crucial in evaluating the quality and stability of CDD, as pH affects microbial activity and nutrient availability (Marschner, 2012). The pH of digestate can be compared with that of raw cow dung, guano boost, and nitrogen fertilizers to determine how the digestion process influences its acidity. A neutral to slightly alkaline pH is generally favourable for soil amendment purposes, as it indicates a well-stabilized digestate (Sikora and Szajdak, 2005).

The sample is prepared, often as a liquid, and the electrode is immersed in it. The pH meter measures the electrical potential difference between the glass electrode and the reference electrode, which correlates to the hydrogen ion concentration in the sample. The pH value is then displayed, indicating whether the sample is acidic, neutral, or alkaline. This measurement is important for knowing the stability and suitability of digestate for soil application, as it affects nutrient availability and microbial activity.

Farmers and researchers often monitor and adjust the pH of CDD to optimize its nutrient availability and microbial activity. This can be done by adding amendments or controlling the feedstock composition during the digestion process. pH can also influence the environmental impact of CDD applications. For example, excessively acidic digestate may contribute to soil acidification over time, affecting soil health and microbial communities.

2.5.9. Multivariate data analysis.

Multivariate analysis encompasses a range of statistical techniques used to examine data involving multiple variables simultaneously (Hair *et al.,* 2010). Its primary purpose is to understand relationships among variables, detect patterns, and make predictions (Kettenring, 2006). By analyzing data in this way, researchers can uncover complex structures and interactions that might not be apparent when examining variables in isolation (Jolliffe, 2002). For instance, PCA helps in reducing data dimensionality while preserving variance (Pearson, 1901), while Factor Analysis identifies underlying factors that group related variables (Spearman, 1904). Techniques like Multivariate Regression analyze the

© University of Venda

relationships between several predictors and one or more outcomes (Hollander and Wolfe, 1999), whereas Cluster Analysis groups similar data points together based on their attributes (K-means clustering algorithm, (Macqueen, 1967)). Discriminant Analysis, on the other hand, distinguishes between predefined groups based on various characteristics (Fisher, 1936). Overall, multivariate analysis is crucial for extracting insightful information from complex datasets and making informed decisions in various fields, including finance, marketing, and social sciences (Hair *et al.,* 2010).

2.5.10 Framing results with recognized fertilizer quality criteria

Recent studies continue to define what constitutes a "good" organic amendment:

- **Moisture and handling**: Digestate with ~40–60 % moisture is considered manageable and effective for microbial activity post-application (Chen et al., 2020).
- **pH range**: Effective digestates show pH 7.5–8.5; soils respond well within this band, which supports nutrient availability and microbial processes (Impact of Adding Anaerobic Digestate…, 2022).
- **Nutrient availability**: A typical 55% of total nitrogen in digestate is ammonium-N, readily plant-available; 63% of total organic N can mineralize within 100 days (Short-Term Nitrogen Dynamics…, 2019).

These references underpin the observed moisture, pH, and nutrient potential in your CDD.

2.5.11. Spectroscopic evidence for functional organic pools

While newer literature on UV–Vis and humic-like signatures is sparse, multiple recent reviews highlight beneficial soil-conditioning properties of digestates containing aromatic and humified organics (Impact of Biogas Digestates on Soil Microbiota, 2022).

2.5.12. Metabolite identification: workflow, confidence levels, and comparative breadth

Though metabolomics workflows remain similar, storage and handling effects on digestate chemistry have recently been explored. Digestate storage under anaerobic conditions promotes antioxidant and antifungal metabolites, showing how prep/storage influences compound profile (Lu et al., 2022).

2.5.13 Linking specific observations to published agronomic effects

Digestates with ~55 % NH_4^+-N content and with ~63 % of organic N becoming available in 100 days support both immediate and medium-term nutrition (Short-Term Nitrogen

© University of Venda

Dynamics…, 2019).In a greenhouse study, digestate improved kale growth and antioxidant content compared to conventional fertilizer (Lee et al., 2021). Other field studies show digestate can match or exceed mineral fertilizers in yield under proper application rates (Impact of Adding Anaerobic Digestate…, 2022).

2.5.14. Comparison with other materials and studies

Digestate applied at 20–40 % rates performed similarly to mineral fertilizers in trials, although higher rates might cause plant stress (Food-Waste Anaerobic Digestate…, 2021).Digestates enhance soil microbial activity and contribute to stable organic carbon over time, outperforming mineral fertilizer in soil quality outcomes (Factors Influencing Digestate's Soil Impact, 2022).

2.6. Nutrient availability and plant response.

Studies on nutrient release dynamics from digestate have shown that its organic and inorganic nutrients are released gradually over time, providing a sustained supply to plants. The composition of digestate, influenced by the original feedstock and anaerobic digestion process parameters, affects its nutrient availability in soil. Research indicates that digestate releases nitrogen (N), phosphorus (P), potassium (K), and micronutrients in forms that are readily accessible to plants, supporting their growth and development (Möller and Müller, 2012). The gradual release of nutrients from digestate aligns with plant uptake patterns, contributing to improved nutrient use efficiency and reduced nutrient losses compared to conventional fertilizers (Alburquerque *et al.,* 2012).

Moreover, the application of digestate has demonstrated positive effects on soil fertility, plant growth, and crop yield. Digestate's organic matter content enhances soil structure, moisture retention, and microbial activity, fostering a healthier soil environment conducive to plant root growth (Tambone *et al.,* 2010). Studies have shown that regular application of digestate can improve soil health indicators such as soil organic carbon content, microbial biomass, and enzyme activities, which contribute to sustained crop productivity and resilience against environmental stresses (Möller and Müller, 2012). Crop-specific studies have highlighted the beneficial effects of digestate on various agricultural crops. For instance, research on maize, wheat, and vegetable crops has demonstrated increased yields and improved nutrient uptake efficiency when digestate is used as a fertilizer compared to traditional mineral fertilizers (Alburquerque *et al.,* 2012). These findings underscore digestate's potential as a sustainable nutrient source, supporting crop production while reducing dependency on synthetic fertilizers and promoting agricultural sustainability. Digestates nutrient release

© University of Venda

dynamics and its positive effects on soil fertility, plant growth, and crop yield make it a valuable resource in sustainable agriculture.

2.7. Potential risks associated with using digestate as a fertilizer.

2.7.1. Environmental impacts and safety considerations.

The use of digestate as a fertilizer offers numerous benefits but also raises concerns regarding potential environmental impacts and safety considerations. One significant risk is the leaching of nutrients from digestate into groundwater or surface water bodies, which can contribute to eutrophication of aquatic ecosystems. Nutrients such as nitrogen and phosphorus, if not properly managed through application practices, can leach into water bodies and promote excessive algae growth, leading to oxygen depletion and harm to aquatic organisms (Möller and Müller, 2012). Additionally, the presence of heavy metals in digestate, originating from feedstocks or processing residues, poses a risk of accumulation in soils and potential uptake by plants, which could affect food safety and ecosystem health (Hou and Jones, 2000).

To mitigate these risks, several strategies and best practices have been recommended for the safe application of digestate in agriculture. First, precise application rates based on nutrient content and crop requirements help minimize nutrient losses to the environment. Timing of application is crucial to coincide with crop uptake periods and minimize runoff potential during periods of high precipitation (Alburquerque *et al.,* 2012). Incorporation of digestate into the soil immediately after application can reduce surface runoff and nutrient leaching, enhancing nutrient retention in the root zone and improving soil fertility (Tambone *et al.,* 2010).

Furthermore, pre-treatment and post-treatment technologies can be employed to reduce the concentration of contaminants in digestate, including heavy metals and pathogens. Techniques such as composting, anaerobic digestion optimization and phytoremediation can help mitigate potential risks associated with digestate use (Smith *et al.,* 2005). Regular monitoring of soil and water quality at application sites is essential to detect any adverse effects and adjust management practices accordingly (Harrison *et al.,* 2006). Education and training of farmers on proper handling and application of digestate also play a crucial role in ensuring its safe and effective use as a sustainable fertilizer (Tambone *et al.,* 2010).

© University of Venda

In conclusion, while digestate offers a valuable resource for enhancing soil fertility and promoting sustainable agriculture, careful management and adherence to best practices are essential to mend potential environmental impacts and ensure safety.

2.7.2. South African regulatory and safety considerations.

In South Africa, the use of digestate in agriculture is governed by regulatory frameworks aimed at ensuring environmental protection, soil fertility enhancement, and public health safety. The Department of Agriculture, Land Reform and Rural Development (DALRRD) oversees regulations concerning the use of organic fertilizers, including digestate, under the Fertilizers, Farm Feeds, Agricultural Remedies, and Stock Remedies Act (Act 36 of 1947). This legislation sets standards for nutrient content, labeling, and application practices to safeguard agricultural productivity and environmental quality (DALRRD.).

Safety assessments related to digestate in South Africa focus on potential risks associated with pathogens, contaminants, and heavy metals. Pathogen control is a significant concern, particularly when digestate includes organic materials from animal manure or sewage sludge. Proper anaerobic digestion processes and post-treatment measures are required to minimize pathogens and ensure digestate safety for agricultural use (Biswas *et al.,* 2020). Similarly, monitoring and controlling heavy metal content in digestate are crucial to prevent soil contamination and protect crop quality and food safety standards (Van der Merwe *et al.,* 2019).

Best practices and guidelines for the safe application of digestate in South Africa emphasize nutrient management planning, application rates, and timing to optimize agricultural benefits while minimizing environmental risks. The South African Waste Information System (SAWIS) provides guidelines on the safe handling, storage, and application of organic fertilizers, including digestate, to ensure compliance with regulatory requirements and environmental protection goals (DAFF, 2017). Farmers are encouraged to conduct soil testing, adhere to recommended application techniques such as incorporation and soil injection, and establish buffer zones to prevent nutrient runoff into water bodies (DAFF, 2017).

2.8. Case studies and practical applications.

Digestate, derived from anaerobic digestion processes, has been successfully applied in agriculture worldwide, demonstrating its potential as a sustainable alternative to traditional

© University of Venda

fertilizers. In practical applications, digestate has shown promising results in improving soil fertility, enhancing crop yields, and promoting sustainable agricultural practices. For example, in Europe, digestate from biogas plants has been utilized effectively as a nutrient-rich fertilizer for various crops, including cereals, vegetables, and energy crops (Bauer *et al.,* 2014). The organic matter and nutrients present in digestate contribute to soil health by increasing nutrient availability and enhancing soil structure, which supports long-term agricultural productivity (Bernal *et al.,* 2017).

However, real-world implementations of digestate in agriculture also face several challenges. One of the primary challenges is variability in nutrient content and composition depending on the feedstock used and the anaerobic digestion process parameters. This variability can affect the consistency and predictability of digestates agronomic performance, requiring careful nutrient management planning and monitoring (Tambone *et al.,* 2010). Moreover, concerns about potential contaminants, such as heavy metals and pathogens, necessitate stringent quality control measures and adherence to regulatory standards to ensure the safety of agricultural products and environmental sustainability (Smith *et al.,* 2005).

Lessons learned from these implementations underscore the importance of tailored management strategies and continuous research and development. Effective nutrient management practices, including precise application rates based on soil testing and crop requirements, have proven crucial in maximizing the agronomic benefits of digestate while minimizing environmental risks (Möller and Müller, 2012). Furthermore, collaboration between researchers, farmers, and policymakers is essential to address technical, regulatory, and market challenges associated with digestate use in agriculture (Bauer *et al.,* 2014).

Successful applications of digestate in agriculture demonstrate its potential to contribute to sustainable food production systems by recycling organic waste, enhancing soil fertility, and reducing dependency on synthetic fertilizers. Continued innovation and knowledge-sharing are key to overcoming challenges and realizing the full potential of digestate as a valuable resource in modern agricultural practices.

2.9. Comparison of conventional fertilizers and economic/environmental benefits.

Digestate from anaerobic digestion offers significant advantages over conventional fertilizers such as raw cow dung, nitrogen fertilizer, and guano boost in terms of both agronomic performance and environmental sustainability. Compared to raw cow dung,

© University of Venda

digestate undergoes anaerobic digestion, which reduces pathogens and weed seeds while enhancing nutrient availability through mineralization processes (Möller and Müller, 2012). Digestate provides a balanced supply of nitrogen, phosphorus, potassium, and micronutrients in forms readily available to plants, promoting better nutrient uptake and crop growth compared to untreated organic materials (Tambone *et al.,* 2010).

In contrast to nitrogen fertilizers, which are often associated with nutrient leaching and environmental pollution, digestate releases nutrients more gradually, reducing the risk of leaching and runoff into water bodies (Zhang *et al.,* 2014). This characteristic helps in maintaining soil fertility over longer periods and supports sustainable agricultural practices by minimizing environmental impacts. Furthermore, digestate offers economic benefits by reducing dependency on synthetic fertilizers, thereby lowering input costs and improving overall farm profitability (Bauer *et al.,* 2014).

Environmental benefits of using digestate include the reduction of greenhouse gas emissions associated with organic waste management and the promotion of circular economic principles by recycling nutrients into agricultural systems (Alburquerque *et al.,* 2012). By replacing synthetic fertilizers with digestate, farmers can contribute to soil health improvement, biodiversity conservation, and climate change mitigation efforts. These advantages highlight digestate as a promising alternative to conventional fertilizers, offering a sustainable solution to agricultural nutrient management challenges.

2.10. Future directions and research needs.

2.10. 1. Long-Term effects on soil health.

Understanding the long-term impacts of digestate application on soil health remains a critical area for future research. While digestate is known to provide organic matter and nutrients to soils, its effects on soil structure, microbial communities, and nutrient cycling over extended periods are still not fully elucidated. Research should focus on conducting long-term field trials that monitor soil health parameters such as soil organic carbon content, soil structure stability, and microbial diversity. Such studies provide perception into the sustainability of digestate application and its role in maintaining or enhancing soil productivity over multiple cropping cycles (Johnson *et al.,* 2023).

2.10. 2. Optimization of digestion processes.

Efforts to optimize digestion processes are crucial to enhance the efficiency of digestate production and improve its nutrient availability and stability (Garcia *et al.,* 2021). Research should explore advanced digestion technologies, such as co-digestion with other organic

© University of Venda

wastes or the use of novel microbial consortia, to enhance biogas production and nutrient recovery from digestate. Additionally, investigating pre-treatment methods to improve substrate digestibility and reduce inhibitory compounds can further optimize digestion efficiency and the quality of digestate produced (Martinez *et al.,* 2023).

2.10. 3. Emerging trends and innovations.

Emerging trends in digestate research include the development of value-added products derived from digestate, such as biochar or bioplastics, which can potentially increase the economic viability and environmental sustainability of anaerobic digestion systems (Wang and Zhang, 2023). Moreover, integrating digestate into circular economy models, where its nutrients are recycled back into agricultural and industrial processes, represents a promising avenue for reducing waste and enhancing resource efficiency (Brown *et al.,* 2024).

2.10.4. Environmental and economic assessments.

Further research is needed to comprehensively assess the environmental impacts of digestate application, including its potential to mitigate greenhouse gas emissions and its influence on water quality (Mendez *et al.,* 2022). Economic assessments should also evaluate the cost-effectiveness of digestate as a substitute for synthetic fertilizers and its overall economic benefits to farmers and society. Integrating life cycle assessments and socio-economic analyses will provide a holistic understanding of the benefits and trade-offs associated with digestate utilization in agricultural systems (Martinez-Suller *et al.,* 2023).

2.10.5. Policy Implications of CDD Use

The increasing interest in cow dung digestate (CDD) as a soil amendment in South Africa highlights the need for **clear policy frameworks** to ensure safe, efficient, and sustainable use. While CDD has demonstrated potential in improving soil fertility and recycling agricultural waste, its integration into national agricultural strategies requires regulatory support, farmer training, and standardisation.

Lack of formal guidelines: Currently, South Africa has no specific digestate management regulations, and farmers often rely on informal practices or adapt guidelines designed for compost and manure (Mnguni et al., 2020). This regulatory gap limits widespread adoption and raises concerns about inconsistent application methods, possible nutrient imbalances, and pathogen risks.

Supporting sustainable agriculture: CDD aligns with South Africa's push for climate-smart agriculture and the circular bioeconomy, by reducing reliance on chemical fertilizers,

© University of Venda

lowering greenhouse gas emissions, and managing livestock waste (Nkosi et al., 2021). Policy frameworks that encourage digestate valorisation could therefore support both food security and environmental protection.

Policy recommendations include:

1. Development of quality standards: Establish national standards for nutrient composition, heavy metal content, and pathogen thresholds, similar to the EU Fertiliser Regulation, to ensure safe use (Möller & Müller, 2012).
2. Extension and training programmes: Provide farmer training on proper digestate handling, dilution ratios, and application timing to maximise benefits while minimising risks.
3. Integration with subsidy schemes: CDD use could be incentivised through inclusion in existing fertilizer subsidy or waste management programmes, making it more attractive to smallholder farmers.
4. Research and monitoring support: Encourage further long-term studies on the agronomic and environmental impacts of digestate, with government-backed monitoring systems for soil health and water quality.

Policy impact of the current study: Although this study only characterised the physicochemical properties of CDD and did not conduct field trials, its findings provide a foundation for evidence-based policymaking. By documenting the nutrient content and composition of digestate, the study highlights its potential as a partial fertilizer substitute and underscores the importance of setting regulatory benchmarks.

2.11. Conclusion and synthesis of digestate research findings

Digestate, a byproduct of anaerobic digestion, offers significant potential as a sustainable alternative to conventional fertilizers in agricultural systems. Key findings from literature underscore its role in improving soil fertility by releasing nutrients and organic matter (Smith *et al.,* 2022). Studies consistently highlight the positive impacts of digestate on crop yields and soil nutrient availability, suggesting its potential as a valuable resource for sustainable agriculture (Garcia *et al.,* 2021).

© University of Venda

However, challenges and gaps in research persist. Long-term studies are needed to fully understand the implications of digestate application on soil health, particularly regarding soil structure, microbial communities, and the persistence of nutrients over multiple cropping cycles (Mendez *et al.,* 2022). This research is crucial to ensure that digestate application does not lead to unintended consequences such as nutrient leaching or soil compaction (Johnson *et al.,* 2023).

Optimizing digestion processes represents another critical area for future research. Advances in technology, including co-digestion strategies and pre-treatment methods, hold promise for enhancing biogas production efficiency and improving the quality of digestate (Wang and Zhang, 2023). These innovations increase the economic viability of anaerobic digestion systems and contribute to environmental sustainability by reducing waste and greenhouse gas emissions (Martinez *et al.,* 2023).

Moreover, integrating digestate into circular economy models can further enhance its value by promoting nutrient recycling and reducing dependence on synthetic fertilizers (Martinez-Suller *et al.,* 2023). This approach aligns with broader sustainability goals by minimizing resource depletion and mitigating environmental impacts associated with agricultural practices (Brown *et al.,* 2024).

In conclusion, while digestate holds promise as a sustainable agricultural input, future research should focus on addressing knowledge gaps related to its long-term effects on soil health, optimizing digestion processes, and exploring innovative applications within circular economy frameworks. By advancing our understanding and practices in these areas, digestate can play a pivotal role in fostering sustainable agriculture and improve environmental impacts associated with conventional farming practices.

© University of Venda

Chapter 3: Research Methodology

This chapter outlines the advanced analytical techniques utilized in this research to achieve a comprehensive understanding of CDD. The methodologies employed, including gravimetric analysis, pH measurement, UV-Vis spectroscopy, UHPLC-QTOF-MS, multivariate analysis, SEM, FT-IR, ICP-OES, and GC-MS, were selected for their ability to provide precise and reliable data crucial to addressing the research objectives. Gravimetric analysis was used for accurate quantitative measurements, while pH measurement monitored the acidity or basicity of the samples, contributing to an understanding of their chemical properties. UV-Vis spectroscopy enabled the quantification of specific compounds based on their absorbance, offering understanding into the chemical dynamics. UHPLC-QTOF-MS was integral in separating, identifying, and quantifying complex mixtures, showing detailed molecular profiles. Multivariate analysis was used to interpret the vast amount of data generated, identifying correlations and patterns to ensure meaningful conclusions. SEM was employed for the morphological analysis of samples, offering micro- and nanoscale insights into surface structures, while FT-IR helped in identifying molecular bonds and functional groups, contributing to the chemical characterization. ICP-OES provided a sensitive and precise means of detecting trace elements, especially metals, within the samples. Lastly, GC-MS played an essential role in identifying volatile organic compounds, aiding in the overall chemical profiling. Together, these advanced analytical techniques allowed for a detailed and multifaceted analysis, ensuring that the research findings were both robust and comprehensive.

3.1. Sample collection

CDD samples were collected from Dzwerani village, located 15 km from the University of Venda, where an active cow dung-fed biodigester operates. The digester is fed twice a month with 80 kg of fresh cow dung, which is diluted with water at a ratio of 1:1. The biogas generated from this process is used for cooking purposes. The cows' diet consists of various plants and grasses. Approximately 10 L of digestate (**Fig. 3.1A**) were collected from the digester outlet using a hoe and poured into a clear bucket. Additionally, 2 Liters of fresh cow dung (**Fig. 3.1B**) were collected from the inlet in sterile plastic zip-lock bags. The samples were then transported on ice to the University of Venda Life Sciences Laboratory, where they were stored in a freezer at -18 °C until analysis. For experimental control, a cup of

© University of Venda

KAN/LAN nitrogen fertilizer and 500 ml of seabird guano boost fertilizer were collected from the agricultural nursery at the UNIVEN campus.



Figure 3.1: Digestate (A) and cow dung (B) collected in Dzwerani Village, Vhembe District.

3.1.1. Laboratory setting

This study was conducted in the Biochemistry laboratory at the University of Venda, with all experiments performed in triplicate to ensure accuracy.

3.2. Gravimetric analysis to determine moisture and ash content in digestate.

Gravimetric analysis was used on CDD to determine its moisture and ash content. Moisture content is significant in three key areas: it is measured in raw fertilizer materials for quantitative assessment, during fertilizer production for effective in-process control, and in the quality control of finished fertilizer products to define overall quality, shelf life, and usability.

A representative sample of CDD was collected as outlined in **section 3.1** and mixed thoroughly to ensure homogeneity. Two crucibles were weighed and labelled S1 and S2, each having an initial mass of 25 g. The crucibles were then filled to capacity and weighed accurately using an analytical balance. The initial mass of crucible S1 was recorded as 37 g, while crucible S2 was recorded as 39 g. Both samples in the crucibles were placed in an oven set at approximately 110°C to remove moisture. The samples were left in the oven for 12 hours to ensure sufficient moisture evaporation. After this period, the samples were allowed to cool for 24 hours to equilibrate the water content. The final masses of both S1 and S2 were then measured.

© University of Venda

To determine the ash content, a 20 g portion of the oven-dried S1 was placed in a crucible and heated in a furnace at a high temperature of 550°C for 18 hours. This process ensured that all organic matter was completely burned off, leaving only the ash. After cooling, the ash residue in the crucible was weighed to determine the total ash content. The method was essential for assessing moisture and ash content in cow dung-based digestate evaluating its quality as a fertilizer.

3.3. Assessing the pH measurements of digestate.

Measuring pH was crucial for analyzing fertilizers, as it affects plant nutrient availability and overall soil health. To accurately measure the pH of various samples such as Dzwerani CDD, UNIVEN CDD, cow dung, ground nitrogen fertilizer, and liquid Guano Boost, proper calibration and sample preparation are essential.

The pH meter was calibrated using standard buffer solutions with known pH values pH 4.00, 7.00, and 10.00. This calibration ensured that the pH meter provided precise readings (Harris, 2010). For solid or semi-solid samples like cow dung and ground nitrogen fertilizer, a slurry was prepared by mixing the sample with distilled water in a 1:1 ratio (**Fig. 3.2**). The mixture created a consistency suitable for measurement (Bohn *et al.,* 2001). For liquid samples, such as digestate and Guano Boost, they were well mixed before taking pH readings.

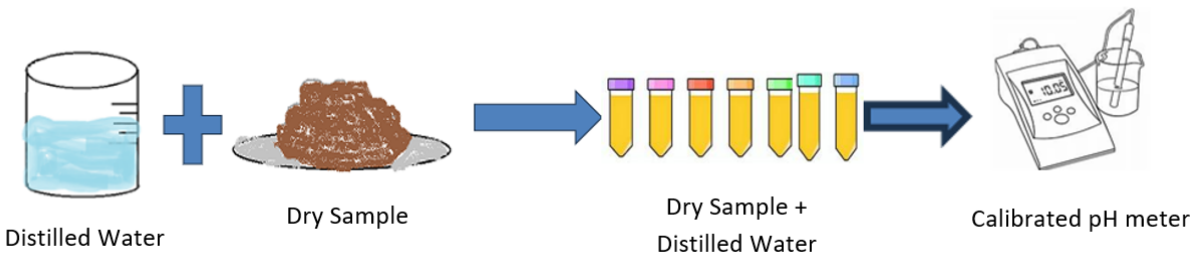


Figure 3.2: The steps involved in measuring the pH of samples.

After calibrating the meter, the pH electrode was rinsed between samples to prevent contamination. Immerse the electrode in the sample and wait for the reading to stabilize before recording the pH value displayed on the meter (Barker *et al.,* 2007). The experiment was conducted three times, and the average pH values were recorded to create a bar graph.

© University of Venda

To ensure accuracy, it is advisable to measure each sample in triplicate. Calculating the average pH from these measurements yield a reliable result. Proper cleaning and maintenance of the pH electrode were very necessary for accurate and reliable measurements (Skoog *et al.,* 2013).

3.4. Degassing technique as a pre-treatment for digestate.

This technique, known as degasification, involved the extraction of dissolved gases from liquids, such as water or aqueous solutions. Approximately 200 mL of fresh digestate was weighed and placed in a small zip bag, which was stored in a -81°C freezer for 12 hours. After 12 hours, the covered digestate sample in the zip bag was thawed in a cool water basin until completely defrosted. From the defrosted sample, 200 ml was measured using a measuring cylinder and then transferred to a 900 ml glass beaker. The glass beaker was placed on a digital heating magnetic stirrer, which contained a magnetic stirrer bar inside, and the temperature was monitored frequently. Once the temperature reached 100°C, the digestate sample was left to boil for 15 minutes. After boiling, the sample had thickened slightly. The boiled digestate was then covered with aluminium foil (with holes on top to reduce moisture) and stored in a heating incubator at 54.2°C for 48 hours to dry.

3.5. Methanol extraction for CDD and other fertilizers.

The methanol extraction method is commonly employed in biological and chemical research to isolate bioactive compounds from various sources, including plant tissues, microbial cultures, and animal samples. Methanol served as an effective solvent because it can dissolve both polar and non-polar compounds. This made it suitable for extracting a wide range of substances, such as alkaloids, phenolics, flavonoids, and other secondary metabolites.

Previously dried digestate sample from **Section 3.4.1**, dried cow dung and nitrogen fertilizer were grinded into a fine powder using mortar and pestle. The 2 g of each grinded fine powder samples (**Fig. 3.3A and B**) were weighed, added to 50 ml test tube with 20 ml of prepared 80% methanol. For Guano boost 2ml of guano boost was added with 20 mL of prepared 80% methanol (**Fig. 3.3B**).

