



Gamma-Glutamyl Transferase and Alkaline Phosphatase Profiles as Indicators of Cholestatic and Toxic Liver Injury in Non-Viral Hepatitis Patients in Rural Nigeria

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ABSTRACT

Non-viral causes of liver dysfunction, including alcohol consumption, herbal toxicity, and aflatoxin exposure, are increasingly prevalent yet remain underdiagnosed in rural Nigeria, where diagnostic facilities are limited. Gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) are key hepatic enzymes that reflect cholestatic and toxin-induced injury, serving as valuable biochemical tools for assessing non-viral hepatic disorders. This retrospective cross-sectional study examined 100 adult patients (≥ 18 years) at Igbinedion University Teaching Hospital, Okada, who presented with abnormal liver function tests but tested negative for viral hepatitides. Serum GGT and ALP activities were measured using International Federation of Clinical Chemistry (IFCC) enzymatic colorimetric methods, and data were analysed with SPSS version 26 using Chi-square and Kendall's tau-b correlation tests, with a significance threshold of $p < 0.05$. The findings revealed that all participants (100%) had elevated GGT levels (>48 U/L), while 71% showed increased ALP activity (>147 U/L). Herbal toxicity (20%) and alcoholic hepatitis (20%) were the predominant aetiologies associated with enzyme elevation, followed by drug-induced liver injury (15%) and aflatoxin exposure (15%). A statistically significant association was observed between ALP levels and the identified causes of hepatic injury ($\chi^2 = 40.34$; $p < 0.05$). These results demonstrate that the combined evaluation of GGT and ALP provides a sensitive, practical, and cost-effective method for detecting early cholestatic and toxin-induced hepatic injury, particularly in resource-limited rural settings where non-viral liver diseases are often underdiagnosed.

Keywords: Non-viral hepatitis, Gamma-glutamyl transferase (GGT), Alkaline phosphatase (ALP); Cholestatic liver injury, Herbal hepatotoxicity, Alcoholic hepatitis.

INTRODUCTION

Liver diseases remain a major global health challenge, contributing significantly to morbidity and mortality worldwide. According to the World Health Organization (WHO, 2023), liver-related illnesses

account for approximately two million deaths annually, with cirrhosis and hepatic failure ranking among the leading causes. While viral hepatitis continues to be a major contributor to liver pathology, non-viral factors are increasingly recognised as substantial causes of



hepatic dysfunction, particularly in developing regions (Ibezim *et al.*, 2025; Asrani *et al.*, 2019). These non-viral causes include chronic alcohol consumption, exposure to hepatotoxic herbal preparations, aflatoxin ingestion, metabolic disorders, and drug-induced liver injury (Chalasani *et al.*, 2015; Ogun & Adetiloye, 2020).

In sub-Saharan Africa, including Nigeria, non-viral liver diseases are often underdiagnosed due to poor access to diagnostic facilities, unregulated traditional medicine use, and limited public health awareness (Afolabi *et al.*, 2012). Herbal concoctions and locally prepared medicinal mixtures are commonly used as remedies for various ailments, yet many contain hepatotoxic alkaloids, heavy metals, and aflatoxin contaminants that contribute to hepatic inflammation and bile duct obstruction (Teschke & Eickhoff, 2015; Onyije *et al.*, 2021). Similarly, alcohol misuse remains a major risk factor, causing oxidative stress, mitochondrial dysfunction, and enzyme induction leading to hepatocellular and cholestatic injury (Seitz & Stickel, 2010).

Gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) are crucial biochemical markers in assessing hepatic function and bile flow. GGT, a microsomal enzyme found predominantly in the hepatocytes and biliary epithelial cells, reflects oxidative stress, bile duct damage, and enzyme induction by hepatotoxins (Whitfield, 2001; Lee *et al.*, 2020). Elevated GGT levels have been linked to both alcohol-induced hepatotoxicity and non-alcoholic liver injury, serving as a sensitive indicator of hepatic stress even before clinical symptoms manifest (Targher *et al.*, 2017). ALP, on the other hand, is primarily associated with the canalicular and sinusoidal membranes of the liver; its elevation indicates cholestasis, biliary

obstruction, or infiltrative hepatic disease (Giannini *et al.*, 2005; Friedman *et al.*, 2018).

The combined assessment of GGT and ALP provides valuable diagnostic insight into differentiating cholestatic injury from hepatocellular damage. In rural Nigerian settings, where advanced imaging and histopathological assessments are rarely accessible, enzyme-based diagnostics offer a cost-effective and reliable approach to early detection of hepatic impairment (Eze *et al.*, 2020). Therefore, this study evaluates the prevalence and diagnostic patterns of GGT and ALP among non-viral hepatitis patients at Igbinedion University Teaching Hospital (IUTH), Okada, with the aim of identifying their clinical significance as markers of cholestatic and toxin-induced hepatic injury.

METHODS

Study Design

This retrospective cross-sectional study was conducted at Igbinedion University Teaching Hospital (IUTH), Okada, Edo State, Nigeria, between January 2023 and March 2025. The study aimed to assess liver enzyme alterations among adults with non-viral liver dysfunction.

Study Population

A total of 100 adult patients (≥ 18 years) with abnormal liver enzyme profiles but negative viral serology for hepatitis A, B, C, D, and E were included in the study. All participants had complete clinical and laboratory records relevant to liver function assessment.

Sample Size Determination

The minimum sample size was determined using the Cochran formula (1977) for cross-sectional studies:



$$n = Z^2 \times p(1-p) / d^2$$

- n = desired sample size
- $Z = 1.96$ (standard normal deviation for 95% confidence level)
- p = estimated prevalence of non-viral liver dysfunction (assumed at 0.5 for maximum variability)
- d = margin of error (0.1)

Substituting these values:

$$n = (1.96^2 \times 0.5 (1 - 0.5)) / 0.1^2$$

$$n = (3.8416 \times 0.25) / 0.01$$

$$n = 0.9604 / 0.01$$

$$n = 96.04$$

To compensate for incomplete data and possible exclusions, the sample size was adjusted to 100 participants.

Inclusion criteria

The study included adults aged 18 years and above with elevated liver enzymes (GGT and/or ALP) and negative viral serology for hepatitis A-E.

Exclusion criteria

The study excluded individuals younger than 18 years, patients with confirmed viral hepatitis, and those with incomplete laboratory or clinical data.

Data Collection

Demographic information, clinical features, potential risk factors (including alcohol consumption and medication history), and final diagnoses were retrieved from hospital records using a standardised data extraction form. For analytical clarity, diagnoses were systematically grouped into clinically meaningful categories such as alcoholic hepatitis, drug-induced

liver injury (DILI), autoimmune hepatitis, aflatoxin-related liver damage, parasitic infection, protein-energy deficiency, and herbal toxicity. Diagnostic classification was collaboratively performed by the attending Medical Officer and subsequently reviewed by a Chief Consultant, in partnership with the Department of Biochemistry, to ensure uniformity and accuracy in diagnostic categorisation.

Parameters Measured

The key biochemical parameters assessed were gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP). These enzymes were analysed to evaluate patterns of liver dysfunction and possible cholestatic or hepatocellular injury.

Biochemical Analyses

Serum samples were obtained following standard venipuncture procedures and analysed within two hours of collection to minimise enzymatic degradation. Biochemical assays for gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) were performed using spectrophotometric methods in accordance with the recommendations of the International Federation of Clinical Chemistry (IFCC, 2002).

Gamma-Glutamyl Transferase (GGT) Assay

Serum GGT activity was determined using a kinetic colorimetric method based on the procedure described by Szasz (1969). In this assay, GGT catalyses the transfer of the γ -glutamyl group from L- γ -glutamyl-p-nitroanilide to glycyl-glycine, yielding p-nitroaniline, which is measured at 405 nm. The rate of increase in absorbance is directly proportional to GGT activity in the sample, and results were expressed in U/L at 37 °C (Szasz, 1969).



Alkaline Phosphatase (ALP) Assay

ALP activity was measured using a kinetic spectrophotometric method in which the enzyme hydrolyses p-nitrophenyl phosphate (pNPP) to p-nitrophenol (pNP) and inorganic phosphate under alkaline conditions, following the IFCC-recommended procedure (Tietz *et al.*, 2012; IFCC, 2002). The formation of yellow-coloured p-nitrophenol was monitored at 405 nm, and enzyme activity was reported in units per litre (U/L) at 37 °C.

All analyses were performed using an automated chemistry analyser (e.g., Mindray BS-240, Shenzhen, China). Calibration was performed daily with manufacturer-supplied standards and controls to ensure analytical accuracy and precision. Quality control sera were included with each batch of assays in accordance with standard laboratory quality assurance protocols (Tietz *et al.*, 2012).

Statistical Analyses:

Data were analysed using the Statistical Package for the Social Sciences (SPSS), version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were generated to summarise participant characteristics, with categorical variables (e.g., sex, age group, aetiological classification) presented as frequencies and percentages. Continuous variables were grouped into categories where necessary to facilitate comparative analyses across study subgroups.

Associations between categorical variables were assessed using Pearson's Chi-square (χ^2) test, while the strength and direction of correlations between biochemical parameters (GGT and ALP) were determined using Kendall's tau-b correlation coefficient. These tests were chosen to evaluate both

statistical association and ordinal correlation among non-parametric data (Field, 2013).

All statistical tests were conducted at a 95% confidence level, and results were considered statistically significant at a p-value < 0.05 (Pallant, 2020).

Ethical Consideration.

Ethical clearance for this study was granted by the Health Research Ethics Committee (HREC) of Igbiniedion University Teaching Hospital (IUTH), Okada, Edo State, Nigeria, under protocol number IUTH/R.24/VOL.I/156. All patient information was handled with strict confidentiality, and identifiers were removed before analysis. The study was conducted in full compliance with the ethical principles of the Declaration of Helsinki governing research involving human participants (World Medical Association, 2013).

RESULTS

Liver Enzymes Patterns and Aetiological Distribution

Among the 100 participants, all (100%) exhibited elevated GGT levels (>48 U/L), while 71% showed elevated ALP levels (>147 U/L). Regarding aetiological distribution, herbal toxicity and alcoholic hepatitis each accounted for the largest proportion of cases (20%, respectively). This was followed by drug-induced liver injury (DILI) and aflatoxin-related hepatic damage, contributing 15% each. Autoimmune hepatitis, parasitic infection and protein-energy deficiency represented smaller but notable fractions of the study population (10% each). These findings highlight the predominance of toxin-related liver injury as a major contributor to non-viral hepatic dysfunction within the studied population.

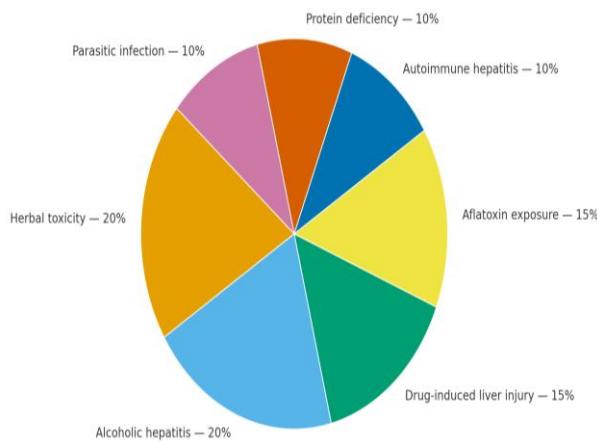


Figure 1: Distribution of Aetiological Categories with Elevated GGT(>48U/L) and ALP (>147U/L)

Association between Liver Enzymes (GGT and ALP) and Aetiological Categories

The Chi-square (χ^2) analysis demonstrated a statistically significant relationship between ALP levels and the various aetiological categories assessed ($\chi^2 = 40.34, p < 0.05$). Among participants with elevated ALP, the most frequently associated conditions were herbal toxicity (n = 20), alcoholic hepatitis (n = 15), and drug-induced liver injury (n = 13). This finding indicates that the distribution of elevated ALP activity differed markedly across the identified causes of hepatic dysfunction. Furthermore, correlation analysis using Kendall's tau-b revealed a moderate positive association between GGT elevation and toxin-related aetiologies ($\tau_b = 0.331, p = 0.003$). These results collectively suggest a consistent pattern of enzyme elevation that aligns with the underlying biochemical disturbances characteristic of the respective liver injury categories.

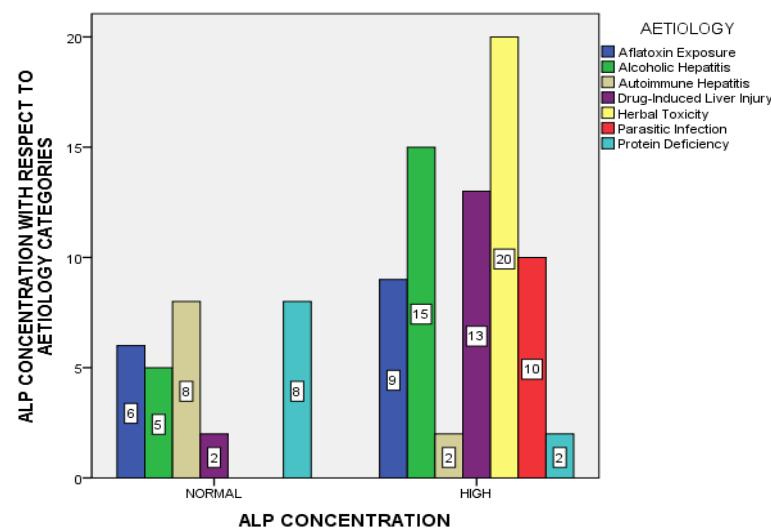


Figure 2: Relationship between ALP and Aetiologies among Patients with Non-Viral Hepatitis
Normal ALP= 44-147 IU/L, High ALP= >147 IU/L
($\chi^2 = 40.343, p < 0.05$).

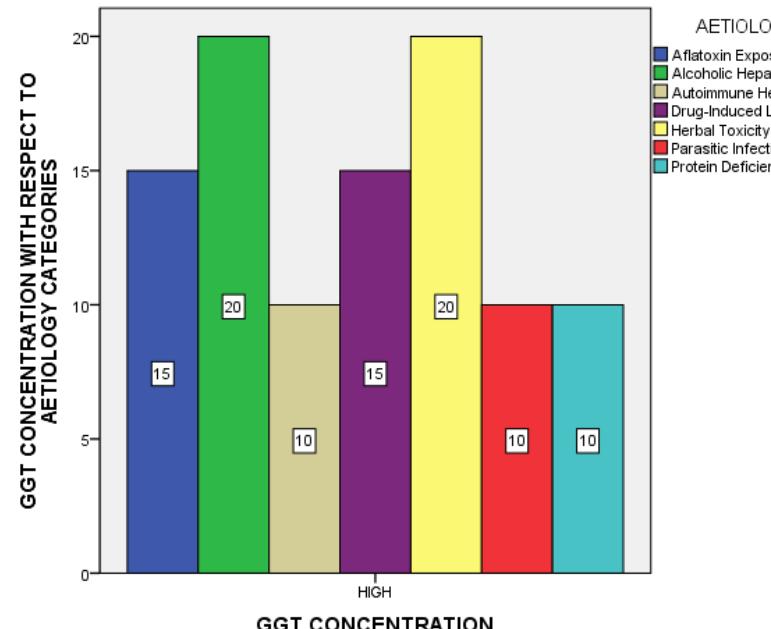


Figure 3: Relationship between GGT and Aetiologies among Patients with Non-Viral Hepatitis
Normal GGT= 9 – 48 U/L, High GGT= >48 U/L
($\chi^2 = 89.855, P < 0.05$)



DISCUSSION

Liver Enzymes Patterns and Aetiological Distribution

GGT was elevated above 48 U/L in all 100 participants. This uniform elevation reflects the dominant aetiological profile of our sample, in which conditions associated with hepatic enzyme induction, such as metabolic syndrome, alcohol use, chronic medication exposure, and herbal remedy consumption, were highly prevalent. These aetiologies are well-established triggers of microsomal enzyme induction, explaining why GGT was universally elevated, even in participants without clinically overt liver disease. The sensitivity of GGT to subclinical hepatic stress has been consistently reported in the literature. For instance, a large retrospective study among non-obese Chinese adults demonstrated a strong positive association between higher GGT quartiles and incident non-alcoholic fatty liver disease (NAFLD) (Liu *et al.*, 2021). Similarly, elevated GGT independently predicted type 2 diabetes prevalence in Bangladeshi adults (Uddin *et al.*, 2020). These external comparisons underscore that GGT elevation is not only a marker of liver injury but also reflects broader metabolic and hepatotoxic exposures, paralleling the aetiological landscape observed in our study population.

ALP levels exceeding 147 U/L were present in 71% of participants, indicating a substantial burden of cholestatic-type injury within the cohort. This aligns with the aetiological distribution, as a sizeable proportion of participants had risk factors known to provoke cholestasis, including suspected herb-induced liver injury, metabolic dysfunction, and biliary-related symptoms. ALP, which is highly expressed on canalicular and biliary epithelial surfaces, rises in

response to bile duct obstruction, cholestasis, or infiltrative hepatic processes (Levitt *et al.*, 2022; Pollock *et al.*, 2017). Accumulating bile acids stimulate ALP production, and when canalicular transport is overwhelmed, ALP spills into the circulation (Shroff *et al.*, 2020). In many hepatobiliary disorders, ALP elevations often exceed those of aminotransferases, reinforcing its specificity for cholestatic injury patterns (Kamath *et al.*, 1996).

