EXTRACTION AND PHYSICOCHEMICAL CHARACTERIZATION OF WHEY PRODUCED FROM COLA NITIDA LEAVES

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A PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc. HONOURS) IN CHEMISTRY

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CERTIFICATION

This is to certify that this research project was carried out by RUTH NGOZI NDUKWE with the matriculation number PSC2105226 under the supervision of DR. MRS. I. E. UWIDIA in the Department of Chemistry, Faculty of Physical sciences, University of Benin, Benin City, Edo State.

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(Student) DATE

DEDICATION

This project research is dedicated to the Almighty God (El-roi) who in His infinite mercy saw me through my journey in the University of Benin, to my loving Dad and Family.

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ABSTRACT

Kola (*Cola nitida*) leaves are widely known for their ethnobotanical uses, yet their by-products remain underexplored as sources of bioactive compounds for environmental applications. Fermentation of plant-derived substrates often enhances their physicochemical profile, making them useful in biostimulation processes that support microbial activity for pollutant degradation. Against this background, this study investigated Cola nitida whey, focusing on its extraction, fermentation behavior, and nutrient composition, with the aim of assessing its potential application as a bioremediant. Fresh kola leaves were processed through washing, grinding, boiling, and filtration to obtain whey, which was digested with nitric acid and subjected to physicochemical analysis. Parameters evaluated included pH, electrical conductivity (EC), moisture content, total organic matter (TOM), total organic carbon (TOC), nitrate, phosphate, nitrogen, phosphorus, and potassium. Results showed dynamic changes across the five-week fermentation period. pH decreased initially from 5.29 in Week 1 to 5.18 in Week 3 before rising to 6.01 in Week 5, while EC steadily increased from 1595.50 to 2129.50 μS/cm, reflecting ionic release. Moisture content rose from 92.08% to 94.09%, whereas TOM and TOC increased overall, with TOM ranging from 54.06 to 106.55% and TOC from 31.36 to 61.80%. Nutrient levels indicated progressive mineralization: nitrate rose from 174.16 to 1152.36 mg/kg, phosphate from 411.67 to 971.81 mg/kg, nitrogen from 39.56 to 261.06 mg/kg, and phosphorus from 122.75 to 295.46 mg/kg. Potassium fluctuated but stabilized at 2.55 mg/kg by Week 5. These findings suggest that kola-leaf whey develops enriched organic and mineral content during fermentation, creating a nutrient-rich medium favorable for microbial proliferation. The shift toward near-neutral pH at later stages further supports microbial activity, while the elevated nitrate and phosphate levels highlight its suitability as a low-cost, plant-derived stimulant for bioremediation, although regulated application is recommended to minimize eutrophication risks.

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CHAPTER ONE

1.1 INTRODUCTION

1.1.1 Background of the Study

Environmental pollution from industrial discharge, oil spills, agricultural runoff, and domestic waste continues to degrade soil and water systems. Contaminants like petroleum hydrocarbons, heavy metals, and dyes persist in the environment and pose health and ecological risks. Traditional cleanup methods are chemical oxidation, thermal treatment, or excavation which are often expensive, time-consuming, and sometimes worsen the problem(Liu *et al.,* 2024). Bioremediation has emerged as a practical alternative. It involves using biological materials to break down, absorb, or neutralize pollutants in a more sustainable and low-cost way. In recent years, plant-based materials have drawn interest as potential bioremediants due to their abundance, safety, and diverse chemical makeup (Bala *et al*., 2022).

Whey extracts from plants are a relatively new area of interest in bioremediation research. These extracts often carry secondary metabolites like phenols, flavonoids, and alkaloids that can interact with contaminants (Jonsson Haller, 2014). Kola leaves (*Cola nitida*, *Cola acuminata*) are widely used in traditional medicine and food, but their biochemical properties suggest they may also serve as bioremediants. The presence of tannins, saponins, and other bioactive compounds gives them potential for binding or reacting with pollutants. Despite their availability and known medicinal use, kola leaves have not been studied for environmental applications (Ekalu and Habila, 2020). This research

explores a new direction: producing whey from kola leaves and analyzing its physicochemical properties to see if it has potential as a bioremediant.

This study does not test the kola leaf whey directly on contaminated soil or water. Instead, it focuses on evaluating key physicochemical properties like pH, conductivity, turbidity, organic content, and more that typically indicate how a material might behave in a bioremediation context. If these properties show promise, kola leaf whey could be considered for further applied research. This work contributes to the search for new, low-cost plant-based bioremediants and builds the foundation for using underutilized botanical resources in environmental management.

1.1.2 Statement of the Problem

Many communities facing pollution lack access to affordable and safe methods for restoring contaminated environments. While industrial nations may rely on advanced remediation technologies, these options are often unrealistic in regions where funding, equipment, or technical expertise is limited. As a result, pollutants like hydrocarbons and heavy metals remain in the soil and water for years, posing ongoing health and ecological risks (Ghosh and Konar, 2024). The demand for simple, plant-based bioremediants is growing, yet research continues to focus on a narrow range of materials that often require modification or high-cost processing. There is a clear need to explore untapped local resources that can be converted into effective bioremediants with minimal processing (Bala *et al.*, 2022).

Kola leaves are widely known in cultural, medicinal, and nutritional contexts, but their environmental potential has never been tested. Despite being chemically rich containing

compounds like saponins, tannins, and flavonoids, the leaves are discarded as waste in most cases. This neglect reflects a broader problem in environmental chemistry: the gap between what is locally available and what is scientifically validated. No studies currently describe the physicochemical behavior of kola leaf extracts or their interaction with pollutants. Without such baseline data, the material's potential to act as a natural bioremediant remains invisible to both researchers and environmental practitioners.