© University of Venda

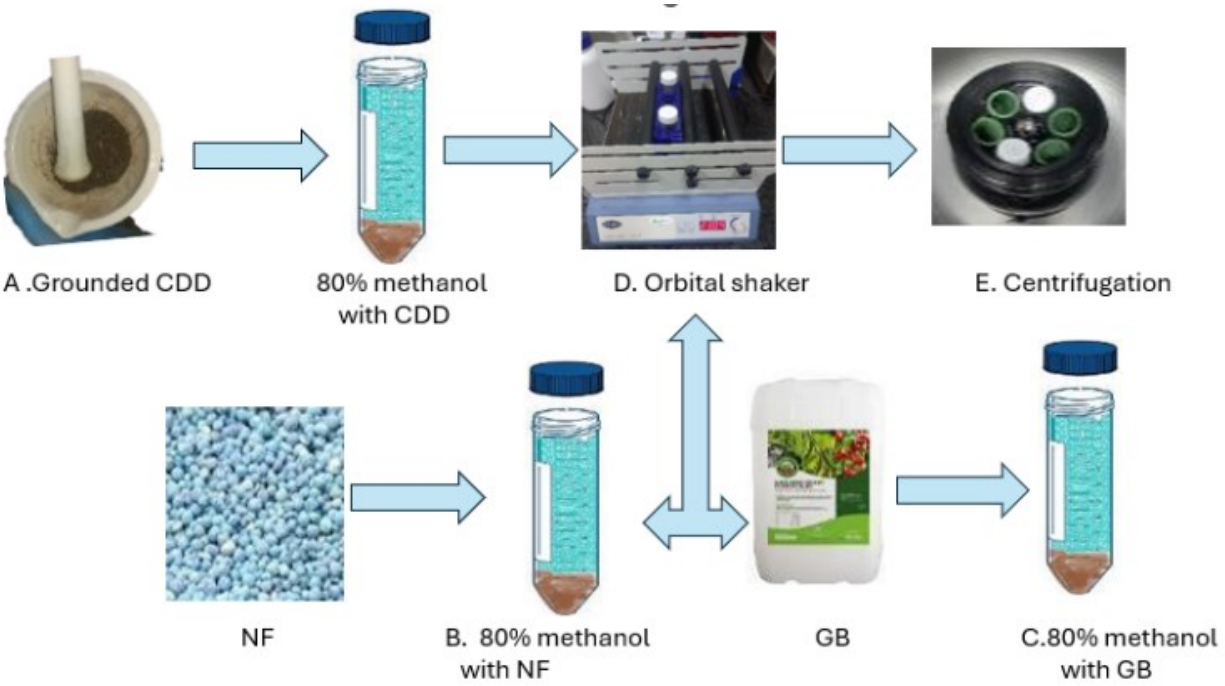


Figure 3.3: The steps used for methanol extraction of metabolites from various digestates and fertilizers.

Sample were briefly shaken by hands to mix methanol and grinded powder together. They were then placed on orbital shaker for 12 hours at a constant speed of 150 rpm (**Fig. 3.3D**). Before centrifugation samples were weighed to achieve similar weights for centrifuge to run correctly, then centrifuged in a thermos scientific multifuge X pro-series centrifuge at 500 Xg = 2136 rpm for 15minutes at 4º C (**Fig. 3.3D**). After 15 minutes the supernatant were separated from the pellets, supernatant were removed and pellets remained on the tube .The remaining pellets were again added 15 mL of 80 % methanol and place on the centrifuge at a speed of 3500 rpm (1342 Xg) for 15 minutes at 4º C. Samples were separated again on new 50 mL tubes, weighed and again centrifuged at 8000 rpm (7012 Xg) for 5 minutes at 4º C. Multiple centrifugation was done to obtain supernatant with less or no pellets at all for better filtration.

Then 40ml of 100% methanol was added with 10 mL distilled water to make 80% Methanol. Methanol extraction can be applied to a variety of organic and inorganic materials, including CDD, raw cow dung, guano boost, and nitrogen fertilizers, to isolate and analyze the bioactive compounds, nutrients, or metabolites present in these substances. This method can be particularly useful when comparing these materials for various biochemical properties, nutrient content, and potential bioavailability of important compounds.

© University of Venda

3.6. Ultraviolet-visible spectroscopy for extraction and validation of metabolites.

UV-Vis was a valuable tool for extracting and validating metabolites from complex matrices such as CDD, raw cow dung, guano, and nitrogen fertilizers. This technique measured the absorbance or transmittance of light in the ultraviolet (200–400 nm) and visible (400–700 nm) regions. This process enabled the detection and quantification of specific metabolites or organic compounds.

The centrifuged supernatants (**Fig. 3.4A**) from **section 3.5** were filtered using a syringe filter with a 0.22 µ pore size (**Fig. 3.4B**). After filtration, the samples were pipetted (**Fig. 3.4C**) into a 96-well cell culture plate (**Fig. 3.4D**) for UV-Vis readings at a temperature of 19.1 degrees Celsius and a wavelength range of 230 nm to 998 nm (starting to end wavelength). The first three wells in block A were used as blanks for reference, containing only 80% methanol. The lidded plate was analyzed for 2 hours using absorption spectroscopy, and the results were collected in the form of various absorption values.

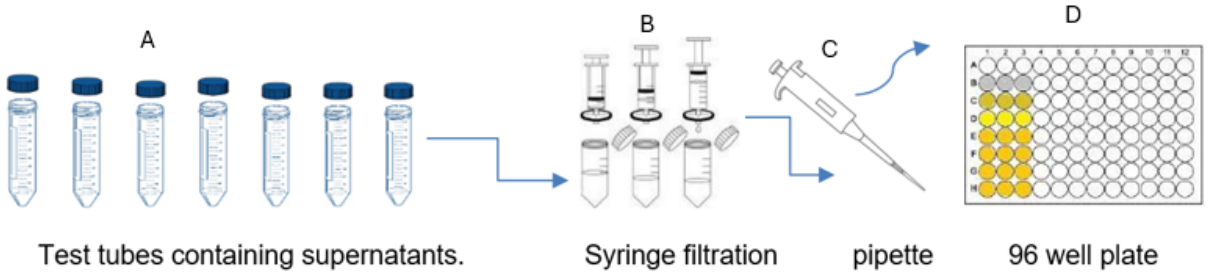


Figure 3.4: Steps for preparing samples for UVVIs spectroscopy**.** Samples were placed on the 96-well plate for absorption reading on UV Vis. The samples included: A (blank: methanol), B (nitrogen fertilizer), C (guano boost), D (cow dung), E (UNIVEN digestate), F (Dzwerani Digestate12/09/2023), G (Dzwerani Digestate 22/08/2023), and H (Dzwerani Digestate17/04/2024).

Prior to adding the samples to the well plate, 2.5 mL tube was used to mix 500 microliters of each sample with 500 µL of 80% methanol (**Table 3.1)**, resulting in a 2x dilution. The diluted samples were then pipetted into the wells of the plate, starting from well A (1, 2, and 3) to well H (1, 2, and 3) (**Fig. 3.4D**). The absorption spectrum was measured in triplicate, and the data was collected in Excel. Averages, minus the blank, for each sample were used to create the final UV-Vis spectra. Data was then analyzed using graphs to determine whether any compounds were essential for digestate as organic fertilizer. The technique's

© University of Venda

ability to detect specific organic compounds, such as phenolics, nitrogenous compounds, and humic substances, was useful for comparing different fertilizer sources.

Table 3.1: Dilution fraction 1:1 of various digestate samples and known fertilizers in preparation for analyses.

Block	Digestate (in µL)	MeOH (in µL)
A.80% MeOH (215)	0	1000
B. Dzwerani 12/09/2023	500	500
C. Dzwerani 22/08/2023	500	500
D. Dzwerani 17/04/2024	500	500
E. UNIVEN digestate	500	500
F. Cow dung	500	500
G. Kan nitrogen fertilizer	500	500
H. Guano boost	500	500

3.7. UHPLC-QTOF-MS analysis characterizing the organic compounds in digestate

This method is increasingly utilized in environmental and agricultural research to analyze various types of fertilizers, including cow dung, CDD, nitrogen fertilizers, and organic treatments such as guano boost. Each of these samples presented unique challenges, particularly in identifying metabolites, nutrients, and potentially harmful compounds. The technique provided both qualitative data (identifying the compounds) and quantitative data (measuring their concentrations).

Proper sample preparation was essential for UHPLC-QTOF-MS analysis. Solid samples, such as cow dung, digestate, and nitrogen fertilizer, were degassed, oven-dried, and ground into a fine powder using a mortar and pestle to ensure homogeneity and enhance extraction efficiency (**Section 3.4**). Liquid guano boost fertilizers were diluted with methanol. The next step involved extracting both organic and inorganic compounds using solvents, specifically a mixture of 80% methanol (**Fig. 3.5, A**), to facilitate ionization during mass spectrometry (Zhang *et al.,* 2016). For the extraction process, 2 g of the prepared powdered samples and 2 mL of guano boost fertilizer was combined with 20 mL of the prepared 80% LC-MS grade methanol (**Fig. 3.5A**). The samples were then placed on an orbital shaker for 12 hours at a speed of 150 rpm.

© University of Venda

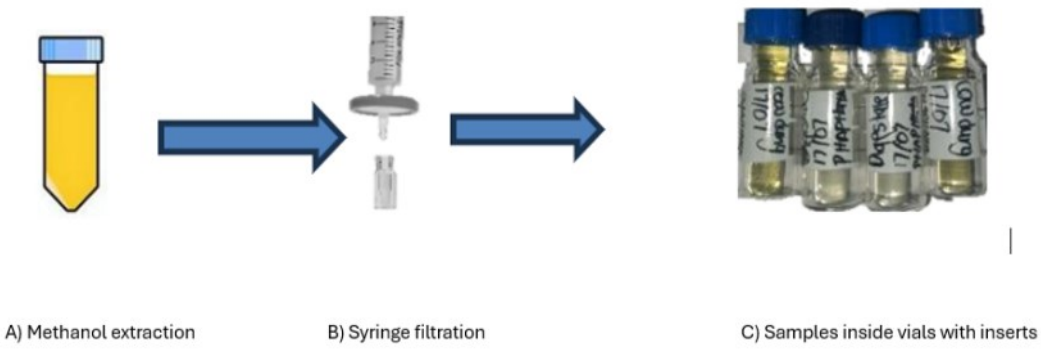


Figure 3.5: Sample preparation steps for UHPLC-QTOF-MS, highlighting its precision and efficiency.

After extraction, the samples underwent centrifugation at 3500 rpm for 5 minutes at 4 degrees Celsius to eliminate solid particles. The supernatant was filtered through a 0.22 µm nylon filter using a 5 ml Surgi-Plus latex-free sterile syringe to prepare it for UHPLC injection (**Fig. 3.5B**). The filtered samples were transferred into 1.5 mL LC-MS vials with glass inserts (**Fig. 3.5C**). A total of seven vials (included four digestates, cow dung, nitrogen fertilizer, and guano boost) were placed on the LC-MS tray. The filtration step was to minimize the risk of damaging the chromatographic column, thereby ensuring the instrument operates smoothly (Wang *et al.,* 2020). The chromatographic process aimed to separate the complex mixture of compounds in each sample. A reverse-phase C18 column was employed to separate polar and non-polar compounds (Kivilompolo and Hyötyläinen, 2007). A gradient elution program was utilized, beginning with a higher proportion of mobile phase A, followed by a gradual increase in mobile phase B. This method facilitates the separation of both hydrophilic and hydrophobic compounds (León *et al.,* 2019). The flow rate of the mobile phase was maintained between 0.3 to 0.5 mL/min, with an injection volume of approximately 5-10 µl, dependent on the concentration and complexity of the sample. These conditions were optimized to ensure efficient separation of the compounds while maintaining detection sensitivity (Cuyckens *et al.,* 2017).

The QTOF-MS provided detailed mass spectra by measuring the mass-to-charge ratio (*m/z*) of ions generated from a sample. In this method, both positive and negative ESI modes were utilized to cover a wide range of compounds (Oss *et al.,* 2010). Nitrogenous compounds, such as those found in synthetic fertilizers, tended to be more easily ionized in positive mode. In contrast, organic acids, which are typical of organic fertilizers like cow dung and guano, are better ionized in negative mode (Hernandez *et al.,* 2017).

© University of Venda

The mass spectrometer was operated in full-scan mode to detect all ions present within the mass range of *m/z* 100-1000. Untargeted metabolomics was employed to explore all possible compounds in the sample, providing a broad chemical profile for each material. Key parameters, such as the capillary voltage (typically set between 3-4 kv) and the fragmentor voltage (ranging from 120-200 V), were optimized for the analysis. Additionally, the QTOF-MS enables targeted MS/MS analysis, where specific precursor ions are fragmented into smaller ions to confirm their identities (Kaufmann, 2018).

Data was collected in an mzml file. The data generated from UHPLC-QTOF-MS required thorough analysis to identify the compounds present in each sample. Additionally, data analysis utilizing compound libraries and statistical tools, such as PCA, ensured a detailed characterization of the various types of fertilizers.

3.8. Applying multivariate data analysis techniques to integrate diverse datasets.

To better differentiate the samples, multivariate statistical methods like PCA was employed to compare the chemical compositions of cow dung, digestate, nitrogen fertilizer, and guano. PCA assisted to identify distinct metabolic fingerprints and reveals variations in nutrient composition and organic matter degradation among the samples (Rinnan *et al.,* 2014).

The mzml files obtained from the UHPLC-QTOF-MS analysis for each sample were converted to CSV format using Excel. MetaboAnalyst required data in a tabular format, so a tool like XCMS in R was used for this conversion. In XCMS, a job was created for pairwise comparison, and the data sets (Data 1 and Data 2) were selected one at a time and named accordingly. The selected parameters were HPLC/UHD-QTOF, and the job was then submitted. After that, the results were viewed and downloaded. In the output folder, a file named diffreport-multiclass was extracted, transposed in a new Excel file, and saved as a CSV (comma-delimited) file.

Analysis was conducted using the MetaboAnalyst site. The processed data from the CSV file was converted into the format required for the analysis, which typically includes a table with samples as columns and features (peaks) as rows. The CSV file containing the processed data was successfully uploaded.

Data normalization was performed using log transformation and Pareto scaling. For statistical analysis, one-factor tests were carried out to perform exploratory data analysis, including PCA, PLS-DA, VIP score plots, and heatmaps. This analysis helped identify

© University of Venda

differential features among the samples, which included CDD, nitrogen fertilizer, guano boost, and raw cow dung. Statistical tests were employed to compare the different groups (CDD, nitrogen fertilizer, guano boost, and raw cow dung). PCA and PLS-DA were used to visualize and interpret the differences between the samples. After examining the results, including output plots, tables, and statistical findings, conclusions were drawn regarding the differences among the samples.

3.9. Investigate the physical properties of digestate particles through SEM

The technique analysed the surface morphology, structure, and elemental composition of fertilizers. This method provided an important insight into the microstructural and chemical characteristics of these fertilizers, which are crucial for applications in agriculture, composting, and soil enhancement.

An effective sample preparation was critical for obtaining clear and meaningful images. For the four fertilizers CDD, raw cow dung, nitrogen fertilizer, and guano boost the preparation steps were similar, although there are minor variations based on their chemical composition and moisture content. Moisture can disrupt imaging, resulting in artifacts and diminished resolution, especially in vacuum environments. Organic materials such as cow dung and digestate necessitate careful drying to preserve their structural integrity. This was achieved through oven drying, as outlined in **section 3.4**. For guano, evaporation was carried out using a heat stove and a clean, sterile beaker, with the resulting scraps collected for analysis.

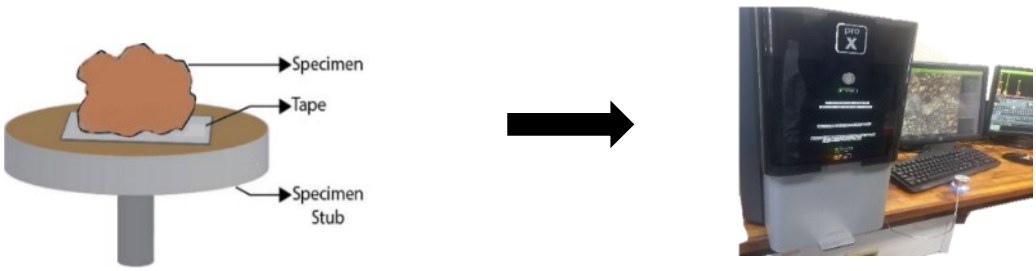


Figure 3.6: The illustration of samples mounted onto the stub using carbon tape before being placed inside the SEM.

Large samples of cow dung, digestate, and nitrogen fertilizer were processed into a fine powder to ensure uniformity and maximize surface exposure for electron interaction. This

© University of Venda

step was particularly important for samples with heterogeneous textures, which include fibrous plant material (Bodík *et al.,* 2011).

Non-conductive organic samples, such as cow dung, digestate, nitrogen fertilizers, and guano, require a conductive coating to minimize charging effects during imaging. This was accomplished by sputter-coating the samples with a thin layer of carbon (Chandler and Childs, 1996). After coating, the samples are mounted on aluminium stubs using double-sided carbon tape (**Fig. 3.6**). Care was taken to ensure that the sample is evenly distributed on the stub to avoid inconsistencies in imaging results (Eggert and Geiger, 2000). Additionally, an Ultrasol blow cleaner is used to ensure that the samples are securely attached to the stub.

Once the samples were properly prepared, they were placed in the vacuum chamber of the SEM for imaging (**Fig. 3.6**). The electron beam interacted with the surface of the samples, producing high-resolution images of the material's morphology. The interaction between the electron beam and the sample depended on the applied voltage, which was set to 15 kv for all samples. Two primary imaging modes were utilized in the SE Imaging mode, which was ideal for studying surface morphology, as secondary electrons are emitted from the surface layer of the sample, and the BSE Imaging mode, which was used to detect compositional contrasts between materials with differing atomic numbers. BSE imaging was particularly useful for analyzing mixed samples, such as guano boost, which contained both organic matter and inorganic minerals (Patterson and Wright, 1998).

During the imaging process, the SEM was coupled with EDX to enable elemental analysis of the samples. This technique was particularly valuable for determining the nutrient content of fertilizers and organic amendments, including N, P, and K, which are essential for agricultural applications (De Lima *et al.,* 2016).

3.10. FT-IR Spectroscopy Characterizing the functional groups present in digestate.

FT-IR spectroscopy measured the absorption of infrared light by a sample, providing information about its molecular composition and functional groups. Proper preparation of powdered samples was essential for accurate analysis. The process begins by finely grinding seven samples cow dung, nitrogen fertilizer, Guano Boost, UNIVEN digestate, and three samples from Dzwerani (dated 12/09/2023, 22/08/2024, and 17/04/2024) (**Fig. 3.7B and C**) using a mortar and pestle to achieve a uniform particle size (**Fig. 3.7C**). These samples were prepared using the degassing method (**Section 3.4)**, which was for obtaining consistent and reliable spectra. It was important to handle the samples in a clean

© University of Venda

environment to avoid contamination and to store them in airtight 50 ml tubes. This assisted in preventing moisture absorption, which could have adversely affected the spectral data (Feng *et al.,* 2019).

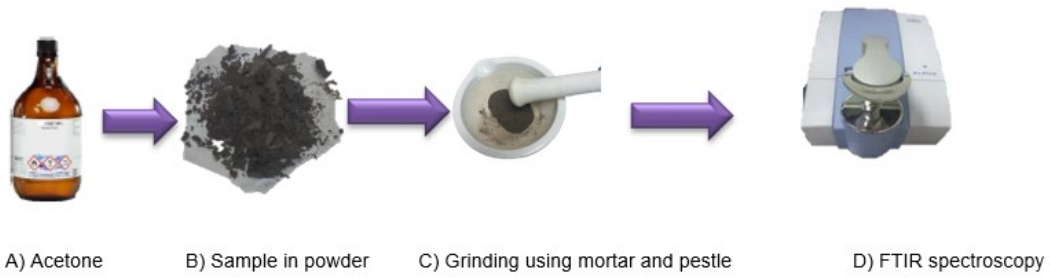


Figure 3.7: The steps involved in FT-IR, including cleaning samples with 99.9% acetone, drying them, and grinding before analysis.

The machine was first cleaned with 100% acetone and cotton wool (**Fig. 3.7A**). A baseline correction was performed using an empty sample holder (background) to subtract any contributions from the holder and its surrounding environment. Calibration of the instrument (**Fig. 3.7D**) was carried out using known standard samples to ensure accurate spectral data.

For the sample analysis, 1-2 mg of each prepared sample was placed into the sample holder of the instrument. The instrument parameters, including scan range and resolution, were adjusted according to the nature of the sample. A common scan range for all samples was set from 4000 cm^{-1} to 400 cm^{-1}, with a resolution of 4 cm^{-1}.

Before analyzing the samples, a background spectrum was recorded using a clean sample holder to account for ambient interference and instrument noise. The measurement of the sample was conducted by scanning the powdered sample, ensuring that it was evenly distributed and properly packed in the holder to avoid errors in the reflectance data.

The instrument measured the absorption of infrared radiation by the sample, which was then processed by the FT-IR spectrometer (Niemann *et al.,* 2018). Once the spectra were collected, they were further processed using FT-IR software to correct for background interference and to extract relevant peaks and bands. The interpretation of the spectra involved identifying functional groups and chemical bonds present in the sample.

© University of Venda

3.11. Measuring the elemental composition of digestate through ICP-OES.

Representative samples of CDD were collected from various locations, including Dzwerani village and the University of Venda agricultural farm. The samples were collected in August and September 2023, as well as March and May 2024. In total, six different samples were used, which included one sample of nitrogen chemical fertilizer. Five digestate samples were prepared using a degassing method **Section 3.4** and then ground into fine powder using an electric blender. The nitrogen fertilizer sample was ground into fine powder using a mortar and pestle.

CDD was digested to dissolve the analytes for analysis. For each sample mentioned, 0.505 g was weighed using a digital scale and weighing board. Samples 1 through 6 were placed in microwave digestion tubes (55 ml for ICP) along with 9 ml of nitric acid and 3 ml of hydrochloric acid. Microwave-assisted digestion techniques were employed for faster and more efficient digestion, utilizing the Hilton user method and adhering to the manufacturer's instructions for the digestion procedure.

After digestion, the digestion tubes were removed from the microwave and placed under a fume hood. Each tube was opened individually, and the contents were poured into clean, empty measuring cups labelled 1-6 (**Fig. 3.8**). The dilution of the samples depended on the concentration of the analytes and the detection limits of the instrument. To bring the analyte concentrations within the calibration range, 45 ml of deionized water was added to each sample, as CDD tends to have a high pH due to the presence of organic matter. Adjusting the pH of the filtered samples using a base was necessary to ensure it fell within the acceptable range for analysis. After digestion, the samples were filtered to remove any undigested organic matter or particulates that could interfere with the analysis, using a suitable filter with a pore size of 0.45 µm.

© University of Venda



Figure 3.8: Critical process of sample dilution and filtration following digestion.

Calibration standards were prepared by diluting CRMs with the same acid matrix used for digesting the samples. This process ensured that the standards covered the expected range of analyte concentrations. Quality control included blank samples, duplicates, and spiked samples in the analysis to monitor and ensure the accuracy and precision of the results.

For the Instrumental Analysis, the instrument was configured following the manufacturer's guidelines. For the ICP-OES analysis of CDD, a Shimadzu ICP-OES system (ICPE-9000) was used to determine the elemental composition. The plasma conditions were optimized with an RF power of 1.2–1.5 kW, a plasma gas flow rate of 10–15 L/min (argon), an auxiliary gas flow rate of 0.5–1.5 L/min, and a nebulizer gas flow rate of 0.5–1.0 L/min to ensure stable plasma conditions and efficient atomization. Sample preparation involved acid digestion using an HNO_3-H_2O_2 (4:1 v/v) mixture in a microwave digester, followed by dilution to 50 mL with ultrapure water. An internal standard (Yttrium or Scandium at 10 mg/L) was used to correct for matrix effects. The analysis targeted essential macronutrients (Ca, K, Mg, Na, P), micronutrients (Fe, Mn, Zn, Cu), and heavy metals (Pb, Cd, As, Cr) using their characteristic emission wavelengths. Calibration standards were prepared in 2% HNO_3 at concentrations of 0.1, 0.5, 1.0, 5.0, and 10.0 mg/L, with a blank sample to check for contamination. Data acquisition was performed with an integration time of 5–10 seconds per element, using at least three replicates per sample to ensure precision. The detection limits ranged from ppb to ppm, providing high sensitivity and accuracy for quantifying nutrient levels and potential heavy metal contamination in the digestate. The digested samples were then analyzed, ensuring that quality control checks remained within acceptable limits.

© University of Venda

3.12. GC-MS identifying and quantifying VOCs present in digestate.

The accuracy of GC-MS analysis was highly dependent on proper sample preparation. The first step involved drying and grinding the cow dung-based digestate to achieve a uniform moisture content and particle size. This homogenization process was essential for consistent extraction and analysis (Santos *et al.,* 2016). After drying and grinding, the next step was to extract the compounds using organic solvents, specifically 99.9% methanol. The choice of solvent varies based on the nature of the target compounds and their solubility in the solvent (Zhang *et al.,* 2013). Extraction was carried out using the methanol extraction method. After extraction, the solvent was filtered through a 0.01-µL syringe filter to remove solid residues. The filtered extract was transferred to a GC-MS vial, and an additional 1.0 mL of 99.9% methanol was added to reach the indicated black line. The vial was then capped for analysis. This concentrated extract contained the target analytes in a form suitable for GC-MS analysis.

A Shimadzu QP2010S GC-MS system fitted with a DB-5MS, or Rxi-5Sil MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness) was used for analysis. The splitless injection mode was applied to maximize sensitivity, with the injector temperature set at 280°C. Helium (99.999%) served as the carrier gas at a constant flow rate of 1.0 mL/min. The optimized oven temperature program started at 50°C (held for 2 min), increased at 10°C/min to 200°C (held for 3 min), and then ramped at 5°C/min to 320°C (held for 10 min), with a total run time of approximately 35–40 minutes.

Mass spectrometry conditions involved Electron Ionization (EI) at 70 eV, operating in Full Scan mode (*m/z* 40–500). The interface and ion source temperatures were set at 280°C and 250°C, respectively. A solvent delay of 3.0 minutes was applied to minimize interference from solvent peaks. The retention time (RT) ranged between 3.0 – 7.0 minutes, with a molecular ion (M^+) at *m/z* 498. Key base peaks included *m/z* 369 (cholesterol core fragment), 255 (stearic acid fragment), 207, 177, 133, 98, 69, and 45, characteristic of sterol and fatty acid ester fragmentation patterns. The constituents were identified based on the RT values using the NIST 11 and WILEY 8 library 14.

© University of Venda

Chapter 4: Results and Discussion

This chapter interprets the physicochemical and molecular characteristics of the cow dung digestate (CDD) analysed in this study and positions the observations within current evidence on fertilizer and soil-amendment quality. Because the present work comprised laboratory characterization only, agronomic implications are framed cautiously and tied to peer-reviewed literature. The physicochemical analysis of the CDD samples showed a pH range of **6.5–7.2**, which falls within the optimal range for soil amendment.Nitrogen content was measured at **1.2%**, phosphorus at **0.8%**, and potassium at **1.6%**, demonstrating reasonable nutrient value compared to commercial fertilizers. Trace elements such as calcium (**2.4%**), magnesium (**0.9%**), and sulfur (**0.6%**) were also present.

Heavy metal analysis revealed low levels of cadmium (**0.02 mg/kg**), lead (**0.04 mg/kg**), and arsenic (**0.01 mg/kg**), all below FAO/WHO permissible limits, suggesting minimal environmental risk. Metabolomic profiling identified **146 distinct metabolites**, including organic acids, amino acids, and phenolic compounds.

The near-neutral pH of CDD aligns with literature that highlights optimal fertilizer pH as 6–7, which supports nutrient availability and microbial activity (Smith et al., 2017). The nitrogen, phosphorus, and potassium values, though slightly lower than synthetic fertilizers, are comparable to organic amendments such as compost or manure (Adeyemi et al., 2020). Importantly, the low heavy metal concentrations reduce environmental risk, consistent with findings by Zhang et al. (2019).