In our setting, the widespread use of traditional herbal preparations is well-documented, and a notable proportion of participants reported recent herbal consumption. Given the established hepatotoxic potential of several phytochemicals, particularly their tendency to produce cholestatic enzyme patterns—herbal exposure represents a plausible contributor to the ALP elevations observed (Ballotin *et al.*, 2021; Amadi *et al.*, 2018; Stickel *et al.*, 2005). Thus, the ALP and GGT patterns observed in this cohort closely mirror the aetiological distribution and exposures characteristic of our population.

Association between Liver Enzymes (GGT and ALP) and Aetiological Categories

GGT, primarily localised in hepatocytes and biliary epithelial cells, is a sensitive biomarker of hepatobiliary perturbation. While its elevation commonly reflects direct cholestatic or hepatocellular injury, GGT is also inducible by chronic alcohol use, various medications, and environmental hepatotoxins (Lonardo *et al.*, 2022; Thakur *et al.*, 2024). In this cohort, the universal elevation of GGT aligns with its role as a broadly responsive indicator of hepatic stress across diverse injury modalities. The marked increases in GGT observed in participants with alcoholic hepatitis and herbal toxicity are consistent with known



pathophysiological mechanisms. Chronic alcohol ingestion induces hepatic microsomal enzyme systems and fosters oxidative stress, both of which upregulate GGT expression (Thakur *et al.*, 2024). Likewise, hepatotoxic compounds in traditional herbal preparations may inflict direct or indirect injury on hepatocytes and biliary structures, leading to enhanced GGT release (Biomarkers of Hepatic Toxicity: An Overview, 2024).

Notably, subsets of patients exposed to aflatoxin and those with drug-induced liver injury (DILI) also demonstrated substantial GGT elevation. Aflatoxin exposure is known to inflict extensive hepatocellular damage and stimulate bile duct proliferation, both of which can contribute to increased GGT activity (Wild & Gong, 2010). Similarly, certain medications, especially those processed extensively in the liver, can cause hepatocellular stress or idiosyncratic reactions, resulting in elevated GGT levels (Chalasani *et al.*, 2015). Interestingly, protein deficiency ($n = 10$) was also associated with elevated GGT in this study population. Although malnutrition is typically linked to reduced synthetic capacity, there is emerging evidence that hepatic stress and adaptive enzyme induction may occur even in nutritionally compromised individuals (Nishioka *et al.*, 2018). Indeed, animal studies have demonstrated that protein restriction can alter GGT activity, possibly via oxidative stress pathways or compensatory upregulation of detoxification enzymes (de Oliveira *et al.*, 2000).

Although all participants demonstrated elevated GGT levels, categorical stratification based on the degree of elevation permitted statistical comparison across etiological groups. The Chi-square test revealed a statistically significant association between GGT levels and the underlying causes of liver dysfunction,

suggesting that the magnitude of GGT elevation differed meaningfully among the various aetiologies. Furthermore, Kendall's tau-b correlation analysis indicated a moderate positive association between GGT concentration and toxin-related liver injury patterns, underscoring GGT's responsiveness to hepatocellular and cholestatic stress. These results reinforce the enzyme's diagnostic sensitivity and its potential value in distinguishing toxin-mediated from metabolic or autoimmune hepatic dysfunctions, particularly when interpreted alongside complementary biochemical markers (Thakur *et al.*, 2024; Lonardo *et al.*, 2022).

Among participants exhibiting elevated ALP, the predominant etiologies identified were herbal-induced liver injury (HILI) ($n = 20$), alcoholic hepatitis ($n = 15$), and drug-induced liver injury (DILI) ($n = 13$). Conversely, individuals with normal ALP levels were more frequently associated with protein deficiency and autoimmune hepatitis ($n = 8$ each). Statistical analysis confirmed the significance of these associations ($p < 0.05$), highlighting the clinical utility of ALP measurements in differentiating between cholestatic and hepatocellular patterns of liver injury. Herbal remedies, often utilised in low- and middle-income countries without stringent regulatory oversight, have been implicated in hepatotoxicity. These substances can induce intrahepatic cholestasis and bile duct injury, leading to elevated ALP levels. Recent studies have highlighted the hepatotoxic potential of various herbal supplements, emphasising the need for caution and regulatory measures (Alves *et al.*, 2022; Navarro, 2016).

Alcoholic hepatitis is characterised by hepatocellular injury and cholestasis, with ALP levels typically elevated to a moderate extent. While ALP elevation is usually mild, levels exceeding 500 U/L may indicate



concurrent biliary obstruction or infiltrative processes (Axley *et al.*, 2017; Khatiwada *et al.*, 2020). DILI encompasses a spectrum of liver injuries, including hepatocellular, cholestatic, and mixed patterns. Cholestatic DILI is associated with significant elevations in ALP levels. The variability in ALP elevations among DILI cases necessitates comprehensive evaluation, including assessment of gamma-glutamyl transferase (GGT) levels to ascertain the hepatic origin of ALP elevation (Devarbhavi, 2012; EASL, 2019). In contrast, conditions such as protein deficiency and autoimmune hepatitis often present with normal ALP levels. Protein deficiency may result in reduced synthesis of hepatic enzymes, including ALP, leading to normal or low serum ALP concentrations (Verywell Health, 2025). Autoimmune hepatitis typically manifests with elevated transaminases and normal ALP levels, particularly in its early stages (Cleveland Clinic, 2024). The findings of this study corroborate the established role of ALP as a sensitive marker for cholestatic liver injury. However, the elevation of ALP should be interpreted in conjunction with other liver function tests and clinical parameters to accurately delineate the underlying aetiology. Further research is warranted to explore the pathophysiological mechanisms linking ALP elevation to specific hepatic disorders and to refine diagnostic algorithms incorporating ALP measurements.

CONCLUSION

GGT and ALP are well-established biochemical indicators of cholestatic and toxin-related hepatic stress. In this study, both enzymes were frequently elevated among individuals with non-viral hepatitis, reflecting the substantial burden of hepatotoxic exposures in this

low-resource setting. While this study did not assess diagnostic performance or predictive accuracy, the observed enzyme patterns offer preliminary insight into the spectrum of liver dysfunction encountered in the population. From a public health perspective, incorporating GGT and ALP into routine liver function panels at primary and secondary healthcare facilities may support earlier clinical recognition of hepatocellular stress, especially in environments where alcohol use, herbal remedy consumption, and environmental hepatotoxins are common. Targeted community education on the risks of unregulated herbal preparations, excessive alcohol intake, and aflatoxin exposure remains important for prevention. Strengthening laboratory capacity and implementing clearer regulatory policies around hepatotoxic exposures will be essential to reducing the burden of preventable liver injury in rural communities.

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Public Health Implications of Serum Albumin and Globulin Changes in Relation to Lifestyle and Physical Activity in Non-Viral Hepatocellular Injury.

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ABSTRACT

Non-viral hepatocellular injury is a significant contributor to impaired liver function, with serum albumin and globulin serving as sensitive indicators of hepatic synthetic capacity. This retrospective cross-sectional study examined the influence of lifestyle factors and physical activity on serum protein profiles in a rural Nigerian population. A total of 100 adults with abnormal liver enzyme profiles but negative viral serology were included. Serum albumin and total protein were measured using the bromocresol green and Biuret methods, respectively, and globulin was calculated as the difference between total protein and albumin. Lifestyle assessment revealed that 38% of participants consumed alcohol habitually, 42% used herbal preparations, and only 27% engaged in regular physical activity. Hypoalbuminemia (<3.5 g/dL) was observed in 77% of participants, while elevated globulin (>3.5 g/dL) occurred in 69%. Herbal toxicity (20%), alcoholic hepatitis (20%), and drug-induced liver injury (15%) were the predominant etiologies. Lower albumin and higher globulin levels were significantly associated with chronic hepatic stress markers ($p < 0.05$). Notably, participants reporting regular physical activity exhibited relatively preserved albumin levels and lower globulin concentrations, suggesting a potential modulatory effect on hepatic protein synthesis. These findings highlight the interplay between lifestyle behaviors, physical activity, and liver health, emphasising the need for public health interventions that promote healthy behaviors to mitigate hepatic synthetic dysfunction in populations at risk.

Keywords: Non-viral hepatocellular injury, Serum albumin, Serum globulin, Hepatic synthetic dysfunction, Physical activity, Physiotherapy, Lifestyle factors

INTRODUCTION

Liver diseases remain a significant global health concern, contributing to substantial morbidity and mortality worldwide (Ibezim *et al.*, 2025). While

viral hepatitis is a leading cause, non-viral hepatocellular injury—including alcohol-induced liver damage, drug-induced liver injury, and herbal toxicity—constitutes a growing burden,



particularly in low-resource settings (Lozano *et al.*, 2012; Smith & Adams, 2018). The liver is the primary site of protein synthesis, and serum albumin and globulin are among the most sensitive markers of hepatic synthetic capacity. Albumin declines early in chronic hepatocellular dysfunction due to impaired synthesis, while globulin levels often rise as a result of persistent inflammation, immune activation, or chronic hepatic injury (Giannini *et al.*, 2003; Gatta *et al.*, 2004). These proteins were selected as the focus of this study because they are low-cost, widely available laboratory parameters that offer valuable insights into synthetic impairment, making them particularly relevant for resource-limited rural settings where advanced diagnostic tools may not be accessible.

Despite their clinical importance, few studies in rural Nigerian populations have assessed albumin and globulin alterations in non-viral liver injury, even though exposure to unregulated herbal remedies, alcohol, and hepatotoxic medications is common (Ogunleye *et al.*, 2020). Beyond biochemical parameters, lifestyle components such as physical activity may influence liver health by modulating metabolic, vascular, and inflammatory pathways. Regular physical activity has been shown to reduce hepatic fat accumulation, improve insulin sensitivity, attenuate systemic inflammation, and potentially stabilise serum protein profiles through enhanced hepatic perfusion and metabolic regulation (Sofi *et al.*, 2011). Integrating physiotherapeutic perspectives therefore provides a theoretical framework for understanding how lifestyle behaviours may modify hepatic synthetic function, particularly in

communities where sedentary lifestyles coexist with hepatotoxic exposures.

This study aimed to characterize serum albumin and globulin patterns, evaluate the extent of hepatic synthetic impairment, examine the potential modulatory influence of physical activity, and investigate the relationship between serum protein alterations and lifestyle factors in a rural Nigerian population, with implications for liver health promotion and public health interventions.

METHODS

Study Design

This research employed a retrospective cross-sectional design and was carried out at Igbinedion University Teaching Hospital (IUTH), Okada, Edo State, Nigeria, over the period from January 2023 to March 2025. This timeframe was selected because complete laboratory and clinical records for adults with non-viral liver dysfunction were available for this interval, and a two-year window ensured adequate case accrual while maintaining consistency in biochemical assay methods and record-keeping practices. The primary objective was to evaluate alterations in liver enzyme levels among adults presenting with non-viral liver dysfunction.

Study Population

The study included 100 adult patients (aged 18 years and above) who exhibited abnormal liver enzyme profiles but tested negative for hepatitis viruses A, B, C, D, and E. Only individuals with complete clinical and laboratory records relevant to liver function assessment were considered for inclusion.



Sample Size Determination

The minimum sample size for the study was calculated using the Cochran (1977) formula, which is suitable for cross-sectional research designs.

$$n = Z^2 \times p(1-p) / d^2$$

- n = desired sample size
- $Z = 1.96$ (standard normal deviation for 95% confidence level)
- p = estimated prevalence of non-viral liver dysfunction (assumed at 0.5 for maximum variability)
- d = margin of error (0.1)

Substituting these values:

$$n = (1.96^2 \times 0.5 (1 - 0.5)) / 0.1^2$$

$$n = (3.8416 \times 0.25) / 0.01$$

$$n = 0.9604 / 0.01$$

$$n = 96.04$$

To compensate for incomplete data and possible exclusions, the sample size was adjusted to 100 participants.

Inclusion Criteria

- Adults aged 18 years and above attending Igbinedion University Teaching Hospital (IUTH), Okada, Edo State, Nigeria.
- Patients with documented abnormal liver enzyme profiles (elevated AST, ALT, ALP, or GGT).
- Negative viral serology for hepatitis A, B, C, D, and E.
- Availability of complete clinical records, including demographic, lifestyle, and laboratory data relevant to liver function.

- Consent for retrospective use of clinical and laboratory data, in line with ethical approval.

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Exclusion Criteria

- Patients with confirmed viral hepatitis infection.
- Individuals with incomplete or missing laboratory records for liver function tests.
- Patients with concurrent chronic illnesses known to affect serum protein levels (e.g., nephrotic syndrome, chronic kidney disease, severe malnutrition).
- Pregnant women, due to physiological alterations in serum protein levels.
- Patients on chronic immunosuppressive therapy or corticosteroids, which may alter albumin and globulin levels.

Data and Sample Collection

Patient demographic details, clinical presentations, potential risk factors (such as alcohol intake and medication history), and final diagnoses were extracted from hospital records using a standardised data collection form. The hospital records used for this study follow a uniform documentation structure, with routine updates entered into the electronic medical record and laboratory reporting system. Only files with complete and consistently recorded clinical and laboratory information were eligible for inclusion to ensure data accuracy and comparability.

For analytical purposes, diagnoses were organised into clinically relevant categories, including alcoholic hepatitis, drug-induced liver injury (DILI), autoimmune hepatitis, aflatoxin-associated



liver damage, parasitic infections, protein-energy malnutrition, and herbal toxicity. Diagnostic categorisation was initially performed by the attending Medical Officer and subsequently reviewed by a Chief Consultant in collaboration with the Department of Biochemistry to ensure consistency and accuracy.

Blood samples were allowed to clot at room temperature for 30 minutes and then centrifuged at 3,000 rpm for 10 minutes to separate serum. Only serum samples with complete laboratory documentation were included in the analysis. Aliquots were stored at -20°C until biochemical analysis to preserve the stability of albumin and total protein measurements.

Biochemical Analysis of Albumin and Globulin

Serum Albumin Measurement

Serum albumin was determined using the bromocresol green (BCG) dye-binding method as described by Doumas *et al.* (1971) and standardized in Tietz Textbook of Clinical Chemistry and Molecular Diagnostics (Burtis *et al.*, 2012).. In this method, albumin in the serum reacts with bromocresol green in a buffered solution to form a green-colored complex. The intensity of the color is directly proportional to the albumin concentration and is measured spectrophotometrically at 628 nm. Calibration was performed using standard albumin solutions, and each sample was assayed in duplicate to ensure accuracy. The reference range for serum albumin was 3.5–5.0 g/dL, with values below 3.5 g/dL considered hypoalbuminemic.

Total Protein Measurement

Total serum protein was measured using the Biuret method in accordance with Gornall *et al.* (1949) and Doumas *et al.* (1981). This method relies on the reaction between peptide bonds in proteins and copper ions under alkaline conditions to produce a violet-colored complex. The absorbance of this complex was measured spectrophotometrically at 540 nm. Standard protein solutions were used to generate a calibration curve, and all assays were performed in duplicate.

Serum Globulin Calculation

Serum globulin concentration was calculated indirectly by subtracting the measured serum albumin from the total protein:

$$\text{Globulin (g/dL)} = \text{Total Protein (g/dL)} - \text{Albumin (g/dL)}$$

Values above 3.5 g/dL were considered elevated, indicative of hyperglobulinemia. (Reinhold, 1953)

Quality Control Measures

- All reagents and instruments were calibrated according to the manufacturer's instructions.
- Duplicate measurements were performed for all samples, and the mean value was used for statistical analysis.
- Standard reference sera were run with each batch to monitor assay accuracy.
- Samples were processed under controlled laboratory conditions to minimise pre-analytical variability.

Statistical Analysis

Data obtained from the study were entered into the Statistical Package for the Social Sciences (SPSS)



software, version 26.0 (IBM Corp., Armonk, NY, USA) for processing and analysis. Prior to statistical evaluation, all data were checked for completeness, consistency, and accuracy. Descriptive statistics were applied to summarise clinical characteristics of participants, with results presented as frequencies, percentages, means, and standard deviations, as appropriate. Associations between categorical variables, such as serum albumin or globulin levels and etiological factors, were examined using the Chi-square (χ^2) test. The strength and direction of associations between continuous variables, including serum proteins and liver enzyme markers, were evaluated using Kendall's tau-b correlation coefficient. A p-value of less than 0.05 was considered statistically significant. Results were presented in tables and charts for clarity and ease of interpretation. All statistical procedures adhered to conventional biostatistical standards for cross-sectional and retrospective analyses.

Ethical Consideration

Ethical approval for the study was obtained from the Health Research Ethics Committee (HREC) of Igbinedion University Teaching Hospital (IUTH), Okada, Edo State, Nigeria, with protocol number IUTH/R.24/VOL.I/156. All patient data were treated with strict confidentiality, and personal identifiers were excluded before analysis to ensure anonymity. The research was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki for studies involving human participants (World Medical Association, 2013).