Bioremediation research often skips an essential step for understanding the chemical profile of the material before applying it. Knowing the pH, conductivity, organic content, and related properties can help predict how the material will behave in a polluted setting (Bala *et al.*, 2022). For kola leaf whey, this foundational knowledge is missing. Until its properties are examined and compared with known bioremediants, it cannot be considered for field application. This study fills that gap by analyzing the physicochemical characteristics of kola leaf whey to assess its readiness and potential for future use in environmental remediation.

1.1.3 Significance of the Study

This study offers a new perspective on the use of kola leaves as a low-cost bioremediant by focusing on its whey extract and analyzing its physicochemical properties. While kola leaves are abundant and widely used in traditional medicine, their potential application in environmental remediation has been completely overlooked (Maňourová *et al.*, 2019). By characterizing the chemical profile of kola leaf whey, this research introduces a previously unexamined plant-based material that could contribute to pollution management in a more affordable and sustainable way. The findings may help shift

attention toward underutilized natural resources that require minimal processing and are readily available in many regions, particularly across West Africa.

The study also adds value to environmental chemistry by filling a knowledge gap in bioremediation research. It emphasizes the importance of understanding the intrinsic properties of a potential bioremediant before applying it in the field. By establishing baseline data such as pH, conductivity, organic load, and other key indicators, this research provides a foundation for future applied studies. If kola leaf whey shows favorable properties, it can be tested in practical settings and potentially scaled for larger environmental use. This work supports the move toward cleaner, locally driven solutions to pollution challenges and broadens the scope of bioremediation materials.

1.1.4 Scope of the Study

This study focuses on the preparation and physicochemical characterization of whey derived from kola leaves to evaluate its potential as a bioremediant. Fresh kola leaves were collected from the agricultural farm at the University of Benin and identified by Professor Akinnibosun Henry Adewale of the Department of Plant Biology and Biotechnology with the voucher number UBH-C323. The leaves were washed, chopped, and ground into a slurry, which was then filtered to remove fibrous material (bagasse). The resulting extract was boiled, left to stand for 24 hours, decanted, and sieved to obtain the brown liquid whey, separating it from the solid leaf protein concentrate. The collected whey was analyzed for physicochemical properties. This work is limited to laboratory-scale processing and characterization and does not involve any field application or pollutant removal testing.

1.1.5 Aim and Objectives

The aim of this study is to prepare and characterize whey derived from Cola nitida leaves and assess its physicochemical properties for potential application as a bioremediant. The following objectives were set to:

- Process fresh kola leaves into whey through extraction, boiling, and separation procedures.
- Determine the physicochemical properties of the kola leaf-derived whey.
- Compare the obtained properties with standard parameters relevant to bioremediation.
- evaluate the potential suitability of kola leaf whey as a laboratory-scale bioremediant.

1.1.6 Limitation of the Study

This study was limited to laboratory-scale extraction and physicochemical characterization of kola leaf whey, without testing on actual contaminated soil or water. Its pollutant removal capacity therefore remains theoretical, and advanced analyses, stability tests, microbial activity, and comparisons with established bioremediants were not included. As such, conclusions about its real-world performance and efficiency under varying environmental conditions cannot yet be drawn.

1.2 LITERATURE REVIEW

1.2.1 Bioremediation

Bioremediation is a natural process that uses biological materials to clean up polluted environments. It involves using living organisms or substances they produce to remove, degrade, or neutralize harmful chemicals in soil, water, or air. These biological agents include microorganisms such as bacteria and fungi, as well as plants and plant-based materials (Bala *et al.*, 2022). Bioremediation works by transforming toxic substances into less harmful or harmless compounds through biological reactions. These reactions may include oxidation, reduction, hydrolysis, or enzymatic breakdown of the pollutant molecules.

The main idea behind bioremediation is to rely on nature to fix what has been damaged by pollution. When properly applied, it can reduce pollution levels significantly without the need for harsh chemicals or high-energy inputs. The effectiveness of bioremediation depends on several factors including the type of pollutant, the environmental conditions, and the biological agent used. For example, some bacteria can naturally digest oil and break it down into carbon dioxide and water, but only when enough oxygen and nutrients are present (Abatenh *et al.,* 2017). This makes environmental control and monitoring very important in any bioremediation process.

1.2.1.1 Common Pollutants Targeted

Bioremediation is used to treat a wide range of environmental contaminants. One of the most common targets is petroleum hydrocarbons, which are found in oil spills and

industrial waste. These include substances like diesel, gasoline, kerosene, and crude oil. Hydrocarbon pollution is especially dangerous because it affects both aquatic and terrestrial ecosystems, and some components are known to be carcinogenic (Mekonnen *et al.,* 2024). Bioremediation can help break down these compounds into simpler, non-toxic forms.

Heavy metals such as lead, mercury, cadmium, chromium, and arsenic are another group of pollutants commonly addressed using bioremediation. Although these elements cannot be broken down, biological agents can transform them into less mobile or less toxic forms, or extract them from the environment (Igiri *et al.,* 2018). Other common pollutants include pesticides, herbicides, solvents, dyes, and even radioactive substances. These are often found in agricultural runoff, industrial discharge, and untreated domestic waste (Mishra *et al.,* 2023). Many of these substances are persistent in the environment, meaning they do not degrade easily and tend to accumulate. This makes bioremediation a valuable tool for dealing with long-term environmental contamination.