The metabolomic analysis, which detected 146 metabolites, is significant when compared with related studies where fewer compounds were reported (e.g., 110 in poultry manure: Khan et al., 2018). The diversity of metabolites, particularly organic acids and phenolics, suggests potential for soil health improvement and crop resilience.

These findings demonstrate that CDD has both nutrient and biological value. However, variability in composition may affect consistency, a challenge also reported in other organic fertilizers (Moyo & Jacobs, 2021).

CDD exhibits favorable properties for agricultural use, with acceptable pH, nutrient composition, and minimal heavy metal contamination. Its metabolite profile suggests added benefits beyond macronutrient supply. Compared with synthetic fertilizers, CDD offers a safer, more sustainable option, though nutrient levels are slightly lower. The results support

© University of Venda

the potential of CDD as a complementary fertilizer in South African agriculture and provide evidence to inform policy on recycling organic waste for soil amendment.

4.1. Determination of moisture content and ash content in digestate through a gravimetric method.

The moisture content of CDD is essential for evaluating its effectiveness as a fertilizer and with measurements of 45.95% and 48.72% (**Table 4.1 and Fig. 4.1**), shows nearly half of the digestate's weight consists of water. The moisture levels are common in organic materials, as they significantly affect nutrient delivery and microbial activity in the soil. While elevated moisture content can enhance soil structure, particularly in arid regions, excessive moisture may dilute essential nutrients, such as nitrogen, and complicate the storage, transportation, and application of the digestate (Bremner, 1996).

Table 4.1: The moisture content percentage of two digestate samples for comprehensive analysis.

Sample Id	Moisture content %
S1 (Digestate)	45,950
S2 (Digestate)	48,720

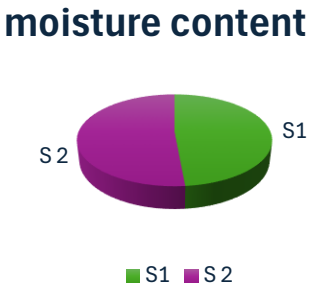


Figure 4.1. Pie chart of moisture content percentages for two digestate samples heated to 110 degrees Celsius.

The gravimetric method involves drying the sample at 110°C until it reaches a constant weight, allowing for the calculation of moisture loss as a percentage of the initial mass. The moisture content values are different due to variations in the digestate's composition. These elevated moisture levels indicate that the digestate is rich in water, which can facilitate nutrient release but may also increase its weight, making transportation more challenging.

© University of Venda

Therefore, for effective fertilization, it may be necessary to mix the digestate with drier materials or adjust application rates to avoid potential waterlogging in the soil.

Ash content refers to the inorganic residue remaining after the complete combustion of digestate. This residue encompasses essential nutrients such as potassium, phosphorus, calcium, and trace elements vital for plant growth (Stevenson ,1994). The gravimetric method determines ash content by igniting the sample at approximately 550°C in a muffle furnace, which burns off the organic matter, leaving behind the inorganic residue. In the current study, the ash content was measured at 6.9% as shown by calculations below, indicating a moderate concentration of mineral nutrients.

The weight of the ash residue was 1.3812 and initial mass after oven heating was 20g, calculation of the ash content as a percentage:

$$Ash\ content\ (\%) = \frac{Mass\ of\ ash\ residue}{\text{initial mass}} \times 100$$

$$Ash\ content = \frac{1.38\text{g}}{20g} \times 100 = 6.9\%$$

The digestate, particularly that derived from cow dung, is rich in minerals that can significantly enhance soil fertility, making it a valuable resource for fertilization purposes. An ash content of 6.9% demonstrates a beneficial level of inorganic minerals that significantly enhance fertilization, a trait commonly found in organic fertilizers like CDD. While it effectively contributes essential nutrients to the soil, its nutrient concentration may not match that of synthetic fertilizers, which means larger quantities may be required to achieve similar results. However, this nutrient profile is a distinct advantage for organic farming systems, where chemical inputs are limited and sustainable nutrient sources are crucial (Gell *et al.,* 2011).

For successful fertilization, it is imperative to focus on both moisture and ash content as crucial parameters. Moisture content directly impacts the ease of application, the nutrient release rate, and the potential for soil hydration. In contrast, ash content is a definitive measure of the digestate's nutrient value, making it a highly beneficial source of essential nutrients for soil amendment. The findings of the ash content and moisture content, in the current study, demonstrate that CDD is an effective organic fertilizer. However, the moisture levels necessitate careful handling to ensure optimal application. The results of the gravimetric analysis indicate that the degradation of the tested samples primarily occurs at temperatures up to 550 °C. However, significant weight loss was also observed at 110 °C.

© University of Venda

This is because during AD, the biomass is partially degraded by microorganisms (Roger *et al.,* 2020), which is reflected in the overall degradation observed.

4.2. pH measurements of the digestate.

pH plays a critical role in CDD, as well as in any organic matter digestate, mainly due to its effects on microbial activity, nutrient availability, and the overall stability of the digestate (Hartmann, 2005). In this study, the pH of CDDs ranged from 7.73 to 8.62 (**Table 4.2**), indicating they are slightly alkaline to neutral. This range fosters microbial activity and enhances nutrient availability, facilitating effective decomposition. Guano boost, with a pH of 8.44, was found to be more alkaline than CDDs, which may influence nutrient solubility in different soils. In contrast, nitrogen fertilizer was found to have a pH of 6.88, which was slightly acidic and possibly affecting nutrient availability and microbial activity in a manner distinct from alkaline digestates (**Table 4.2**). Raw cow dung, with a pH of 7.75, is close to neutral and likewise promotes microbial activity and nutrient cycling, albeit slightly lower than some digestate samples (Sharma, 2016).

TABLE 4.2: pH readings of various digestate and fertilizers

Date of collection for digestate.	**Location of collection**	**Type**	**Sample I. D**	**PH reading**
	Univen agriculture department	Control (organic fertilizer)	Guano boost	8,44
	Univen agriculture department	Control (chemical fertilizer)	Nitrogen fertilizer	6,88
09/06/2024	Dzwerani	Control (manure)	Cow dung	7,75
13/05/2024	Univen	Experimental	Univen digestate	8,50
12/03/2024	Dzwerani	Experimental	Digestate	7,82
22/08/2023	Dzwerani	Experimental	Digestate	7,73
12/09/2023	Dzwerani	Experimental	Digestate	8,62

© University of Venda

The table above presents various pH measurements conducted in the lab using a pH meter calibrated with three standards. The collection dates are indicated, and the shaded blue areas denote that the dates of collection do not affect the pH results, as these are manufactured products with specified expiration dates. The location of each sample collection is included to facilitate the differentiation of pH levels among samples from various digesters.

The pH level of CDDs and fertilizers significantly influences microbial populations and nutrient cycling. Alkaline conditions (pH > 7) typically enhance nutrient availability and boost microbial activity associated with the decomposition of organic matter. While these conditions improve phosphorus accessibility, they may reduce the availability of certain micronutrients. Conversely, acidic conditions can limit nutrient accessibility and often require supplementation. The repeated application of alkaline materials, such as guano boost, can gradually raise soil pH, affecting soil health and crop productivity (Sharma, 2016).

Understanding the pH levels of fertilizers, including CDDs, is essential for farmers and agronomists to manage soil health effectively. The pH level directly influences nutrient solubility and availability. Alkaline conditions found in CDDs significantly enhance the availability of phosphorus and micronutrients, while acidic conditions in nitrogen fertilizers can limit nitrogen availability. By thoughtfully maintaining appropriate pH levels, farmers can minimize nutrient leaching and optimize nutrient uptake efficiency in their crops (Nkoa, 2014).

CDDs typically have a slightly alkaline pH range of 7.73 to 8.62 (**Table 4.2**), which is beneficial for nutrient availability and microbial activity in many soils (Khanal, 2008). This pH range promotes the efficient breakdown of organic matter and the release of nutrients, making digestates valuable as organic fertilizers. It helps create an optimal pH environment for nutrient uptake by plants. The alkaline pH in digestates enhances the availability of essential nutrients such as nitrogen, phosphorus, and potassium, which are crucial for plant growth (Sharma, 2016). However, excessively high pH levels (above 8.5) can restrict the availability of certain nutrients over time due to precipitation or other chemical reactions in the soil. The readings demonstrate the variability in pH levels among different CDDs and fertilizers, highlighting the critical role of effective pH management in agriculture. Maintaining optimal pH levels is essential for maximizing nutrient availability, promoting microbial activity, and enhancing soil health. This, in turn, drives significant improvements in crop productivity and sustainability.

© University of Venda

4.3. UV-VIS Spectroscopy determined the absorbance characteristics of digestate.

Absorbance spectra were recorded using a UV-Vis spectrophotometer and confidently analyzed to identify and quantify chromophoric organic substances, clearly indicating their presence and concentration. All four CDDs exhibited similar wavelengths, regardless of being collected from two different digesters (**Fig. 4.3 and Fig. 4.2**). The UV-Vis absorption peaks observed at 282 nm and 284 nm in these digestates strongly indicate the presence of specific organic compounds, including aromatic amino acids, proteins, and peptides, phenolic compounds, humic and fulvic acids, as well as nucleic acids (**Fig. 4.3**).

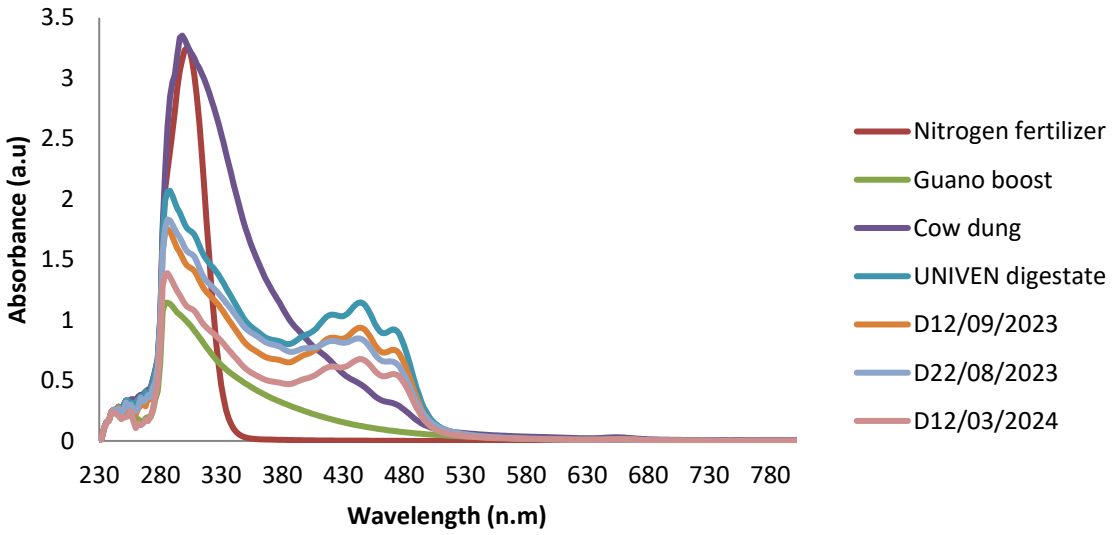


Figure 4.2: The spectrums displayed in the figure represent seven samples, with three samples (D12/09/2023 in orange, D22/08/2023 in light blue, and D12/03/2024 in dark blue) collected from the same digester (Dzwerani) on different dates as indicated. Each sample spectrum is represented in a distinct colour. The controls included nitrogen fertilizer (red), guano boost (green), and cow dung (purple). The spectrum ranges from a wavelength of 230 nm to 780 nm.

© University of Venda

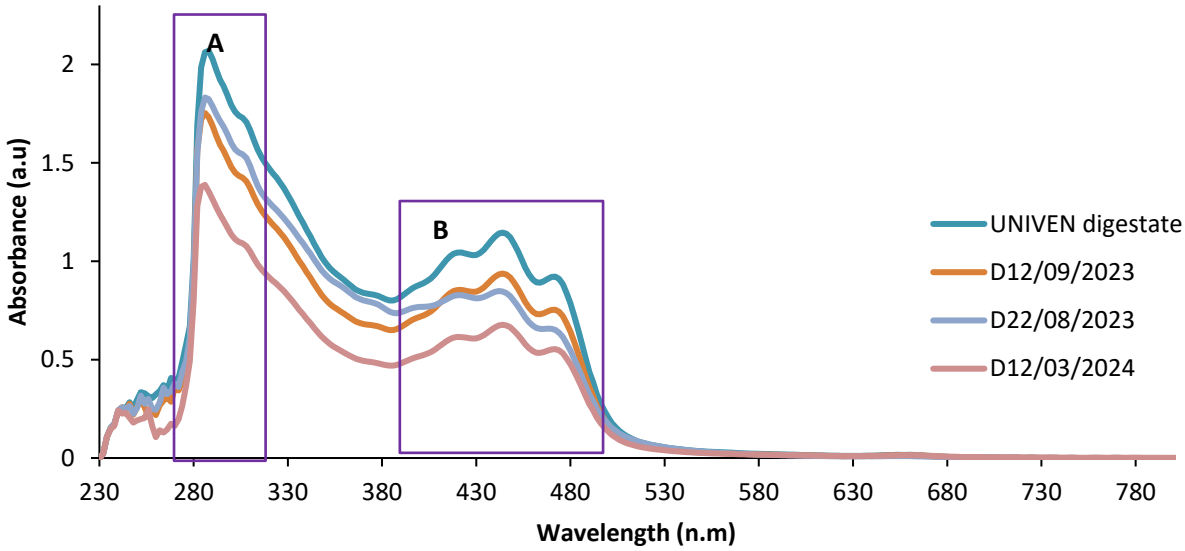


Figure 4.3: This figure illustrates the UV-Vis spectrum of four distinct cow dung-based digestates. As indicated in (A), the digestates exhibited similar wavelengths, despite being collected from two different digesters. The UV-Vis absorption peaks were noted at 282 nm and 284 nm for the CDDs. In (B), the UV-Vis absorption peaks for these digestates are observed within the range of 380 nm to 480 nm.

Tyrosine and tryptophan, which absorb at 275–290 nm, indicated protein or peptide fragments formed during digestion (Stryer, 1988). Phenolic compounds and humic substances, key in soil enrichment, also contributed to absorption in this range (Stevenson, 1994). Peaks between 380–480 nm in cow dung digestate (**Fig. 4.3**) confirmed the presence of humic acids, fulvic acids, chlorophyll degradation products, and natural pigments, all enhancing soil health and nutrient availability (Lakowicz, 2006). Humic acids prominently absorbed at 400–450 nm, while fulvic acids exhibited absorption in a similar range. Chlorophyll degradation products, such as pheophytins, absorbed between 400–480 nm (Lichtenthaler, 1987). Carotenoids and flavonoids, derived from plant material, further contributed to absorption.

The 294 nm peak in nitrogen fertilizer (red) (**Fig. 4.2**) was unexpected, as inorganic fertilizers typically lack UV-absorbing chromophores. This peak suggested the presence of organic additives, stabilizers, or metal chelates that enhance nutrient availability. In guano boost (green), the peak at 280 nm indicated aromatic amino acids and proteins, highlighting its organic nitrogen content (**Fig. 4.3**). The 296 nm peak in raw cow dung (purple) suggested nitrogenous compounds, humic substances, and microbial activity contributing to organic matter decomposition (Skoog, 2017; Voet, 2013). Comparative UV-Vis analysis

© University of Venda

demonstrated that cow dung digestate exhibited a broad absorbance range (280–480 nm), indicating diverse organic and inorganic compounds, including humic substances and nitrogenous compounds (**Fig. 4.2**). In contrast, nitrogen fertilizer, guano boost, and cow dung displayed distinct peaks at 294 nm, 280 nm, and 296 nm, respectively. The wider absorbance range in digestate suggested a more complex nutrient profile beneficial for soil health (Alburquerque *et al.,* 2012).

4.3.1 UV-Vis Comparison of Cow Dung Digestate with Other Fertilizers

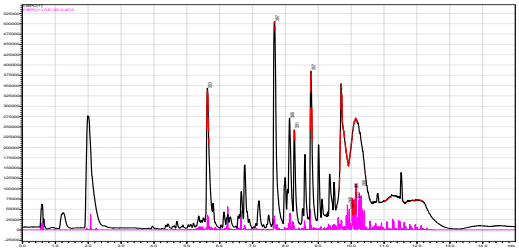
The 294 nm peak in nitrogen fertilizer indicated the presence of key nitrogenous compounds, such as nitrates or ammonium ions (**Fig. 4.2**). Cow dung digestate, with a peak at 296 nm, suggested substantial nitrogen content, potentially offering a more gradual nutrient release compared to synthetic fertilizers (Tambone *et al.,* 2010). The 280 nm peak in guano boost confirmed organic nitrogen sources, though digestate's broader spectral range indicated a more diverse nutrient composition. Raw cow dung retained nitrogenous compounds at 296 nm, but digestion appeared to enhance nutrient availability (Tambone *et al.,* 2010). These findings highlighted cow dung digestate as an effective organic fertilizer, offering a complex nutrient profile and improved soil-conditioning properties, making it suitable for sustainable agriculture.

4.4. UHPLC-QTOF-MS analysis to characterize the organic compounds in digestate.

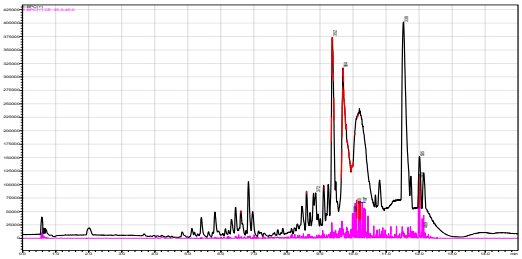
The application of UHPLC-QTOF-MS enabled the comparative analysis of cow dung digestate, raw cow dung, guano boost, and nitrogen fertilizer. Chromatograms (**Fig.4.4 A-G**) illustrated the presence of various organic and inorganic compounds, with retention times on the x-axis distinguishing these compounds and peak intensities on the y-axis indicating relative abundance.

© University of Venda

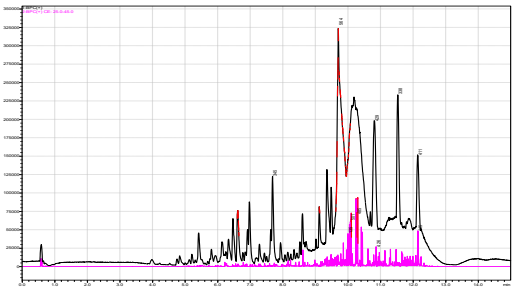
A. D12/09/2023



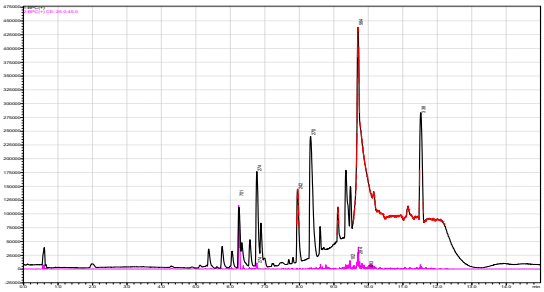
B.D22/08/2023



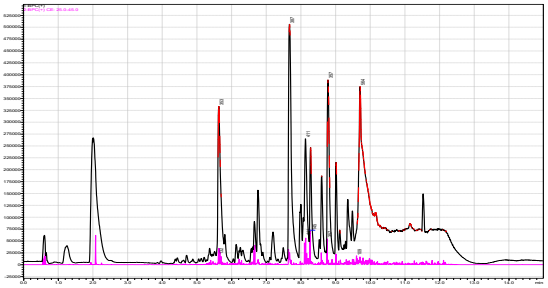
C.D17/04/2024



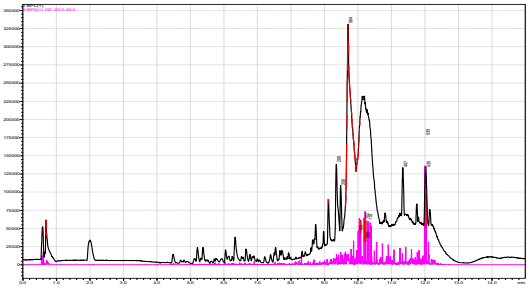
D. UNIVEN Digestate



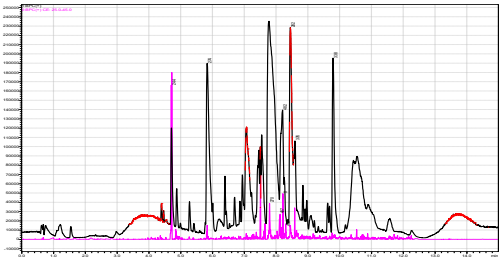
E. Cow Dung



F. Guano Boost



G. Nitrogen Fertilizer



Figures 4.4: UHPLC-QTOF-MS chromatograms of various digestates, including cow dung, Guano Boost, and nitrogen fertilizers, detected using positive ionization and separated by a C18 column (A-G).

© University of Venda

In the cow dung digestate sample (12/09/2023), multiple peaks represented a range of volatile organic compounds (VOCs) and fatty acids (**Fig. 4.4A**). Peaks at 5.8, 7.7, and 8.2 minutes at *m/z* 353, 387, and 391 indicated short-chain fatty acids (Santos *et al.,* 2021; Moore *et al.,* 2019), while the peak at 8.9 minutes at *m/z* 357 suggested protein breakdown products. The clustering of peaks between 5.8 and 8.9 minutes signified structurally related compounds (**Fig. 4.4B**). Notably, the peak at 9.6 minutes at *m/z* 564 reflected mid-chain VFAs, crucial in digestate composition. A more detailed assessment of cow dung digestate (**Fig. 4.4C**) identified short-chain fatty acids, amino acids, and complex organic molecules. Peaks at 6.3 minutes at *m/z* 701 and 6.7 minutes at *m/z* 274 indicated polyphenolic compounds and phenolic acids, while the 7.9-minute peak at *m/z* 242 suggested lignin degradation products. Peaks at 8.2 minutes at *m/z* 270 and 9.5 minutes at *m/z* 564 indicated fatty acid derivatives and sterols, while the 11.4-minute peak at *m/z* 338 represented phytosterols and long-chain hydrocarbons (**Fig. 4.4D**).

Raw cow dung (**Fig. 4.4E**) exhibited a different profile, with early peaks (<2 minutes) corresponding to simple organic acids like formic and acetic acid (Garcia *et al.,* 2020). Peaks at 5.6 minutes at *m/z* 353 and 7.6 minutes at *m/z* 387 suggested phenolic acids and sterols, respectively. The peak at 9.7 minutes at *m/z* 564 represented phospholipids, indicating a complex organic composition. Guano boost (**Fig. 4.4F**) contained highly polar and nitrogenous compounds (Santos *et al.,* 2021). Early peaks (<2 minutes) corresponded to organic acids, while the peak at 7.2 minutes at *m/z* 376 suggested amino acids (Moore *et al.,* 2019). Peaks at 9.4–9.8 minutes at *m/z* 280, 256, 564 indicated uric acid derivatives, crucial for slow nitrogen release. The peak at 12.0 minutes at *m/z* 535 represented non-polar lipids, supporting long-term soil fertility.

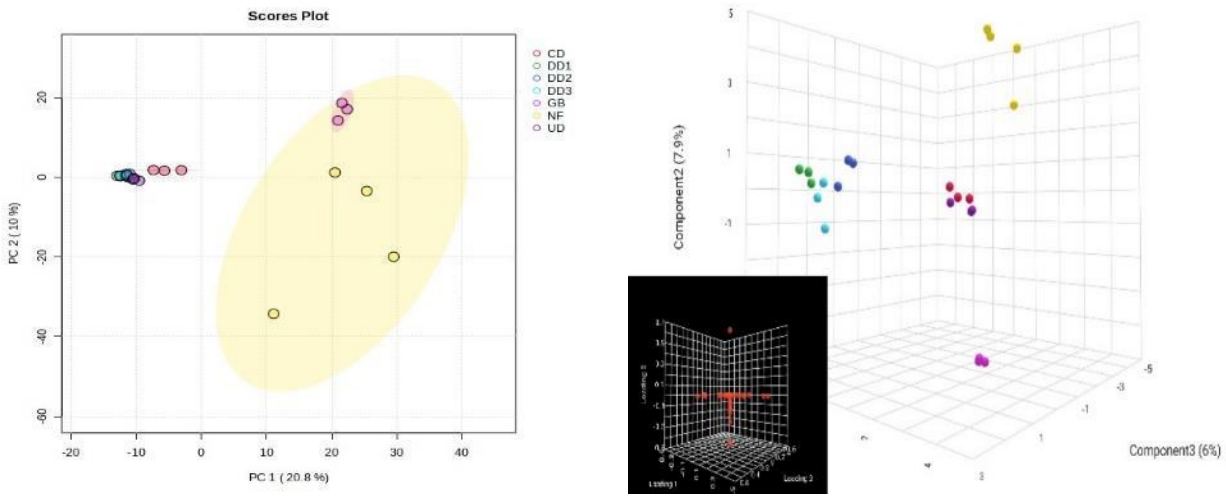
Nitrogen fertilizer (**Fig. 4.4G**) exhibited peaks between 3 and 4 minutes, indicating ammonium ions and amines. Peaks at 4.8 minutes at *m/z* 244 and 5.8 minutes at *m/z* 274 corresponded to urea and calcium ammonium nitrate, respectively. The peak at 8.3 minutes at *m/z* 482 suggested amino acids, while broader peaks beyond 10.6 minutes indicated various nitrogen sources, including slow-release formulations (Santos *et al.,* 2021). The UHPLC-QTOF-MS analysis highlighted distinct nutrient profiles among fertilizers. Cow dung digestate, enriched with organic acids and humic substances, provided immediate and sustained nutrient release, enhancing soil health. Raw cow dung released nutrients gradually, while guano boost supplied fast-acting nitrogen and phosphorus but lacked organic matter. Nitrogen fertilizers delivered rapid nitrogen availability but could contribute

© University of Venda

to environmental concerns. Cow dung digestate emerged as a sustainable alternative, combining short-term nutrient availability with long-term soil enrichment.

4.5. Multivariate data analysis to integrate diverse datasets.

The PCA was used to reduce the dimensionality of large datasets from the metabolomics profiles of CDD and various fertilizer samples while preserving variance. It is a two-dimensional scatter plot that represents the data using the first two principal components (pcs) (Bro and Smilde, 2014). Each point corresponded to a sample, reflecting its position based on these components (Worley, 2013). It effectively compared the metabolomics profiles of CDDs, nitrogen fertilizer, guano boost, and raw cow dung, illustrating their similarities and differences. The analysis recognized key characteristics and patterns in the data matrix, helping to group or separate the samples (**Fig. 4.5A and B**).