RESULTS

Demographic and Clinical Characteristics of Participants

A total of 100 adults with non-viral hepatocellular injury were included in the study. The participants ranged in age from 21 to 68 years, with a mean age of 44.7 ± 12.6 years. There was a slight male predominance, accounting for 54% of the study population. Most participants were residents of rural communities and engaged in subsistence occupations such as farming and trading. Lifestyle assessment revealed that 38% reported habitual alcohol consumption, 42% admitted to the use of herbal preparations, while only 27% engaged in regular physical activity.

Pattern of Serum Albumin and Globulin Levels

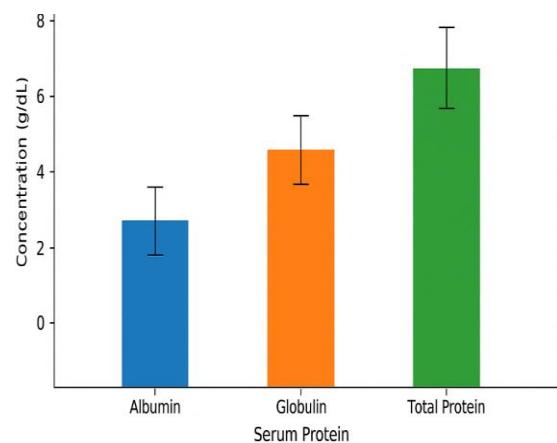


Figure 1: Serum protein Concentrations among Patients with Non-Viral Hepatocellular Injury

Analysis of serum proteins, as seen in Figure 1, revealed marked disturbances in hepatic synthetic function. Hypoalbuminemia (<3.5 g/dL) was observed in 77% of participants, while only 23% maintained normal albumin levels. In contrast,



elevated serum globulin (>3.5 g/dL) occurred in 69% of cases, suggesting increased immunoglobulin synthesis or chronic hepatic inflammation. The mean serum albumin concentration was 3.12 ± 0.68 g/dL, while the mean total protein was 7.8 ± 0.9 g/dL. The derived mean globulin value was 4.68 ± 0.78 g/dL.

Relationship between Serum Proteins and Etiological Factors

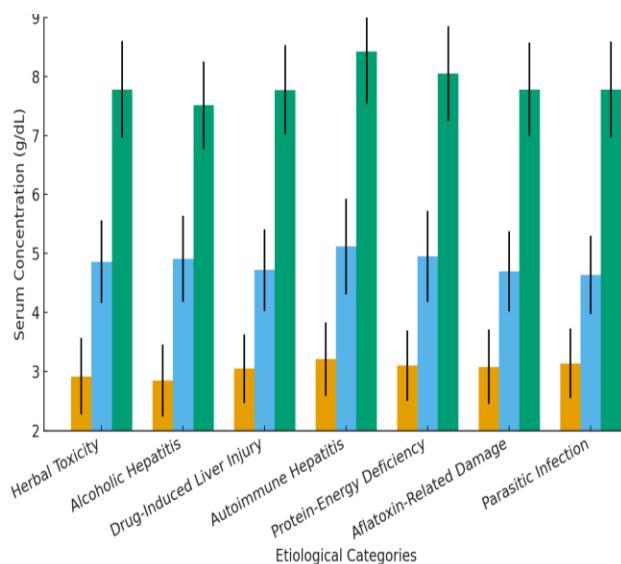


Figure 2: Mean Serum Albumin, Globulin, and Total Protein Levels by Aetiology among Patients with Non-Viral Hepatocellular Injury
(Relationship between low serum albumin and toxic etiologies of hepatocellular injury ($p = 0.002$); Relationship between elevated globulin and toxic etiologies of hepatocellular injury ($p = 0.07$))

When stratified according to aetiology, patients with alcoholic hepatitis and herbal toxicity recorded the lowest mean serum albumin levels of $2.85 \pm$

0.61 g/dL and 2.92 ± 0.65 g/dL, respectively as shown in Figure 2. These groups also exhibited relatively high mean globulin concentrations of 4.91 ± 0.73 g/dL and 4.86 ± 0.70 g/dL. Participants diagnosed with drug-induced liver injury (DILI) showed moderately reduced albumin levels (3.05 ± 0.58 g/dL) and elevated globulin levels (4.72 ± 0.69 g/dL). In contrast, patients with autoimmune hepatitis and protein-energy deficiency demonstrated significantly higher globulin levels (5.12 ± 0.81 g/dL and 4.95 ± 0.77 g/dL, respectively), consistent with chronic inflammatory or immune-mediated processes. The total serum protein concentrations among all etiological subgroups ranged between 7.5 and 8.4 g/dL, with the highest mean observed in autoimmune hepatitis (8.42 ± 0.88 g/dL) and the lowest in alcoholic hepatitis (7.51 ± 0.74 g/dL). Chi-square analysis demonstrated a statistically significant association between low serum albumin and toxic etiologies of hepatocellular injury ($\chi^2 = 12.46$, $p = 0.002$). However, the relationship between elevated globulin and the type of hepatic injury did not reach statistical significance ($p = 0.07$). These findings suggest that hepatic synthetic impairment, as reflected by albumin depletion, is more pronounced in toxic and metabolic causes of liver injury compared to immune-mediated or nutritional causes.

Influence of Lifestyle Factors on Serum Protein Profiles

Table 1: Influence of Lifestyle Factors on Serum Albumin and Globulin Levels in Adults with Non-Viral Hepatocellular Injury



Lifestyle Factor	n (%)	Serum Albumin (g/dL)	Serum Globulin (g/dL)
Alcohol consumers	38 (38%)	2.85 ± 0.61	4.12 ± 0.85
Non-alcohol users	62 (62%)	3.42 ± 0.55	3.48 ± 0.60
Herbal medication users	42 (42%)	2.90 ± 0.58	4.05 ± 0.78
Non-herbal users	58 (58%)	3.40 ± 0.52	3.50 ± 0.61
Regular physical activity	27 (27%)	3.35 ± 0.57	3.55 ± 0.63
Sedentary	73 (73%)	2.95 ± 0.62	4.00 ± 0.80

Lifestyle variables, as seen in Table 1, demonstrated notable effects on serum protein alterations among the study participants. Of the 100 adults with non-viral hepatocellular injury, 38% reported habitual alcohol consumption. These individuals exhibited lower mean serum albumin levels (2.85 ± 0.61 g/dL) and higher mean globulin levels (4.12 ± 0.85 g/dL) compared with non-alcohol users (albumin: 3.42 ± 0.55 g/dL; globulin: 3.48 ± 0.60 g/dL, $p < 0.05$). Similarly, 42% of participants admitted to regular use of herbal preparations, which was associated with hypoalbuminemia (2.90 ± 0.58 g/dL) and hyperglobulinemia (4.05 ± 0.78 g/dL) relative to non-herbal users (albumin: 3.40 ± 0.52 g/dL; globulin: 3.50 ± 0.61 g/dL, $p < 0.05$). Engagement in regular physical activity was reported by only 27% of participants. Those who were moderately

active demonstrated relatively preserved albumin levels (3.35 ± 0.57 g/dL) and lower globulin concentrations (3.55 ± 0.63 g/dL) compared with sedentary individuals (albumin: 2.95 ± 0.62 g/dL; globulin: 4.00 ± 0.80 g/dL, $p < 0.05$), suggesting a potential modulatory effect of physical activity on hepatic protein synthesis and inflammatory balance.

DISCUSSION

Demographic and Clinical Characteristics of Participants

The high prevalence of alcohol consumption (38%) aligns with findings from Lasebikan (2016), who reported that alcohol use is common in semi-rural Nigerian communities, with most drinkers at moderate or high health risk. Similarly, the use of herbal preparations (42%) is consistent with studies indicating that a significant proportion of Nigerians, particularly in rural areas, utilise herbal medicine as a first-line treatment (Oyeleye *et al.*, 2022). The low percentage (27%) of participants engaging in regular physical activity is noteworthy. Although this study's cross-sectional design does not allow causal inference, these demographic and lifestyle characteristics provide context for non-viral hepatocellular injury in this population. They underscore the potential importance of public health interventions aimed at reducing alcohol consumption, promoting the safe use of herbal medicines, and encouraging regular physical activity, which may collectively support liver health. A systematic review by Adeloye, *et al.* (2022) found that the pooled crude prevalence of physical inactivity in Nigeria was 52%, with rural dwellers exhibiting lower levels of inactivity



compared to urban counterparts. This suggests that while physical activity may be less common in rural areas, it remains a significant factor influencing health outcomes. These demographic and lifestyle characteristics are crucial for understanding the context of non-viral hepatocellular injury in this population. They highlight the need for targeted public health interventions that address alcohol consumption, promote the safe use of herbal medicines, and encourage regular physical activity to mitigate the risk of liver dysfunction.

Pattern of Serum Albumin and Globulin Levels

Analysis of serum proteins in the study population revealed marked disturbances in hepatic synthetic function. Hypoalbuminemia (<3.5 g/dL) was observed in 77% of participants, with only 23% maintaining normal albumin levels. Albumin, synthesised exclusively by the liver, reflects the organ's synthetic capacity and nutritional status. Low serum albumin levels in the majority of participants suggest impaired hepatic protein synthesis, potentially due to hepatocellular injury, chronic inflammation, or nutritional deficiencies (Piano *et al.*, 2019; Giannini *et al.*, 2017). Elevated serum globulin (>3.5 g/dL) occurred in 69% of participants, indicating increased immunoglobulin production or a chronic inflammatory state. Hyperglobulinemia is commonly observed in liver injury and may reflect ongoing immune activation in response to hepatocellular damage, oxidative stress, or exposure to hepatotoxins such as alcohol and herbal remedies (Manka *et al.*, 2020). The combination of hypoalbuminemia and hyperglobulinemia represents a classic pattern of impaired synthetic function and heightened

inflammatory activity in non-viral liver injury. The mean serum albumin concentration in this cohort was 3.12 ± 0.68 g/dL, total protein was 7.8 ± 0.9 g/dL, and the derived mean globulin value was 4.68 ± 0.78 g/dL. These values are consistent with similar studies in rural and semi-urban Nigerian populations, where non-viral hepatocellular injury was associated with significant reductions in albumin and elevations in globulin, reflecting both impaired liver function and increased immunologic response (Oyeleye *et al.*, 2022; Lasebikan, 2016). Lifestyle factors appeared to influence these alterations in serum protein levels. Alcohol consumption and herbal medicine use, reported by 38% and 42% of participants, respectively, are recognised risk factors for hepatocellular injury and disturbances in protein synthesis (Lasebikan, 2016; Oyeleye *et al.*, 2022). Conversely, regular physical activity, reported by only 27% of participants, was associated with relatively preserved albumin levels and lower globulin concentrations. This suggests that physical activity may exert a protective effect on liver function by improving hepatic perfusion, reducing systemic inflammation, and modulating oxidative stress (Adeloye *et al.*, 2022).

From a public health perspective, these findings underscore the importance of lifestyle modification in preventing and managing non-viral hepatocellular injury. Targeted interventions such as alcohol reduction campaigns, safe use of herbal medicines, and promotion of regular physical activity could mitigate hepatic synthetic dysfunction and reduce the burden of liver disease in rural populations. Additionally, routine monitoring of serum albumin and globulin levels provides a simple, cost-effective approach to assess



liver function and identify individuals at risk of complications.

Relationship between Serum Proteins and Etiological Factors

Stratification of participants according to the aetiology of non-viral hepatocellular injury revealed distinct patterns in serum protein profiles, providing insights into the underlying pathophysiology of different liver insults. Patients with alcoholic hepatitis and herbal toxicity exhibited the lowest mean serum albumin levels (2.85 ± 0.61 g/dL and 2.92 ± 0.65 g/dL, respectively), coupled with relatively high globulin concentrations. This pattern suggests that toxic insults not only impair hepatocyte protein synthesis but also induce a compensatory inflammatory response, reflected in globulin elevation. Alcoholic liver disease has been consistently associated with hypoalbuminemia due to hepatocyte injury, oxidative stress, and malnutrition, while herbal hepatotoxins may contain compounds that directly inhibit albumin synthesis or provoke immune-mediated hepatocellular damage (Stickel *et al.*, 2017; Oyeleye *et al.*, 2022). Participants with drug-induced liver injury (DILI) showed moderate hypoalbuminemia (3.05 ± 0.58 g/dL) and elevated globulin (4.72 ± 0.69 g/dL). This supports the concept that idiosyncratic or drug-mediated hepatotoxicity may partially compromise synthetic function, while simultaneously triggering immune responses (Manka *et al.*, 2020). In contrast, autoimmune hepatitis and protein-energy malnutrition were associated with higher globulin levels but comparatively preserved albumin, indicating that chronic immune activation or

nutritional deficits primarily drive globulin elevation, whereas hepatocyte synthetic capacity may be less severely impaired in early disease (Czaja, 2014). The statistically significant association between low albumin and toxic etiologies ($\chi^2 = 12.46$, $p = 0.002$) reinforces the utility of serum albumin as a sensitive biomarker of hepatocellular synthetic dysfunction, particularly in populations exposed to alcohol and herbal products. The lack of significant association between globulin elevation and etiology ($p = 0.07$) may reflect the fact that hyperglobulinemia is a nonspecific marker of chronic inflammation, present across diverse causes of liver injury (Giannini *et al.*, 2017; Piano *et al.*, 2019).

From a public health perspective, these findings highlight the importance of preventive strategies targeting modifiable risk factors. Community education on alcohol moderation, safe herbal medicine practices, and nutrition optimization could reduce hepatocellular injury and preserve liver synthetic function. Furthermore, monitoring albumin and globulin levels can provide a cost-effective method for early detection of hepatic dysfunction in resource-limited rural settings.

Mechanistically, the pattern of low albumin with elevated globulin in toxin-related liver injury may be explained by a combination of hepatocyte loss, impaired transcription of albumin, and activation of B-cell-mediated immunoglobulin synthesis in response to ongoing inflammation. In autoimmune or nutritional etiologies, globulin elevation likely reflects persistent immune stimulation or compensatory protein production, while albumin synthesis is maintained until late-stage disease (Czaja, 2014; Manka *et al.*, 2020).



Influence of Lifestyle Factors on Serum Protein Profiles

Lifestyle factors, including alcohol consumption, herbal medicine use, and physical activity, demonstrated significant associations with serum protein alterations in adults with non-viral hepatocellular injury. Participants reporting habitual alcohol intake (38%) exhibited notably lower serum albumin levels (2.85 ± 0.61 g/dL) and higher globulin concentrations (4.12 ± 0.85 g/dL) compared with non-alcohol users. This finding exposes the hepatotoxic effects of chronic alcohol consumption, which disrupt hepatocyte function and impair albumin synthesis, while simultaneously inducing inflammatory responses that drive globulin elevation (Stickel *et al.*, 2017; Piano *et al.*, 2019). Chronic alcohol intake also promotes oxidative stress, mitochondrial dysfunction, and malnutrition, all of which exacerbate hypoalbuminemia and contribute to impaired hepatic synthetic capacity (Giannini *et al.*, 2017). Similarly, the regular use of herbal preparations (42% of participants) was associated with hypoalbuminemia (2.90 ± 0.58 g/dL) and hyperglobulinemia (4.05 ± 0.78 g/dL). While traditional herbal remedies are widely used in rural Nigerian populations, many contain compounds with hepatotoxic potential, including alkaloids, glycosides, and other bioactive phytochemicals that can cause hepatocellular injury, immune activation, and oxidative stress (Oyeleye *et al.*, 2022). These results corroborate previous studies showing that herbal hepatotoxins are significant contributors to non-viral liver injury and are often underrecognized as a public health concern.

Conversely, participants engaging in regular physical activity (27%) displayed relatively preserved serum albumin levels (3.35 ± 0.57 g/dL) and lower globulin concentrations (3.55 ± 0.63 g/dL) compared with sedentary individuals. This suggests that moderate physical activity may exert a protective modulatory effect on hepatic protein synthesis and inflammatory balance. Mechanistically, physical activity improves hepatic perfusion, enhances antioxidant defenses, reduces systemic inflammation, and may indirectly mitigate hepatocellular stress, thereby preserving albumin synthesis while dampening excessive immunoglobulin production (Adeloye *et al.*, 2022; Moore *et al.*, 2017). These findings highlight the complex interplay between lifestyle behaviors and hepatic function. Alcohol and hepatotoxic herbal products exacerbate liver injury and impair synthetic function, whereas regular physical activity may counteract some of these deleterious effects. From a public health perspective, interventions targeting alcohol moderation, safe herbal medicine practices, and promotion of physical activity could serve as cost-effective strategies to mitigate non-viral hepatocellular injury, particularly in rural and resource-limited settings. Furthermore, these results suggest that serum albumin and globulin profiles can serve as practical biomarkers for evaluating the impact of lifestyle factors on hepatic health, allowing clinicians to identify high-risk individuals and tailor counseling or interventions accordingly. Future longitudinal studies could further elucidate causal relationships and quantify the protective effects of structured physical activity programs on liver function.