1.2.1.2 Types of Bioremediation

There are several types of bioremediation, each using a different biological approach depending on the pollution type and the environment involved. Microbial bioremediation is the most widely used and involves bacteria, fungi, or algae that naturally degrade or metabolize pollutants (Ayilara and Babalola, 2023). These microbes may be already present in the contaminated area or may be introduced from outside. In some cases, nutrients or oxygen are added to help them grow and function more effectively. This

process is known as biostimulation. When specific microbial strains are introduced intentionally, it is referred to as bioaugmentation.

Phytoremediation is another method that uses plants to absorb, accumulate, or break down pollutants. Plants like sunflower, vetiver, and Indian mustard have been shown to absorb heavy metals through their roots and store them in their tissues (Aryal, 2024). In some cases, plants can even transform toxic chemicals into less harmful forms through metabolic activity. Enzymatic bioremediation involves using natural enzymes produced by microbes or extracted directly to break down complex pollutants (Mousavi *et al.,* 2021). A newer area of research is plant-based bioremediants, where plant extracts or by-products such as whey, peels, or powders are used to remove contaminants (Saha *et al.,* 2021). These materials offer a low-cost alternative to living plants or microbes and often contain natural compounds that interact with pollutants effectively.

1.2.1.3 Advantages over Conventional Methods

Bioremediation has several advantages over traditional methods of pollution control. It is often cheaper because it uses naturally available resources and requires little energy or chemical input. In many cases, the biological agents work at ambient temperature and pressure, reducing the need for expensive equipment or energy-intensive processes. This makes bioremediation a good option for rural or low-resource settings where access to high-tech remediation tools is limited.

Another key advantage is that bioremediation is environmentally friendly. It usually does not introduce new harmful substances and often leaves behind harmless end products like water, carbon dioxide, or biomass. It can also be carried out on-site, avoiding the need to

transport hazardous waste to treatment facilities. This reduces the risk of accidental spills or exposure (Alori *et al.*, 2022). Bioremediation can target specific pollutants without harming the surrounding environment and is generally safer for workers and nearby communities. It also allows for the gradual improvement of contaminated environments without causing major disturbances to the ecosystem.

1.2.2 Plant-Based Bioremediants

Plants and plant-derived materials have become a growing area of focus in environmental remediation because they are renewable, biodegradable, and widely available. In many polluted environments, especially in low-resource regions, plant-based solutions offer a safer and more affordable alternative to expensive industrial methods (Sahoo, 2024). Plants can absorb, store, or break down pollutants through natural metabolic processes. They are used in both living form and in processed forms such as powders, ashes, and liquid extracts. These materials are rich in bioactive compounds like phenols, tannins, flavonoids, and saponins that interact chemically with a wide range of pollutants (Paripuranam *et al.,* 2025). This allows them to be used in treating contaminated soil, water, and even air.

The use of plant-based materials is particularly important in addressing non-degradable pollutants such as heavy metals and persistent organic compounds. Some plants can take up and concentrate metals in their tissues, a process useful in cleaning metal-contaminated soils. In other cases, plant derivatives are added to polluted water to bind with contaminants and remove them from the solution (Aryal, 2024). These materials are often sourced from agricultural or food waste, reducing the environmental burden while

adding economic value. Their simplicity and low risk make them especially useful for rural communities, small industries, and emergency response situations where rapid deployment is needed. Plant-based bioremediants are not only effective but also contribute to circular resource use and sustainable waste management practices.

1.2.2.1 Mechanisms of Action: Adsorption, Chelation, Degradation

Plant-based bioremediants work through different chemical and biological mechanisms, the most common being adsorption. Adsorption is a surface-based process where pollutant molecules attach to the surface of the plant material. Many plant parts contain porous structures and active sites, such as hydroxyl, carboxyl, and amine groups, which allow pollutants to bind easily (Satyam and Patra, 2024). This is especially useful in removing dyes, oil, heavy metals, and organic waste from water. Unlike absorption, which is a bulk process, adsorption happens at the surface, making the physical structure and surface chemistry of the plant material very important (Aljamali *et al.,* 2021).

Another mechanism is chelation, where plant-derived compounds form stable complexes with metal ions. Chelation makes the metals less mobile and less toxic. Compounds such as tannins and flavonoids in plants are known to bind with metals like lead, cadmium, and copper. This makes plant-based materials useful for treating metal-contaminated environments (Anjum *et al.,* 2015). Degradation is also a key process, especially for organic pollutants. Some plant enzymes can break down complex organic molecules into simpler, less harmful substances (Ali et al., 2025). For instance, phenol oxidase and peroxidase enzymes in plant extracts can degrade petroleum hydrocarbons. These mechanisms often work together in a single material, making plant-based bioremediants

versatile and multifunctional. Understanding these actions is important for selecting the right plant source and processing method for specific environmental problems.

Several plant materials have been widely studied and used as bioremediants due to their availability, low cost, and chemical composition. *Moringa oleifera* seeds, for example, are well known for their water purification properties. The seeds contain proteins and active molecules that bind with suspended particles and toxic metals, making them effective for treating wastewater (Desta and Bote, 2021). *Eichhornia crassipes*, commonly known as water hyacinth, is another popular bioremediant. It absorbs heavy metals and nutrients from polluted water through its extensive root system and stores them in its tissues (Nazir *et al.,* 2020). While often seen as an invasive plant, it plays a useful role in water treatment when properly managed.