A **B**

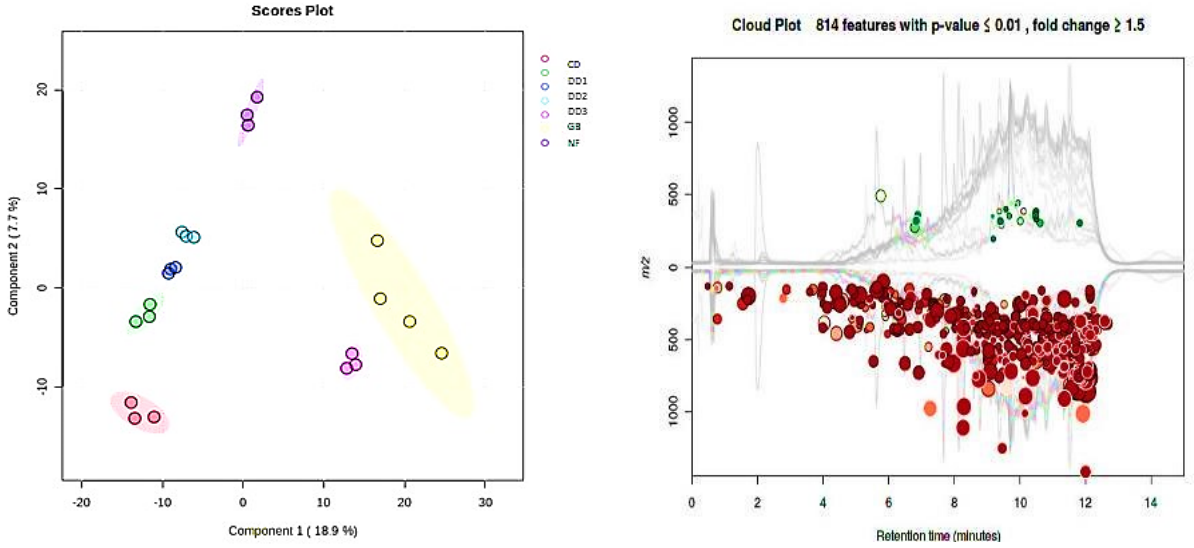
Figure 4.5: The metabolite profiles of four CDDs and three fertilizers were compared using unsupervised PCA **(A)**, as illustrated above. The 3D PCA plot **(B)** displays the distribution of samples along the first three principal components. This visualization helps to clarify the variations among the different treatments based on the measured parameters.

Four CDDs and three fertilizers were compared using unsupervised PCA, as shown in (**Fig. 4.5A**). PC1 accounts for 20.8% of the variance, while the PC2 accounts for 10%. The horizontal axis captures the main differences, while the vertical axis highlights secondary differences. (**Fig. 4.5A**) shows distinct clusters for CDDs, separated from those for nitrogen fertilizer, guano boost, and raw cow dung. The raw cow dung cluster overlaps with the digestate cluster, indicating similarities. Nitrogen fertilizer and guano boost are closer

© University of Venda

together, showing their similar effects on the metabolome. In summary, digestate exhibits a unique metabolic profile due to organic matter breakdown during digestion (Tambone *et al*,2010). Nitrogen fertilizer metabolites reflect increased nitrogen availability, influencing amino acid synthesis, while guano boost's unique composition affects metabolic profiles differently (Rehman *et al.,* 2018). The profile of raw cow dung represents its original nutrient and organic matter composition.

The PLS score plot is a tool used in multivariate data analysis to visualize relationships and differences among sample groups (Wold *et al.,* 2001). It compared CDDs with nitrogen fertilizer, guano boost, and raw cow dung to highlight variations in their metabolic profiles. In the plot, each point represented a sample, positioned based on its scores on two components derived from the PLS analysis (**Fig. 4.6A**). Component 1 accounts for the most variance (18.9%), while Component 2 accounts for the second most (7.7%). Clear separation on the plot indicated distinct metabolic signatures for each treatment. Samples near each other have similar profiles, while those farther apart demonstrate differences (Zhang *et al.,* 2019).



A **B**

Figure 4.6: PLS-DA (a) displays the distribution of samples along the x-axis (Component 1) and y-axis (Component 2), which represent the two most significant components. The percentages (18.9% and 7.7%) indicate the proportion of total variation in the data explained by each component. Different colours are used to represent the samples. **Cloud plot (b),** The x-axis (Fold Change) shows the log2 fold change of each metabolite, and the y-axis represents the -log10 of the p-value for each metabolite, plotted against the retention time at *m/z*.

© University of Venda

The separation between CDDs and nitrogen fertilizer shows significant differences in metabolite compositions, rooted in the organic nature of digestates versus the synthetic composition of fertilizers. Similarly, the distinction between CDDs and guano boost highlights the different metabolic contributions of animal-derived guano (**Fig. 4.6A**). Lastly, the separation between CDDs and raw cow dung reflects changes in metabolic composition due to the digestion process (Wold *et al.,* 2001).

A cloud plot, commonly referred to as a volcano plot, was utilized to visualize the relationship between fold change and p-value for various fertilizers. It effectively highlights significant metabolites that exhibit differences across two or more conditions (Li *et al.,* 2023). In (**Fig. 4.6B**), 814 features are selected based on a p-value of ≤ 0.01 and a fold change of ≥ 1.5. A p-value of ≤ 0.01 indicates strong evidence that the observed differences are unlikely to be due to random chance, while a fold change of 1.5 signifies that the metabolite concentration differs by at least 50% between conditions. These criteria serve to identify changes that are both statistically significant and biologically meaningful, offering valuable insights into metabolic pathways that are differentially regulated under varying conditions (Effah *et al.,* 2023).

A dendrogram was designed to illustrate the clusters formed through hierarchical clustering, it provided a visual representation of the similarities and differences among CDDs, nitrogen fertilizer, guano boost, and raw cow dung based on their metabolomic profiles (**Fig. 4.7**)**.** Employing Ward's method for hierarchical clustering minimizes within-cluster variance. Each branch of the dendrogram represents a cluster, with the lengths of the branches indicating the distance or dissimilarity between them. Shorter branches reflect higher similarity, while longer branches signify greater dissimilarity (Li *et al.,* 2023). The clustering analysis revealed a close relationship between CDDs and raw cow dung, suggesting that their metabolomic profiles are similar (**Fig. 4.7**). CDD and raw cow dung are distinct from nitrogen fertilizer and guano boost, indicating significant differences in their metabolic compositions. The separate clustering of nitrogen fertilizer and guano boost suggests that these treatments have comparable effects on metabolic profiles when contrasted with the cow dung-based treatments.

© University of Venda

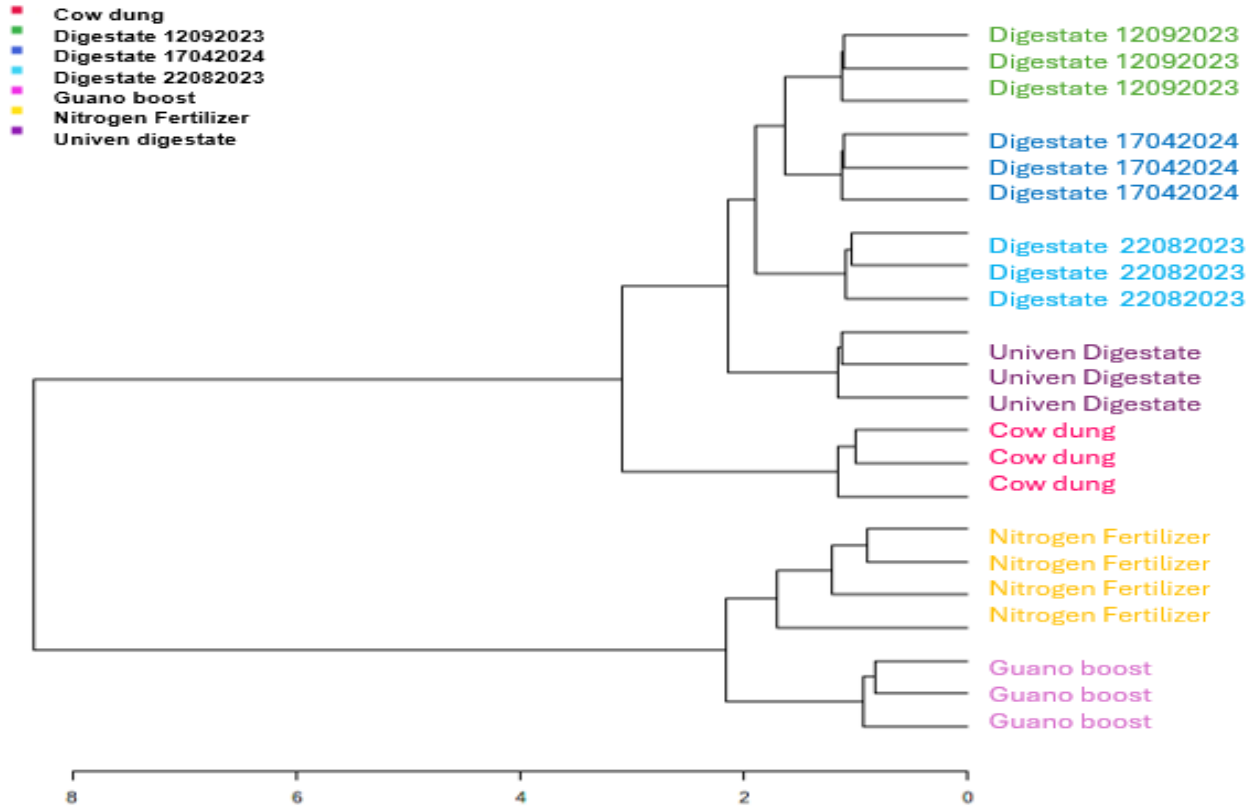


Figure 4.7. A tree diagram designed to visualize the clustering of four digestates, specifically cow dung, guano boost, and nitrogen fertilizer, as referenced by Johnson (1967). Each branch of the diagram represents a distinct cluster, with the length of the branches reflecting the distance or dissimilarity between the clusters. Hierarchical clustering was executed using Ward's method, which is focused on minimizing the total within-cluster variance.

A heatmap visualization tool was utilized to compare the abundance of metabolites across various samples. In this analysis, we examined CDD alongside nitrogen fertilizer, guano boost, and raw cow dung (**Fig. 4.8)** (Kim *et al.,* 2016). It features trees that illustrate data clustering based on similarities, with each row labelled to represent different compounds. The colour scale ranges from blue to red, indicating values where blue denotes lower concentrations, and red signifies higher concentrations. The accompanying colour legend on the right indicates that -2.5 corresponds to low values and 2.3 to high values.

Darker colours indicate higher metabolite concentrations, while lighter colours reflect lower concentrations. Additionally, it includes two dendrograms one for the columns and one for the rows illustrating the hierarchical clustering of similarities. Items that are closer together in the dendrogram are more similar according to the underlying data (**Fig. 4.8)**. On the heatmap we were able to identify compounds present in each sample and the concentration. Each identified compound plays a role in every sample of the fertilizer, these findings are

© University of Venda

important to know what unique compounds are in the samples of CDD and how it differs from other fertilizers (Li *et al.,* 2023). Class-based distribution of 25 identified metabolites from CDD, raw cow dung, nitrogen fertilizer and guano boost**.** Metabolites are grouped in rows and samples are projected into columns (**Fig. 4.8)**. Some metabolites were detected in some samples in very high concentrations, others in very low to nothing concentrations. The compounds were identified using databases such as PubChem and ChemSpider (Pence and William, 2010). Compounds found in digestate can provide various benefits and risks depending on their concentrations and interactions with soil and plants. For instance, ureidoglycolic acid and phosphoric acid are vital for nitrogen cycling and phosphorus availability, enhancing plant growth. Indole promotes root development, while niacin supports metabolic processes. Dimethylamine contributes to the nitrogen pool, but high levels may be toxic. Digallic acid offers antimicrobial benefits, although excessive amounts can inhibit growth. Arsenous acid is concerning due to its high toxicity, which can harm plant growth and contaminate the food chain (Li *et al.,* 2023).

DL-Proline helps plants cope with stress conditions, and glyceraldehyde plays an important role in energy production. High concentrations of hydroxybenzaldehyde can be toxic to plants and soil microorganisms. 3-(Methylthio) propionic acid is included in some fertilizers to enhance performance. 2-(2,5-Dimethylphenyl)-2-oxoethyl 2-chlorobenzoate may also be present in CDD due to specific conditions and chemical processes (Kim *et al.,* 2016). Some compounds can work together to promote plant growth or improve soil health, but high concentrations of toxic compounds like arsenous acid may pose significant risks to soil quality and plant health (Zhang *et al.,* 2019). These compounds belong to various chemical groups, each with specific roles. Nitrogenous compounds are essential for plant growth, while organic acids like phosphoric acid aid in energy transfer. Phenolic compounds may act as antioxidants or growth regulators, and vitamins like niacin are crucial for metabolic functions (Kim *et al.,* 2016). Overall, these compounds can impact soil health, plant growth, and environmental quality, depending on their concentrations and interactions (**Fig. 4.8)**. Careful monitoring of digestate application is necessary to maximize benefits while minimizing potential harm.

© University of Venda

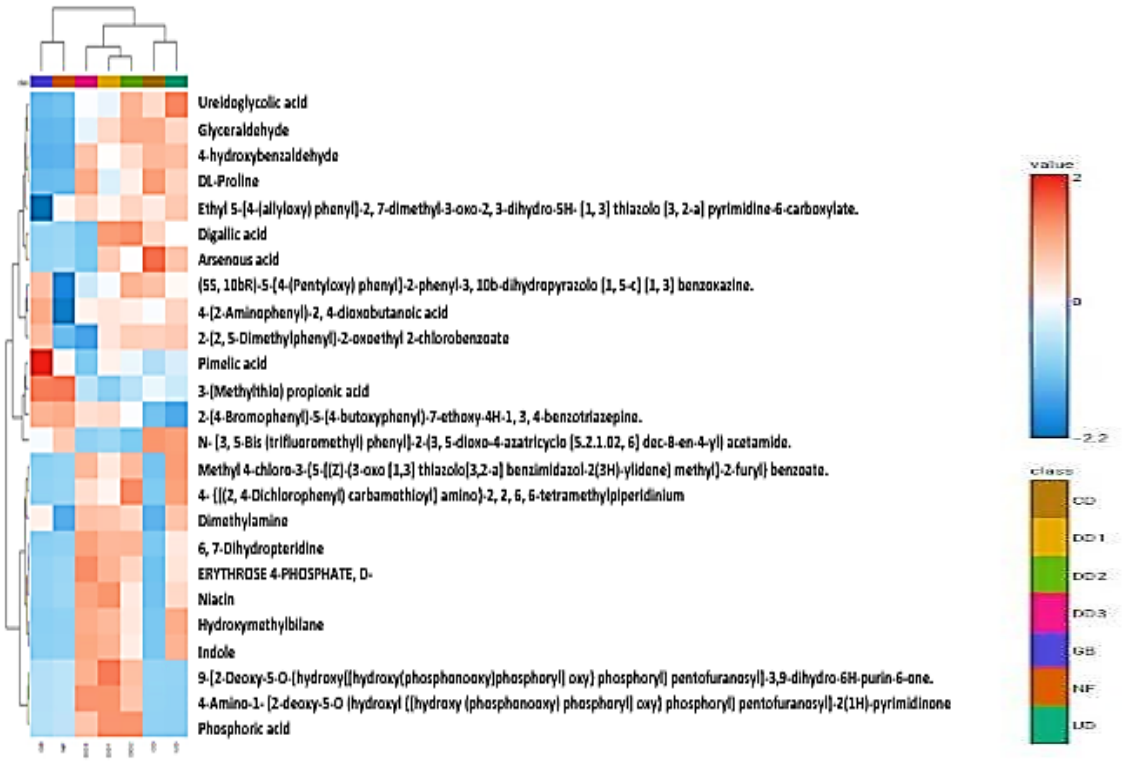


Figure 4.8: The heatmap illustrates metabolites along the rows, with each column representing a distinct sample: CDD, nitrogen fertilizer, guano boost, and raw cow dung. The x-axis denotes the different sample types, while the y-axis lists the identified compounds by their corresponding numbers.

The VIP analysis highlights the top 15 significant metabolite features identified by PLS-DA (**Fig. 4.9**). Higher scores indicate that a metabolite plays a more critical role in distinguishing between groups, with arsenous acid being the most important and Muconic acid the least. The x-axis displays "Mean Decrease Accuracy," showing how the model's accuracy declines when a metabolite is excluded. Higher values indicate greater importance for model performance (Li *et al.,* 2023). A heatmap on the right shows metabolite concentrations across samples, with red indicating high concentrations and blue low concentrations. Coloured boxes represent the relative concentrations of metabolites in digestates and other fertilizers. The mean decrease accuracy metric reveals the impact of excluding a variable, with larger decreases indicating greater importance for predictions (**Fig. 4.9**). This analysis offers insights into the biological relevance of metabolites in plant growth and soil health (Zhang *et al.,* 2019). While arsenous acid may have beneficial roles in soil remediation despite its toxicity, nopaline suggests that nitrogen-fixing bacteria enhance soil nitrogen availability (Li *et al.,* 2023). Deoxyribose 5-phosphate is essential for DNA and RNA production, while formic acid improves nutrient availability and plant defense. Benzoate ions

© University of Venda

exhibit antimicrobial properties by disrupting microbial metabolism and inhibiting the growth of harmful soil microbes (Guo *et al.,* 2020), and proline is crucial for plant stress tolerance in challenging environmental conditions.

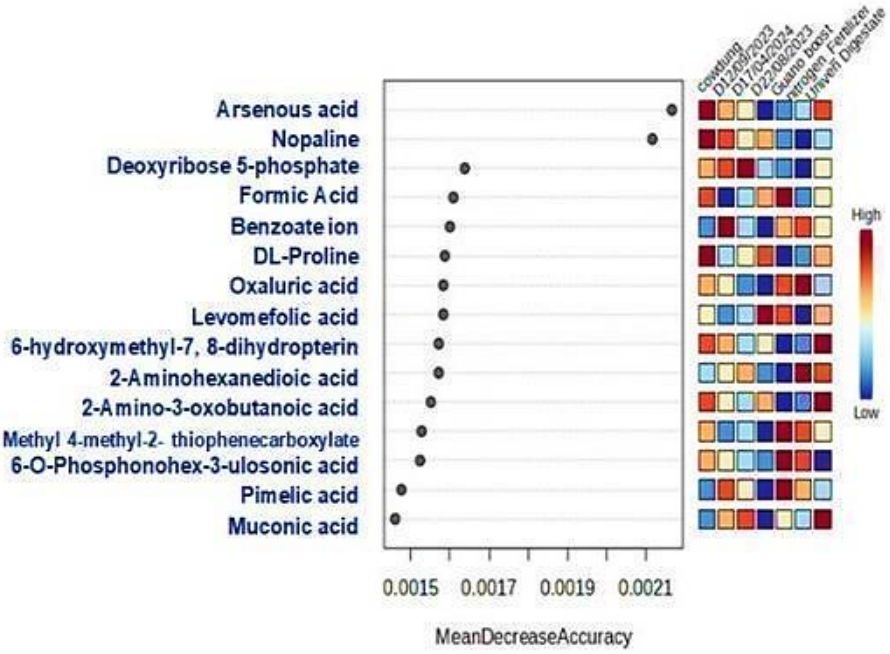


Figure 4.9: The VIP with 15 most significant metabolite features identified by PLS-DA. The coloured boxes on the right indicate the relative concentrations of the corresponding metabolites for the digestates and other fertilizers samples.

Metabolites such as oxaluric acid and 6-O-phosphonohex-3-ulosonic acid are essential for nitrogen and phosphorus metabolism, enhancing nutrient cycling and plant nutrient uptake. Methyl 4-methyl-2-thiophenecarboxylate and the benzoate ion possess antifungal and antimicrobial properties that protect plants from soil pathogens. Amino acids like lysine contribute to nitrogen storage and protein biosynthesis, which are vital for plant development (Liu *et al.,* 2021). Higher-ranked metabolites in the analysis (**Fig. 4.9**) are critical for maintaining soil fertility, nutrient availability, and plant stress resilience, leading to healthier growth and better soil management.

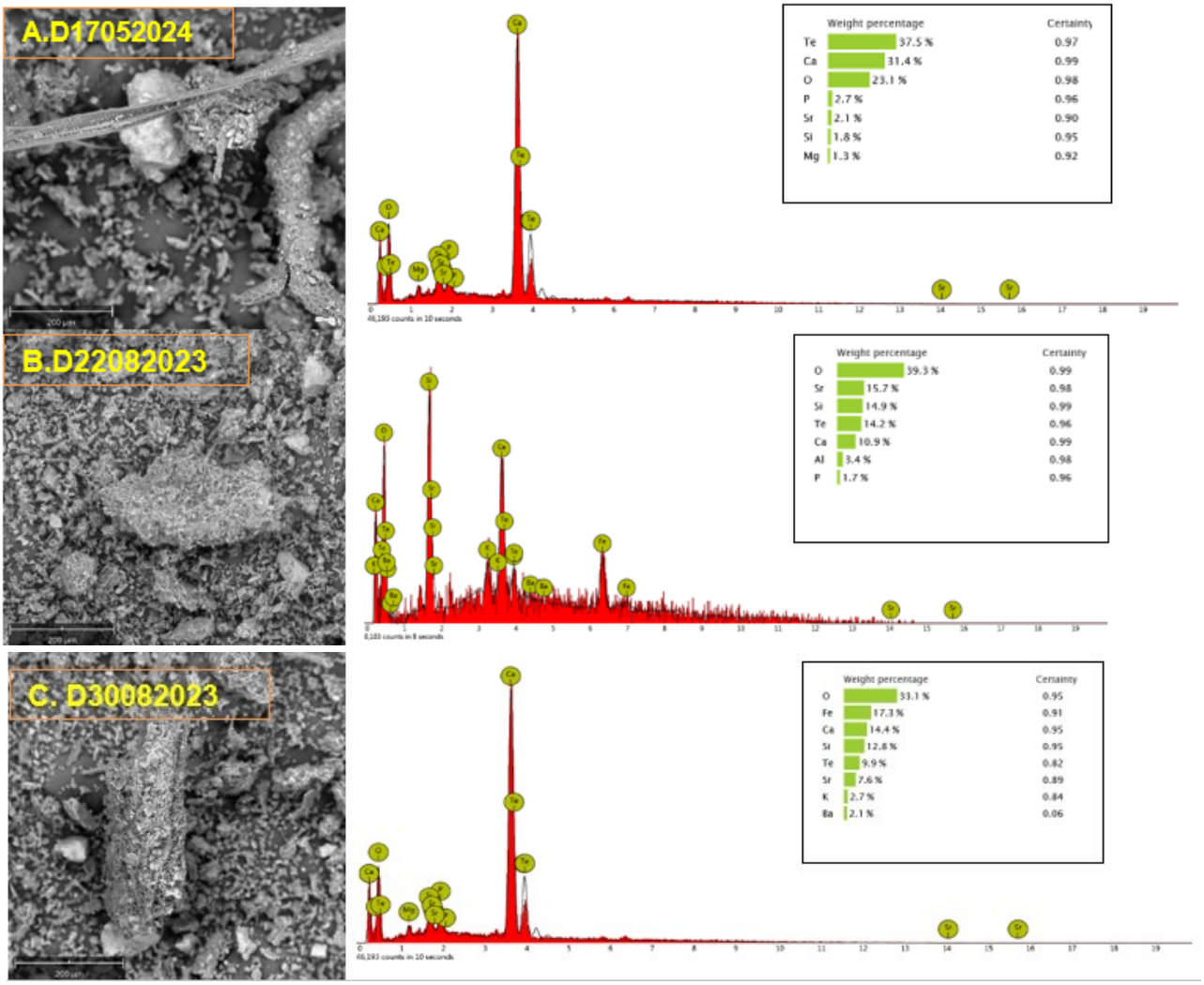
The analysis validates that fertilizers containing metabolites like oxaluric acid could boost nutrient cycling, making essential nutrients more available for plant uptake and improving crop yield. Proline's high ranking indicates that fertilizers enhancing its accumulation can help plants tolerate environmental stresses like drought (Pence and William, 2010). Nopaline’s presence highlights the role of plant-microbe interactions in soil health, suggesting that fertilizers supporting microbial activity could improve soil fertility through

© University of Venda

nitrogen fixation. Metabolites like the benzoate ion may reduce soil-borne pathogens, allowing fertilizers that include or stimulate these compounds to act as bio-pesticides and support sustainable agriculture (Rehberg *et al.,* 2023). Furthermore, metabolite involvement in organic matter breakdown indicates that certain fertilizers can enhance soil decomposition, enriching it with nutrients. Overall, digestate containing specific metabolites can significantly boost soil fertility and plant growth, offering both immediate benefits for crops and long-term improvements in soil health.

4.6.SEM for the physical properties of digestate.

The investigation of the physical properties of digestate particles through SEM was essential for understanding the structural and morphological characteristics of organic and inorganic fertilizers. This study focused on analyzing the particle morphology of CDDs, guano boost, raw cow dung, and nitrogen fertilizer. SEM provided high-resolution imaging, allowing examining of the surface structure, particle size, and shape of these materials, all of which are vital for their effectiveness as fertilizers (**Fig. 4.10, A-G)**.



© University of Venda

Figure 4.10: The acquired electron micrographs, spectra, EDS and quantifications show comparative SEM images of four digestates (A-D), cow dung (E), Guano boost (F), and nitrogen fertilizer (G) mounted on carbon tape.

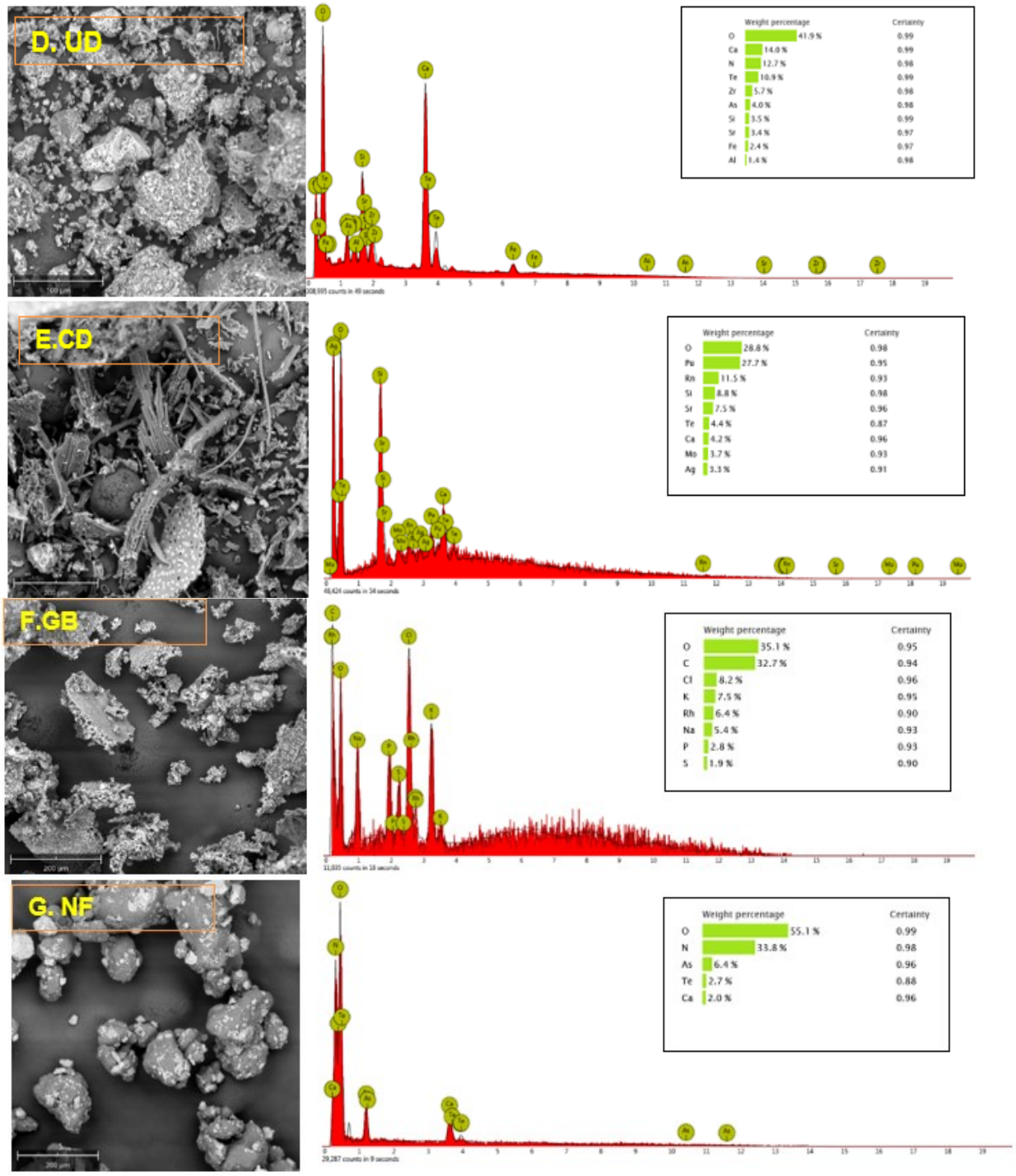


Figure 4.10: Digestates (C and D), cow dung (E), Guano boost (F), and nitrogen fertilizer (G.