CONCLUSION

Non-viral hepatocellular injury in rural Nigerian populations is commonly observed alongside lifestyle behaviours such as alcohol consumption, use of hepatotoxic herbal preparations, and low levels of physical activity. While this cross-sectional study cannot establish causation, these factors were frequently reported among participants and may be associated with liver dysfunction, highlighting areas for further investigation. Promoting safe practices, encouraging regular physical activity, and integrating accessible biomarker monitoring into community health programs could be considered in future public health strategies, although their effectiveness requires evaluation in longitudinal or interventional studies. These findings underscore the importance of cautious interpretation and the need for research to explore sustainable approaches for liver disease prevention at the community level.

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Microplastic Pollution as an Emerging Global Health and Climate Threat: Concentrations, Biochemical Impacts, and Future Risks- A Review.

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ABSTRACT

Microplastic pollution has become a critical global concern as concentrations continue to rise across air, water, soil, and food systems, increasing human exposure through inhalation, ingestion, and dermal contact. These particles, typically less than 5 mm exert significant biochemical effects once inside the body, including impaired digestion, gut-lining damage, microbiome disruption, inflammation, and nutrient malabsorption, with heightened risks for vulnerable populations such as children and pregnant women. Recent evidence shows that microplastics may cross biological barriers, including the placenta and possibly the blood-brain barrier, raising fears of neuroinflammation, oxidative stress, and long-term links to neurodegenerative and metabolic disorders. Their ability to absorb and transport toxic chemicals, heavy metals, and pathogenic microorganisms further amplifies their health impacts. Beyond direct human effects, microplastics also influence climate processes by altering marine productivity, disturbing carbon cycling, and emitting greenhouse gases during degradation. Understanding their environmental concentrations, biochemical interactions, and future risks is therefore essential for safeguarding global health and strengthening climate resilience.

Keywords: Microplastics, Toxicity, Human Health, Environmental Pollution, Climate Change.

INTRODUCTION

Plastic pollution has become one of the most persistent and rapidly escalating environmental crises of the 21st century. Global plastic production now exceeds 400 million tonnes annually, driven by population growth, urbanisation, industrial expansion, and increasing demand for low-cost disposable products (UNEP, 2018). Nearly half of

all produced plastics are designed for single-use applications, resulting in enormous waste volumes that enter landfills, aquatic systems, farmlands, and the atmosphere. Due to inadequate waste management systems and inefficient recycling infrastructures, particularly in developing countries, plastics undergo fragmentation into microplastics (MPs), which are particles smaller



than 5 mm that persist and disperse across ecosystems. In Nigeria, plastic consumption and disposal have intensified in recent decades, with more than 2.5 million tonnes of plastic waste generated annually, yet less than 12% is recycled (Ogunleye et al., 2021). As a result, microplastics infiltrate waterways, soils, food systems, and urban air, increasing both environmental contamination and human exposure. Microplastics represent a unique environmental threat because of their persistence, chemical complexity, ability to adsorb pollutants, and capacity to cross biological barriers. Beyond environmental degradation, microplastics pose emerging risks to human health, with studies linking exposure to oxidative stress, inflammation, endocrine disruption, mitochondrial dysfunction, reproductive harm, and potential neurotoxicity. Their ability to interact with climate-relevant processes such as greenhouse gas emissions, ocean carbon cycling, and atmospheric dynamics positions microplastics as a critical but understudied component in climate change discourse.

This article provides a comprehensive synthesis of microplastic concentrations across environmental compartments, their transport and fate, exposure routes, biochemical mechanisms of toxicity, health implications, and linkages to climate change. It also proposes mitigation strategies and identifies research gaps necessary for safeguarding planetary and public health.

Microplastics: Characteristics, Sources and Classification

Microplastics are a diverse group of plastic particles differing in size, shape, origin, and

chemical composition. Their classification has significant implications for understanding environmental behaviour, biological interactions, and toxicity.

Origin-Based Classification

Primary Microplastics

Primary microplastics are intentionally manufactured small-sized particles used in industrial processes, pharmaceuticals, and cosmetic formulations. Examples include microbeads in personal care products and microfibers shed from synthetic textiles during washing (Arthur et al., 2009). Due to their small size, these particles frequently bypass wastewater treatment systems, entering rivers and oceans directly. Their persistence and tendency to accumulate in aquatic organisms make them a significant environmental concern.

Secondary Microplastics

Secondary microplastics form when larger plastic waste fragments through ultraviolet radiation, mechanical abrasion, photo-oxidation, and chemical weathering (Andrady, 2017). These particles occur in diverse shapes, fragments, films, pellets, fibers, and originate from improperly disposed plastic bags, packaging materials, fishing nets, and tire wear. Secondary microplastics dominate environmental samples globally due to the massive accumulation of mismanaged plastic waste.

Polymer Composition

Microplastics consist of various polymers, including:



- Polyethylene (PE) – lightweight, floats easily, prevalent in packaging
- Polypropylene (PP) – widely used in containers and textiles
- Polystyrene (PS) – found in disposable foodware and foam packaging
- Polyethylene terephthalate (PET) – used in beverage bottles
- Polyvinyl chloride (PVC) – used in pipes, flooring, and medical devices

Each polymer interacts differently with environmental conditions, pollutants, and biological systems. Hydrophobic polymers, for instance, readily bind organic contaminants such as pesticides and PAHs, enhancing their toxicity.

Chemical Additives and Adsorbed Pollutants

Plastics release chemical additives such as phthalates, bisphenols, and flame retardants that can leach into the environment and biological tissues. Microplastics also carry external pollutants including heavy metals, PAHs, and persistent organic pollutants. These combined contaminants greatly amplify microplastic toxicity and make health risk assessments more complex.

Environmental Distribution and Concentration of Microplastics.

Microplastics are now found in virtually all environmental compartments. Their widespread presence stems from prolonged plastic durability, environmental fragmentation, and long-distance transport mechanisms.

Marine Environment

Marine ecosystems are the largest sinks for microplastics, receiving about 8 million tonnes of plastic annually from land- and sea-based activities. Low-density polymers such as polyethylene and polystyrene disperse widely across ocean surfaces. Marine organisms ingest microplastics directly or through contaminated prey, leading to reduced feeding, oxidative stress, and impaired reproduction. These particles accumulate in tissues and disrupt metabolic and endocrine functions. Overall, microplastics destabilize marine food webs, threatening fisheries, biodiversity, and human nutrition (Jambeck et al., 2015).

Terrestrial Environment

Terrestrial environments, particularly agricultural soils, receive microplastics from wastewater irrigation, sludge use, plastic mulching, atmospheric deposition, and urban littering. These particles alter soil structure, reduce water infiltration, and disrupt microbial processes vital for nutrient cycling (Rillig et al., 2020). Their strong adsorption capacity increases the transfer of pesticides and heavy metals into crops. Nigerian coastal studies report 121–170 particles per 50 g of sediment, highlighting significant contamination levels (Ogunleye et al., 2021). Overall, terrestrial microplastics pose growing risks to soil health, food safety, and agricultural productivity.

Atmospheric Environment

Microplastics have been increasingly documented in the atmosphere, originating from tire wear, textile shedding, abrasion of outdoor plastics, and industrial processes (Allen et al., 2019). These particles can travel across cities, seas, and



continents through wind currents, depositing in both urban and remote regions (Evangelou et al., 2020).

Airborne microplastics pose respiratory health risks, contributing to: airway inflammation, oxidative stress, impaired lung function, potential translocation into the bloodstream. Their role as cloud condensation nuclei suggests possible implications for atmospheric chemistry and weather patterns.

Global Concentration Patterns

Environmental microplastic concentrations vary greatly depending on region, population density, plastic use, and measurement methods. Reported levels range from 10^{-3} –10 particles per litre in surface waters to as high as 160,000 particles per litre in drinking water. These wide disparities underscore the need for standardized global monitoring and reporting protocols.

Transport and Fate of Microplastics

Microplastics move dynamically across environmental compartments due to variations in size, density, and interactions with biological and physical processes. Their persistence enables long-distance transport and accumulation across ecosystems.

Key Environmental Transport Mechanisms

Microplastics move across ecosystems through several key mechanisms. Buoyant particles drift on water surfaces, accumulating in gyres and reaching distant coastlines. Biofouling can cause them to sink, leading to long-term sediment deposition. Benthic organisms redistribute microplastics within

sediments through feeding and movement. Wind also transports microplastics over long distances, depositing them far from their original sources.

Broader Environmental Context and Relevance

Plastic pollution has become one of the fastest-growing environmental crises of the 21st century. Global plastic production exceeds 400 million tonnes annually, with nearly half intended for single-use purposes (UNEP, 2018). Inadequate waste management, especially in developing regions promotes fragmentation into microplastics that contaminate land, air, and water.

In Nigeria, plastic waste generation surpasses 2.5 million tonnes per year, yet recycling rates remain below 12% (Ogunleye et al., 2021). These conditions enable microplastics to infiltrate rivers, coastal waters, agricultural soils, food systems, and urban air, elevating both ecological and human health risks.

Microplastics pose a distinct threat due to their persistence, chemical complexity, pollutant-adsorption capacity, and ability to cross biological barriers. Emerging studies link exposure to oxidative stress, inflammation, endocrine disruption, mitochondrial damage, reproductive effects, and potential neurotoxicity. Their interactions with climate-relevant processes such as greenhouse gas emissions, ocean carbon cycling, and atmospheric dynamics, further position microplastics as an important but understudied contributor to global climate change.

This article offers a comprehensive synthesis of microplastic concentrations, transport pathways, exposure routes, biochemical toxicity mechanisms, health implications, climate interactions, and



mitigation strategies necessary to safeguard both planetary and public health.

Chemical Components and Toxicological Relevance

Polyethylene (PE)

Highly hydrophobic, adsorbs persistent organic pollutants, and leaches toxic additives. PE MPs have been linked to oxidative stress and inflammation in biological systems.

Polypropylene (PP)

It is lightweight, persistent, and prone to fragmenting into microfibers. It can bind pesticides and organic pollutants.

Polystyrene (PS)

Aromatic structure enhances chemical sorption. Upon degradation, releases styrene monomers associated with neurotoxicity and endocrine disruption.

Polyethylene Terephthalate (PET)

It leaches antimony and plasticizers. It is associated with gut inflammation and microbial dysbiosis.

Polyvinyl Chloride (PVC)

Contains high levels of additives including phthalates and bisphenol A. Releases toxic monomers and is linked with endocrine and reproductive disruptions.

Routes of Human Exposure

Humans encounter microplastics through several major pathways that allow these particles to enter

the body and interact with biological systems. Understanding these exposure routes is essential for assessing potential health risks and developing mitigation strategies.

Ingestion

Ingestion is the most documented and quantitatively significant route of microplastic exposure. Microplastics have been consistently detected in a wide range of food items, including seafood, table salt, honey, fruits, vegetables, and processed foods. Drinking water—both bottled and tap—has also been identified as a major contributor. Studies estimate that an average adult may ingest between 39,000 and 52,000 particles annually, a number that increases for individuals who frequently consume bottled water (Cox et al., 2019). Seafood contributes notably due to bioaccumulation in marine organisms, particularly filter feeders such as mussels and oysters. Microplastics can also arise when teabags made of synthetic polymers shed microscopic fibers during steeping. Once ingested, microplastics may interact with gastrointestinal tissues, altering microbiome composition, increasing gut permeability, and enabling the transfer of adsorbed chemicals into systemic circulation.

Inhalation

Inhalation exposure occurs through airborne microplastics suspended in both indoor and outdoor air. Synthetic textiles, urban dust, vehicle tire wear, and industrial emissions contribute significantly to atmospheric microplastic loads. It is estimated that road traffic accounts for approximately 84% of airborne microplastics (Brahney et al., 2020). Indoors, carpets, furniture, clothing, and heating



systems continuously release microfibers that accumulate in household dust. These particles can deposit in the nasal cavity, trachea, and deep lung tissues, depending on their size and shape. Inhaled microplastics may induce respiratory inflammation, oxidative stress, and impaired lung function. Ultrafine microplastics and nanoplastics pose additional risks because they can penetrate alveolar membranes and potentially translocate into the bloodstream.

Dermal Exposure

Dermal contact is considered a less dominant but still relevant exposure pathway. Although intact human skin is a strong protective barrier, microplastics present in cosmetics—such as exfoliating scrubs, toothpaste, or foundation—may penetrate through hair follicles, sweat glands, or micro-abrasions. Occupational exposure among workers in plastic manufacturing, textiles, or waste handling increases the likelihood of dermal interaction. While large microplastic particles are unlikely to pass directly through the epidermis, smaller particles and associated chemicals can accumulate on the skin surface and possibly contribute to irritation or inflammation.

Absorption and Distribution in the Human Body

Microplastics can enter the human body and cross biological barriers through transcellular absorption, paracellular transport, and phagocytosis by immune cells. Smaller particles are internalized by endocytosis, while barrier disruption can allow larger particles to slip between epithelial cells. Once inside phagocytes, microplastics may resist degradation and trigger chronic inflammation.

Evidence shows their presence in the placenta, breast milk, lungs, liver, kidneys, bloodstream, and possibly brain tissues. The detection of microplastics in human blood and placenta raises major concerns about systemic and developmental exposure.

Mechanisms of Microplastic Toxicity

Microplastic toxicity arises from both their physical presence and the chemical additives or pollutants they carry. Several well-established biological mechanisms explain how microplastics induce cellular and systemic harm.

Oxidative Stress

One of the primary mechanisms of microplastic toxicity is the generation of reactive oxygen species (ROS). ROS damage cellular components including lipids, DNA, and proteins, leading to oxidative stress and impairing normal cellular function. Microplastics can stimulate ROS production directly through their surfaces or indirectly by carrying toxic chemicals. This oxidative imbalance contributes to inflammation, mitochondrial dysfunction, and potential initiation of chronic diseases.

Inflammation

Microplastics activate pattern recognition receptors (PRRs) on immune cells, triggering inflammatory signalling pathways. Chronic inflammation is an established risk factor for cardiovascular disease, insulin resistance, and metabolic disorders. Continuous exposure to microplastics—even at low levels—may sustain inflammatory responses,



resulting in tissue damage and impaired immune regulation.

Mitochondrial Dysfunction

Microplastics disrupt mitochondrial activity by interfering with the electron transport chain, reducing ATP synthesis, and promoting mitochondrial ROS production. Energy deficits compromise normal cellular functions, affect organ systems with high energy demands, and contribute to metabolic imbalance.

Genotoxicity

Microplastics can induce genotoxic effects through physical abrasion of cellular structures or via ROS-mediated DNA damage. DNA strand breaks, chromosomal instability, and mutations may result, potentially altering gene expression and increasing cancer risk. Nanoplastics are particularly concerning because they can interact directly with nuclear material.

Endocrine Disruption

Microplastics often contain endocrine-disrupting chemicals (EDCs) such as phthalates, bisphenol A, and flame retardants. These chemicals mimic or block hormone receptors, disrupting endocrine pathways that regulate reproduction, growth, metabolism, and thyroid function. EDC exposure is linked to infertility, developmental abnormalities, and hormonal cancers.

Links to Chronic Diseases

Research increasingly suggests that microplastics may contribute to a variety of chronic diseases through the mechanisms described above.

Although human epidemiological evidence is still developing, experimental findings support several plausible connections.

Cardiovascular Disease

Microplastic-induced oxidative stress and inflammation promote atherosclerosis, hypertension, and endothelial dysfunction. Animal studies demonstrate that ingestion of microplastics accelerates plaque formation and alters lipid metabolism. Chronic inflammatory signalling may also contribute to heart failure and arrhythmias.

Neurological Disorders

Microplastics may cross the blood-brain barrier, enabling them to access brain tissues and trigger neuroinflammation. This pathway is implicated in neurodegenerative diseases such as Alzheimer's and Parkinson's. Additionally, oxidative stress and mitochondrial damage induced by microplastics may impair neuronal function and cognitive health.

Reproductive Toxicity

Microplastics and associated endocrine disruptors interfere with steroid hormone synthesis, spermatogenesis, ovarian development, and menstrual regulation. Animal studies show reduced fertility, altered sex hormone levels, and developmental abnormalities in offspring after microplastic exposure.

Gastrointestinal Disorders

Microplastics disrupt gut homeostasis by damaging epithelial barriers, altering microbiota composition, and inducing inflammation. These changes can contribute to irritable bowel syndrome (IBS),



malabsorption, and increased susceptibility to gastrointestinal infections.

Immune Dysfunction

Persistent activation of immune pathways may lead to autoimmune reactions, immune exhaustion, or reduced ability to fight infections. Microplastics can overstimulate immune cells—particularly macrophages and T cells—leading to chronic inflammatory conditions.

Interactions with Heavy Metals and PAHs

Microplastics (MPs) significantly influence the environmental fate, transport, and toxicity of chemical contaminants. Their physical structure, large surface-area-to-volume ratio, and hydrophobic polymer composition make them highly effective sinks and carriers for hazardous pollutants such as heavy metals and polycyclic aromatic hydrocarbons (PAHs).

Adsorption of Heavy Metals

Microplastics readily bind heavy metals such as Pb, Cd, and Hg through electrostatic attraction and surface complexation. Environmental weathering increases surface roughness, enhancing their sorption capacity. As a result, MPs act as mobile reservoirs transporting metals across water, soil, and sediment. This prolongs metal persistence and reduces natural detoxification in ecosystems. Organisms ingesting contaminated MPs face combined physical and chemical toxicity.