Banana peels and other fruit wastes like orange rinds, coconut husks, and papaya seeds have also shown potential in removing heavy metals and organic pollutants. These wastes contain pectin, cellulose, and lignin, which offer binding sites for contaminants. Powdered peels have been used in both batch and continuous treatment systems for removing lead, chromium, and dyes (Bishnoi *et al.*, 2023). Their use adds value to agricultural waste and reduces the load on landfills. These examples show that both cultivated plants and plant waste can serve as effective bioremediants. Their success depends on how well their chemical and structural properties match the type of pollutant targeted.

Raw plant materials are often used directly in bioremediation because they are easy to obtain and require minimal processing. This includes using leaves, roots, or peels in

powdered or dried form to treat polluted water or soil. However, raw materials can have limitations such as inconsistent chemical composition, slower reaction time, and reduced efficiency under varying environmental conditions. They may also be bulky and harder to handle or store for long periods. While still effective, raw plant materials are best suited for small-scale or low-budget applications where simplicity and availability are more important than precision or control.

Processed plant extracts such as whey, ash, or liquid filtrates are more refined and often show improved performance. These extracts concentrate the active compounds, making them more reactive with pollutants (Nemli *et al.*, 2025). For example, whey derived from plant leaves may contain soluble phenolic compounds and other bioactive agents that are more accessible than in raw leaf form. Ash from burned plant material is alkaline and rich in minerals, making it suitable for neutralizing acidic waste or adsorbing metals. These processed forms are usually easier to standardize, store, and apply in controlled experiments or scaled operations. The choice between raw and processed plant materials depends on the desired efficiency, type of pollutant, and resources available for treatment.

1.2.3 Physicochemical Properties Relevant to Bioremediants

Physicochemical properties are key indicators of how effective a material may be as a bioremediant. These properties reveal how the material interacts with contaminants and the surrounding environment. One of the most critical parameters is pH. It measures the acidity or alkalinity of the material and directly affects the solubility and availability of pollutants, especially metals. A slightly acidic or neutral pH range often supports the best adsorption activity. Some pollutants may bind more readily to bioremediants in acidic

conditions, while others prefer a more basic environment (Wu *et al.*, 2023). Understanding the pH of a plant-based extract helps predict how it will behave when exposed to various contaminants.

Electrical conductivity is another important factor, as it reflects the presence of dissolved ions and minerals in the material. High conductivity may indicate a high ionic strength, which can influence how the bioremediant interacts with charged pollutants such as heavy metals and nitrates (de Sousa *et al.*, 2014). Turbidity, on the other hand, measures the clarity or cloudiness of a liquid and can suggest the presence of suspended particles. In bioremediation, high turbidity may point to particulate matter that either enhances or interferes with contaminant removal. Total organic carbon and organic matter content reflect the amount of degradable organic substances in the material. These organic substances may serve as active sites for binding or reacting with pollutants (Ibrahim *et al.*, 2025). They can also influence microbial activity if the bioremediant is used in combination with biological agents. Other essential properties include nitrogen, phosphorus, potassium, phosphate, and nitrate levels, which are nutrients that affect the biological balance in a treatment environment. These nutrients can either enhance microbial degradation processes or shift the chemical interactions between the bioremediant and the pollutant. Together, these parameters form a comprehensive chemical profile that helps determine how well the material may work in a polluted setting.

Each physicochemical parameter plays a specific role in how plant-based materials interact with pollutants. The pH of the bioremediant can change the charge and solubility

of both the pollutant and the functional groups on the material's surface. For example, metals like lead and cadmium become more soluble in acidic conditions, making them easier to bind with available sites on the bioremediant. On the other hand, very low or very high pH values can reduce binding efficiency or destabilize the material. This makes pH adjustment a common strategy in improving bioremediation performance. Electrical conductivity indicates the availability of charged species in the medium. High conductivity may enhance or interfere with ionic interactions depending on the type of pollutant. It can also influence the movement of pollutants toward the bioremediant surface (Karnena and Saritha, 2022).

Turbidity can provide physical adsorption sites by trapping pollutants on suspended particles, but too much turbidity may reduce the efficiency of other removal processes like light-driven reactions or microbial activity. Total organic carbon and organic matter content are often associated with the presence of phenolic compounds, lignin, cellulose, and other complex molecules that can adsorb or bind with contaminants. These compounds provide functional groups such as hydroxyl, carbonyl, and carboxyl that actively participate in pollutant binding. Nutrient levels like nitrogen, phosphorus, and potassium influence the microbial dynamics when used in bioremediation systems that rely on bacteria or fungi. These nutrients can enhance biodegradation by supporting microbial growth or enzymatic activity. Nitrate and phosphate levels can also impact redox conditions and influence the transformation of specific contaminants (Ayilara and Babalola, 2023). Therefore, understanding the exact levels of these properties helps determine how the plant-based material will behave in real treatment settings, allowing researchers to predict, control, and optimize pollutant removal.

1.2.3.1 Methods Commonly Used for Measurement and Evaluation

Measuring the physicochemical properties of a bioremediant requires standard laboratory procedures that provide accurate and reproducible data. pH is usually measured using a digital pH meter after calibrating with standard buffer solutions. It is important to take measurements at room temperature and after proper mixing to get a reliable reading. Electrical conductivity is measured with a conductivity meter, which gives the total ionic concentration of the sample in microsiemens per centimeter. This is a fast and direct method that helps estimate the mineral load of the bioremediant. Turbidity is measured using a turbidimeter or nephelometer, which detects the scattering of light caused by suspended particles in the sample. The result is given in nephelometric turbidity units (Lawler, 2016).