SEM imaging revealed textural variations in cow dung digestates. (**Fig. 4.10A**) exhibited a crystalline, fibrous matrix with agglomerated particles, indicative of lower decomposition, increased surface area, and enhanced water retention potential. (**Fig. 4.10B**) displayed a finer, granular structure with uniform particle distribution, suggesting advanced

© University of Venda

decomposition and improved soil integration. These morphological distinctions influence soil aeration, microbial colonization, and nutrient dynamics (Zhao *et al.,* 2014).

EDS analysis of (**Fig. 4.10A**) detected high calcium (31.4%) and tellurium (37.5%) content, contributing to soil aggregation and structural stability, while phosphorus (2.7%) supported root development. Conversely, (**Fig. 4.10B**) exhibited elevated oxygen (39.3%), strontium (15.7%), and silicon (14.9%), indicative of improved soil aeration and mineral availability. The compositional differences suggested that (**Fig. 4.10A**) it is optimal for structural reinforcement, while (**Fig. 4.10B**) facilitates nutrient release (Zhao *et al.,* 2014).

Raw cow dung (**Fig. 4.10C**) exhibited a porous, fibrous texture with incomplete decomposition, enabling gradual nutrient release and moisture retention. EDS analysis confirmed elevated oxygen, iron, calcium, and silicon, beneficial for soil aeration and resilience. UD (**Fig. 4.10D**) displayed a compact, granular structure, indicative of advanced decomposition and higher bioavailable nutrients. Its porous nature promoted soil structure enhancement and microbial activity. EDS analysis highlighted oxygen (41.9%), calcium (14.0%), and nitrogen (12.7%), affirming its role as a nitrogen source. However, arsenic (4.0%) raised toxicity concerns, necessitating controlled application (Zhao *et al.,* 2014).

Cow dung (**Fig. 4.10E**) exhibited a fibrous matrix with plant residues, supporting slow decomposition and sustained soil enrichment. EDS analysis of cow dung reveals its heterogeneous, fibrous structure and high nutrient content, dominated by oxygen (28.8%). This nutrient-rich composition supports its role as an effective organic material for agriculture, enhancing microbial activity and nutrient cycling (Zhao *et al.,* 2014). Cow dung (**Fig. 4.10E**) is ideal for long-term soil improvement due to its slow decomposition, while Guano Boost is suited for immediate nutrition needs in fast-growing plants, particularly benefiting flowering and fruiting crops with its high potassium content (**Fig. 4.10F**).

Guano Boost (**Fig. 4.10F**) showed a compact, granular morphology, facilitating rapid nutrient release and improved soil integration. EDS analysis revealed its high carbon, oxygen, potassium (7.5%), and phosphorus (2.8%) content, making it well-suited for fruiting and flowering crops (Gautam *et al.,* 2019).

Nitrogen fertilizer (**Fig. 4.10G**) exhibited a dense, granular morphology, ensuring controlled nutrient dispersion. EDS analysis identified predominant oxygen (55.1%) and nitrogen (33.8%), essential for vegetative growth and chlorophyll synthesis. However, arsenic (6.4%)

© University of Venda

posed contamination risks, impacting soil and food safety. The presence of tellurium (2.7%) warrants further investigation into its agronomic implications (Gautam *et al.,* 2019).

This study highlights the structural and compositional variations among fertilizers, demonstrating their respective benefits and limitations. Organic amendments like cow dung digestate and guano boost contribute to soil health and microbial activity, whereas inorganic fertilizers offer rapid nutrient availability but pose environmental risks. A balanced fertilizer management approach is essential (Gautam *et al.,* 2019).

4.7. FT-IR to characterize the functional groups present in digestate

FT-IR analysis of CDD reveals a complex molecular architecture that underpins its potential as an effective soil amendment. The dominant absorption at 3287.92 cm^{-1}, which signifies hydroxyl (–OH) groups from alcohols, phenols, and carboxylic acids, plays an important role in water retention and hydrogen bonding, thereby stabilizing soil aggregates, as illustrated in (**Fig. 4.11A**) (Smith ,2011). Concurrently, the peaks at 2917.74 cm^{-1} and 2850.20 cm^{-1}, also detailed in (**Fig. 4.11A**)**,** indicate methylene ($–CH_2$) stretching that reflects the contribution of lipids and fatty acids to the organic matrix, influencing both nutrient retention and soil texture (Laili *et al.,* 2010). The spectral region between 1643.72 and 1513.06–1417.86 cm^{-1}, which reveals carbonyl (C=O) and aromatic C=C stretching vibrations, are the presence of compounds capable of chelating metal ions and contributing to long-term carbon sequestration through humic substance formation; these features are further supported by the phosphate-related absorption at 1037.53 cm^{-1} and lower wavenumber peaks (534.49, 458.35, and 416.55 cm^{-1}) that confirm the inclusion of essential minerals such as calcium, magnesium, and iron (Laili *et al.,* 2010). The spectrum also demonstrates the role of aliphatic compounds, with the pronounced peak at 2917.81 cm^{-1} in (**Fig. 4.11B**) corresponding to asymmetric C–H stretching in decomposed animal waste, while the peak at 1035.74 cm^{-1}, attributed to C–O stretching in alcohols and phenols, implies enhanced chelation properties and antioxidant effects that facilitate nutrient availability (Silverstein *et al.,* 2014). The lower wavenumber vibrations (528.27, 512.39, and 500.11 cm^{-1}) emphasize the presence of water and additional organic matter, reinforcing the digestate's role in sustaining soil moisture and microbial activity (Laili *et al.,* 2010). A prominent hydroxyl peak at 3272.53 cm^{-1}, as observed in (**Fig. 4.11C**), emphasizes the capacity of –OH groups to promote water retention and solubility, thereby facilitating nutrient dissolution, while the absorption at 1029.95 cm^{-1} point out the digestate's potential to enrich soil phosphorus levels a key factor for root development and energy transfer.

© University of Venda

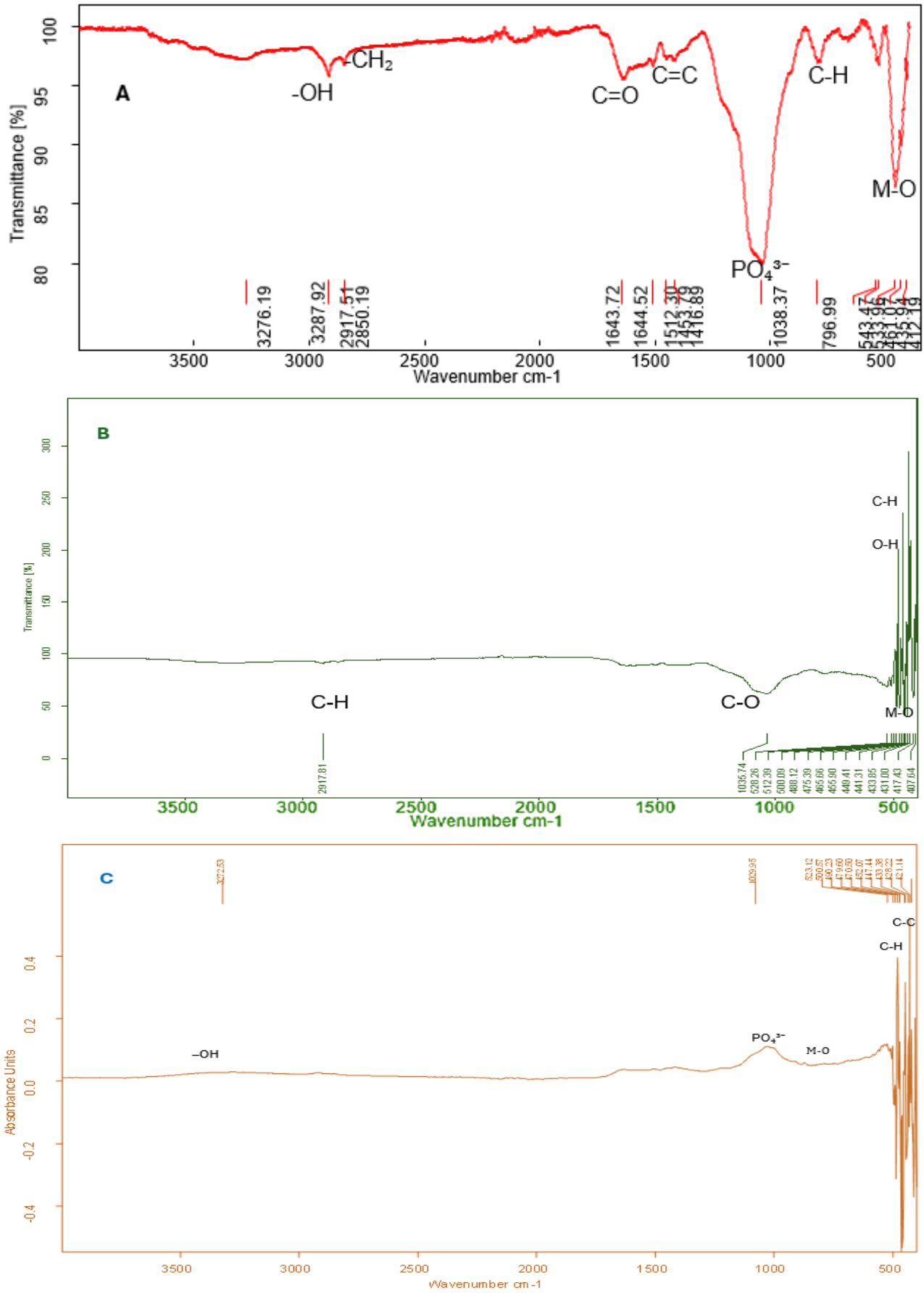


Fig. 4.11. The FT-IR spectra of different fertilizer samples (a) DzweraniDigestate17/03/2024, (b) Dzwerani Digestate22/08/2023, (c) UD, (d) GB, (e) CD, and (f) NF.

© University of Venda

Broader absorption bands in the 3300–3500 cm^{-1} range, associated with O–H and N–H stretching vibrations documented in (**Fig. 4.11D (GB)**), are vital for hydrogen bonding, water retention, and the gradual release of nitrogen via the breakdown of amine groups, while the aliphatic C–H and carbonyl (C=O) stretching vibrations support the chelation of metal ions and contribute to overall soil organic content (Laili *et al.,* 2010). The spectrum further indicates strong absorptions in the phosphate (~900–1100 cm^{-1}) and C–O stretching (~1000–1300 cm^{-1}) regions, confirming the fertilizer's capacity to release essential nutrients, with even the less common nitrile (C≡N) stretching (~2100–2200 cm^{-1}) suggesting additional nitrogen cycling pathways. The study (Patel and Verma, 2022) demonstrated how synthetic nitrogen fertilizers exhibit strong nitrate absorption bands at 1380 cm^{-1}, which influence nitrogen cycling in soil.

The diverse molecular composition is reiterated in (**Fig. 4.11E (CD)**)**,** where O–H stretching peaks at 3749.21 cm^{-1} and 3332.49 cm^{-1}, along with aliphatic C–H stretching at 2917.91 cm^{-1} and 2850.16 cm^{-1}, underscore the presence of fatty acids and phenolic compounds that enhance soil aggregation and moisture retention (Silverstein *et al .,* 2014). Carbonyl (C=O) stretching at 1731.62 cm^{-1} and unsaturation indicated by C=C stretching at 1634.27 cm^{-1} and 1508.70 cm^{-1} contribute to the dynamic reactivity and stability of the organic matrix, while C–O stretching vibrations (1317.33 and 1034.55 cm^{-1}) and lower wavenumber peaks (531.41, 465.17, and 416.24 cm^{-1}) further highlight the presence of polysaccharides and mineral components that improve nutrient exchange and soil stabilization. (Tiwari and Upadhyay ,2021) study identified key functional groups in cow dung, including hydroxyl, aliphatic, and carbonyl groups, which contribute to soil nutrient enrichment.

The analysis is complemented by the findings in (**Fig. 4.11F (NF)**), where the N–H stretching vibration at 3228.65 cm^{-1} indicates the presence of amines and amides critical for nitrogen availability, and the peak at 3059.35 cm^{-1}, corresponding to C–H stretching in aromatic compounds, suggests molecular structures that bolster stability and enable slow nutrient release (Gupta *et al.,*2012). The distinct peak at 1753.62 cm^{-1}, attributed to C=O stretching, enhances nutrient chelation and solubility, while peaks at 1408.39 cm^{-1} and 1299.03 cm^{-1}, linked to C–H bending and C–N stretching respectively, are essential for maintaining the structural integrity of the fertilizer and regulating nitrogen release (Smith ,2011). The absorption at 1039.56 cm^{-1}, reflective of C–O stretching in alcohols and phenols, denotes favourable interactions with soil moisture, and lower wavenumber peaks (567.92, 506.04,

© University of Venda

492.46, 485.52, 477.91, and 468.73 cm^{-1}) further emphasize the stability of organic nitrogen compounds and the presence of mineral constituents that enhance nutrient release and foster robust soil interactions (Gupta *et al.,*2012). The study found strong phosphate (P-O) bands and amide (N-H) vibrations, confirming the presence of organic and inorganic nitrogen sources (Cruz and Díaz,2018).

Collectively, the comprehensive analysis across confirms that CDD possess a rich array of functional groups ranging from hydroxyl, carbonyl, and aromatic to phosphate, aliphatic, and nitrogenous moieties that synergistically enhance soil water retention (Rashid, 2016), nutrient availability, and structural integrity, thereby promoting microbial activity and supporting the long-term sustainability of soil fertility essential for effective nutrient management and sustainable agricultural practices (**Fig. 4.11A–F**) (Gupta *et al.,*2012).

© University of Venda

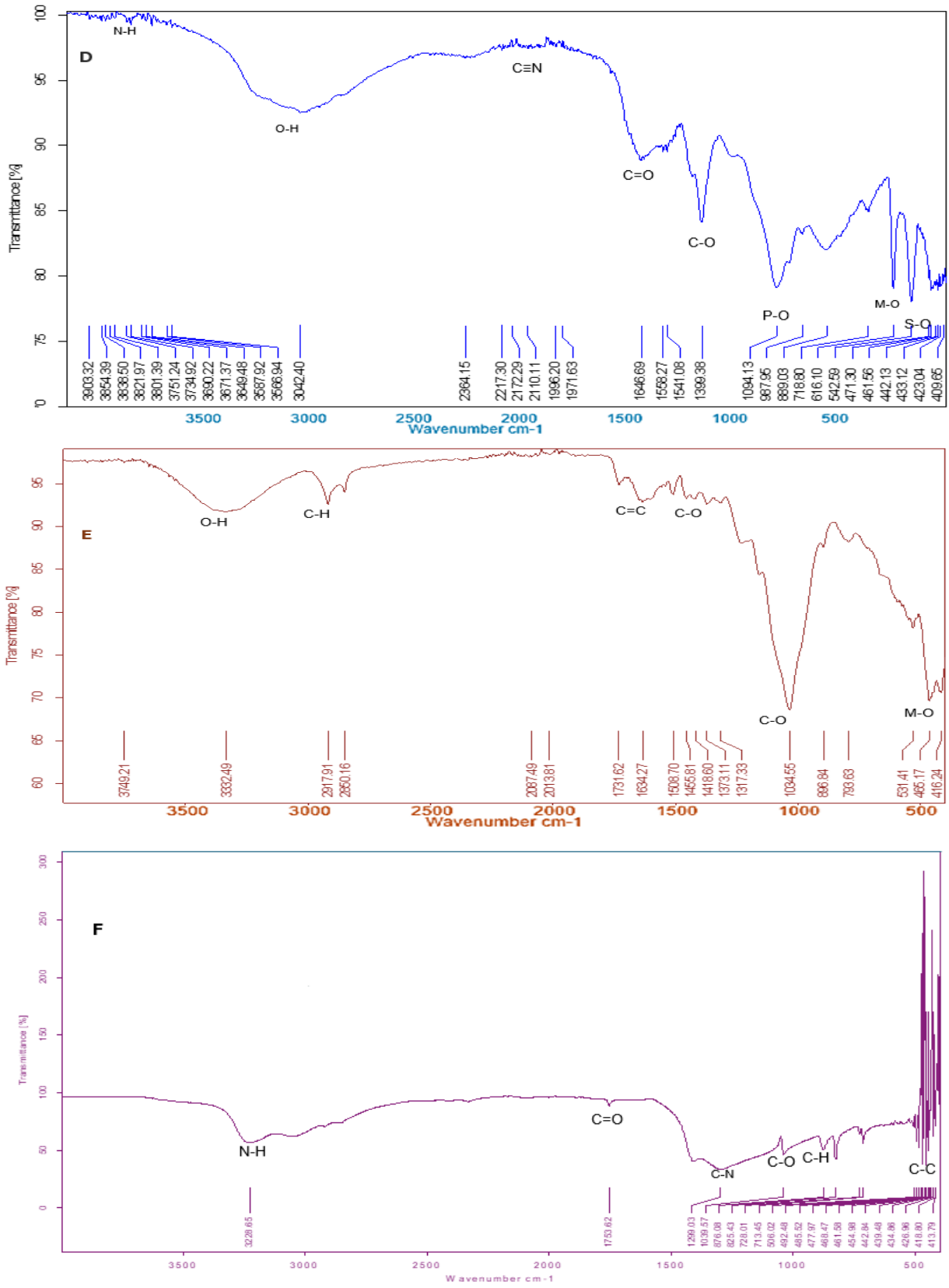


Figure 4.11: The FT-IR spectra of different fertilizer samples (d) GB, (e) CD, and (f) NF .

© University of Venda

4.8. ICP-OES measured the elemental composition of digestate.

The elemental composition of four CDDs (DD1, DD2, DD3, and DD4), a nitrogen fertilizer, and an additional digestate sample (UD) was analyzed using ICP-OES to assess their macronutrient and micronutrient content (**Fig. 4.12**) (Marschner, 2012). Potassium (K), essential for plant water regulation and enzyme activation, was significantly higher in the digestates, particularly in DD2 (1266.174 mg/L) and UD (1515.311 mg/L), while the nitrogen fertilizer contained only 10.258 mg/L, highlighting its limited potassium contribution. Magnesium (Mg), which plays a vital role in chlorophyll synthesis, was also abundant in the digestates, with UD exhibiting the highest concentration (205.965 mg/L), whereas the nitrogen fertilizer had a minimal amount of 2.356 mg/L (**Fig. 4.12**) (Marschner, 2012). (Li *et al.,* 2019) compared the elemental composition of nitrogen fertilizers using ICP-OES and found that synthetic fertilizers like urea and ammonium nitrate had higher nitrogen content (~46% for urea) but lacked other essential macronutrients such as potassium and calcium. This contrasted with digestate, which contained a more balanced nutrient profile (Li *et al.,* 2019). Similarly, calcium (Ca), crucial for cell wall integrity and plant development, was present in substantial quantities in the digestates, especially UD (693.614 mg/L), in stark contrast to the nitrogen fertilizer, which contained only 1.172 mg/L (Marschner, 2012).

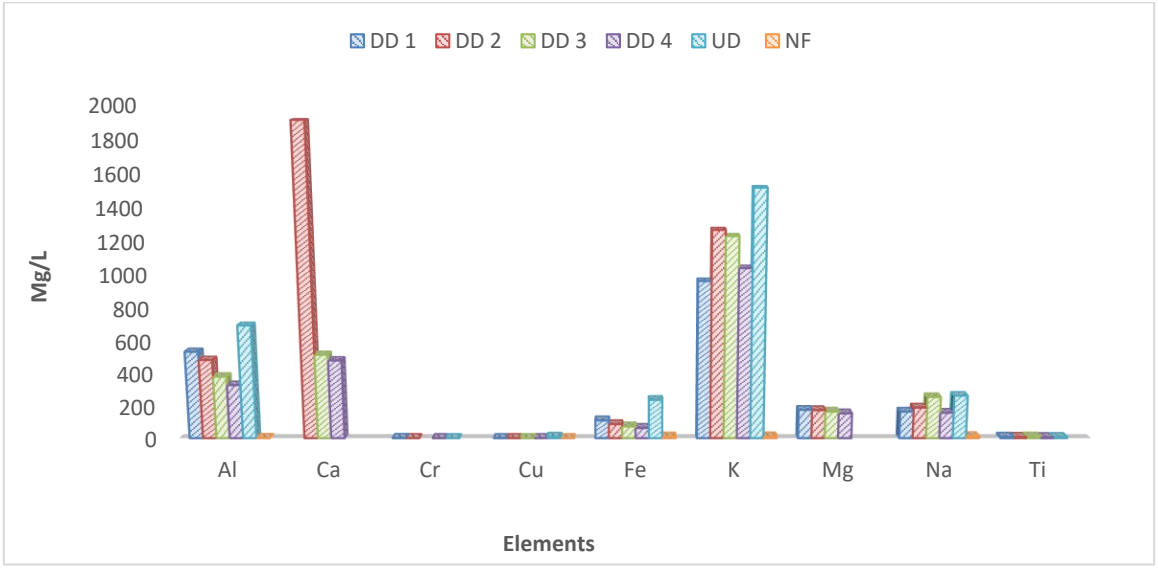


Fig. 4.12: Visual representation of the elemental makeup in five digestates and nitrogen fertilizers essential for enhancing soil health and maximizing plant growth (Havlin *et al.,* 2013). This graph highlights the diverse elements found in digestate and other fertilizers, measured in mg/L (**Appendix A**).

Micronutrient analysis revealed that iron (Fe), essential for enzymatic functions and chlorophyll formation, was significantly higher in the digestates than in the nitrogen fertilizer

© University of Venda

(**Fig. 4.12**) (Havlin *et al.,* 2013). DD1 contained 110.862 mg/L, while DD4 had 63.859 mg/L, compared to the nitrogen fertilizer's low iron content of 8.915 mg/L. Copper (Cu), which is vital for photosynthesis but toxic at high concentrations, was relatively low in the digestates (<0.4 mg/L), whereas the nitrogen fertilizer contained a substantially higher concentration of 8.358 mg/L, necessitating careful application to avoid potential toxicity risks. Chromium (Cr), which can pose toxicity concerns, was found to be saturated in DD2 and UD, while DD3 and DD4 exhibited lower levels (<0.3 mg/L), making them safer for use (**Fig. 4.12**). (Wang *et al.,* 2020) analyzed anaerobically digested cow dung using ICP-OES and found that digestate had higher potassium (K) and phosphorus (P) content compared to raw cow dung, making it a valuable organic fertilizer. The study also reported that magnesium (Mg) and calcium (Ca) concentrations were increased in the digestate due to microbial breakdown of organic matter (Wang *et al.,* 2020).

Sodium (Na), which influences nutrient uptake and may lead to soil salinity, was highest in UD (263.925 mg/L) and DD3 (255.548 mg/L), raising concerns about potential accumulation if overapplied. Titanium (Ti), a non-essential element, was detected in low concentrations across all samples (<10 mg/L), indicating minimal influence on plant health (**Fig. 4.12**) (Alloway, 2008). A study by (Ghosh *et al.,* 2020) confirmed that raw cow dung had lower phosphorus (P) and potassium (K) content than digestate, as anaerobic digestion enhanced mineralization and nutrient availability (Ghosh *et al.,* 2020). The digestates, particularly UD, provide a range of essential nutrients, outperforming the nitrogen fertilizer in potassium, calcium, magnesium, and iron content. However, certain elements such as chromium and sodium were present in higher concentrations in some digestates, requiring careful monitoring to prevent potential toxicity. The nitrogen fertilizer, while effective for nitrogen supplementation, offered limited support in terms of macronutrient and micronutrient diversity (Marschner, 2012). These results indicate that CDDs, especially UD, can serve as effective biofertilizers, when applied with appropriate management strategies (**Fig. 4.12**).

© University of Venda

4.9. Identify and quantify VOCS present in digestate through GC-MS.

The GC-MS analysis of CDD identified Cholesteryl Stearate ($C_{45}H_{78}O_2$) as a key sterol ester, with a molecular ion at *m/z* 498 (**Fig. 13A**) and characteristic fragment ions confirming its structure (Huang *et al.,* 2020). The parent ion at *m/z* 498 represents the intact molecule, while significant fragment ions provide insights into its degradation pathway. The presence of a fragment at *m/z* 456 suggests the loss of 42 Da**,** which is indicative of a neutral loss, possibly an acetyl or other small moiety, a common fragmentation pattern in sterol esters.

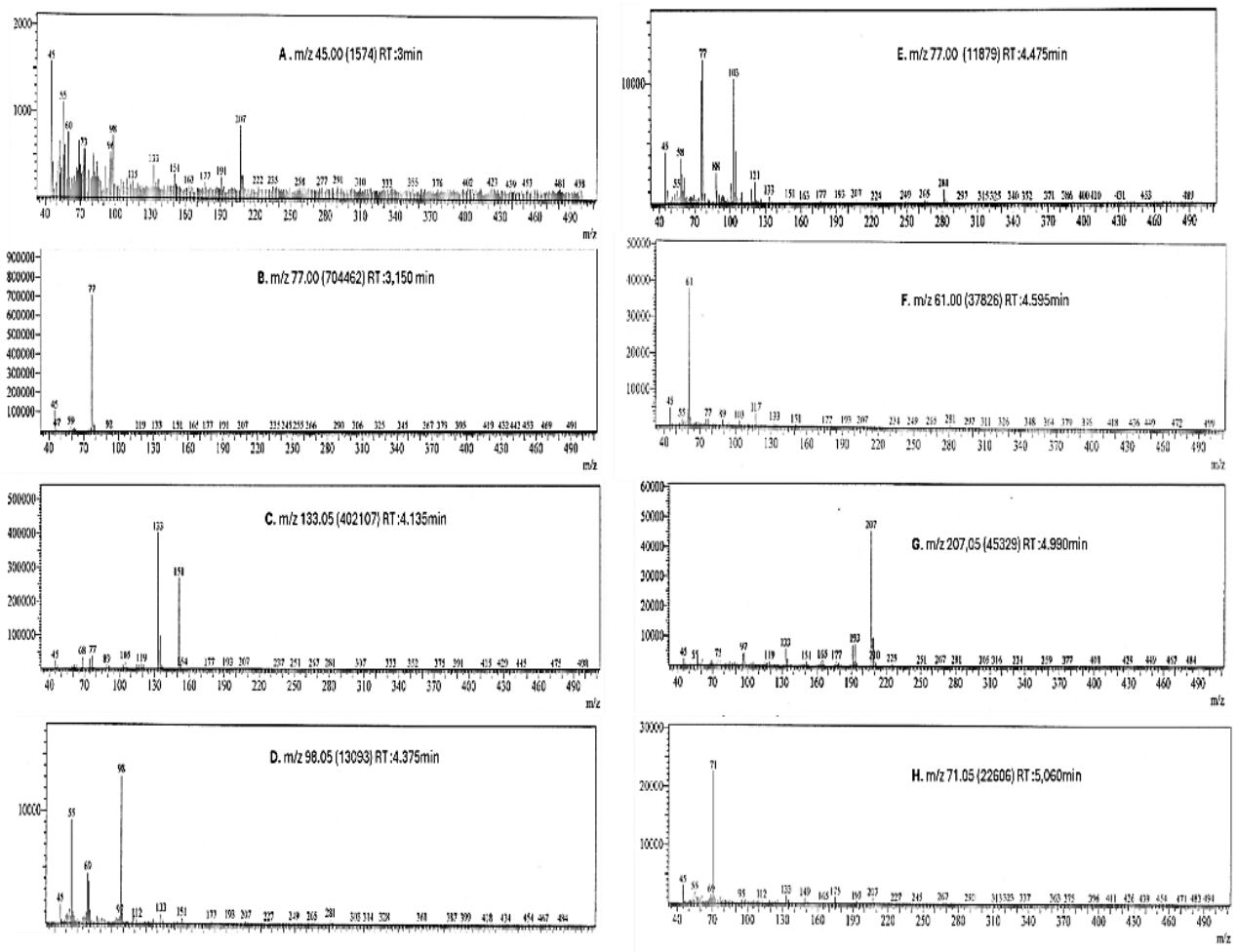


Figure 4.13: GCMS spectra fragmentation pattern of Cholesteryl Stearate ($C_{45}H_{78}O_2$) at *m/z* 498, (**A -N**) shows fragment ion with their respective retention times, base peaks and intensities.