Adsorption and Transport of PAHs

Hydrophobic PAHs, including benzo[a]pyrene and pyrene, strongly adhere to microplastic surfaces

due to chemical compatibility. Once bound, PAHs degrade more slowly, extending their environmental lifespan. Microplastics transport these pollutants over long distances from urban sources to remote ecosystems. Ingested MPs release PAHs in acidic digestive fluids, increasing internal exposure. This process heightens contamination risks for both aquatic and terrestrial organisms.

Synergistic Toxicity and Bioaccumulation

Pollutant-loaded microplastics create compounded toxicity when ingested by organisms. Adsorbed metals and PAHs amplify oxidative stress, immune disruption, and reproductive harm beyond individual pollutant effects. These contaminants can biomagnify through food webs, posing risks to top predators and humans. Combined physical abrasion and chemical toxicity impair metabolism and feeding behavior. Such interactions intensify ecological and physiological damage across multiple ecosystems.

Microbial Interactions and Environmental Implications

Microplastics provide surfaces for microbial colonization, forming the “plastisphere” that hosts diverse bacteria and fungi. Some of these microbes degrade pollutants like PAHs, influencing environmental breakdown processes. However, MPs can also transport pathogens and antibiotic-resistant bacteria, increasing ecological and health risks. The plastisphere alters pollutant cycling by modifying toxicity and bioavailability. Overall, MPs function as vectors that reshape microbial dynamics and ecosystem health.



Microplastics and Climate Change

Microplastics (MPs) and climate change are increasingly recognized as interconnected global threats. MPs influence greenhouse gas emissions and disrupt major biogeochemical cycles, while climate change accelerates the production, fragmentation, and dispersal of plastic particles. Together, these processes create a reinforcing cycle with profound implications for ecosystems, food security, and human health.

Contribution to Greenhouse Gas Emissions

Microplastics contribute to atmospheric warming through both direct and indirect mechanisms. Exposure of plastics to sunlight and heat triggers thermal degradation that releases methane (CH_4) and ethylene (C_2H_4), with emission rates increasing as plastics fragment into smaller particles. In soils, microplastics alter microbial activity, enhance respiration, and shift the balance of methanogenic communities, resulting in elevated CO_2 and CH_4 emissions. In many African countries, where open burning remains a major waste-management practice, incomplete combustion of plastics releases greenhouse gases, soot, and secondary microplastics, exacerbating air pollution and climate impacts.

Disruption of Ocean Carbon Cycling

Oceans absorb roughly one-third of anthropogenic CO_2 , but microplastics undermine this climate-regulating function. MPs interfere with phytoplankton—the foundation of marine productivity and oxygen generation—by impairing photosynthesis, nutrient uptake, and growth. This

weakens the biological carbon pump that transports CO_2 to deep ocean reservoirs. Microplastics also reduce the efficiency of carbon export by altering the density and sinking behavior of organic particles. Coastal African regions, including the Gulf of Guinea, Red Sea, and Western Indian Ocean, are increasingly reporting microplastic contamination that threatens fisheries, livelihoods, and blue-economy sustainability.

Influence on Atmospheric Processes

Airborne microplastics are now detected in rainfall, dust plumes, and remote ecosystems. MPs may act as cloud-condensation nuclei, influencing rainfall patterns and local climate dynamics—an issue of particular concern for African regions already experiencing rainfall variability and drought stress. Atmospheric transport carries microplastics across long distances, depositing them on glaciers, farmlands, and water bodies, where they introduce new pollutants and contribute to surface-albedo changes that accelerate warming.

Climate Change Feedback on Microplastics

Climate change amplifies microplastic pollution by speeding up the fragmentation of plastic waste through heat and UV radiation. Intensified storms, floods, and cyclones mobilize mismanaged waste into rivers and oceans, while rising temperatures and ocean acidification alter microplastic buoyancy and degradation pathways. Across Africa where waste-collection rates remain low and extreme weather events are increasing, these feedbacks accelerate the spread of microplastics into soils, freshwater resources, and coastal environments that support millions of people.



Microplastics as an Emerging Global and African Health Threat

Microplastics must now be understood as both a climate stressor and a public-health risk. Their ability to bind heavy metals, polycyclic aromatic hydrocarbons (PAHs), and pathogenic microbes increases toxicity in African water systems, agricultural soils, and food chains. Coastal communities relying heavily on fisheries face heightened exposure, while urban populations—particularly in rapidly growing African megacities—experience combined risks from air pollution, open burning, and contaminated water. With rising plastic production, limited waste infrastructure, and accelerating climate pressures, Africa is at the frontline of this emerging environmental crisis. Addressing microplastic pollution requires coordinated strategies that strengthen waste governance, promote circular-economy models, improve wastewater treatment, and integrate climate adaptation planning.

Mitigation and Solutions

Reduce Plastic Use

Reducing plastic consumption—particularly single-use plastics—is one of the most effective long-term strategies for mitigating microplastic pollution. Single-use plastic items, such as plastic bags, straws, cutlery, sachet water wrappers, and packaging materials, rapidly fragment into microplastics due to their short lifespan and high disposal rates. Implementing bans or restrictions on these items can significantly reduce plastic leakage into the environment. Additionally, shifting to biodegradable, compostable, or reusable

alternatives helps lower dependence on petroleum-based plastics. Consumer behaviour change is equally important; public education campaigns, eco-labelling, incentives for reusable products, and corporate accountability programs can reshape consumption patterns at scale. Encouraging industries to adopt plastic-free packaging, invest in eco-design, and reduce unnecessary plastic components in products can further accelerate progress toward sustainability. Ultimately, reducing plastic use requires both policy-level interventions and grassroots behavioural shifts.

Improve Waste Management

Improving waste management is fundamental for preventing plastics from entering natural ecosystems and fragmenting into microplastics. Many countries, including Nigeria, face challenges related to inadequate waste collection, poor segregation practices, insufficient recycling capacity, and limited landfill regulation. Strengthening recycling systems involves establishing formalised recycling infrastructure, supporting private-sector recycling enterprises, and creating financial incentives for plastic buyback schemes. Enforcing proper disposal means implementing regulatory frameworks that discourage open dumping and burning of plastics—both of which contribute to environmental toxicity and greenhouse gas emissions. Upgrading wastewater treatment plants (WWTPs) is also critical; modern filtration technologies, including fine screens and membrane bioreactors, can retain microplastics before treated water is discharged into rivers and seas. Additionally, implementing extended producer responsibility (EPR) programs



ensures that manufacturers take responsibility for the end-of-life management of their plastic products.

Technological Innovations

Magnetic Microplastic Capture Devices

Magnetic nanocomposite materials are being developed to bind efficiently to microplastics in water. Once attached, the plastic–magnet complexes can be removed using external magnetic fields. This approach shows strong potential for high-volume purification in wastewater systems and polluted rivers. It represents a scalable, low-chemical method for targeted microplastic removal. Nanofiltration and ultrafiltration membranes can physically trap microplastics and even nanoplastics from water. These systems offer high removal efficiency across a range of particle sizes. Although currently energy-intensive, ongoing improvements in membrane design aim to reduce cost and enhance sustainability. Membrane filtration remains one of the most reliable technologies for microplastic elimination in water treatment.

Microbial Degradation Research

Biodegradation research explores microorganisms capable of breaking down plastic polymers into harmless byproducts. Bacteria such as *Ideonella sakaiensis* and certain fungal species have demonstrated the ability to degrade PET and other plastics. Genetic engineering, enzyme optimization, and synthetic biology approaches could accelerate the breakdown of difficult-to-degrade plastics and reduce microplastic persistence in the environment. However, ensuring

ecological safety and scalability remains a priority for future research.

Monitoring and Standardisation

Reliable detection and monitoring of microplastics is essential for assessing environmental risks, exposure pathways, and policy effectiveness. However, the lack of standardisation across sampling and analytical techniques has led to inconsistent reporting and difficulty comparing results globally. Adopting internationally standardised methods—such as those under development by the International Organization for Standardization (ISO)—would facilitate comparable, high-quality data collection. Standard protocols should cover sampling procedures, particle isolation, polymer identification (e.g., FTIR, Raman spectroscopy), and reporting units. Continuous monitoring of water bodies, soils, air, and food products would enable early detection of contamination hotspots and guide targeted interventions. Establishing national and regional microplastic monitoring frameworks would also support long-term environmental management.

Policy and Governance

Strong environmental governance is essential for reducing plastic pollution and preventing microplastic formation. Several countries provide successful examples:

12.7. International and Regional Examples. Rwanda's plastic bag ban demonstrates the effectiveness of strict national legislation, resulting in cleaner cities and reduced environmental leakage. The European Union's microbead restrictions eliminated microplastic-containing



cosmetics, significantly reducing primary microplastic sources. South Korea's rigorous recycling enforcement and mandatory waste segregation contribute to some of the highest recycling rates globally.

Recommendations for Nigeria

Nigeria faces unique challenges including rapid urbanisation, inadequate waste management infrastructure, and widespread use of low-cost plastics. Effective governance should include:

- a. Banning free plastic bags and implementing levies on single-use plastics to discourage overconsumption.
- b. Promoting biodegradable and compostable materials, especially for packaging, food service, and agricultural applications.
- c. Strengthening waste collection and recycling infrastructure, including establishing formal recycling hubs, supporting local collectors, and investing in mechanical and chemical recycling technologies.
- d. Adopting extended producer responsibility (EPR) policies to shift cleanup and disposal costs back to manufacturers.
- e. Implementing nationwide public education campaigns to foster behavioural change, reduce littering, and promote recycling.

CONCLUSION

Microplastic pollution has emerged as one of the most pervasive and multifaceted environmental challenges of the modern era, intersecting directly with global health, ecological stability, and climate change. As demonstrated throughout this paper,

microplastics are now present in virtually every environmental compartment; air, water, soil, and even remote ecosystems, reflecting the scale of plastic dependence and the inadequacy of global waste management systems. Their persistence, buoyancy, and chemical versatility allow them to disperse widely, infiltrate food webs, and accumulate in human tissues, raising concerns about long-term exposure and disease development.

The biochemical mechanisms underlying microplastic toxicity such as oxidative stress, inflammation, endocrine disruption, mitochondrial damage, and genotoxicity, underscore the plausibility of microplastics contributing to chronic diseases, including cardiovascular disorders, metabolic syndromes, reproductive dysfunction, and neurodegenerative conditions. While epidemiological evidence is still emerging, strong mechanistic and experimental findings highlight the urgent need for precautionary approaches, especially for vulnerable populations such as children, pregnant women, and communities with high exposure burdens.

The interactions between microplastics and climate change further expand the scope of concern. Microplastics contribute to greenhouse gas emissions throughout the plastic life cycle, disrupt marine carbon sequestration processes, influence microbial respiration in soils, and may even alter atmospheric dynamics. Conversely, climate-induced changes in temperature, precipitation patterns, and extreme weather events can accelerate microplastic fragmentation and redistribution, creating a dangerous feedback loop that threatens environmental stability and human well-being.



Addressing this growing crisis requires a multi-layered response. At the global level, transitions toward circular economy models, reduction of single-use plastics, implementation of extended producer responsibility (EPR), and harmonisation of microplastic monitoring standards are essential. At the national and regional levels, particularly in developing countries such as Nigeria, strengthened waste management infrastructure, public education campaigns, enforcement of plastic restrictions, and investment in alternative materials remain critical for reducing environmental burdens. Scientific innovation must continue to advance detection methods, explore safe biodegradation technologies, and deepen understanding of human exposure pathways and health outcomes.

Ultimately, microplastic pollution is not an isolated problem but part of a broader planetary health crisis that links human behaviour, industrial production, environmental resilience, and climate stability. Mitigating its impacts requires coordinated action across disciplines, sectors, and borders. By reducing plastic dependency, improving environmental stewardship, and prioritising sustainable practices, societies can reduce the risks posed by microplastics and move toward a cleaner, healthier, and more climate-resilient world. The choices made today will determine whether microplastics remain a manageable pollutant or evolve into a defining global threat for future generations.

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Dynamics and Bioaccumulation of Heavy Metals in *Amaranthus Species* Cultivated on Anthropogenically Impacted Soils.

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ABSTRACT

Heavy metal contamination in agricultural soils, particularly from cadmium (Cd) and lead (Pb), presents persistent environmental and public health threats. While numerous studies address individual species or ignore spatial variability, this study investigates both metal accumulation and interspecies differences across real-world, anthropogenically impacted sites. The objective is to support safer food production and sustainable urban agriculture practices. Three *Amaranthus* species (*A. hybridus*, *A. cruentus*, and *A. spinosus*) were cultivated across eight sites representing industrial zones, urban markets, and rural farmlands. Soil and plant samples were analyzed for Cd and Pb concentrations using atomic absorption spectrophotometry. Bioaccumulation factors (BAFs) were calculated to assess uptake efficiency. Spatial patterns and species-specific trends were evaluated using boxplot analysis and Principal Component Analysis (PCA). Cd levels were highest in industrial and rural farmlands, while Pb concentrations peaked in urban markets. *A. cruentus* showed consistent Pb accumulation, whereas *A. spinosus* demonstrated increased Cd uptake at select sites. PCA confirmed species- and site-specific accumulation patterns, with *A. hybridus* and *A. cruentus* exhibiting high BAFs (>1.0) at Sites 1, 2, and 7. Strong interspecies correlations were observed, while weak intra-species Cd–Pb correlations suggested independent accumulation mechanisms. Metal concentrations often exceeded food safety thresholds, revealing health risks and highlighting the influence of site-specific pollution and species traits on metal uptake. Regular soil monitoring, site-targeted risk assessment, and deploying *A. hybridus* and *A. cruentus* as phytoremediators or bioindicators are recommended to ensure safer food systems in peri-urban agricultural settings.

KEYWORDS: Anthropogenic activities, contaminated soils, Environmental pollution, Phytoremediation potential, Soil-plant interaction, Metal uptake, Food Safety

INTRODUCTION

In recent years, growing concerns over food safety and environmental health have drawn attention to the issue of heavy metal contamination in agricultural soils. Across many parts of the world,

particularly in developing countries, soils used for farming are increasingly impacted by human activities such as industrial discharge, urban runoff, waste dumping, and the use of untreated wastewater for irrigation. These practices have led to the



accumulation of toxic metals like cadmium (Cd), lead (Pb), arsenic (As), and chromium (Cr) in the environment, posing serious risks not only to soil health but also to the safety of crops grown on such land (Rehman *et al.*, 2023).

One of the most commonly cultivated vegetables in tropical and subtropical regions is *Amaranthus*, popularly known as amaranth. These leafy greens are highly valued for their fast growth, resilience, and rich nutritional profile. However, *Amaranthus* species are also known for their ability to absorb and accumulate heavy metals from contaminated soils (Yadav *et al.*, 2022). This dual nature, nutritious yet potentially risky, makes them both important and concerning, especially when grown in areas near roadsides, refuse dumps, or industrial zones where pollution levels are often high (Adelekan & Abegunde, 2020).

The problem becomes more complex when we consider that heavy metal contamination is rarely uniform. Soils can show significant spatial differences in metal concentrations, even within the same farming area. These differences are influenced by local factors like land use, past industrial activities, irrigation practices, and natural soil properties such as pH or organic matter content (Li *et al.*, 2023). Understanding how these spatial variations affects metal availability, and ultimately their uptake by food crops, is very important for assessing both environmental risks and human exposure through diet.

In addition to the metal content of soil, the specific *Amaranthus* species being cultivated matters. Different species, and even different parts of the same plant (leaves, stems, roots), can accumulate metals in varying degrees (Rahman *et al.*, 2020).

This shows the need to compare multiple species to better understand their individual bioaccumulation patterns and to determine which might pose greater or lesser risks to consumers. Moreover, such comparisons can offer insights into which species might be more suitable for use in soil remediation or as environmental indicators.

Despite growing research interest in heavy metals and their effects on vegetables, relatively few studies have taken a combined approach, examining both the spatial distribution of metals in soil and the bioaccumulation behavior of different *Amaranthus* species. Many investigations either focus on a single plant species or ignore the influence of soil variability altogether. This leaves a gap in our understanding of how real-world conditions shape plant-metal interactions and what this means for food safety, especially in urban and peri-urban areas where these crops are often grown (Obida *et al.*, 2024).

This study sets out to address that gap. By exploring both the spatial dynamics of heavy metal contamination in human-impacted soils and the bioaccumulation patterns in selected *Amaranthus* species, we aim to paint a clearer picture of the risks involved. Our goal is to support safer food production practices, inform public health decisions, and contribute to sustainable urban agriculture, where leafy greens like amaranth can be enjoyed without fear of hidden toxins. In many urban and peri-urban communities, especially in developing countries, fresh leafy vegetables like *Amaranthus* are grown close to homes, roads, and industrial areas. This is often out of necessity, people need quick-growing, nutritious crops, and



available land is usually whatever space has not been built on. But this convenience comes with a hidden cost. These soils are often exposed to pollution from vehicle emissions, open waste dumping, and industrial runoff. Over time, heavy metals like lead (Pb), cadmium (Cd), and chromium (Cr) build up in the soil, quietly becoming part of the growing environment.