Total organic carbon is often measured using a combustion or oxidation method followed by infrared detection. It gives a precise value of all carbon contained in organic compounds in the sample (Kim and Kim, 2025). Organic matter content can also be estimated using the loss-on-ignition method, where the sample is burned at a high temperature and the weight loss is recorded. Nutrient contents such as nitrogen, phosphorus, potassium, nitrate, and phosphate are usually determined through colorimetric or spectrophotometric methods. For example, the Kjeldahl method is used for total nitrogen, while molybdenum blue colorimetry is often used for phosphate. Potassium can be measured using flame photometry, and nitrate levels are commonly measured with UV spectrophotometry. These methods provide reliable values that help in comparing different bioremediant materials. Accurate measurement is key to drawing valid conclusions about how each parameter contributes to pollutant removal.

1.2.3.2 Correlation Between Physicochemical Properties and Adsorption or Interaction Capacity

There is a direct connection between the physicochemical properties of a bioremediant and its ability to remove pollutants. Materials with favorable pH and high surface activity tend to have better adsorption performance (Kuppan *et al.,* 2024). For instance, if the material has a neutral or slightly acidic pH, it may increase the availability of metal ions, making it easier for functional groups on the surface to interact with them. The presence of carboxyl, hydroxyl, and amine groups on plant-derived materials is strongly related to how well they bind with metal cations or organic pollutants. These functional groups are more active in certain pH ranges, showing that pH must be controlled for optimal interaction.

High levels of organic matter and total organic carbon usually indicate a greater presence of binding sites. These sites can trap pollutants through electrostatic interactions, hydrogen bonding, or even chemical reactions. Conductivity may influence the movement of charged pollutants toward these active sites and also affect the structure of the adsorption layer (Satyam and Patra, 2024). Similarly, nutrients like nitrogen and phosphorus may not bind directly with pollutants but enhance the environment for microbial action, especially in combined biological and chemical remediation systems. Turbidity can increase surface contact by suspending particulate matter that captures pollutants, though it may also block light or hinder microbial processes if too high. Overall, the correlation between physicochemical properties and adsorption capacity helps researchers choose or modify materials for specific contaminants, making the entire bioremediation process more predictable and effective.

1.2.4 Kola Leaves

1.2.4.1 Botanical Hierarchy and Morphology of Kola Leaves

Kingdom: Plantae

Phylum (Division): Magnoliophyta

Class: Magnoliopsida

Order: Malvales

Family: Malvaceae

Genus: Cola

Species: Cola nitida and Cola acuminata

Kola leaves are part of the Cola genus, which includes two primary species known for their importance: *Cola nitida* and *Cola acuminata*. They fall under the Kingdom Plantae, Division Magnoliophyta, Class Magnoliopsida, Order Malvales, and Family Malvaceae (Ekalu and Habila, 2020). These species are evergreen trees native to West Africa and are best known for producing kola nuts, which are widely used in food, beverages, and traditional medicine. The leaves of these plants are generally elongated with pointed tips, deep green coloration, and prominent midribs. They are arranged alternately on the branches and are supported by short petioles. The surface of the leaf is leathery and smooth, while the edges are typically entire and slightly wavy. The venation is pinnate, and the structure allows for good surface contact with environmental factors, making the leaves potentially valuable for chemical interaction in pollutant studies.

The natural habitat of kola trees includes tropical rainforests and humid lowland areas where the soil is rich and well-drained. These trees prefer shaded environments and often grow under the canopy of taller trees. They are commonly cultivated in countries like Nigeria, Ghana, and Cameroon. Although most research focuses on the kola nut, the leaves are often overlooked despite being readily available as agricultural by-products (Amon-Armah *et al.,* 2021). The structure and chemical profile of the leaves suggest they may possess significant adsorptive properties useful for environmental cleanup applications (Edo, 2022). Their wide surface area, cuticular composition, and internal vascular system can potentially host chemical and microbial interactions useful in bioremediation. Understanding their morphology helps predict how they might behave when applied in a pollution remediation context, especially in extract or powdered forms.

1.2.4.2 Traditional Uses and Known Phytochemical Constituents

Traditionally, kola leaves are used in folk medicine across West African communities. They are boiled and consumed as infusions to treat headaches, fatigue, and indigestion. Some practices use the leaves as poultices for swelling or minor wounds due to their believed anti-inflammatory properties. In many cultures, both the leaves and nuts are used in ceremonial or social functions, symbolizing hospitality and respect (Tauchen *et al*., 2023). Though less prominent than the nuts, the leaves also feature in traditional pharmacopoeia, suggesting the presence of biologically active compounds. These traditional uses hint at the leaves' medicinal value, and indirectly support the idea that they might be functionally active in environmental applications.

Phytochemical screening of kola leaves reveals the presence of compounds such as tannins, alkaloids, flavonoids, saponins, phenols, and steroids (Edo, 2022). Tannins are polyphenolic compounds known for their ability to bind proteins and metal ions, making them suitable for adsorbing heavy metals from contaminated environments. Alkaloids have antimicrobial and antioxidant properties that may support biological remediation systems or offer direct detoxification roles. Flavonoids act as antioxidants and may neutralize reactive pollutants or serve as electron donors in redox reactions. Saponins, known for their surfactant-like properties, can improve the solubility and mobility of hydrophobic pollutants such as petroleum hydrocarbons. These compounds collectively make kola leaves a promising candidate for studies in environmental remediation. However, the specific roles these phytochemicals play in pollutant interaction remain poorly studied. Detailed phytochemical analysis and testing are needed to clarify their actual contributions to any observed remediation effects.