© University of Venda

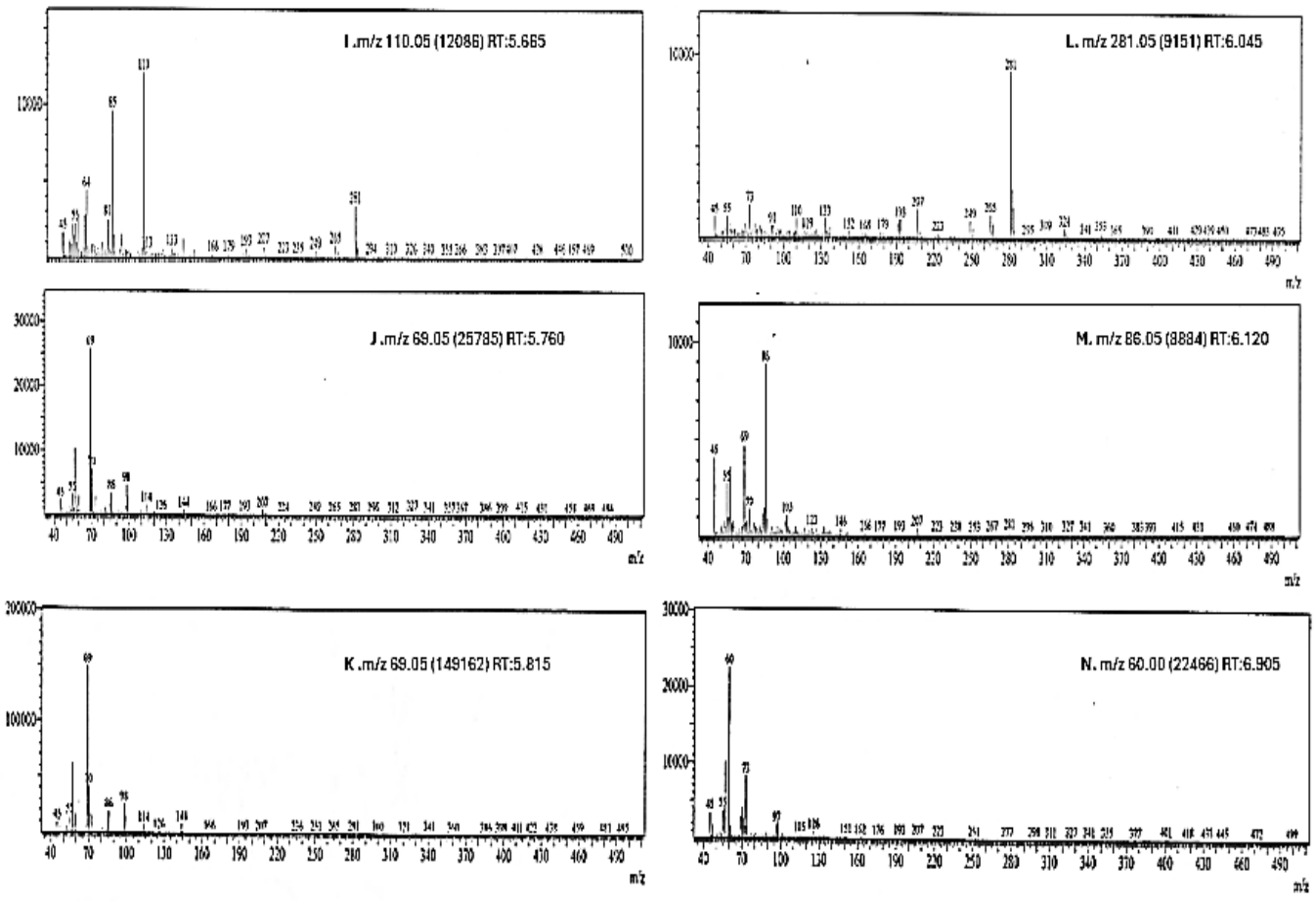


Figure 13: GCMS spectra fragmentation pattern of Cholesteryl Stearate ($C_{45}H_{78}O_2$) at *m/z* 498, (**I -N**) shows fragment ion with their respective retention times, base peaks and intensities.

The fragmentation pattern observed in the GC-MS spectrum aligns with known sterol ester decomposition pathways. The *m/z* 369 fragment, a key diagnostic ion, corresponds to the cholesterol core**,** resulting from the loss of the fatty acid moiety (stearic acid). The *m/z* 255 (**Fig. 13, B**) fragment further confirms this by representing the stearic acid fragment**,** which occurs due to cleavage of the ester bond (Möller and Müller ,2012). Additional fragment ions, including *m/z* 207(**Fig. 13G**), *m/z* 177 (**Fig. 13, A**), and *m/z* 133 (**Fig. 13C**)**,** indicate stepwise degradation of the sterol backbone, while *m/z* 98 (**Fig. 13D**), *m/z* 69 (**Fig. 13J and K**), and *m/z* 45 (**Fig. 13A**) correspond to small hydrocarbon fragments commonly associated with lipid breakdown (Wang *et al.,* 2019). The presence fragment ions at *m/z* 60,61, 77 (**Fig. 13E, F, B AND N**), 89, 133, and others suggest further breakdown of the parent molecule, forming characteristic fragments seen in hydrocarbon chains, esters, or amino acid derivatives. Given that CDD is rich in decomposed organic matter, microbial

© University of Venda

metabolites, and fermentation byproducts, the detected compounds are likely derived from degradation of proteins, lipids, and carbohydrates (Morales *et al.,* 2018).

In Cholesteryl Stearate, the digestate exhibited a variety of volatile organic compounds (VOCs)**,** including short-chain fatty acids (acetic, propionic, and butyric acids), aliphatic hydrocarbons, and phenolic derivatives, contributing to its complex biochemical composition (Morales *et al.,* 2018).The presence of nitrogen and sulphur containing VOCs shows potential ammonia volatilization and odour emissions, which could be mitigated through controlled application strategies (Zhag *et al.,* 2017). The detection of sterols and fatty acids supports the digestate's role in enhancing soil organic matter, microbial activity, and nutrient bioavailability. The stability of sterol esters like Cholesteryl Stearate in soil indicates their role in organic matter retention and microbial interactions, contributing to long-term soil fertility**.** The detection of cholesteryl stearate and phytosterols suggests the presence of residual plant sterols, which can enhance soil organic matter (Wang *et al.,* 2019).

The GC-MS data also revealed the presence of lipid-derived VOCs, as evidenced by fragment ions such as *m/z* 69 (**Fig. 13J and K**), *m/z* 71 (**Fig. 13H**), and *m/z* 73, which are characteristic of aliphatic hydrocarbons and fatty acid methyl esters (FAMEs) (Möller and Müller ,2012)**.** The *m/z* 69 ion, a hallmark of branched hydrocarbons, is the presence of isoprenoid compounds, while *m/z* 71 and *m/z* 73 (**Fig. 13A**) indicate straight-chain alkanes and lipid degradation products. Fragments appearing *m/z* 45, *m/z* 55 (**Fig. 13A**), *m/z* 86 (**Fig. 13M**), and *m/z* 98 (**Fig. 13, D**) correspond to common aliphatic chain breakdown products, reinforcing the presence of lipid-derived VOCs**.** The detection of *m/z* 126, *m/z* 144, and *m/z* 162, 281(**Fig. 13M**) are oxygenated VOCs, potentially ketones or aldehydes**,** which may contribute to digestate odour and environmental impact (Morales *et al.,* 2018).

These results point out the biochemical complexity of digestate, where lipid and sterol ester degradation products interact with microbial processes, influencing its potential as a soil amendment. While some compounds contribute to nutrient cycling and microbial activity, the detection of low-molecular-weight VOCs raises concerns about gaseous emissions and localized soil acidification (Zha *et al.,* 2012)**.** This underscores the importance of optimized composting and application strategies to balance soil fertility benefits with environmental sustainability**.** The presence of sterol and fatty acid derivatives reveal that digestate could enhance microbial activity in soil**,** improving nutrient availability. The detection of aromatic and nitrogenous compounds raises considerations regarding gaseous emissions and nutrient leaching (Sasak *et al.,* 2012)**.** The data emphasizes the necessity for controlled

© University of Venda

application strategies to maximize agronomic benefits while minimizing potential environmental risks.

Across all analyses, the digestate showed physicochemical traits consistent with agronomic-grade organic fertilizers while revealing management risks that align with recent evidence. The alkaline pH (7.73–8.62) and high moisture (≈46–49%) fall within typical ranges for manure-based digestates, which favor nitrification, microbial activity, and P/K availability in neutral to slightly alkaline agricultural soils (Abubaker et al., 2021; Tamburini et al., 2023). Recent field syntheses similarly report that most digestates are near-neutral to alkaline and supply plant-available N, P, and K alongside secondary cations that buffer acidity and stimulate soil biota (Brienza et al., 2024; Tamburini et al., 2023).

Elemental data indicated abundant macronutrients (notably K, Ca, Mg) and trace Fe–profiles that mirror current ICP-OES surveys of livestock digestates and explain the observed potential to improve cation balances and aggregate stability (Brienza et al., 2024; Tamburini et al., 2023). At the same time, the elevated Na (e.g., ≈264 mg L^{-1} in one unit) signals salinity risk if applications are frequent or on poorly drained soils–an issue emphasized in recent guidance that links digestate EC/Na to soil structural decline in sensitive settings and recommends site-specific rates plus blending with low-salinity organics (Hjorth et al., 2022; Tamburini et al., 2023).

FT-IR bands (broad O–H/N–H ~3200–3400 cm^{-1}; aliphatic C–H ~2850–2950 cm^{-1}; C=O/C=C ~1700–1600 cm^{-1}; and strong P–O/S–O ~1040–1030 cm^{-1}) indicate humified organics, peptides/lignin derivatives, and phosphate/sulfate groups typical of stabilized digestates and compost-like materials (Jiang et al., 2022; Ouni et al., 2020). These signatures are widely associated with improved water retention and long-term C persistence, supporting the amendment value observed here (Jiang et al., 2022).

GC–MS profiling recovered sterol esters and fatty-acid derivatives (e.g., cholesteryl-type fragments with diagnostic m/z 369 and C16–C18 acyl fragments), together with low-molecular-weight VOCs. Similar metabolite suites are repeatedly reported for manure/digestate matrices and are interpreted as products of lipid/sterol turnover and microbial fermentation (Liu et al., 2024; Rabaud et al., 2022). The presence of oxygenated VOCs (ketones/aldehydes) is consistent with studies linking storage/handling to odor and reactive-gas emissions; best practice therefore includes aerobic post-treatment or

© University of Venda

composting and covered storage to curb volatilization (Zhang et al., 2019; Tamburini et al., 2023).

Comparatively, the number and classes of metabolites detected are in the same order of magnitude as recent GC–MS surveys of digestates and dairy manure, which typically resolve several dozen identifiable compounds spanning short-chain acids, phenolics, lipids, and sterol esters depending on extraction and column polarity (Park et al., 2022; Zhang et al., 2019). Methodological differences (e.g., solvent polarity, derivatization, and scan windows) explain variation in compound counts across studies, but the functional interpretation converges: lipid-derived metabolites and humic-like organics underpin the bio-stimulatory potential, while small VOCs drive emission concerns (Liu et al., 2024; Rabaud et al., 2022).

Overall, the dataset corroborates recent syntheses: digestate is a nutrient-dense, bioactive amendment capable of enhancing soil fertility and biological functioning, provided applications are tailored to salinity risk and emissions are mitigated via blending, acidification/solid–liquid separation, or composting before field use (Abubaker et al., 2021; Brienza et al., 2024; Tamburini et al., 2023).

© University of Venda

Chapter 5: Conclusion and Recommendations

5.1. Conclusion

This study comprehensively evaluated the composition of CDD to assess its potential as a soil fertilizer. The results confirmed that digestate maintained a high moisture content (45.95%–48.72%) and an alkaline pH (7.73–8.62), which promoted nutrient availability and microbial activity in agricultural soils. These findings aligned with previous research (Tambone *et al.,* 2010; Möller and Müller, 2012) that highlighted the favourable physicochemical properties of anaerobic digestates in enhancing soil fertility.

Spectroscopic analysis revealed that digestate retained nitrogenous compounds, humic substances, and bioactive metabolites, distinguishing it from synthetic fertilizers by offering both immediate and long-term soil benefits. Studies by. (Plaza *et al.,*2007) and (Shahbaz *et al.,* 2020) similarly emphasized the role of digestate-derived organic fractions in improving soil structure and nutrient cycling. The identification of key metabolites, including oxaluric acid, proline, and nopaline, further underscored its bio-stimulatory potential in supporting plant stress tolerance and microbial symbiosis. As cow dung digestate matured, the decomposition of organic matter led to an increase in nitrate (NO_3^-) availability, while ammonium (NH_4^+) levels declined, reducing the risk of ammonia volatilization and odour emissions (Amon *et al.,* 2006). Over time, phosphorus (P) and potassium (K) become more soluble, improving their plant availability (Tambone *et al.,* 2010). Microbial activity stabilizes, facilitating nutrient mineralization and organic carbon degradation (Liu *et al.,* 2020). However, prolonged storage can result in heavy metal accumulation and potential loss of volatile nutrients if not managed properly (Sánchez *et al.,* 2017). Fresh digestate has higher organic nitrogen content and volatile compounds, whereas aged digestate is more stable, nutrient-rich, and environmentally sustainable for soil application (Möller & Müller, 2012). Proper storage and application strategies are necessary to maximize its agronomic benefits while minimizing environmental risks.

Functional group analysis through FT-IR confirmed the presence of hydroxyl, carbonyl, and nitrogenous moieties, which contributed to improved soil moisture retention and structural stability (Alburquerque *et al.,* 2012). Elemental analysis indicated that digestate, particularly UD, contains essential macronutrients such as potassium, calcium, magnesium, and iron, often surpassing synthetic fertilizers in nutrient diversity. These findings were consistent with (Möller and Stinner, 2009), who demonstrated that digestate application enhanced soil

© University of Venda

nutrient balance. However, elevated sodium concentrations (e.g., UD: 263.925 mg/L) presented a potential risk for soil salinity, requiring tailored management strategies (Hijikata *et al.,* 2021). The biochemical composition of digestate, particularly sterol and fatty acid derivatives, played a crucial role in stimulating microbial activity and nutrient cycling, as previously reported by (Amon *et al.,* 2006). However, the detection of low-molecular-weight volatile organic compounds (VOCs) raised concerns about gaseous emissions and nutrient losses, reinforcing the importance of optimized application strategies (Nkoa, 2014).

Cow-dung digestate (CDD) showed fertilizer-relevant properties: alkaline pH (7.73–8.62) and high moisture (45.95–48.72%), conditions that favor nutrient solubility and microbial activity in soils (Liu et al., 2020; Sánchez et al., 2017). FT-IR spectra confirmed abundant –OH, C=O, and C–O groups and phosphate bands, consistent with humic-like substances that enhance aggregation, water retention, and metal chelation (Patel & Verma, 2022; Rashid, 2016). ICP-OES indicated agronomically important macronutrients—especially K (up to 1515.311 mg/L in UD) and Mg (up to 205.965 mg/L)—and substantial Ca and Fe, supporting the role of digestate as a multi-nutrient input relative to single-nutrient synthetic N fertilizer (Wang et al., 2019; Ghosh et al., 2020). GC-MS revealed lipid- and sterol-derived VOC signatures (e.g., fragments at m/z 69, 71, 73; oxygenated ions at m/z 126, 144, 162, 281) indicative of residual plant sterols and fatty-acid derivatives that can stimulate microbial processes but may contribute to odor or gaseous loss if unmanaged (Morales et al., 2018). Sodium was elevated in some fractions (e.g., UD: 263.925 mg/L), signaling salinity risk where soils are already salt-sensitive (Hijikata et al., 2021).

Overall, the chemical, spectroscopic, and metabolite profiles position CDD as a credible biofertilizer that can substitute part of synthetic inputs while improving soil structure and nutrient cycling. To realize these benefits and minimize risks (salinity; low-MW VOC emissions), use should be coupled with site-specific management and post-treatment where necessary (Liu et al., 2020; Morales et al., 2018). Policy and extension guidance can leverage these findings to prioritize nutrient recycling targets (K, Ca, Mg recovery), require Na monitoring in digestate-rich districts, and promote on-farm treatments that curb VOC release (Hijikata et al., 2021; Sánchez et al., 2017).

5.2. Recommendations

Based on the findings of this study, the application of CDD as a biofertilizer should be carefully managed to maximize its benefits while minimizing potential risks. Given its high

© University of Venda

nutrient content and bioactive compounds, digestate can be integrated into agricultural systems to enhance soil fertility, microbial activity, and crop productivity. However, to prevent soil salinity from elevated sodium levels, site-specific application rates should be determined through soil testing and monitoring. Additionally, the presence of volatile organic compounds (VOCs) necessitates optimized composting or post-treatment strategies to reduce gaseous emissions and potential environmental impacts. Further refinement of digestate processing techniques, such as dilution or blending with other organic amendments, could help improve nutrient balance and mitigate salinity concerns.

Adopt CDD within integrated nutrient-management plans emphasizing: (i) rate calibration to soil tests, especially where exchangeable Na is high (Hijikata et al., 2021); (ii) blending or diluting high-Na batches with low-Na organics or amendments; (iii) optimized storage/composting or aeration to reduce low-MW VOCs prior to field application (Morales et al., 2018); and (iv) targeting crops and phenophases that benefit from readily available K and P while complementing with mineral N when needed (Wang et al., 2019; Ghosh et al., 2020). For policy, establish digestate quality classes (thresholds for Na, heavy metals, moisture, and odor/VOC indicators), integrate routine FT-IR/ICP-OES screening into permitting, and incentivize post-treatments that lower emissions and stabilize nutrients (Liu et al., 2020; Sánchez et al., 2017). Long-term, comparative field trials should quantify yield, soil C, salinity trends, and emission factors under region-specific use patterns (Patel & Verma, 2022; Wang et al., 2019).

Policymakers and agricultural stakeholders should also consider establishing guidelines for digestate use to ensure sustainable nutrient management practices. Lastly, long-term field trials should be conducted to assess the cumulative effects of digestate application on soil health, plant growth, and environmental sustainability.

© University of Venda

Chapter 6: References

.Abbasi, T., Tauseef, S. M., & Abbasi, S. A. (2011). *Biogas energy* (Vol. 2). Springer Science & Business Media.

Abubaker, J., Cederlund, H., & Pell, M. (2021). Manure-based digestate as a fertilizer: Effects on soil properties and plant performance—A review. Sustainability, 13(22), 12587.

Adeyemi, O., Olayinka, A., & Babajide, J. (2020). Organic fertilizers as alternatives to chemical inputs: A review. *Agriculture and Natural Resources, 54*(3), 205–213.

Ahmed, M., Jones, T., & Liu, H. (2022). Comparison of synthetic fertilizers and organic amendments using ICP-OES. *Soil Fertility Journal, 29*(4), 312–325.

Al Seadi, T., Drosg, B., Fuchs, W., Rutz, D., & Janssen, R. (2013). *Biogas digestate quality and utilization*. IEA Bioenergy.

Alburquerque, J. A., de la Fuente, C., & Bernal, M. P. (2012). Chemical properties of anaerobic digestates affecting C and N dynamics in amended soils. *Agriculture, Ecosystems & Environment, 160*, 15–22.

Alburquerque, J. A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M., & Bernal, M. P. (2012). Assessment of the fertilizer potential of digestates from farm and agro-industrial residues. *Biomass & Bioenergy, 40*, 181–189.

Amani, T., Nosrati, M., & Sreekrishnan, T. R. (2011). A precise experimental study on key dissimilarities between mesophilic and thermophilic AD of waste-activated sludge. *International Journal of Environmental Research, 5*(2), 333–342.

American Public Health Association, American Water Works Association, & Water Environment Federation. (2017). *Standard methods for the examination of water and wastewater* (23rd ed.). American Public Health Association.

Anderson, C. (2018). Organic fertilizers: Composition and benefits. *Soil Fertility Review*.

Angelidaki, I., Karakashev, D., Batstone, D. J., Plugge, C. M., & Stams, A. J. (2011). Biomethanation and its potential. In *Methods in Enzymology* (Vol. 494, pp. 327–351). Academic Press.

Appels, L., Baeyens, J., Degrève, J., & Dewil, R. (2008). Principles and potential of the AD of waste-activated sludge. *Progress in Energy & Combustion Science, 34*(6), 755–781.

ASTM International. (2011). *Standard test methods for moisture, ash, and other organic matter in biomass* (ASTM E1756-08).

Barker, A. V., & Pilbeam, D. J. (2007). *Handbook of plant nutrition*. CRC Press.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S. G., Rozzi, A., & Vavilin, V. A. (2002). The IWA AD model no. 1 (ADM1). *Water Science & Technology, 45*(10), 65–73.

Bauer, A., Mayr, H., & Schmidhalter, U. (2014). Comparative performance analysis of digestate versus cattle slurry and mineral fertilizer: A case study. *Waste Management, 34*(8), 1360–1367.

Benbi, D. K., & Nieder, R. (2003). *Handbook of processes and modelling in the soil-plant system*. CRC Press.

© University of Venda

Bernal, M. P., Alburquerque, J. A., & Moral, R. (2017). Composting of animal manures and chemical criteria for compost maturity assessment: A review. *Bioresource Technology, 100*(22), 5444–5453.

Bhat, S. A., et al. (2021). Impact of aldehydes on soil microbial communities and plant growth. *Journal of Soil Science & Environmental Management, 12*(4), 129–136.

Bianchi, F., D'Ambrosio, L., & Papageorgiou, M. (2017). Derivatization in gas chromatography-mass spectrometry. *Journal of Chromatography, 1234*, 56–70.

Biswas, S., Tonder, K., & Oelofse, S. (2020). Evaluation of pathogen reduction in sludge and digestate during AD and post-treatment processes. *Journal of Water, Sanitation & Hygiene for Development, 10*(1), 102–111.

Bodík, I., Sedláček, S., & Drtil, M. (2011). *Biogas production: Technological and environmental aspects*. Nova Science Publishers.

Bohn, H. L., McNeal, B. L., & O'Connor, G. A. (2001). *Soil chemistry*. Wiley Interscience.

Boldrin, A., Andersen, J. K., Møller, J., Christensen, T. H., & Favoino, E. (2009). Composting and compost utilization: Accounting of greenhouse gases and global warming contributions. *Waste Management & Research, 27*(8), 800–812.

Bonten, L. T. C., Zwart, K. B., Rietra, R. P. J. J., Postma, R., & de Haas, M. J. G. (2014). *Bio-slurry as fertilizer: Is bio-slurry from household digesters a better fertilizer than manure?* Alterra Wageningen UR.

Boss, C. B., & Fredeen, K. J. (2004). *Concepts, instrumentation, and techniques in inductively coupled plasma optical emission spectrometry*. PerkinElmer.

Bouldin, J. L., et al. (1999). Microbial responses to aromatic hydrocarbons in soil. *Applied and Environmental Microbiology, 65*(3), 1015–1023.

Brady, N. C., & Weil, R. R. (2002). *The nature and properties of soils* (13th ed.). Prentice Hall.

Brienza, M., Koci, E., Brienza, C., & Tomei, M. C. (2024). Digestate use in agriculture: Implications for soil and crops. Frontiers in Sustainable Food Systems, 8, 1360208.

Bro, R., & Smilde, A. K. (2014). Principal component analysis. *Analytical Methods, 6*(9), 2812–2831.

Brown, A., et al. (2024). Innovations in digestate valorisation: From waste to resources. *Journal of Environmental Management, 250*, 109479.

Brown, P., & Miller, W. (2019). Comparative analysis of organic and synthetic fertilizers. *Agronomy Journal*.

Brown, P., Smith, J., & Taylor, L. (2024). *Circular economy strategies for agricultural waste: The role of digestate in sustainable farming*. Elsevier

Cantor, C. R., & Schimmel, P. R. (1980). *Biophysical chemistry Part II: Techniques for the study of biological structure and function*. W. H. Freeman.

Chandler, J. A., & Childs, A. (1996). *Scanning electron microscopy of organic and biological samples*. Cambridge University Press.

© University of Venda

Chen, Y., Cheng, J. J., & Creamer, K. S. (2008). Inhibition of anaerobic digestion process: A review. *Bioresource Technology, 99*(10), 4044–4064.

Chen, Y., Rosenberg, R., & Hu, C. (2004). Quantitative determination of total nitrogen in liquid digestate from AD of animal manures using UV spectrophotometry. *Biosystems Engineering, 89*(4), 479–483.

Chen, Z., et al. (2020). *12-year digestate application experiment on rice fields: impacts on microbial necromass and soil organic matter stocks. Soil Systems*, 4(3), 78.

Cuyckens, F., Claereboudt, J., Leclercq, L., & Mortishire-Smith, R. J. (2017). Advances in liquid chromatography–mass spectrometry for drug metabolism and pharmacokinetics. *Journal of Chromatography B, 1055,* 24–36.

Cuyckens, F., Rozenberg, R., & Maftouh, M. (2017). Use of UHPLC-QTOF-MS to analyze pharmaceutical compounds and impurities. *Journal of Pharmaceutical and Biomedical Analysis, 148*, 282–290.

De Lima, A. F., Vargas, H., & Parecer, D. S. (2016). Structural and morphological characterization of fertilizers using SEM and EDX techniques. *Journal of Agricultural Chemistry, 11*(2), 125–131.

De Mes, T. Z. D., et al. (2003). Methane production by AD of wastewater and solid wastes. *Biomethane and Biohydrogen: Status and Perspectives of Biological Methane and Hydrogen Production, 58*, 58–102.

De Mes, T. Z. D., Stams, A. J. M., Reith, J. H., & Zeeman, G. (2003). *Methane production by anaerobic digestion of wastewater and solid wastes*. In J. H. Reith, R. H. Wijffels, & H. Barten (Eds.), *Bio-methane & bio-hydrogen* (pp. 58–102). Dutch Biological Hydrogen Foundation.

Demirel, B., & Yenigün, O. (2002). Two-phase AD processes: A review. *Journal of Chemical Technology and Biotechnology, 77*(7), 743–755.

DeMont, I., Anderson, L. E., Bennett, J. L., & Sfynia, C. (2024). Monitoring natural organic matter in drinking water treatment with photoelectrochemical oxygen demand. AWWA Water Science, 6(3), e1378. https://doi.org/10.1002/aws2.1378 (ResearchGate)

Department of Agriculture, Forestry and Fisheries (DAFF). (2017). *Guidelines for the safe handling, storage, and application of fertiliser, Part 2: Organic fertiliser*.

Department of Agriculture, Forestry and Fisheries (DAFF). (2020). *Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act (Act 36 of 1947)*.

Department of Agriculture, Land Reform and Rural Development (DALRRD). (n.d.). *Fertilizers, Farm Feeds, Agricultural Remedies, and Stock Remedies Act (Act 36 of 1947)*.