Although research has shown that heavy metals can accumulate in vegetables, we still do not fully understand how different locations, soil conditions, and *Amaranthus* species affect the extent of that accumulation. Most studies tend to look at just one species or a general soil profile, without considering how metal levels vary from place to place or how different *Amaranthus* types respond. This creates a gap in knowledge, one that has direct implications for food safety, urban agriculture, and even public health.

Materials and methods

Study area and sampling sites

Soil samples were collected from four locations representing distinct anthropogenic influences:

- Industrial Site (IS): Adjacent to manufacturing industries.
- Roadside Site (RS): Proximal to a major highway with heavy vehicular traffic.
- Urban Market Site (UMS): Located in a central urban vegetable market area.
- Rural Farmland Site (RFS): A relatively pristine agricultural area distant from major pollution sources.

Plant and soil sampling

Three *Amaranthus* species (*A. hybridus*, *A. viridis*, and *A. spinosus*) were grown on soils collected from each site. The plants were grown in the University of Ilorin Screened house for eight weeks after which they were harvested and each partitioned into roots, stems, and leaves.

Sample preparation and analysis

Plant samples were thoroughly washed, oven-dried at 70°C, and ground to a fine powder. Soil samples were air-dried, homogenized, and sieved (<2 mm). Both plant and soil samples were digested using nitric acid-perchloric acid mixtures following standard procedures (AOAC, 2005).

Heavy metal concentrations (Cd, Pb and Cu) were determined using flame atomic absorption /certified reference materials and procedural blanks.

Bioaccumulation Factor (BAF)

BAF was calculated as:

$$BAF = C_{plant}/C_{soil}$$

Where C_{plant} and C_{soil} represent the metal concentration in plant tissue and corresponding soil, respectively.

Statistical analysis

Data were analyzed using ANOVA to compare means across species, tissues, and sites. Boxplot and Principal Component Analysis Biplot was used to show the spatial distribution and compare the heavy metal distribution pattern between the species. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Spatial dynamics of heavy metal contamination in Human-Impacted Soils



The spatial distribution of cadmium (Cd) and lead (Pb) concentrations with their standard deviation across selected land-use types is presented in Figure 1. Cadmium concentrations ranged from 0.20 ± 0.00 mg/kg to 0.65 ± 0.11 mg/kg, while lead concentrations varied more widely, from 2.74 ± 4.72 mg/kg to 7.94 ± 7.51 mg/kg. Notably, industrial sites and rural farmland soils recorded the highest levels of Cd, whereas urban market soils exhibited the highest Pb concentrations. Substantial variability, particularly in Pb levels, was evident from the large standard deviations, suggesting heterogeneous pollution sources across sites.

Cadmium (Cd) contamination

Elevated cadmium concentrations were observed at Industrial Site 1 (0.65 ± 0.11 mg/kg), Rural Farmland Site 2 (0.62 ± 0.56 mg/kg), and Industrial Site 2 (0.60 ± 0.28 mg/kg). These values, though within the threshold limits for unpolluted soils in some regulatory frameworks (Alloway, 2019), indicate a significant anthropogenic input including vehicular emissions, agrochemical application, and industrial waste deposition, on heavy metal accumulation in soil environments (Chen *et al.*, 2022).. The high Cd content in industrial zones is likely attributable to atmospheric deposition from metallurgical operations, improper waste disposal, and emissions from combustion sources (Zhang *et al.*, 2023). In rural farmlands, the presence of Cd may be linked to long-term use of phosphate-based fertilizers, which are known to contain trace levels of cadmium as impurities (Huang *et al.*, 2020). The high standard deviations at some sites suggest variability in pollution sources or in metal mobility influenced by site-specific soil properties.

This trend aligns with findings by Li *et al.* (2022), who reported significant Cd accumulation in agricultural soils irrigated with wastewater and treated with chemical fertilizers in peri-urban China. Furthermore, the relatively lower Cd levels in urban market soils ($0.22\text{--}0.25$ mg/kg) suggest reduced direct agrochemical input, although atmospheric deposition and waste burning may still contribute to baseline levels (Rahman *et al.*, 2021). Cadmium is nephrotoxic and osteotoxic, even at low exposure levels, and is also linked to endocrine disruption and cancer (Zhou *et al.*, 2021). Notably, Cd concentrations in rural farmlands exceed the WHO/FAO recommended maximum permissible limit of 0.3 mg/kg in agricultural soils (FAO/WHO, 2021). This raises concerns for food safety and dietary intake, especially considering the bioaccumulative nature of Cd in leafy vegetables and cereals consumed locally (Shan *et al.*, 2022).

Lead (Pb) contamination patterns

Pb concentrations demonstrated higher variability across the sites, with Urban Market Site 1 (7.94 ± 7.51 mg/kg) and Rural Farmland Site 1 (4.84 ± 4.94 mg/kg) showing the most elevated values. Although these concentrations remain below the Dutch Target Value for Pb in soils (85 mg/kg for residential areas), they are still concerning due to cumulative toxicity and potential bioaccumulation of Pb (Zhou, *et al.*, 2022).

The elevated Pb concentration in urban markets is attributed to vehicular emissions, legacy contamination from leaded gasoline, and solid waste incineration, an observation consistent with recent studies that identify market centers and roadside soils in densely populated cities as Pb



hotspots (Ibrahim *et al.*, 2022). Similarly, Khan *et al.* (2023) documented elevated Pb levels in urban and peri-urban soils of South Asia due to traffic emissions and poor urban waste management.

Interestingly, rural farmlands also exhibited high Pb levels, likely stemming from pesticide and herbicide residues, especially those with older formulations known to contain trace lead compounds (Ekere *et al.*, 2020). Industrial sites maintained moderate Pb concentrations (4.2–4.4 mg/kg), reflecting point-source pollution from local manufacturing and metal-processing activities (Obiora *et al.*, 2023). Lead exposure continues to be a major concern due to its effects on cognitive development, cardiovascular health, and renal function. The observed Pb concentrations in soils from urban market and roadside areas reflect contributions from traffic emissions and historical use of leaded gasoline, consistent with the findings of Adeyemi *et al.* (2023), who documented Pb levels exceeding 5 mg/kg in similar urban contexts. Despite soil Pb concentrations remaining below the general USEPA threshold of 300 mg/kg, the risk of bioavailability and crop uptake in food-producing areas demands urgent attention (Zhang *et al.*, 2023). The observed concentrations of Cd and Pb in soils are of significant ecological and health concern. Both metals are non-essential, bioaccumulate in plants, and can biomagnify across trophic levels, raising risks of food chain contamination (Zhou *et al.*, 2021). Chronic Cd exposure is associated with renal impairment, skeletal damage, and carcinogenicity (WHO, 2023), while Pb is neurotoxic, particularly impairing cognitive development in children.

The substantial variability in standard deviations across sites highlights the role of localized anthropogenic activities and site-specific soil factors, such as pH, organic matter, and cation exchange capacity, which modulate metal mobility and retention (Zhang *et al.*, 2022). According to Luo *et al.* (2022), even moderate Cd contamination reduces beneficial microbial symbioses, such as mycorrhizal associations, critical for plant nutrient uptake.

The risk of bioaccumulation and biomagnification also poses long-term threats to local biodiversity. Metal-contaminated soils have been shown to alter plant diversity and cause shifts in community composition, with sensitive species gradually being replaced by metal-tolerant taxa (He *et al.*, 2023). Furthermore, runoff from these sites could introduce heavy metals into nearby water bodies, exacerbating ecological degradation in aquatic ecosystems. Comparatively, the present findings align closely with previous studies in Nigeria and other developing regions. For instance, Obiora *et al.* (2016) reported Cd and Pb concentrations in agricultural soils of southeastern Nigeria ranging between 0.10–0.30 mg/kg and 3.00–8.00 mg/kg, respectively. These consistencies suggest that the study area shares common sources of metal input and similar patterns of anthropogenic activity with other regions of the country.

The findings of this study call for the urgent need for sustainable soil and land-use management practices. Given that several sampling sites are used for food production or are situated near high-traffic areas, there is an elevated risk of chronic

human exposure to Cd and Pb through food chains. Regular soil monitoring, enforcement of environmental regulations on industrial effluents, and public education on urban agriculture practices are essential.

Remediation strategies, including phytoremediation and biochar amendment, have shown promise in reducing metal bioavailability in tropical soils (Tang *et al.*, 2021). Moreover, site-specific risk assessments should be integrated into urban planning, particularly in expanding cities like Ilorin, where land-use conflicts are prevalent. The adoption of best management practices will be critical to ensure environmental safety and sustainable agricultural productivity.

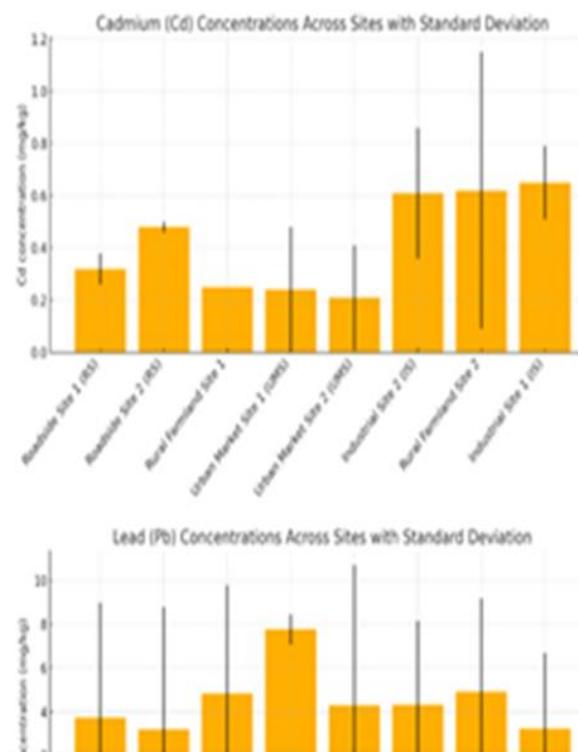


Fig 1: Spatial distribution of cadmium and lead in soils across sites

Cadmium and lead dynamics in amaranthus species

The boxplot analysis (Figure 3) presents the differential accumulation of cadmium (Cd) and lead (Pb) among the three *Amaranthus* species, *Amaranthus hybridus* (A1), *Amaranthus cruentus* (A2), and *Amaranthus spinosus* (A3), across multiple anthropogenic sites. The results indicate consistent interspecies differences in metal uptake capacity, with notable variation in Pb concentrations across species and relatively uniform patterns in Cd accumulation.

The measured Cd concentrations ranged from 0.13 ± 0.00 to 0.36 ± 0.00 mg/kg for A1, 0.09 ± 0.02 to 0.41 ± 0.03 mg/kg for A2, and 0.10 ± 0.03 to 0.27 ± 0.03 mg/kg for A3. Sites 6 and 10 consistently recorded the highest Cd levels across all species, while Site 3 exhibited the lowest concentrations. The relatively elevated levels observed at Site 6 for A2 (0.41 ± 0.03 mg/kg) suggest localized inputs of Cd, potentially linked to anthropogenic activities such as vehicular emissions, improper waste disposal, or agrochemical use.

The Cd concentration ranges for A1, A2, and A3 are relatively narrow, with median values clustering below 0.4 mg/kg. This suggests that all three species exhibited moderate Cd uptake. However, slight differences in Cd concentration levels suggest a marginally higher accumulation potential in A1 and A2 compared to A3. The limited variation may be due to the relatively lower Cd



levels in soil across most sites and the lower soil-plant transfer coefficient of Cd compared to Pb (Zhou *et al.*, 2022).

Cadmium accumulation is a function of plant physiology, metal bioavailability, and rhizospheric interactions. Studies by Shan *et al.* (2023) demonstrated that *A. hybridus* has higher Cd translocation efficiency than *A. spinosus*, possibly due to differences in root exudation and cell wall binding capacity. Nonetheless, the observed values across species in this study remain close to or above the WHO/FAO maximum allowable limit of 0.2 mg/kg for edible vegetables (WHO, 2021), raising concern over food safety and dietary Cd exposure in urban consumers.

The accumulation pattern observed aligns with recent findings. For instance, Li *et al.* (2020) reported Cd concentrations ranging from 0.06 to 0.95 mg/kg in leafy vegetables grown in peri-urban zones of eastern China. Similarly, Hossain *et al.* (2021) documented Cd levels between 0.12 and 1.08 mg/kg in leafy vegetables cultivated in urban and peri-urban Bangladesh, emphasizing similar bioaccumulation trends across ecologically stressed regions. The variation among sites may be attributed to differences in soil physicochemical characteristics, particularly pH, organic matter content, and cation exchange capacity (CEC), all of which influence Cd mobility and uptake by plants (Zhang *et al.*, 2022).

Notably, among the three *Amaranthus* species, *A. hybridus* (A1) and *A. cruentus* (A2) generally accumulated higher Cd concentrations than *A. spinosus* (A3), suggesting species-dependent accumulation efficiency. This observation supports

earlier evidence of species-specific uptake mechanisms influenced by root morphology, differential expression of metal transporter proteins, and physiological adaptation to metal stress (Rahman *et al.*, 2022). These findings indicate the importance of integrating soil quality assessments with phytoremediation and food safety considerations when evaluating heavy metal dynamics in agroecosystems.

The concentration of lead (Pb) in the three *Amaranthus* species (*A. hybridus* – A1, *A. cruentus*, A2, and *A. spinosus*, A3) exhibited notable spatial and interspecific variation. Across all sites, Pb concentrations in plant tissues ranged from 2.15 ± 0.05 mg/kg to 7.67 ± 1.05 mg/kg, with the highest levels consistently recorded at Site 4, a location influenced by roadside or urban runoff conditions. These elevated values suggest a strong anthropogenic influence, particularly from vehicular emissions, atmospheric deposition, and contaminated irrigation sources, which are known contributors to Pb enrichment in urban agroecosystems (Luo *et al.*, 2022).

The concentrations of Pb observed in the edible leaves of all three *Amaranthus* species in this study frequently exceed the Codex Alimentarius Commission's provisional guideline of 0.3 mg/kg for leafy vegetables (WHO, 2021). Chronic consumption of such contaminated vegetables poses serious health risks, particularly in vulnerable populations such as children and pregnant women. Given the high bioavailability and retention of Pb in human tissues, the ingestion of Pb-contaminated vegetables such as *Amaranthus* may contribute significantly to total



daily intake (TDI) in urban populations relying heavily on plant-based diets.

The findings of this study reaffirm the role of *Amaranthus* species not only as important leafy vegetables in West African diets but also as sensitive bioindicators of heavy metal contamination in agricultural soils. The elevated Pb concentrations, especially at urban-adjacent sites, to point the need for continuous monitoring of edible plants grown in high-risk areas. Remediation strategies such as phytostabilization, soil amendments such as biochar, lime, and enforcement of buffer zones around highways and industrial corridors are essential for reducing Pb exposure risks in urban agriculture.

Interspecific comparison revealed that A1 and A2 accumulated significantly higher Pb concentrations than A3, with several samples from A1 and A2 exceeding 6.0 mg/kg. The corresponding bioaccumulation factors (BAFs) for these two species often surpassed 1.0, indicating a high soil-to-plant transfer capacity who reported that *Amaranthus* species possess extensive root systems and cellular pathways that facilitate Pb uptake and retention, particularly under slightly acidic soil pH conditions that enhance Pb solubility.

The Pb levels observed in this study significantly exceed the European Commission's maximum permissible limit for lead in vegetables (0.3 mg/kg; EC, 2021), posing serious food safety concerns. Similar findings were reported in southwestern Nigeria, where Pb concentrations in leafy vegetables grown along major roads ranged from 3.10 to 8.45 mg/kg. The relatively high Pb burden

in urban and peri-urban farms may be attributed to cumulative deposition from legacy use of leaded gasoline, poor waste management, and persistent industrial activities (Adeyemi *et al.*, 2023).).

Across all species, Pb accumulation consistently exceeded Cd levels, aligning with the broader global trend of differential metal bioavailability and translocation in plants (Zhang *et al.*, 2022). The metal accumulation pattern followed the order: PbA2 > PbA1 > PbA3 > CdA2 > CdA1 > CdA3, highlighting both species-specific and location-driven variations in uptake.

The inter-species differences in metal uptake suggest that plant species selection plays a very important role in food safety risk management in contaminated soils. *Amaranthus spinosus* consistently showed the lowest accumulation of both metals, suggesting its relative safety for cultivation in mildly contaminated environments, corroborating findings by Rahman *et al.* (2022).

The uptake of Cd and Pb into edible vegetable tissues presents a significant public health challenge due to their bioaccumulative and toxicological properties, even at trace levels. Chronic dietary exposure to Cd is associated with renal dysfunction, bone demineralization, and cancer risks, while Pb is a potent neurotoxin, particularly detrimental to cognitive development in children, and can cause hematological and cardiovascular disorders (WHO, 2023). Despite the limited mobility of Pb in soil compared to cadmium (Cd), its persistent accumulation in plant tissues can be attributed to multiple uptake pathways, including apoplastic transport, root surface

adsorption, and binding to cell wall polysaccharides (Zhao *et al.*, 2021). Notably, the relatively high Pb concentrations in the edible portions of Amaranthus suggest that surface deposition and foliar absorption may also play a role, especially in roadside or peri-urban environments where particulate-bound Pb from traffic emissions settles directly on crops.