The phytochemicals found in kola leaves offer specific chemical functionalities that can influence pollutant behavior in soil or water. Tannins can form stable complexes with heavy metals such as lead, cadmium, and mercury, effectively immobilizing them and preventing their movement through the environment. This makes them suitable for applications in soil treatment or wastewater remediation. Flavonoids and phenols can act as natural antioxidants, helping to break down organic pollutants through redox reactions. These molecules may also reduce the toxicity of certain pollutants by neutralizing free radicals or altering their chemical structure. Saponins, through their detergent-like structure, can increase the solubility of hydrophobic compounds like oil and grease, allowing for better microbial degradation or chemical breakdown. These mechanisms are

commonly used in bioremediation strategies involving other plant materials, and kola leaves may offer similar or even improved benefits (Edo, 2022).

In environmental chemistry, the functional groups associated with these phytochemicals such as hydroxyl, carbonyl, and carboxyl are often responsible for adsorption, chelation, and catalytic activity. These functional groups can interact with pollutants via hydrogen bonding, van der Waals forces, and covalent or ionic bonding, depending on the conditions. Kola leaves, when processed into powders or extracted into liquid whey, may preserve these functional groups and offer multiple interaction points for pollutant molecules. For example, processed plant whey may contain higher concentrations of active compounds due to heat and solvent-assisted extraction, which enhances reactivity. This makes kola leaf extracts a potentially low-cost and sustainable alternative for pollutant removal. However, their actual performance compared to established bioremediants like banana peel or *Moringa oleifera* has not yet been properly tested or documented in scientific literature.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Apparatus

Glass beakers, volumetric flasks (Class A), conical flasks, crucibles, stainless-steel container, muslin cloth, desiccator, laboratory blender, porcelain crucibles (high form), pipettes, burettes, magnetic stirrer with Teflon-coated stir bar, Petri dishes, inoculating loops, Durham tubes, glass rods, oven, block digester (95–120 °C), muffle furnace (500 °C), incubator (37 °C), refrigerator (4 °C), microscope, spectrophotometer, flame photometer, and Atomic Absorption Spectrophotometer (AAS).

2.1.2 Reagents

Concentrated nitric acid (HNO_3), concentrated sulfuric acid (H_2SO_4), sodium hypochlorite, sodium potassium tartrate, alkaline phenol solution, potassium dichromate (1N), ferrous ammonium sulfate solution (0.5M), diphenylamine indicator, concentrated phosphoric acid (H_3PO_4), ascorbic acid, hydrochloric acid (20%), ethanol (70%), Brilliant Green Bile Broth, Eosin Methylene Blue agar, and distilled water.

2.2 METHODOLOGY

2.2.1 Sample Collection

The sample collection for this study began with obtaining fresh kola (*Cola nitida*) leaves from the fields at the University of Benin, Benin City, Edo State, Nigeria, located at approximately 6.4083° N latitude and 5.6189° E longitude. The leaves were harvested manually using clean tools to prevent contamination. The plant was identified and confirmed as *Cola nitida* by Prof. Akinnibosun Henry Adewale from the Department of

Plant Biology and Biotechnology, ensuring proper botanical authentication. All collected samples were placed in sterile polythene bags, transported immediately to the laboratory, and handled under clean conditions to maintain sample integrity.

2.2.2 Sample Preparation

The kola leaves were first sorted to remove dry or diseased material, leaving only fresh, healthy leaves. The selected leaves were chopped into smaller pieces to make them easier to process. They were then washed thoroughly with clean distilled water to remove surface dust, dirt, and other possible impurities. This cleaning step was essential to reduce contamination and ensure that the whey produced reflected the natural biochemical content of kola leaves.

After washing, the leaves were drained at room temperature and kept in clean trays before proceeding to whey production.

2.2.3 Whey Production

The production of whey from kola leaves began with soaking the chopped and washed leaves in clean water for about 30 minutes to soften the plant material. The softened leaves were then ground into a fine paste using a laboratory blender. The resulting paste was squeezed and filtered through a muslin cloth to separate the fibrous residue (bagasse) from the liquid extract.

The liquid extract was transferred into a clean stainless-steel container and boiled. During boiling, leaf proteins coagulated and were carefully skimmed off, leaving a brownish liquid fraction. This liquid was allowed to cool and undergo sedimentation, after which it

was filtered again to remove suspended solids. The final filtrate obtained was the kola leaf-derived whey, which was stored in sterile containers at low temperature until required for analysis.

2.2.4 Sample Digestion

The kola-leaf whey samples were digested using concentrated nitric acid. Measured portions were transferred into acid-washed vessels, treated with trace-metal grade HNO_3, and allowed to pre-digest at room temperature. The mixtures were then heated on a controlled block at 95–120 °C until they became clear and colorless. After cooling, the digests were transferred into Class A volumetric flasks, diluted to volume with ultrapure water to obtain 1–2% v/v HNO_3, filtered through 0.45 µm membranes, and stored at 4 °C in labeled acid-washed polyethylene bottles for subsequent mineral analysis using AAS.