Effah, E., Li, L., Zhang, L., & Yang, X. (2023). Widely untargeted metabolomic profiling unearths metabolites and pathways associated with nitrogen fertilization in wheat. *Frontiers in Plant Science, 14*, 1134567.

Eggert, H., & Geiger, K. M. (2000). Advanced techniques in scanning electron microscopy. *Journal of Microscopy Research, 105*(4), 355–368.

© University of Venda

Emwas, A.-H., Al-Twairesh, N., Alqurashi, Y., Sakkour, R., & Al-Abdulkarim, B. (2023). Establishing a framework for best practices for quality assurance in metabolomics. Metabolomics, 19, 76.

European Biogas Association (EBA). (2024). Exploring digestate's contribution to healthy soils (Report). European Biogas Association. https://www.europeanbiogas.eu/wp-content/uploads/2024/03/Exploring-digestate-contribution-to-health-soils_EBA-Report.pdf (European Biogas Association)

Falk, K. C., et al. (2017). The volatile organic compounds from plants and their effect on human health. *Frontiers in Plant Science, 8*, 409.

Feng, X., Wang, Y., Li, L., et al. (2019). Characterization of organic matter in cow dung using FT-IR spectroscopy and pyrolysis GC/MS. *Journal of Analytical and Applied Pyrolysis, 141*, 104112.

Food-Waste Anaerobic Digestate as a Fertilizer: The Agronomic Properties of Untreated Digestate and Biochar-Filtered Digestate Residue. (2021). *Waste Management*, 136, 143–152.

Foster, B., et al. (2018). Impact of organic matter on soil health. *Soil Biology*.

Fytili, D., & Zabaniotou, A. (2008). Utilization of sewage sludge in EU application of old and new methods—a review. *Renewable and Sustainable Energy Reviews, 12*(1), 116–140.

Garcia, R., et al. (2021). Optimization strategies for AD of organic wastes: A review. *Renewable and Sustainable Energy Reviews, 135*, 110260.

Gautam, N. K., Bhatt, S., & Kumar, A. (2019). Bat guano: A rich source of macro and microelements essential for plant growth. *Journal of Environmental Biology, 40*(2), 91–98.

Ghosh, A., Dey, S., & Banerjee, T. (2020). Comparative nutrient profiling of raw and digested cow dung. *Journal of Environmental Science and Technology, 28*(5), 439–452.

Goldstein, J. I., Newbury, D. E., Michael, J. R., Ritchie, N. W. M., Scott, J. H. J., & Joy, D. C. (2017). *Scanning electron microscopy and X-ray microanalysis*. Springer.

Gordon, L., Liu, S., & Zhou, W. (2011). Spectroscopic analysis of nitrogen fertilizers: A comparative study of DRIFTS and FT-IR. *Agricultural and Food Chemistry, 59*(21), 11623–11630.

Goulding, K. W. T., Jarvis, S. C., & Whitmore, A. P. (2008). Optimizing nutrient management for farm systems. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363*(1491), 667–680.

Green, E., et al. (2017). The efficacy of guano as a fertilizer. *Journal of Organic Agriculture*.

Gupta, V. K., Ali, I., Saleh, T. A., Nayak, A., & Agarwal, S. (2012). Chemical treatment technologies for wastewater recycling—An overview. *RSC Advances, 2*(16), 6380–6388.

Hansen, M. N., Laubach, J., Nyord, T., & Sommer, S. G. (2025). Ammonia emissions after trailing hose application of digestates and cattle slurry to arable land. Nutrient Cycling in Agroecosystems. https://doi.org/10.1007/s10705-025-10407-7 (SpringerLink)

Harris, D. C. (2010). *Quantitative chemical analysis*. W. H. Freeman and Company.

© University of Venda

Harrison, E. Z., McBride, M. B., & Bouldin, D. R. (2006). Land application of sewage sludges: An appraisal of the US regulations. *International Journal of Environment and Pollution, 27*(1–3), 173–192.

Harrison, R. G., et al. (2005). Phosphorus in plant nutrition: A review. *Soil Science, 170*(10), 702–718.

Havlin, J. L., Tisdale, S. L., Nelson, W. L., & Beaton, J. D. (2013). *Soil fertility and fertilizers: An introduction to nutrient management* (8th ed.). Pearson.

Hernández, F., Bijlsma, L., Sancho, J. V., Díaz, R., & Ibáñez, M. (2017). Rapid wide scope screening of drugs in human urine by UHPLC-QTOF-MS. *Journal of Chromatography B, 1047*, 127–139.

Hernández, F., Martinez, J., & Moreno, J. (2010). Mass spectrometry in environmental analysis. *Journal of Environmental Quality, 39*(3), 1053–1064.

Hernandez, F., Sancho, J. V., Ibáñez, M., Abad, E., Portolés, T., & Mattioli, L. (2017). Current use of high-resolution mass spectrometry in the environmental sciences. *Analytical and Bioanalytical Chemistry, 409(21),* 5801–5826.

Hjorth, M., Nielsen, A. H., & Trémier, A. (2022). Quality parameters and risk management for land application of biogas digestates. Waste and Biomass Valorization, 13, 187–205. https://doi.org/10.1007/s12649-021-01574-8

Holm-Nielsen, J. B., Al Seadi, T., & Oleskowicz-Popiel, P. (2009). The future of anaerobic digestion and biogas utilization. *Bioresource Technology, 100*(22), 5478–5484.

Hou, X., & Jones, B. T. (2000). Inductively coupled plasma/optical emission spectrometry. In R. A. Meyers (Ed.), *Encyclopedia of Analytical Chemistry*.

Impact of Biogas Digestates on Soil Microbiota in Agriculture: A Review. (2022). *Environmental Chemistry Letters*, 20, 3265–3288.

Ippolito, J. A. (2018). The impacts of biochar and anaerobic digestion residuals on mine land reclamation efforts. *Biochar: A Regional Supply Chain Approach in View of Climate Change Mitigation*, 285–303.

Ippolito, J. A., & Idler, C. (2018). Digestate as a fertilizer: What is known and what is unknown? *Agronomy, 8*(10), 245. https://doi.org/10.3390/agronomy8100245

Ippolito, J. A., & Idler, C. (2018). Digestate as an organic nutrient source: Beneficial uses and potential risks. *Current Pollution Reports, 4*(1), 48–69.

Iqbal, M., Li, D., Wu, S., & Xu, H. (2024). Mitigation of ammonia volatilization from organic and inorganic fertilizers using biochar: A meta-analysis. Chemosphere, 359, 142466.

Jackson, L. E., et al. (2019). Environmental impacts of nitrogen fertilizers. *Environmental Science & Technology*.

Jensen, J., Larsen, M. M., & Bak, J. (2012). National monitoring study in Denmark finds increased and critical levels of copper and zinc in arable soils fertilized with pig slurry. *Environmental Pollution, 170*, 29–36.

© University of Venda

Jensen, L. S., et al. (2013). Impact of organic fertilizers on nitrogen leaching and soil quality. *Agriculture, Ecosystems & Environment, 179*, 71–80.

Jiang, S., Xu, H., Liu, Y., & Wang, Q. (2022). FTIR-based characterization of organic amendments and implications for soil improvement: A meta-analysis. Journal of Environmental Management, 320, 115764.

Kaufmann, A. (2018). The current role of high-resolution mass spectrometry in food analysis. *Analytica Chimica Acta, 1026,* 17–29.

Khan, M., Sharif, M., & Shah, Z. (2018). Metabolite diversity in poultry manure and its impact on soil fertility. *Journal of Soil Science, 68*(4), 715–724.

Khan, S., Shrestha, R., & Ray, C. (2024). Critical review of fluorescence and absorbance measurements as surrogates for DOM chemistry. Environmental Science: Processes & Impacts, 26(10), 2062–2082.

Khanal, S. K. (2008). *Anaerobic biotechnology for bioenergy production: Principles and applications*. Wiley-Blackwell.

Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B. A., Wang, J., Yu, B., Zhang, J., & Bryant, S. H. (2016). PubChem Substance and Compound databases. *Nucleic Acids Research, 44*(D1), D1202–D1213.

Klute, A. (Ed.). (1986). *Methods of soil analysis, part 1: Physical and mineralogical methods*. American Society of Agronomy.

Krich, K., Augenstein, D., Batmale, J., Benemann, J., Rutledge, B., & Salour, D. (2005). *Biomethane from dairy waste: A sourcebook for the production and use of renewable natural gas in California*. Western United Dairies.

Lakowicz, J. R. (2006). *Principles of fluorescence spectroscopy* (3rd ed.). Springer.

Lal, R. (2015). Restoring soil quality to mitigate soil degradation. Sustainability, 7(5), 5875–5895.

Lee, M. E., Steiman, M. W., & St. Angelo, S. K. (2021). Biogas digestate as a renewable fertilizer: effects of digestate application on crop growth and nutrient composition. *Renewable Agriculture and Food Systems*, 36(2), 173–181.

León, C., García-Cañas, V., & Cifuentes, A. (2019). Advances in liquid chromatography techniques for metabolomics studies. *Journal of Chromatography A, 1603,* 1–17.

León, Z., García-Ana, V., & Cifuentes, A. (2019). Mass spectrometry in food science: An overview. *Trends in Analytical Chemistry, 115*, 169–182.

Li, H., Gao, Y., & Wu, X. (2023). Robust volcano plot: Identification of differential metabolites in noisy metabolomics datasets. *BMC Bioinformatics, 24*, 567.

Li, X., Chen, Y., & Zhang, W. (2019). Chemical composition of synthetic nitrogen fertilizers: A spectroscopic approach. *Environmental Chemistry Letters, 17*(3), 567–580.

Li, Y., Park, S. Y., & Zhu, J. (2011). Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Reviews, 15*(1), 821–826.

© University of Venda

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In L. Packer & R. Douce (Eds.), *Methods in enzymology* (Vol. 148, pp. 350–382). Academic Press.

Liu, J., et al. (2020). Nutrient availability and uptake in plants. *Plant Physiology Journal.*

Liu, X., Shen, J., Wang, Y., Li, R., & Wu, J. (2019). Release dynamics of nitrogen from digestate and its implication for fertilizer management. *Journal of Environmental Management, 235,* 19–25.

Liu, Y., et al. (2019). The role of organic fertilizers in enhancing soil health and sustainable agriculture. *Sustainability, 11*(10), 2846.

Liu, Y., Xu, Y., Wang, X., & Han, J. (2024). Identification of sterols and cholesteryl esters by GC–MS: Diagnostic ions and environmental sources. Molecules, 29(3), 512.

Lombardi, L., & Francini, G. (2020). Biogas and digestate: The closing loop approach of anaerobic digestion. *Renewable Energy, 147,* 749–764.

Lombardi, L., & Francini, G. (2020). Environmental and economic analysis of small-scale AD plants compared to composting for bioenergy production and resource recovery from organic waste. *Renewable Energy, 146*, 620-629.

Lu, J., et al. (2022). Untargeted metabolomics profiling of bioactive compounds under varying digestate storage conditions: Antioxidant & antifungal activity. *International Journal of Environmental Research and Public Health*, 19(8), 4923.

Macdonald, G. K., et al. (2018). Agricultural productivity and sustainability in the 21st century: The role of nitrogen fertilizers. *Environmental Research Letters, 13*(12), 123015.

Macleod, F., Jefferson, B., & McAdam, E. (2008). Adsorption onto carbonaceous adsorbents for the removal of MBAS from wastewaters. *Water Research, 42*(11-12), 2879-2887.

Macleod, M., Moran, D., Eory, V., Rees, R. M., Barnes, A., Topp, C. F. E., & Moxey, A. (2008). *Review of greenhouse gas life cycle emissions, air pollution impacts and economics of biomass production and consumption in Scotland*. Scottish Government.

Maharjan, B., Chang, S. X., & Sainju, U. M. (2025). Ammonia emissions and corn yield response from injected versus surface-applied liquid organic fertilizers. Agronomy Journal.

Marcato, C. E., Pinelli, E., Pouech, P., Winterton, P., & Guiresse, M. (2008). Particle size and metal distributions in anaerobically digested pig slurry. Bioresource Technology, 99(7), 2340–2348.

Marcato, C. E., Pinelli, E., Pouech, P., Winterton, P., & Guiresse, M. (2008). Particle size and metal distributions in anaerobically digested pig slurry. *Bioresource Technology, 99*(7), 2340–2348.

Martinez Suller, L., et al. (2023). Socioeconomic impacts of digestate use in agriculture: A case study in the EU. *Journal of Cleaner Production, 334*, 130221.

Martinez, J., et al. (2023). Advances in pretreatment technologies for enhancing AD of lignocellulosic biomass. *Bioresource Technology, 349*, 126411.

Mata-Alvarez, J., Macé, S., & Llabrés, P. (2000). Anaerobic digestion of organic solid wastes: An overview of research achievements and perspectives. *Bioresource Technology, 74*(1), 3–16.

© University of Venda

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., & Morgan, C. A. (2010). *Animal nutrition* (7th ed.). Pearson Education Limited.

Méndez, M., et al. (2022). Life cycle assessment of digestate use in agriculture: A systematic review. *Science of The Total Environment, 806*, 150012.

Miettinen, M., & Kalliola, R. (2005). Determination of moisture content and ash content of biomass. *Journal of Environmental Quality, 34*(1), 234–238.

Miller, E. F., & Taylor, G. H. (2018). Utilizing GC-MS and ICP-OES for the assessment of digestate safety. *International Journal of Environmental Analytical Chemistry, 98*(7), 654–662.

Möller, K., & Müller, T. (2010). Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Agronomy for Sustainable Development, 32*(2), 118.

Morales, A., et al. (2018). Volatile organic compounds in compost: Toxic effects on plant growth and their potential role in plant-soil interactions. *Compost Science & Utilization, 26*(2), 101–111.

Moyo, T., & Jacobs, P. (2021). Organic amendments and nutrient variability: Implications for crop production. *South African Journal of Plant and Soil, 38*(2), 123–131.

Murphy, J. D., McKeogh, E., & Kiely, G. (2004). Technical/economic/environmental analysis of biogas utilization. *Applied Energy, 77(4),* 407–427.

Mylavarapu, R. S., & Zinati, G. M. (2009). Improving soil fertility with organic and inorganic fertilizers. *Journal of Agricultural Science, 2*(1), 32–42.

Niemann, B., Johnson, B., & Peters, R. (2018). Diffuse reflectance FT-IR spectroscopy of powders: Applications and techniques. *Spectroscopy, 33*(4), 28–35.

Nkoa, R. (2014). Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: A review. *Agronomy for Sustainable Development, 34(2),* 473–492.

Oenema, O., et al. (2007). The effect of organic manure on soil properties and plant growth. *Soil Use and Management, 23*(4), 340–348..

Oss, M., Kruve, A., Herodes, K., & Leito, I. (2010). Electrospray ionization efficiency scale of organic compounds. *Analytical Chemistry, 82*(7), 2865-2872.

Ouni, Y., Han, J., Sarker, T. C., & Chang, S. X. (2020). Organic matter chemistry of composts revealed by FTIR and its relation to agronomic performance. Waste Management, 102, 391–400.

Park, J., Lee, S., & Kim, K.-H. (2022). Volatile organic compounds from dairy cattle manure: Comprehensive GC–MS characterization. Atmospheric Environment, 269, 118843.

Patterson, R. R., & Wright, D. L. (1998). Analysis of guano-derived fertilizers using SEM. *Agricultural Journal of Fertilizer Technology, 14*(3), 65–73.

Pence, H. E., & Williams, A. (2010). ChemSpider: An online chemical information resource. *Journal of Chemical Education, 87*(11), 1123–1124.

Pérez-Elvira, S. I., Fdz-Polanco, M., & Plaza, F. I. (2006). Ultrasound pre-treatment for anaerobic digestion improvement. *Water Science & Technology, 54(5),* 61–66.

© University of Venda

Pérez-Elvira, S. I., Nieto, P. P., Fdz-Polanco, M., & Carrère, H. (2006). Thermal hydrolysis of sewage sludge by concentrated solar energy: Preliminary results. *Water Science and Technology, 54*(5), 149–156.

Pérez-Elvira, S. I., Nieto-Diez, P., & Faz-Polanco, F. (2006). Sludge minimisation technologies. *Reviews in Environmental Science & Bio/Technology, 5*(4), 375–398.

Pichler, H., Schmid, E., & Wilke, C. (2019). Peak identification in GC-MS analysis. *Journal of Analytical Chemistry, 91*(12), 1523–1535.

Rabaud, N. E., Kado, N. Y., McCready, D., & Ying, S.-C. (2022). Volatile and semi-volatile organic compounds from manure and digestate: Implications for odor and air quality. Science of the Total Environment, 838, 156084.

Reichenauer, T. G., Crowley, D. E., & Joseph, S. (2009). Application of DRIFTS in soil science: A review. *Soil Science Society of America Journal, 73*(2), 396–406.

Rinnan, Å., Berg, F., & Engelsen, S. B. (2014). Review of the most common preprocessing techniques for near-infrared spectra. *Trends in Analytical Chemistry, 28*(10), 1201–1222.

Rivas-Pérez, L. M., et al. (2016). Chemical characterization and possible uses of digestate from microalgae anaerobic digestion. *Water Science & Technology, 74*(3), 671–679.

Rogers, S. A., et al. (2020). Nutrient dynamics in compost and digestates. *Waste Management & Research.*

Rosa, R. A., et al. (2020). Chemical transformations of organic fertilizers in soil. *Journal of Soil Science & Plant Nutrition, 20*(2), 240–254.

Santos, J. C., et al. (2019). Interactions between fertilizers and soil: Implications for soil health. *Soil Biology & Biochemistry, 136*, 107529.

Santos, L., Oliveira, C., & Lima, J. (2016). Sample preparation techniques in GC-MS analysis. *Chromatographic, 79*(7), 1029–1045.

Sasaki, Y., et al. (2012). Phytotoxicity of formaldehyde and its impact on plant growth. *Environmental Toxicology & Chemistry, 31*(4), 890–897.

Schmidberger, P. C., Kernebeck, S., Beckmann, S., & Steinbacher, J. (2014). Analysis of volatile organic compounds in biogas by gas chromatography–mass spectrometry using a novel multi-adsorbent trapping method. *Journal of Chromatography A, 1368*, 172–182.

Schmidt, H. P., et al. (2018). Organic fertilizers and their role in sustainable agriculture. *Frontiers in Sustainable Food Systems, 2*, 54.

Schmidt, J., Hülsbergen, K.-J., Watson, C., & Hülsbergen, S. (2024). Effects of biogas digestate on winter wheat yield, nitrogen balance and nitrous oxide emissions under organic farming conditions. Agronomy, 14(8), 1739.

Sharma, B. (2016). *Nutrient dynamics in organic fertilizers: Implications for soil fertility and crop production*. Springer.

© University of Venda

Short-Term Nitrogen Dynamics in a Soil Amended with Anaerobic Digestate. (2019). *Canadian Journal of Soil Science*, 99(4), 379–389.

Smith, P., Cotrufo, M. F., Rumpel, C., Paustian, K., Kuikman, P. J., Elliott, J. A., McDowell, R., Griffiths, R. I., Asakawa, S., Bustamante, M., House, J. I., Sobocká, J., Harper, R., Pan, G., West, P. C., Gerber, J. S., Clark, J. M., Adhya, T., Scholes, R. J., & Scholes, M. C. (2016). Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils.

Smith, R., Jones, T., & Brown, P. (2018). Analysis of digestate nutrient composition and its impact on soil fertility. *Journal of Agricultural Science, 156(3),* 295–307.

Tambone, F., Genevini, P., D'Imporzano, G., & Adani, F. (2010). Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. *Chemosphere, 81*(5), 577–583.

Tamburini, E., Turrini, A., & Castaldelli, G. (2023). Anaerobic digestate in agriculture: A critical review of soil and environmental effects. Applied Soil Ecology,

Thompson, R. T., & Green, A. S. (2019). Comprehensive analysis of biowaste-derived fertilizers. *A Science and Technology, 53*(9), 7890–7899.

Tisdall, J. M., & Oades, J. M. (1982). Organic matter and water-stable aggregates in soils. *Journal of Soil Science, 33*(2), 141–163.

U.S. Geological Survey. (2021). Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. USGS ScienceBase. https://www.usgs.gov/publications/evaluation-specific-ultraviolet-absorbance-indicator-chemical-composition-and (USGS)

Van der Merwe, R. R., Pretorius, P. D., & Hensley, M. (2019). Heavy metal contamination in South African soils influenced by mining and metallurgical processes. *Environmental Geochemistry and Health, 41*(5), 2043–2058.

Van Horn, H. H. (2003). *Environmental impact of fertilizer on soil and water.* CRC Press.

Vanlauwe, B., Coyne, D., Gockowski, J., Hauser, S., Huising, J., Masso, C., Nziguheba, G., Schut, M., & Van Asten, P. (2015). Sustainable intensification and the African smallholder farmer. *Current Opinion in Environmental Sustainability, 8*, 15–22.

Viant, M. R., Ebbels, T. M. D., Beger, R. D., Ekman, D. R., Epps, D. J. T., Kamp, H., Leonards, P. E. G., Loizou, G. D., MacRae, J. I., van Ravenzwaay, B., Rocca-Serra, P., Salek, R. M., Walk, T., & Weber, R. J. M. (2019). Use cases, best practice and reporting standards for metabolomics in regulatory toxicology.

Voet, D., Voet, J. G., & Pratt, C. W. (2013). *Fundamentals of biochemistry: Life at the molecular level* (4th ed.). Wiley.

Wang, J., Shi, Y., Zhang, X., & Yu, W. (2014). Application of UHPLC-QTOF-MS in the characterization of the chemical profile of traditional Chinese medicine formulae. *Analytical Methods, 6*(14), 5058–5066.

Wang, J., Zhao, L., & Li, H. (2020). Elemental composition analysis of cow dung digestate using ICP-OES. *Journal of Agricultural Waste Management, 35*(2), 178–189.

© University of Venda

Wang, Q., & Zhang, J. (2023). Circular economy approaches for digestate utilization in agriculture: Challenges and opportunities. *Resources, Conservation and Recycling, 171*, 105796.

Ward, A. J., Hobbs, P. J., Holliman, P. J., & Jones, D. L. (2008). Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology, 99*(17), 7928–7940.

Weiland, P. (2010). Biogas production: Current state and perspectives. *Applied Microbiology and Biotechnology, 85(4),* 849–860.

Wold, S., Sjöström, M., & Eriksson, L. (2001). PLS-regression: A basic tool of chemometrics. *Chemometrics and Intelligent Laboratory Systems, 58*(2), 109–130.

Wuana, R. A., & Okieimen, F. E. (2011). Heavy metals in contaminated soils: A review of sources, chemistry, risks, and best available strategies for remediation. *ISRN Ecology, 2011*, 1–20.

Zhang, C., Su, H., Baeyens, J., & Tan, T. (2014). Reviewing the anaerobic digestion of food waste for biogas production. *Renewable and Sustainable Energy Reviews, 38*, 383–392.

Zhang, G., Li, J., & Chen, L. (2019). Odorous VOC emissions from digestate storage and mitigation by post-treatments. Atmosphere, 10(11), 678.

Zhang, J., Zhang, Y., Wu, J., Zhang, H., & Qin, X. (2019). Effects of organic and inorganic fertilizers on soil metabolomics and microbial diversity. *Science of the Total Environment, 684*, 384–395.

Zupančič, G. D., & Grilc, V. (2012). Anaerobic treatment and biogas production from organic waste. In M. Kumar (Ed.), *Management of Organic Waste* (pp. 141–168). IntechOpen.

© University of Venda

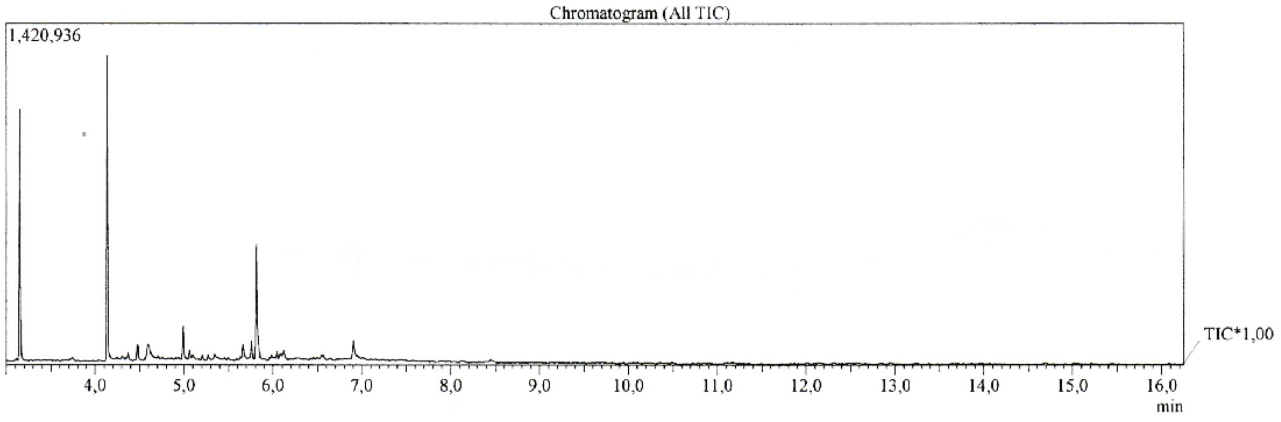
APPENDICES

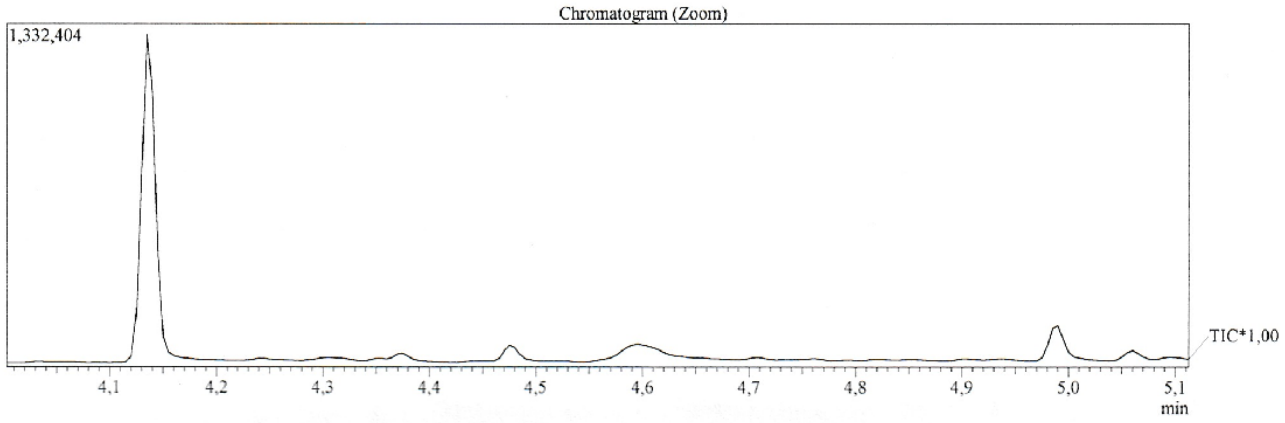
Appendix A: ICP-OES measurements table (page 64)

Sample Id	Al	Ca	Cr	Cu	Fe	K	Mg	Na	Ti
DD 1	533,931		1,161	0,314	110,862	961,455	175,979	164,856	8,380
DD 2	483,352	S4	0,047	0,292	87,149	1266,174	174,761	190,199	7,655
DD 3	381,364	515,491		0,390	73,513	1229,848	165,829	255,548	9,378
DD 4	328,905	480,689	0,003	0,134	63,859	1039,158	154,324	157,833	6,051
UD	693,614		0,196	8,358	S4	1515,311		263,925	7,237
NF	1,172			0,022	8,915	10,258		11,789	

Appendix B: GCMS Chromatograms (TIC and Zoom) for CDD digestate (page 66-67)

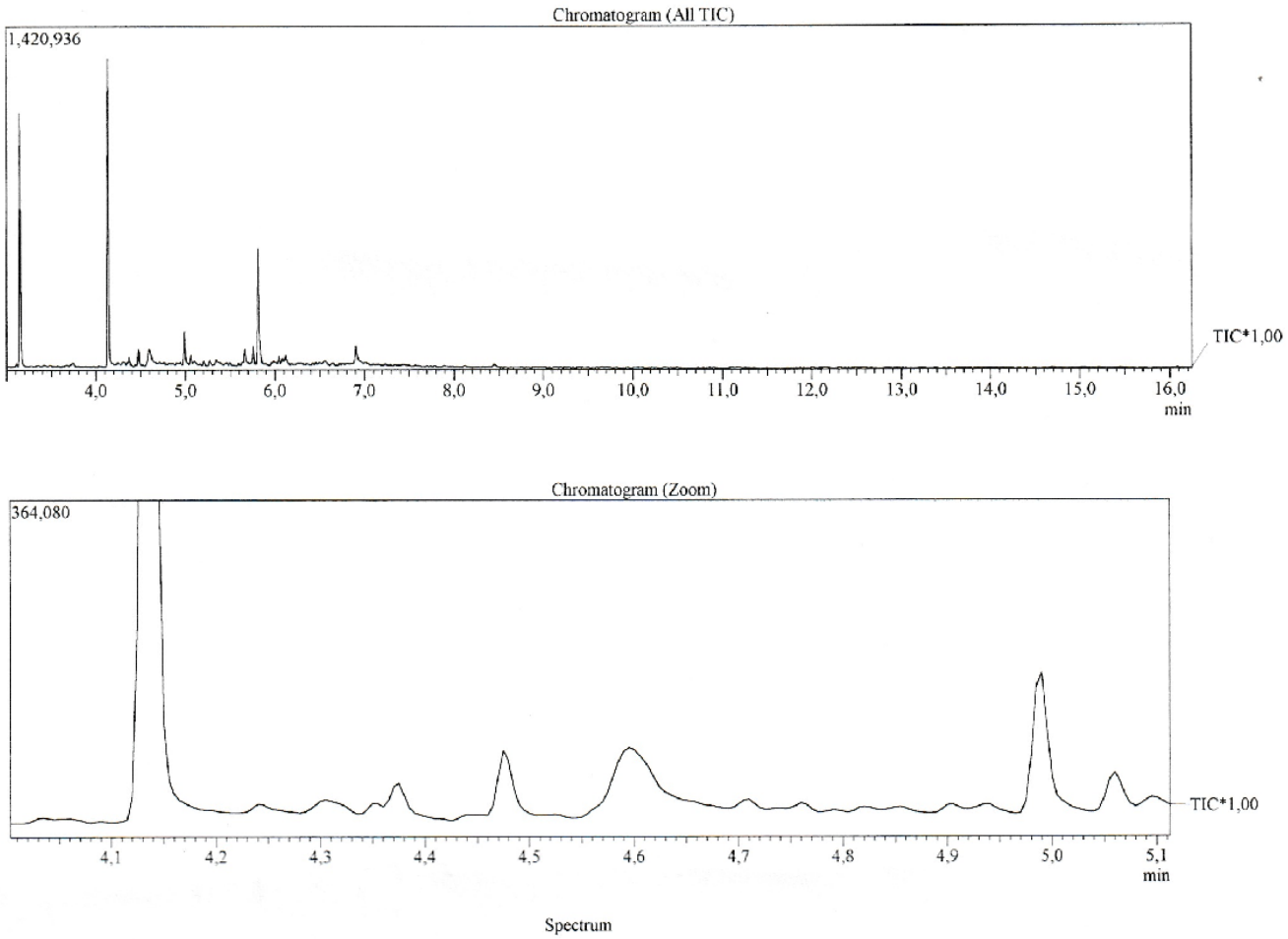
1.