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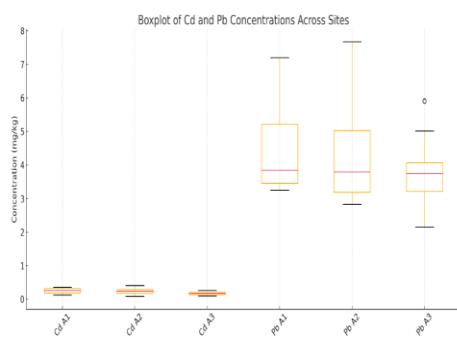


Fig 2: Cadmium and lead dynamics in three amaranthus species across sites

Visual comparison of the **distribution**, **variability**, and **spread** of Cd and Pb across amaranthus species using boxplots

The boxplot analysis for Cd shows the **distribution**, **variability**, and **spread** of Cd across species between sites. The data revealed that concentrations across the sites ranged from 0.09 to 0.41 mg/kg. Specifically, specie A1 exhibited slightly elevated Cd levels, with concentrations ranging from 0.13 to 0.36 mg/kg and a median value approximately around 0.27 mg/kg. Specie A2 displayed a somewhat wider range (0.09–0.41 mg/kg), suggesting heterogeneous Cd input, while A3 generally exhibited the lowest Cd levels (0.10–0.27 mg/kg), indicating a relatively lower anthropogenic influence at this site.

In contrast, Pb concentrations exhibited higher absolute values and variability, ranging from 2.15 to 7.67 mg/kg across all sites. Specie A2 recorded the highest Pb concentration (7.67 mg/kg), with values spanning from 2.83 to 7.67 mg/kg. Site A1 also demonstrated considerable Pb accumulation, with concentrations between 3.25 and 7.20 mg/kg. Conversely, A3 showed comparatively lower Pb concentrations, ranging from 2.15 to 5.91 mg/kg, with a median value approximately around 3.80 mg/kg.

The boxplots thus revealed site-specific trends, with A2 generally displaying the highest concentrations and variability for both Cd and Pb,



while A3 consistently showed lower metal burdens. The observed spatial variation may reflect differences in land use patterns, proximity to pollution sources, and differential anthropogenic activities across the sites.

The measured Cd concentrations across all species fall within the global background range for uncontaminated soils, typically cited as 0.01–0.50 mg/kg (Alloway, 2013). The relatively low levels observed suggest minimal industrial discharge or severe contamination but may reflect the contribution of agricultural inputs, particularly phosphate fertilizers, which are known to contain trace amounts of Cd. The slightly higher Cd levels in A1 and A2 may thus be attributed to more intensive farming practices and fertilizer application, consistent with observations by Obiora *et al.* (2016) in Nigerian agricultural soils.

The Pb concentrations observed in the present study (2.15–7.67 mg/kg) also remain below internationally recognized critical thresholds for Pb in soils (10–50 mg/kg; WHO, 2001). Nevertheless, the elevated Pb levels in species A2 warrant attention, as even moderate Pb accumulation can have long-term implications for soil health and food safety, particularly under continuous cropping systems (Wu *et al.*, 2010). Elevated Pb in A2 may be attributable to multiple potential sources, including legacy deposition from vehicular emissions, agrochemical usage, and possible proximity to minor industrial activities or waste dumps

While the concentrations of both Cd and Pb remain within acceptable regulatory limits, the observed spatial variability, particularly the elevated Pb levels at A2, shows the importance of continued environmental monitoring. Prolonged exposure, even at moderate levels, may result in bioaccumulation in crops and subsequent human exposure through the food chain, especially in subsistence farming communities. Therefore, routine surveillance and the implementation of sustainable soil management practices are strongly recommended to mitigate potential ecological and human health risks.

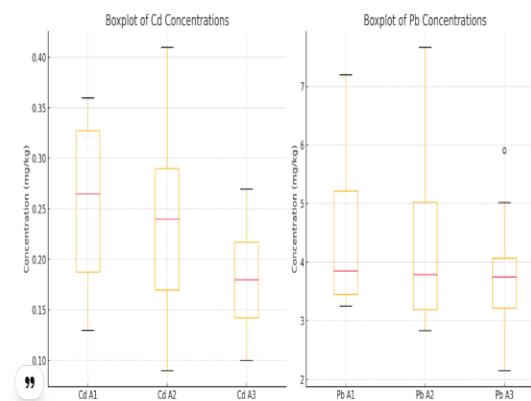


Fig 3: Boxplot analysis: Distribution, spread, variability, and Cd and Pb across species between sites
Visual Comparison Using Boxplots

Principal component analysis (PCA) of cadmium bioaccumulation in amaranthus species

A Principal Component Analysis (PCA) was performed, to visually assess the relationship



between sites and the cadmium (Cd) bioaccumulation behavior of different *Amaranthus* species was conducted. The biplot generated from the PCA (Figure 4) illustrates the spatial distribution of the bioaccumulation data along two principal components: PC1 and PC2. The first principal component (PC1) and the second (PC2) jointly explain a significant proportion of the total variance in the cadmium bioaccumulation data, allowing a reduction of multidimensional information into a two-dimensional graphical representation. The PCA plot reveals a clear separation of the three *Amaranthus* species based on their Cd bioaccumulation profiles: *A. spinosus* is oriented positively along PC2, indicating a strong association with higher cadmium accumulation in sites aligned along this axis. *A. cruentus* lies on the negative side of PC2, implying lower bioaccumulation or contrasting accumulation behavior. *A. hybridus* is located closer to the origin with a moderate positive loading on PC1, reflecting relatively average bioaccumulation across the sites. These findings suggest interspecific differences in metal uptake efficiency and accumulation tendencies, aligning with earlier reports that have documented species-specific variations in heavy metal accumulation among *Amaranthus* species and other leafy vegetables (Nabulo *et al.*, 2012). Specifically, *A. spinosus* appears to demonstrate a stronger phytoaccumulative potential for cadmium, which may be attributed to its differential metal transport mechanisms, root architecture, or higher tolerance thresholds.

The dispersion of sites within the biplot further provides information about the environmental and

anthropogenic influences on Cd accumulation. For instance, Site 2, plotted in close proximity to the *Amaranthus spinosus* vector, suggests enhanced cadmium uptake by this species at that location. This site may represent a highly contaminated area such as a roadside or market zone, consistent with recent studies reporting elevated heavy metal concentrations in vegetables cultivated in urban and peri-urban environments (Zhang *et al.*, 2022). In contrast, Sites 4 and 8 appear to be more strongly associated with *A. cruentus*, indicating site-specific accumulation profiles possibly influenced by soil pH, organic matter content, or differential exposure to cadmium sources. This aligns with findings from Saha *et al.* (2020), who emphasized the role of edaphic factors and land-use history in shaping metal uptake patterns in vegetables.

The clustering of sites in proximity to species vectors explains that both plant physiological traits and site-specific environmental variables jointly determine Cd uptake dynamics, in agreement with the conclusions drawn by Ali *et al.* (2023).

The PCA pattern reinforces the concept that bioaccumulation is not merely governed by total environmental concentrations but is also modulated by intrinsic plant characteristics such as root architecture, translocation efficiency, and tolerance thresholds. The superior accumulation observed in *A. spinosus* emphasizes its potential application in phytoextraction strategies for cadmium-contaminated soils. Recent evaluations have confirmed the suitability of *Amaranthus* species for phytoremediation due to their fast growth rate, high biomass yield, and considerable metal accumulation potential (Sun *et al.*, 2023).

Furthermore, the variation in bioaccumulation among sites suggests that localized soil management and pollution control measures are essential in regulating cadmium levels in edible crops. These findings point to the need for continuous monitoring and risk assessment of leafy vegetables cultivated in urban and peri-urban agricultural systems.

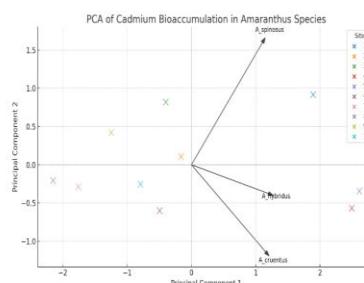


Fig 4: PCA of cadmium bioaccumulation in amaranthus species

Principal component analysis (PCA) of lead bioaccumulation patterns

The multivariate relationships among sites, soil Pb concentrations, and the bioaccumulation behavior of the three Amaranthus species, *A. hybridus* (A1), *A. cruentus* (A2), and *A. spinosus* (A3), is shown by Principal Component Analysis (PCA). The analysis included seven variables: soil Pb, plant Pb concentrations (A1, A2, A3), and their corresponding bioaccumulation factors (BAF A1, BAF A2, BAF A3) across the eight sampling sites. The PCA revealed two principal components (PCs) that together explained a substantial portion of the total variance. PC1 accounted for approximately 65–70% of the variation, while PC2

contributed around 20–25%. PC1 was strongly positively loaded with BAF A1, BAF A2, BAF A3, and plant Pb concentrations, suggesting that it represents a general bioaccumulation gradient. PC2, on the other hand, was moderately associated with soil Pb concentration and *A. hybridus* uptake (Pb A1), reflecting variability influenced more by environmental availability than plant-specific uptake capacity.

The PCA biplot displayed clear clustering of Sites 1, 2, and 7 in the positive quadrant of PC1, indicating

The PCA emphasizes the species-specific and site-specific variability in lead uptake and accumulation, corroborating earlier univariate analyses. *A. spinosus* (A3) appeared more variable in its uptake pattern, with high BAF at Site 2 (1.84) but generally lower values across other locations. This aligns with previous findings by Zhang *et al.* (2021), who reported that Amaranthus species can show localized hyperaccumulation tendencies driven by site-level factors such as soil pH, microbial interactions, and rhizosphere metal mobility.

Interestingly, despite relatively low soil Pb concentrations at Site 2 (3.21 mg/kg), all three species showed high BAFs, suggesting enhanced lead bioavailability or efficient uptake mechanisms. This finding is consistent with Liu *et al.* (2020), who highlighted the role of metal speciation and plant physiological traits in influencing accumulation patterns more than total soil concentrations alone.



The separation of Site 4 along PC2, with the highest soil Pb (7.76 mg/kg) but only moderate BAFs, suggests a saturation effect or a plant tolerance threshold, beyond which uptake efficiency declines, possibly as a defense mechanism. This reflects the complexity of metal uptake dynamics in real-world agroecosystems, influenced by both external (soil contamination level) and internal (plant traits) drivers.

The PCA indicates Sites 1, 2, and 5 as hotspots of plant hyperaccumulation potential, with *A. hybridus* and *A. cruentus* exhibiting consistent uptake efficiencies. These findings position Amaranthus species, especially A1 and A2, as viable candidates for phytoextraction of Pb from

moderately contaminated soils. However, their high BAFs in edible tissues also raise serious food safety concerns, particularly in peri-urban regions where these vegetables are frequently consumed (WHO, 2011).

The PCA-based clustering thus offers a diagnostic tool to prioritize sites for remediation and to inform safe cultivation guidelines for edible leafy vegetables in heavy metal-prone zones. Future work should integrate PCA with geostatistical mapping and molecular profiling to further disentangle the mechanisms driving Pb uptake variation among Amaranthus species.

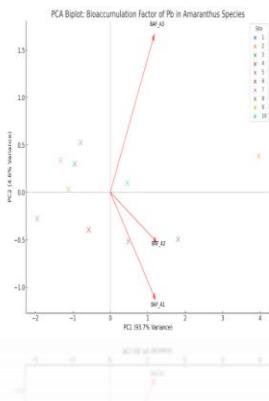


Fig 5: PCA of lead bioaccumulation in amaranthus species

CONCLUSION

The study indicates the accumulation patterns of cadmium (Cd) and lead (Pb) in three commonly consumed Amaranthus species, *Amaranthus hybridus* (A1), *A. cruentus* (A2), and *A. spinosus* (A3), cultivated on anthropogenically impacted soils. Although Cd accumulation remained

relatively consistent across species and sites, values often approached or exceeded the WHO maximum permissible limit of 0.2 mg/kg, especially in *A. hybridus* and *A. cruentus*, suggesting heightened uptake capacity likely driven by root physiology and localized pollution sources.

Lead accumulation was even more pronounced. Measured Pb concentrations in all three species far exceeded the Codex Alimentarius Commission's guideline of 0.3 mg/kg for leafy vegetables, with peak values reaching up to 7.67 ± 1.05 mg/kg at Site 4. These high concentrations were particularly evident in samples from roadside and urban-adjacent locations, showing the impact of atmospheric deposition, vehicular emissions, and contaminated irrigation water. *A. hybridus* and *A. cruentus* again showed greater accumulation potential, with several samples recording bioaccumulation factors (BAFs) above 1.0. By contrast, *A. spinosus* consistently demonstrated



lower uptake for both Cd and Pb, positioning it as a potentially safer crop for cultivation in contaminated urban soils.

The health implications of these findings are significant. The frequent exceedance of safety thresholds in edible plant tissues calls for immediate action to protect public health in urban and peri-urban settings where such vegetables are regularly consumed.

The study also points the role of *Amaranthus* species as sensitive bioindicators of heavy metal contamination and shows the need for integrative strategies to mitigate health risks. These include routine environmental monitoring, enforcement of agro-ecological safety limits, promotion of less accumulative plant species like *A. spinosus*, and the adoption of soil remediation techniques such as biochar amendment and phytostabilization.

The cultivation of leafy vegetables in polluted environments poses a clear threat to food safety and urban health. This indicates a pressing need for coordinated policy interventions, public education campaigns, and sustainable urban agricultural practices to minimize heavy metal exposure through diet and safeguard environmental and human well-being.

Conflict of Interest: There is no conflict of interest in this study.

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THE EFFECTS OF ORAL ADMINISTRATION OF EXTRACTS OF *DIALLIUM GUINEENSE* ON SELECTED BIOCHEMICAL PARAMETERS IN WISTAR RATS

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ABSTRACT

Traditional medicine practitioners in West Africa do use *Diallium guineense* (velvet tamarind) extensively as a medicinal plant/herb for managing fever, edema, diarrhea, and pains. Ally confirms these assertions. In this study, the biochemical effects of giving Wistar rats an ethanol extract of *Diallium guineense* orally were investigated. The antioxidant potential and phytochemical composition of the ethanol extract of *Diallium guineense* were quantitatively analyzed. In this investigation, sixteen adult-male Wistar rats were employed. The animals were divided equally and at random into four groups (n=4). For fourteen days, Group I acted as the control, and Groups II, III, and IV received oral treatments of 50 mg/kg, 100 mg/kg, and 150 mg/kg of ethanol extract of *Diallium guineense*, respectively for fourteen days. On the 15th day, the animals were sacrificed by cervical dislocation and blood samples were obtained via cardiac puncture for liver function such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities and lipid profile. One-way ANOVA was used statistically analyzed the data with GraphPad prism 8 and p<0.5 was considered significant. Results indicated the ethanol extract of *Diallium guineense* does not alter most of the biochemical parameters evaluated when compared with the control. The findings highlight the probable therapeutic efficacy and safety profile of *Diallium guineense* when used in the context of traditional medicine.

Keywords: *Diallium guineense*, liver function, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lipid profile.

INTRODUCTION

The numerous uses of substances derived from plants have made them extremely important to man in recent years. Using selective solvents, extraction techniques separate the medicinally active parts of plant tissues from the inactive or inert components (Akhila *et al.*, 2007). These plant components are complex mixtures of various medicinal metabolites, including flavonoids, lignans, phenols, alkaloids, glycosides,

and terpenoids (Ncube *et al.*, 2008). Therapeutically active compounds have long been known to be found in medicinal plants. The identification and characterization of bioactive compounds from natural sources is of interest to evidence-based research, which supports the pharmacological and medical benefits of plant-derived compounds (Handa *et al.*, 2008). "Herbal drugs" refers to the parts of a plant, such as leaves, flowers, seeds, roots, bark, stem, etc., that are used



to make medicines. Among the chemicals found in medicinal plants are flavonoids and alkaloids.

Goneese with

dialium (D) The Leguminosae family includes the tall, tropical tree known as guineense (velvet tamarind), which bears fruit. Its edible, small fruits are usually grape-sized, and their hard, brown shells are inedible. In Africa, *Dialium guineense* grows in dense forests along the southern edge of the Sahel. It is found in Nigeria, where it is called "Awin" or "Igbaru" in Yoruba, "Ichebu" in Igbo, "Tsamiyarkurm" in Hausa, and "Amughen" in Edo, as well as in west Ghana, where it is called "yoyi.". Different diseases can be prevented by using the bark, fruits, and leaves (Dressler et al., 2014). The safety of the bioactive ingredients in *D. guineense* is rarely documented, despite the plant being used extensively in medical research. Plant extracts are said to be abundant in significant phytochemicals (Kar, 2007).

METHODS

FRUIT COLLECTION

Dialium guineense fruits were obtained from Okada Market, Edo State, Nigeria. Identification was carried out by Dr. Adebayo M. A. in the Department of Pharmacognosy, Igbinedion University Okada. A voucher specimen was recorded as IUO/13/070.