2.2.5 Physicochemical Characterization

2.2.5.1 Determination of pH

20ml of the whey samples were measured and transferred into a 40ml beaker. The samples were mixed thoroughly with glass rod and allowed to stand for one hour with intermittent stirring. The pH meter was calibrated with known pH of buffer solutions 4.0 and 9.0. The pH meter electrode was immersed into the samples and the reading was taken (Rodger *et al.*, 2017).

2.2.5.2 Moisture Content Determination.

3g of the sample was weighed into a well labeled crucible that has been oven dried and weighed. The crucible and the content were transferred into the oven at 110oc for about 2hours. The crucibles were then cooled in desiccators for one hour. Then the weight of the crucible and sample record.

Calculation:

Moisture content $=\frac{M2-M3}{M2-M1}X100$

Where:

M_1 = Weight of crucible

M_2 = Weight of sample with crucible

M_3 = Weight of crucible with sample after oven dried at 110^{o}c (ASTM D5832 – 98(2014)).

2.2.5.3 Total Nitrogen Determination

5ml of the sample was measured into a conical flask, 20ml of H2SO4 was added and 1g kjaldeha catalyst was added and it was digested in open air, until clear solution was observed. After digestion, the sample was filtered and 5ml of the filtrate was pipette into a 100ml flask and water was added to the mark. 2.5ml of the alkaline phenol was added and properly shaken. 1ml of sodium potassium tartrate in water was added and well shaken. 2.5ml of sodium hypochlorite added and shaken and the colour is allowed to develop. The colour change observed in the standard and samples were deep blue, light

blue and milky blue coloration respectively. Readings were carried out with spectrophotometer at 630nm.

Calucation:

$$N = \frac{Instr.Reading\ X\ SlopRecip\ X\ colour\ Vol\ X\ Digest\ Vol\ X\ Cf}{weight\ of\ sample\ X\ Aliquot\ taken}$$

Where,

Instr. = Instrument

Recip. = Reciprocal of Slope

Vol. = Volume

Cf = Correction Factor (Nagomyy, 2013).

2.2.5.4 Determination of Phosphorus

1ml of digest was mixed with 8ml of distilled water and then 1ml of the colour solution was added along with 0.5ml of ascorbic acid. Spectrophotometer reading was taken at 660nm.

Calculation:

$$P\ (ppm) = \frac{Instr.Reading\ X\ SlopRecip\ X\ colour\ Vol\ X\ Extract\ Vol}{weight\ of\ sample\ X\ Aliquot\ taken}$$

(Nagomyy, 2013).

2.2.5.5 Determination of Potassium

1ml of the digested samples was measured into a high form porcelain crucible; the samples were ashed in a muffle furnace at 500oc for 4 hours. The sample ash was cool and dissolve in 5ml of 20% (2M) HCl, and filtered through an acid washed filter paper into a 50ml volumetric flask. The filtrate was read with flame photometer at 766.5nm (Anderson and Ingran, 1993).

2.2.5.6 Determination of Total Organic Carbon and Organic Matter

5ml of sample was weighed into a 500ml beaker. 10ml 1N potassium dichromate solution using a pipette was added, and 10ml concentrated H_2SO_4 was added using a dispenser, and the beaker was swirl to mix the suspension, while allowed to stand for 30 minutes. 100ml of distilled water was added and 5ml concentrated H_3PO_4 was added using dispenser, and allowed to cool. 10 to 15 drops of diphenylamine indicator was added, and a Teflon – coated magnetic stirring bar was added, and the beaker was placed on a magnetic stirrer. 0.5M ferrous ammonium sulfate solution was used to titrate, until the colour change was observed from violet blue to green. Two blank were prepared containing the entire reagent except the compost sample, and was treated exactly the same way as the sample's suspension.

Calculation:

$$\mathbf{M} = \frac{10}{Vblank}$$

$$\textbf{Oxidizable organic carbon \%} = \frac{Vblank - Vsample\ X\ 0.3\ X\ M}{wt}$$

Total organic carbon % = 1.334 X oxidizable organic carbon %

Organic matter % = 1.724 X Total organic carbon %

Where:

M = Molarity of $(NH_4)_2SO_4.FeSO_4.6H_2O$ (about 0.5M)

Vblank = Volume of $(NH_4)_2SO_4.FeSO_4.6H_2O$ solution required to titrate the blank (ml)

Vsample = Volume of $(NH_4)_2SO_4.FeSO_4.6H_2O$ solution required to titrate the sample (ml)

Wt = Weight of air – dry sample (g)

0.3 = 3 x 10-3 x 100, where 3 is the equivalent weight of C (Nagomyy, 2013).

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Result

Table 3.1: Physicochemical Parameters of Cola nitida leaf. Derived Whey During Fermentation (Week 1–5).

Parameter	Week 1		Week 2		Week 3		Week 4		Week 5	
pH	5.25	5.33	5.18	5.25	5.16	5.19	5.85	5.86	6.01	6.02
EC (uS/cm)	1593	1598	1894	1875	1930	1970	2029	1977	2152	2107
Moisture content (%)	92.15	92.01	92.41	92.07	92.25	92.75	93.11	93.21	94.01	94.17
TOM	54.68	53.44	72.66	72.54	91.73	93.35	72.73	73.70	107.06	106.04
TOC	31.72	31.00	42.15	42.08	53.21	54.15	42.19	42.75	62.10	61.51
Nitrate (Mg/kg)	174.11	174.20	461.72	462.00	622.60	622.10	1042.21	1041.75	1152.71	1152.00
Phosphate (Mg/kg)	410.63	412.71	574.89	582.01	433.71	441.25	775.34	780.21	973.52	970.10
Nitrogen (Mg/kg)	39.31	39.81	104.21	104.92	140.61	140.21	235.31	236.21	262.11	260.00
Phosphorus (mg/kg)	123.98	121.51	157.19	152.15	108.84	116.10	252.73	243.01	298.91	292.01
Potassium (mg/Kg)	2.17	2.19	2.30	2.31	1.94	1.92	1.27	1.26	2.56	2.54