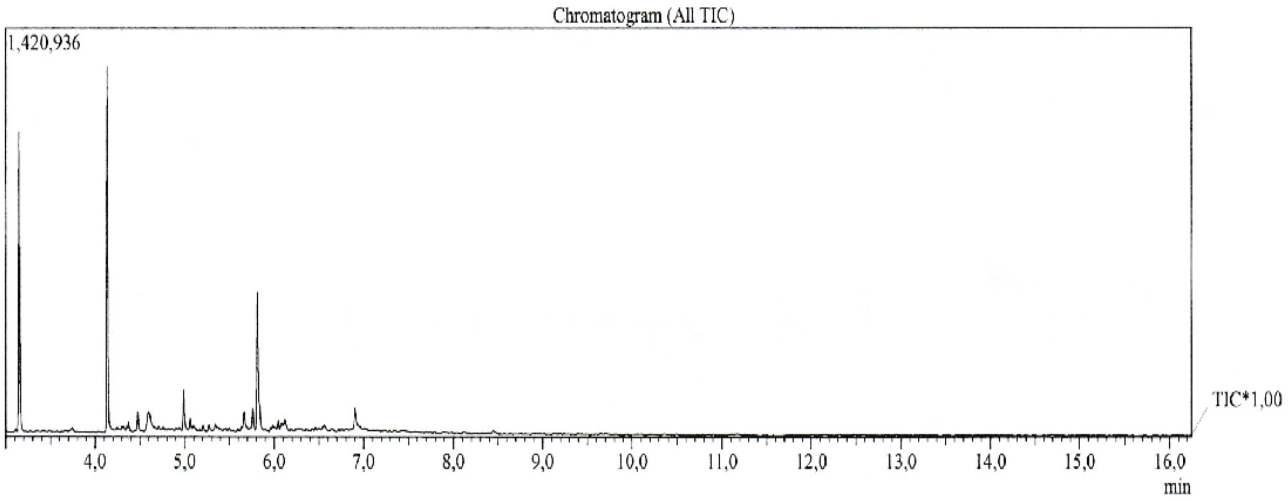


© University of Venda

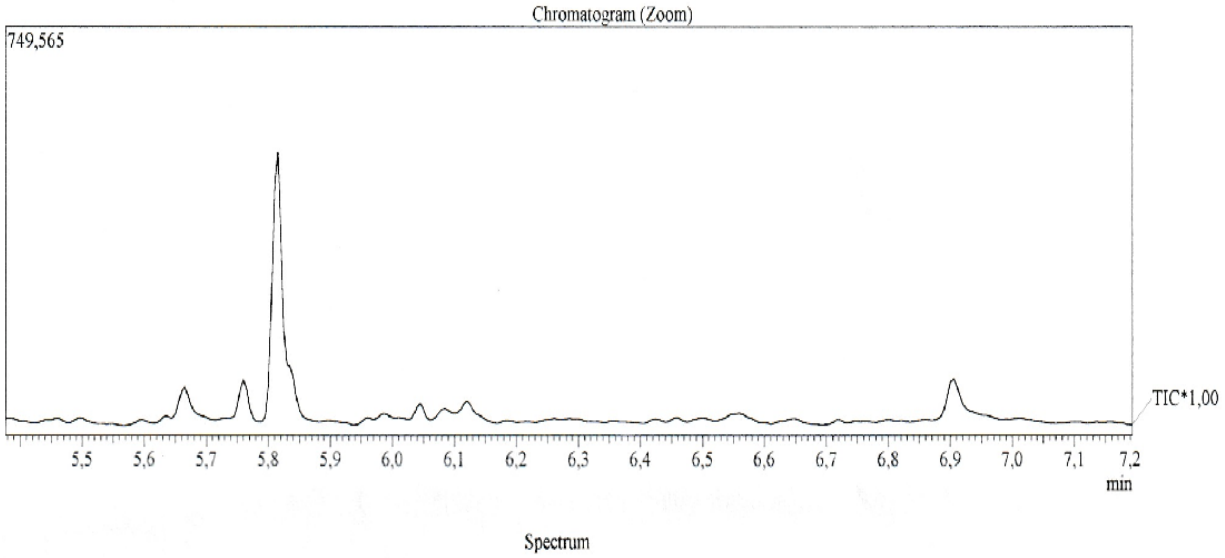
2.



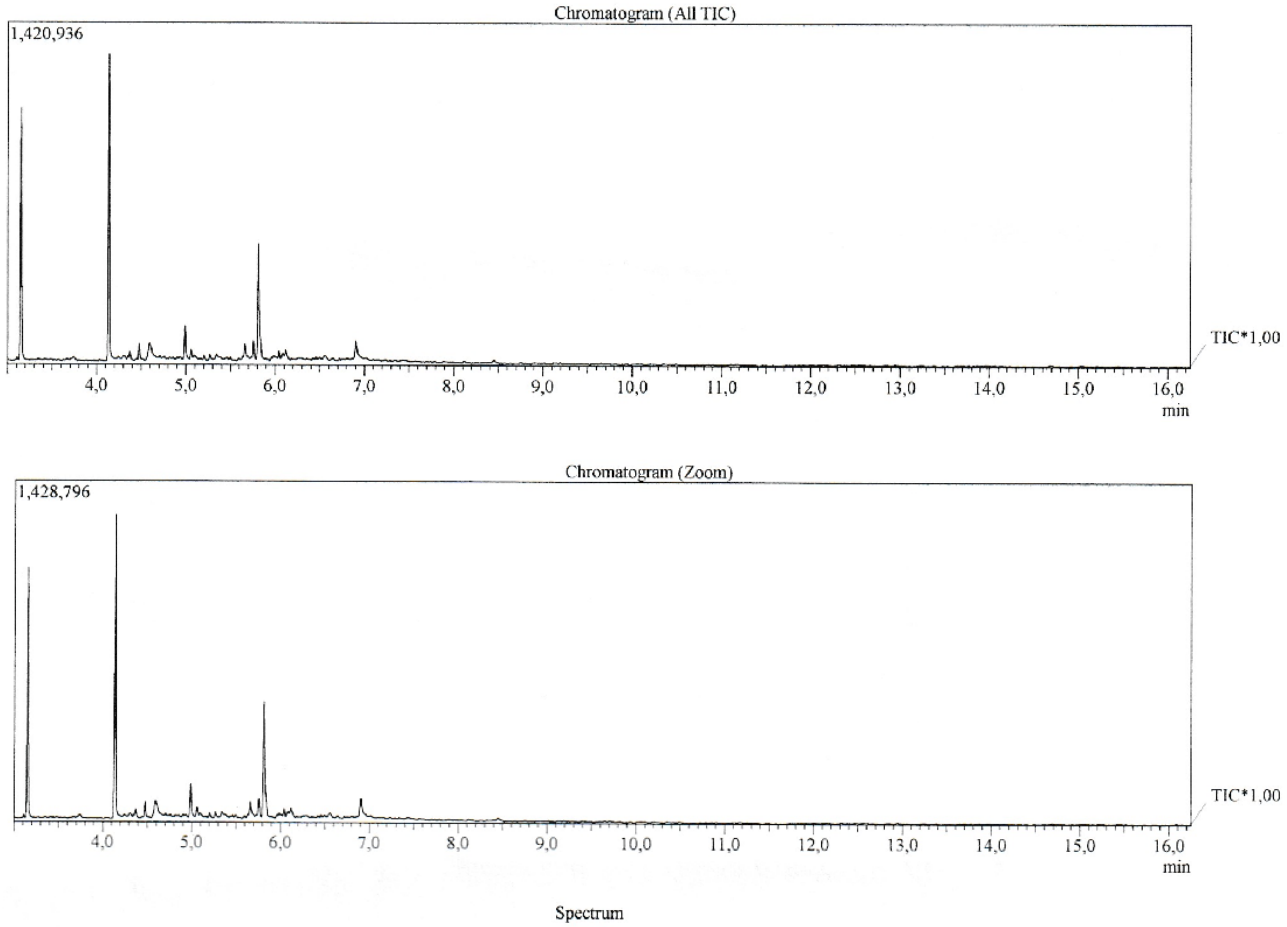
3.



© University of Venda

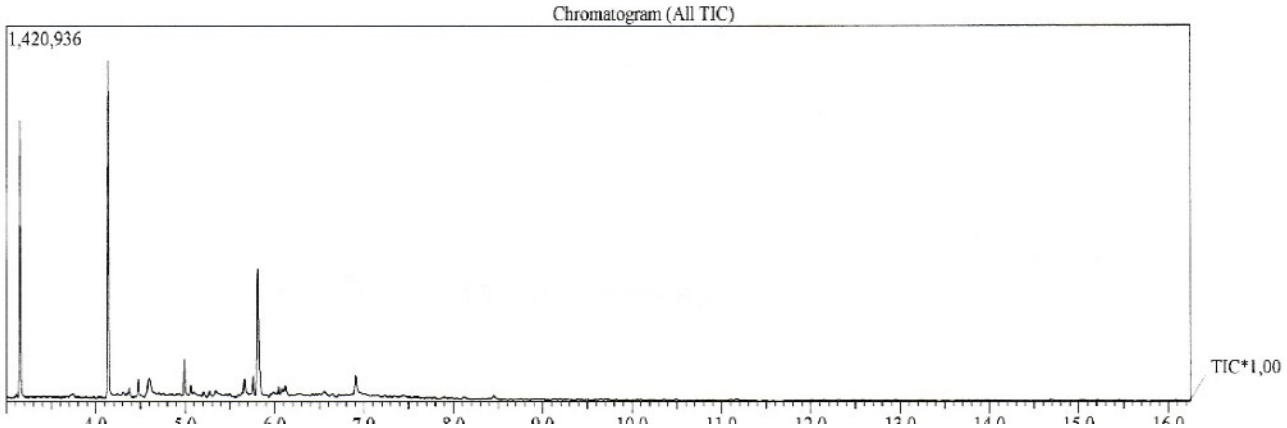


4.



© University of Venda

5.



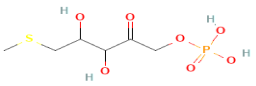
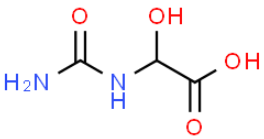
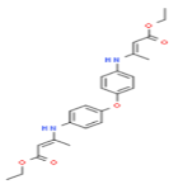
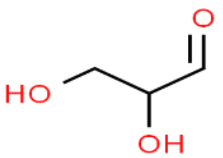
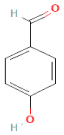
Appendix C: FT-IR table with functional groups for CDD (page 63 and 60)

Sample	Wavenumber (cm^{-1})	Functional Group	Significance
CDDs	3339.60 3272.53 cm^{-1}	Hydroxyl (-OH)	• Improves nutrient solubility and soil moisture retention.
	2198.22 -2106.91	Nitrile (C≡N)	• Source of organic nitrogen for plant growth.
	1639.89	Carbonyl (C=O)	• Contributes to soil organic matter stability.
	1449.52 2917.81	Aliphatic (C-H)	• Indicates the presence of aliphatic compounds, enhancing soil structure
	1362.07	Nitrate (-NO_3^-)	• Readily available nitrogen source for plants.
	1079.50 - 988.20 1029.95 cm^{-1}	Phosphate (PO_4^{3-})	• Essential for energy transfer and root development
	426.56 - 415.08 488.34 - 417.45	Metal-Oxygen (M-O) M-O bonds	• Presence of micronutrients for plant health. • Enhances soil fertility, essential for plant growth. • Improves nutrient retention and availability. • Supports beneficial microbial communities.
	1035.74	C-O stretching (alcohols, phenols)	• Improves nutrient availability and potential antioxidant properties
	500.57 cm^{-1}, 490.23 cm^{-1}, and 479.60 cm^{-}	Bending vibrations of metal-ligand bonds or functional groups associated with silicates	• These compounds can enhance soil structure and contribute to nutrient retention by improving soil aggregation, leading to increased aeration and drainage (Deng *et al.,* 2020).

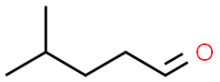
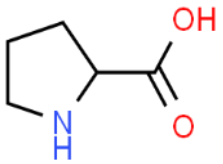
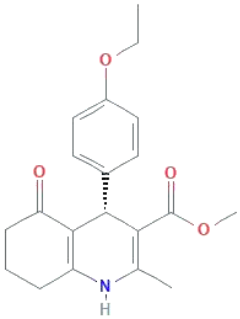
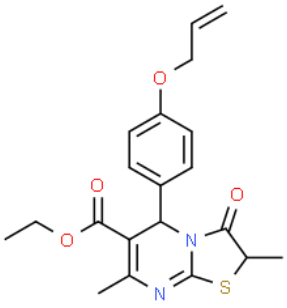
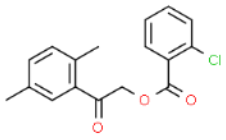
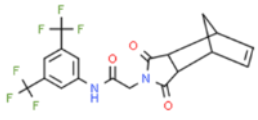
© University of Venda

	470.50 cm^{-1} to 421.14 cm^{-1}	Polysaccharides, proteins, and other organic compounds	• These compounds contribute to the overall stability of the digestate and its interactions with soil organic matter. The presence of these functional groups is beneficial for promoting soil microbial activity and enhancing nutrient cycling within the soil ecosystem.

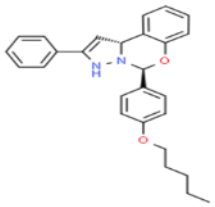
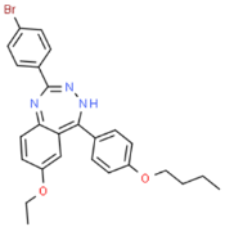
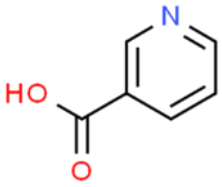
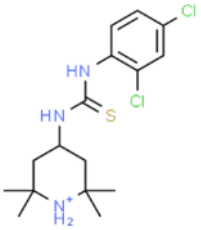
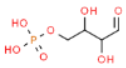
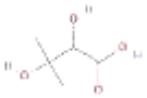
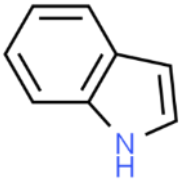
APPENDIX D: UHPLC-QTOF-MS identified compounds in digestate and other fertilisers (page 45).

COMPOUND NUMBER	MOLECULAR FORMULA	COMPOUND NAMES	STRUCTURE
329,2944836	C6H13O7PS	(3,4-Dihydroxy-5-methylsulfanyl-2-oxopentyl) dihydrogen phosphate	
	C3H6N2O4	Ureidoglycolic acid	
731,4905634	$C_{24}H_{28}N_2O_5$	2-Butenoic acid, 3,3'[oxybis(4,1phenyleneimino)] bis-, diethyl ester, (2E,2'E)	
	C3H6O3	Glyceraldehyde	
126,0907722	C7H6O2	4-hydroxybenzaldehyde	
	C6H12O	4-Methylpentanal	

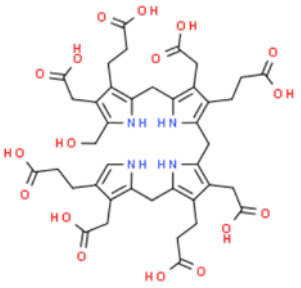
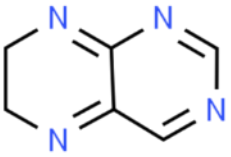
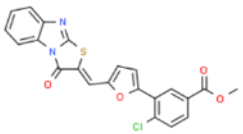
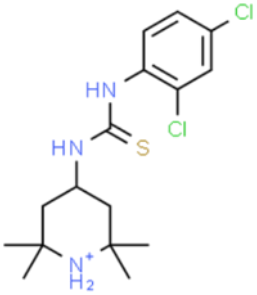
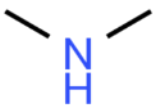
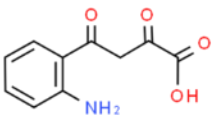
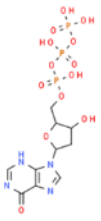
© University of Venda

			
594,2785616	C5H9NO2	**DL-Proline**	
341,018811	C14H10O9	**Digallic Acid**	
320,2554046	C20H22N2O4S	Ethyl 5-[4-(allyloxy) phenyl]-2,7-dimethyl-3-oxo-2,3-dihydro-5H- [1,3] thiazolo[3,2-a] pyrimidine-6-carboxylate	
263,0734689	C17h15clo3	2-(2,5-Dimethylphenyl)-2-oxoethyl 2-chlorobenzoate	
407,3310195	C19H14F6N2O3	N-[3,5-Bis(trifluoromethyl)phenyl]-2-(3,5-dioxo-4-azatricyclo [5.2.1.02,6] dec-8-en-4-yl) acetamide	

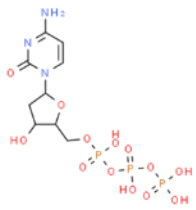
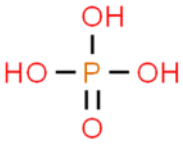
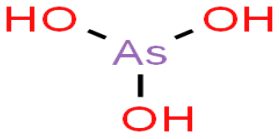
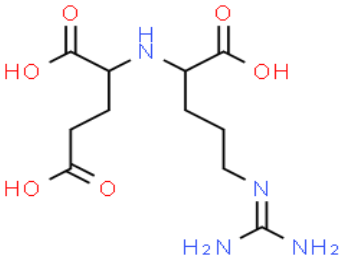
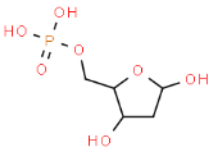
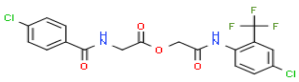
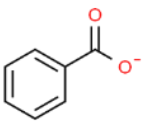
© University of Venda

149,1280008	C27H28N2O2	(5S,10br)-5-[4-(Pentyloxy) phenyl]-2-phenyl-3,10b-dihydropyrazolo[1,5-c] [1,3] benzoxazine	
355,2417516	C26h26brn3o2	2-(4-Bromophenyl)-5-(4-butoxyphenyl)-7-ethoxy-4H-1,3,4-benzotriazepine	
376,2711223	C7H12O4	Pimelic acid	
913,819513	C6H5NO2	Niacin	
201,1705295	C16H24Cl2N3S	4-[2,4-Dichlorophenyl) carbamothioyl] amino-2,2,6,6-tetramethylpiperidinium	
677,8384927	$C_4H_9O_7P$	ERYTHROSE 4-PHOSPHATE, D-	
	$C_5H_{10}O_4$	2,3-Dihydroxy-3-methylbutanoic acid	
776,2775204	C8H7N	Indole	

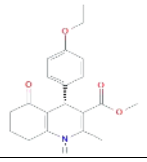
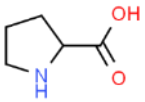
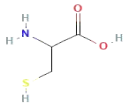
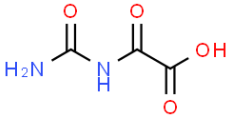
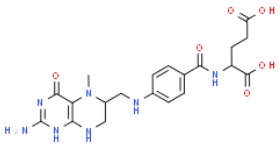
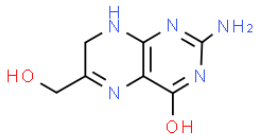
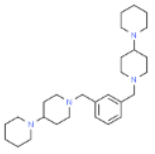
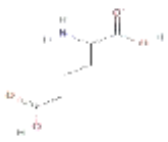
© University of Venda

767,4064854	$C_{40}H_{46}N_4O_{17}$	Hydroxymethylbilane	
162,0884811	C6H6N4	6,7-Dihydropteridine	
568,459668	C22H13Cln2o4s	Methyl 4-chloro-3-{5-[(Z)-(3-oxo [1,3] thiazolo[3,2-a] benzimidazol-2(3H)-ylidene) methyl]-2-furyl} benzoate	
201,1705295	C16H24Cl2N3S	4-[2,4-Dichlorophenyl) carbamothioyl] amino-2,2,6,6-tetramethylpiperidinium	
654,3019732	C2H7N	Dimethylamine	
459,2795776	C10H9NO4	4-(2-Aminophenyl)-2,4-dioxobutanoic acid	
658,5793107	C10H15N4O13P3	9-[2-Deoxy-5-O-(hydroxy{[hydroxy(phosphonooxy)phosphoryl] oxy} phosphoryl) pentofuranosyl]-3,9-dihydro-6H-purin-6-one	

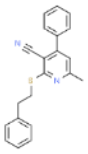
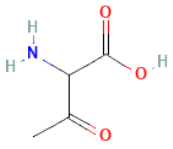
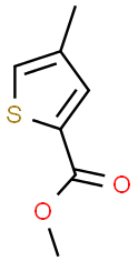
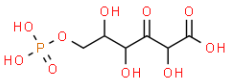
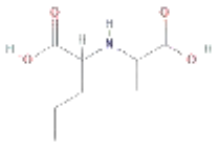
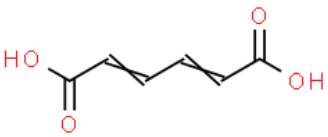
© University of Venda

605,5608447	C9H16N3O13P3	4-Amino-1-[2-deoxy-5-O-(hydroxy{[hydroxy(phosphonooxy)phosphoryl] oxy} phosphoryl) pentofuranosyl]-2(1H)-pyrimidinone	
979,9354155	H3O4P	Phosphoric acid	
530,0933551	H3aso3	Arsenous acid	
413,1204331	$C_{11}H_{20}N_4O_6$	Nopaline	
615,1391756	C5H11O7P	Deoxyribose 5-phosphate	
284,1790493	C18H13Cl2F3N2O4 CH202	2-[4-Chloro-2-(trifluoromethyl) phenyl] amino-2-oxoethyl N-(4-chlorobenzoyl) glycinate Formic acid /methanoic	 
237,0752605	C7H5O2 C23H30ClN3O	BENZOATE ION Quinacrine	

© University of Venda

			
594,2785616	C5H9NO2 C3H7NO2S	DL-Proline DL-Cysteine	 
443,3348979	C3H4N2O4	Oxaluric acid	
143,1061821	C20H25N7O6	Levomefolic acid	
213,2319672	C7H9N5O2	6-HYDROXYMETHYL-7,8-DIHYDROPTERIN	
469,401035	C28H46N4 C6H11NO4	1,3-Bis[4,1'-bipiperidin-1-yl) methyl] benzene 2-Aminohexanedioic acid	 

© University of Venda

219,0624099	C21H18N2S C4H7NO3	6-Methyl-4-phenyl-2-[2-phenylethyl) sulfanyl] nicotinonitrile 2-Amino-3-oxobutanoic acid	 
381,2057871	C7H8O2S	Methyl 4-methyl-2-thiophenecarboxylate	
384,2035092	C6H11O10P C8H15NO4	6-O-Phosphonohex-3-ulosonic acid 2-(1-Carboxyethylamino) pentanoic acid	 
376,2711223	C7H12O4	Pimelic acid	
304,2605943	C6H6O4	Muconic acid	

© University of Venda

563,5504807	C4H8O2S	3-(Methylthio) propionic acid	

APPENDIX E: Table provides mass fragmentation, and significance of Cholesteryl Stearate in GC-MS**.**

***m/z* Value**	**Fragment ions/Compound**	**Significance**
498	Cholesteryl Stearate (M^+, Molecular Ion)	Parent ion, confirms molecular weight
369	Cholesterol Core Fragment	Characteristic of sterol esters
255	Stearic Acid Fragment ($C_{18}H_{35}O_2^+$)	Loss of cholesterol backbone
207	Cholesterol Side Chain Fragment	Common sterol ester fragment
177	Cholesterol Derivative Fragment	Indicates sterol structure
133	Hydrocarbon Fragment	Possible lipid breakdown product
98	Alkyl Chain Fragment	Lipid-related fragmentation
69	Small Hydrocarbon Ion	Common in lipid analysis
45	Small Alkyl Fragment	General hydrocarbon breakdown

© University of Venda