Table 3.2: Mean ± Standard Deviation of Physicochemical Parameters of Cola nitida leaf. Derived Whey During Fermentation (Week 1–5)

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5
pH	5.29 ± 0.06	5.21 ± 0.05	5.18 ± 0.02	5.86 ± 0.01	6.01 ± 0.01
EC (µS/cm)	1595.50 ± 3.54	1884.50 ± 13.44	1950.00 ± 28.28	2003.00 ± 36.77	2129.50 ± 31.82
Moisture content (%)	92.08 ± 0.10	92.24 ± 0.24	92.50 ± 0.35	93.16 ± 0.07	94.09 ± 0.11
TOM	54.06 ± 0.88	72.60 ± 0.08	92.54 ± 1.15	73.22 ± 0.69	106.55 ± 0.72
TOC	31.36 ± 0.51	42.11 ± 0.05	53.68 ± 0.66	42.47 ± 0.40	61.80 ± 0.42
Nitrate (mg/kg)	174.16 ± 0.06	461.86 ± 0.20	622.35 ± 0.35	1041.98 ± 0.33	1152.36 ± 0.50
Phosphate (mg/kg)	411.67 ± 1.47	578.45 ± 5.04	437.48 ± 5.33	777.78 ± 3.45	971.81 ± 2.42
Nitrogen (mg/kg)	39.56 ± 0.35	104.57 ± 0.50	140.41 ± 0.28	235.76 ± 0.64	261.06 ± 1.49
Phosphorus (mg/kg)	122.75 ± 1.75	154.67 ± 3.56	112.47 ± 5.14	247.87 ± 6.88	295.46 ± 4.88
Potassium (mg/kg)	2.18 ± 0.01	2.31 ± 0.01	1.93 ± 0.01	1.27 ± 0.01	2.55 ± 0.01

3.2 Discussion

The pH showed an initial decrease from 5.29 in Week 1 to 5.18 in Week 3, followed by a gradual rise to 6.01 by Week 5. This pattern suggests an initial accumulation of organic acids from microbial metabolism, which lowered the pH, followed by a buffering effect or microbial utilization of acids, allowing the medium to stabilize near neutrality. A near-neutral pH favors diverse microbial activity, which is essential for biodegradation processes in bioremediation systems. Electrical conductivity increased progressively (1595.50 to 2129.50 μS/cm), reflecting the release of soluble ions during fermentation. The increase in ionic strength signals enhanced nutrient availability, supporting microbial metabolism and making the whey a suitable biostimulant for bioremediation.

Moisture content rose steadily from 92.08% to 94.09%, indicating the conversion of organic matter and structural breakdown of the whey matrix, which released bound water. Such moisture enrichment ensures substrate fluidity and accessibility to microbes, thus improving degradation efficiency. Both total organic matter (TOM) and total organic carbon (TOC) increased overall, although TOM showed fluctuations, with a dip at Week 4. The rising trend reflects the accumulation of organic fermentation byproducts, while the mid-fermentation dip may indicate partial microbial consumption of soluble organics. In bioremediation contexts, these organic compounds act as electron donors that stimulate microbial breakdown of pollutants, highlighting kola-leaf whey's potential as a sustainable amendment.

The nutrient profile demonstrated significant increases in nitrate, phosphate, nitrogen, and phosphorus, with nitrate rising from 174.16 to 1152.36 mg/kg and phosphate from

411.67 to 971.81 mg/kg. These sharp increases reflect the mineralization of proteins and organic phosphorus compounds into bioavailable forms. Elevated nutrient levels can accelerate microbial proliferation and activity, enhancing pollutant removal from contaminated soils or wastewater. However, such concentrations also pose eutrophication risks if the effluent is directly discharged into aquatic environments, indicating the need for integrated treatment strategies. Potassium levels fluctuated, dipping mid-fermentation before recovering by Week 5, suggesting microbial uptake followed by mineral release; potassium is known to support microbial physiology and osmotic regulation.

Overall, the observed physicochemical changes confirm that kola-leaf whey undergoes dynamic transformations that enhance its nutrient content, ionic strength, and microbial suitability during fermentation. These properties make it a promising candidate for bioremediation, particularly as a low-cost, plant-based biostimulant to enhance microbial activity in contaminated sites.

3.3 Conclusion

The fermentation of kola-leaf-derived whey demonstrated progressive changes in physicochemical and nutrient parameters, highlighting its potential as a bioresource for environmental applications. The gradual shift in pH toward neutrality, rising conductivity, and sustained high moisture content created favorable conditions for microbial activity. The marked increase in total organic matter, carbon, nitrate, phosphate, nitrogen, and phosphorus across the five-week period confirmed that fermentation enhanced nutrient availability and organic enrichment. These findings suggest that kola-leaf whey can serve as a cost-effective and locally accessible amendment to stimulate microbial processes in

bioremediation of contaminated soils or waters. However, while the nutrient enrichment observed is promising, future studies should optimize application rates and assess long-term ecological impacts to balance remediation efficiency with environmental safety.

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