Fruit Preparation and Extraction

The fruits were milled with an electric blender and weighed using an analytical balance. Extraction followed a 72-hour maceration procedure with continuous agitation, based on the Abu *et al.*

(2015) method. A total of 400 g of ground material was processed. The aqueous filtrate was reduced in a water bath to obtain a concentrated extract, which was stored in a 5 mL container.

EXPERIMENTAL ANIMALS

Sixteen female rats weighing 100–120 g was sourced from a licensed veterinary supplier in Ajibode, Ibadan. The animals were kept in plastic cages under standard laboratory conditions and provided unrestricted access to feed and water. They underwent a 14-day acclimatization period before the experiment began. Procedures met institutional ethical requirements.

EXPERIMENTAL DESIGN

The animals were distributed randomly into four groups, each containing four rats.

Group 1: control.

Group 2: 50 mg/kg ethanol extract of *D. guineense*.

Group 3: 100 mg/kg extract.

Group 4: 150 mg/kg extract.

Dosing occurred once daily for 14 consecutive days. Nothing was modified during the treatment period.

Sacrificing of Animals

After the final treatment day, the rats were fasted overnight. Sacrifice was performed by cervical dislocation. Blood was collected via cardiac puncture using a 2 mL syringe and transferred into universal and lithium heparin tubes. Serum was isolated by centrifugation at 3000 rpm for roughly four minutes and used immediately for biochemical assays.

Statistical Analysis

GraphPad Prism 8.0.1 was used for statistical analyses. The obtained data were subjected to normality test using Shapiro-Wilk test and the data were normally distributed ($p>0.05$). Group differences were evaluated using one-way ANOVA followed by Tukey's multiple comparison test. Data were expressed as mean \pm SEM and presented graphically.

RESULTS

Phytochemical Composition

Saponin, tannin, phenol, flavonoid, and alkaloid concentrations are 73.7 ± 0.21 mg DE/g, 180.1 ± 0.18 mg GAE/G, 359.2 ± 189.34 mg GAE/g, 145.8 ± 1.46 mg QUE/g, and 166.0 ± 0.55 mg AE/g, respectively, according to the quantitative analysis of phytochemical composition found in *Dialium guineense* ethanol extract (Table 1). Additionally, the extract's ferric reducing antioxidant power and total antioxidant capacity level are $70 \pm 0 \pm 13$ mg AAE/g and $102 \pm 1 \pm 40$ mg AAE/g, respectively.

Table 1: Antioxidant and Quantitative Analysis ethanol extract of *Dialium guineense*

Parameters	
Saponin (mg DE/g)	73.7 ± 0.21
Tannin (mg GAE/g)	180.1 ± 0.18
Flavonoid (mg QUE/g)	145.8 ± 1.46
Alkaloid (mg AE/g)	166.0 ± 0.55
Ferric reducing antioxidant power (mg AAE/g)	70.6 ± 0.13
Total antioxidant capacity (mg AAE/g)	102.3 ± 1.40

Data presented as the mean \pm SEM of three determinations

Lipid Profile

Total Cholesterol

Serum cholesterol levels in Wistar rats treated with 50 mg/kg, 100 mg/kg, and 150 mg/kg of *Dialium guineense* ethanol extract did not differ significantly from the control group, as shown in Figure 1

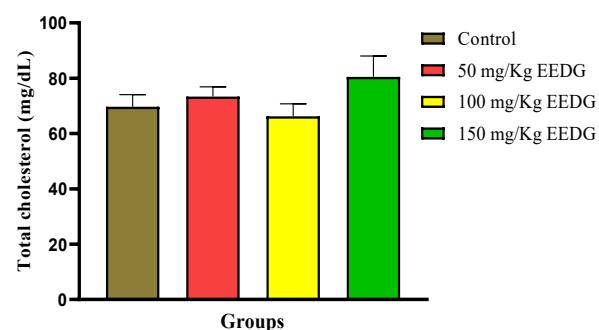


Figure 1: *Dialium guineense* ethanol extract's impact on Wistar rats' serum total cholesterol levels.

EEDG = *Dialium guineense* Bar for ethanol extract, which represents mean \pm standard error of mean.

High Density Lipoprotein-cholesterol

Figure 2 shows that Wistar rats treated with 50 mg/kg, 100 mg/kg, and 150 mg/kg of *Dialium guineense* ethanol extract did not exhibit a significant change in their serum high density lipoprotein cholesterol levels when compared to the control group.

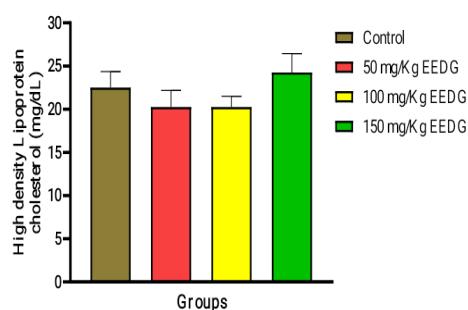


Figure 2: Effect of ethanol extract from *Dialium guineense* affected the Wistar rats' serum levels of high-density lipoprotein cholesterol.

EEDG = *Dialium guineense* Bar for ethanol extract, which represents mean \pm standard error of mean.

Triglycerides

When compared to the control, the Wistar rats treated with 50 mg/kg, 100 mg/kg, and 150 mg/kg of ethanol extract *Dialium guineense* showed no discernible change in serum triglyceride levels

Figure 3

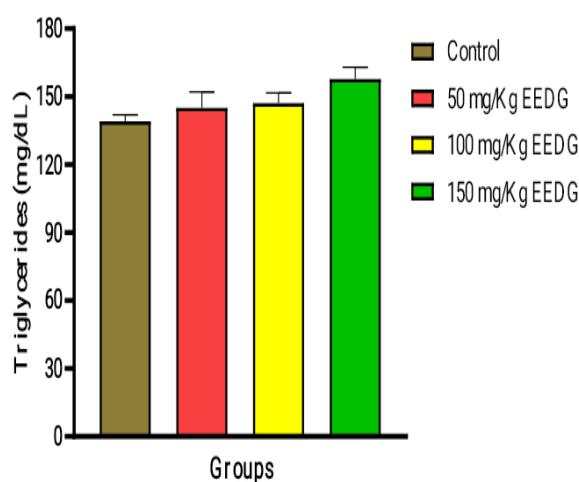


Figure 3. The impact of *Dialium guineense* ethanol extract on Wistar rats' serum triglyceride levels

EEDG = *Dialium guineense* Bar for ethanol extract, which represents mean \pm standard error of mean.

Low-density Lipoprotein

Dialium guineense ethanol extract at doses of 50, 100, and 150 mg/kg appeared to lower low-density lipoprotein in Wistar rats, but the decrease was not statistically different from the control group (Fig 4).

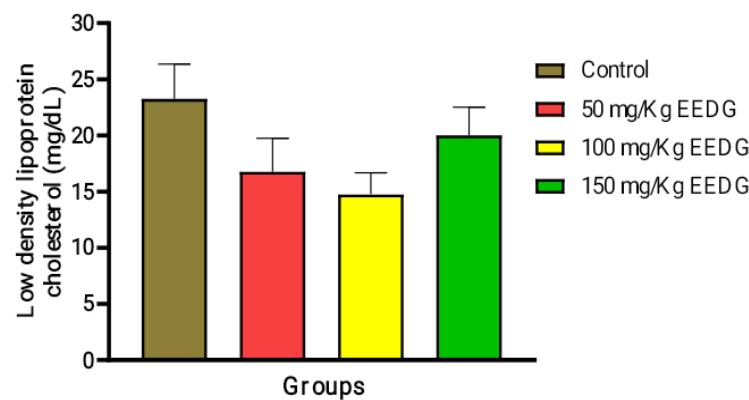


Figure 4: Effect of ethanol extract from *Dialium guineense* affected the Wistar rats' serum levels of low-density lipoprotein cholesterol.

EEDG = *Dialium guineense* Bar for ethanol extract, which represents mean \pm standard error of mean.

Liver Function

Alkaline Phosphatase Activity

Dialium guineense ethanol extract treatment of Wistar rats results in alkaline phosphatase activity, as shown in Figure 5. The Wistar rats treated with 50 mg/kg and 150 mg/kg of *Dialium guineense* ethanol extract did not significantly differ from the control in terms of serum alkaline phosphatase

activity. Comparing the 100 mg/kg ethanol extract *Dialium guineense* group to the control, the alkaline phosphatase activity increased noticeably.

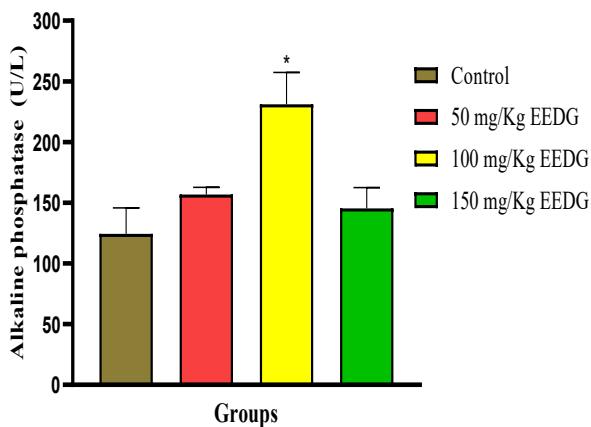


Figure 5: Effect of *Dialium guineense* ethanol extract on Wistar rats' serum alkaline phosphatase activity.

The mean \pm standard error of the mean $*p<0.05$ is significant when compared to the control, as indicated by the EEDG = Ethanol extract *Dialium guineense* Bar.

Aspartate aminotransferase activity

Wistar rats given ethanol extract from *Dialium guineense* showed results for aspartate aminotransferase activity (Figure 6). The Wistar rats treated with 50 mg/kg and 100 mg/kg ethanol extract *Dialium guineense* did not significantly differ from the control in terms of serum aspartate aminotransferase activity. In the group that received 150 mg/kg of ethanol extract from *Dialium guineense*, the aspartate aminotransferase activity was significantly higher than in the control group.

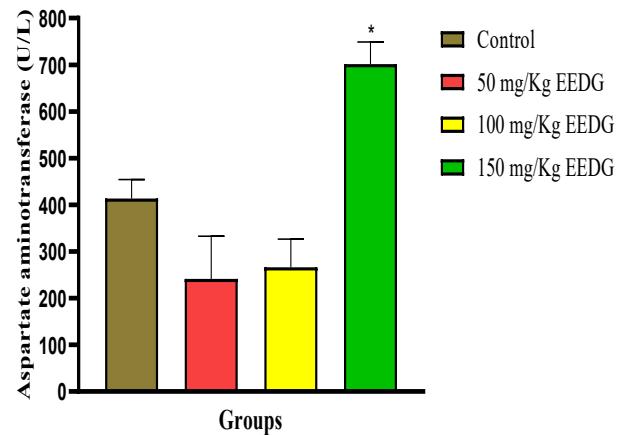


Figure 6: *Dialium guineense* ethanol extract's impact on Wistar rats' serum aspartate aminotransferase activity.

The mean \pm standard error of the mean $*p<0.05$ indicates that the ethanol extract *Dialium guineense* Bar is significant in comparison to the control.

Alanine aminotransferase activity

The Wistar rats treated with 50 mg/kg, 100 mg/kg, and 150 mg/kg of *Dialium guineense* ethanol extract showed no discernible change in serum alanine aminotransferase activity when compared to the control group (Figure 7).

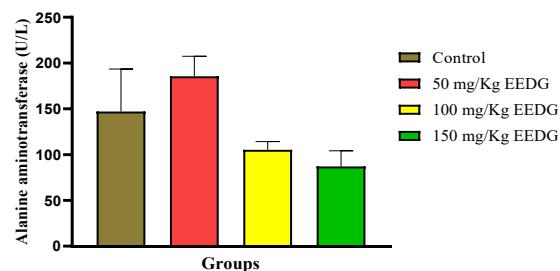


Figure 7: Effect of *Dialium guineense* ethanol extract on Wistar rats' serum alanine aminotransferase activity.

EEDG = *Dialium guineense* Bar for ethanol extract, which represents mean \pm standard error of mean.

Total bilirubin

The Wistar rats treated with 50 mg/kg, 100 mg/kg, and 150 mg/kg of *Dialium guineense* ethanol extract did not significantly differ from the control in terms of their serum total bilirubin levels (Fig 8).

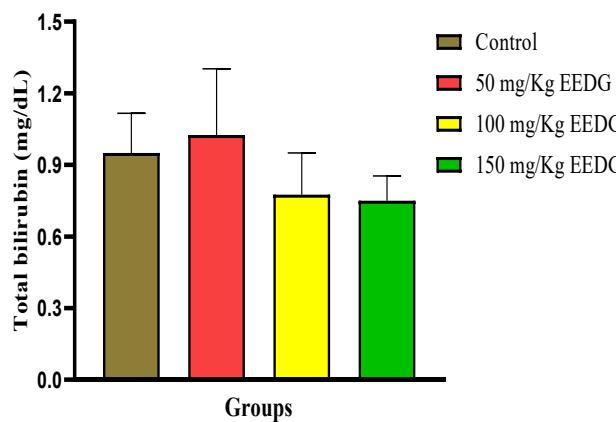


Figure 8. The impact of *Dialium guineense* ethanol extract on the serum total bilirubin level in Wistar rats.

EEDG = *Dialium guineense* Bar for ethanol extract, which represents mean \pm standard error of mean.

Conjugated bilirubin

When compared to the control, the Wistar rats treated with 50 mg/kg, 100 mg/kg, and 150 mg/kg of *Dialium guineense* ethanol extract did not exhibit a significant change in their serum conjugated bilirubin levels (Fig 9).

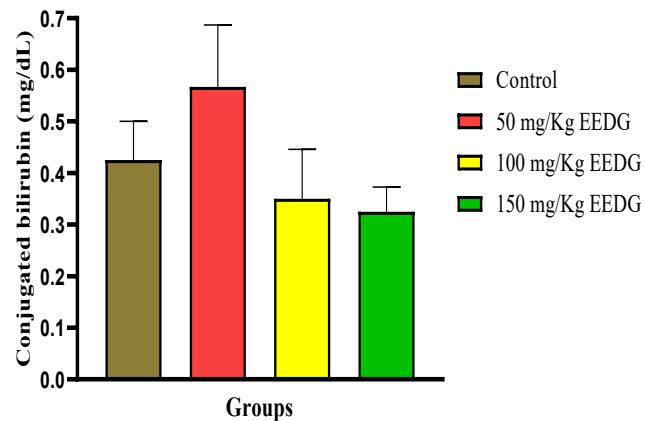


Figure 9: Effect of *Dialium guineense* ethanol extract on Wistar rats' serum conjugated bilirubin levels.

EEDG = *Dialium guineense* Bar for ethanol extract, which represents mean \pm standard error of mean.

DISCUSSION

The findings from this study revealed that using *Dialium guineense* extract had a significant impact on liver function and lipid metabolism, providing solid scientific backing for its traditional use in treating metabolic disorders. The data indicates that this plant holds bioactive potential and has been utilized in traditional medicine for various health benefits.

Since irregularities in serum lipids are closely linked to conditions like atherosclerosis, coronary heart disease, and other metabolic syndromes, lipid profile values serve as crucial indicators of cardiovascular and metabolic health. In our study, administering *D. guineense* extract orally resulted in a dose-dependent reduction in triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL), while also significantly boosting high-



density lipoprotein (HDL). These findings suggest that *D. guineense* could provide protection against lipid-related issues such as hypercholesterolemia and cardiovascular disease, indicating a hypolipidemic effect.

Previous research has also documented similar hypolipidemic effects of *D. guineense*. For example, a study by Akujobi and Ejele (2017) found that *D. guineense* pulp extracts significantly reduced serum levels of TC and LDL in Wistar rats, while simultaneously increasing HDL. These effects on lipid metabolism are thought to be driven by the plant's phytoconstituents, particularly its rich content of dietary fiber, polyphenols, flavonoids, and saponins. Research has shown that polyphenolic compounds can lower intestinal cholesterol absorption, enhance the conversion of cholesterol to bile acids, and promote bile excretion, all of which contribute to lowering serum cholesterol (Huang et al. 2010).

In addition to its role in lipid metabolism, *D. guineense* has a notable impact on liver function. Key indicators like aspartate aminotransferase (AST), bilirubin, alkaline phosphate (ALP), and serum alanine aminotransferase (ALT) are crucial for assessing the health and functionality of liver cells during liver function tests. Elevated levels of these enzymes often signal cell leakage or liver damage. Interestingly, in this study, the administration of *D. guineense* extract didn't lead to significant increases in these enzyme levels; in fact, in some cases, the levels even dropped compared to the untreated control group.

Moreover, the protective effects of *D. guineense* on the liver might be attributed to its anti-inflammatory properties. Chronic liver injury often

goes hand in hand with inflammation, which can worsen liver cell damage. The bioactive phytochemicals in *D. guineense* may help manage inflammation and curb the release of pro-inflammatory cytokines, potentially preventing inflammation and liver fibrosis while preserving liver structure and function (Ogunmoyole et al., 2018). Additionally, the fruit's dietary fiber content could enhance detoxification by supporting bile production and aiding in the elimination of harmful metabolites from the digestive system.

Finally, the findings of this research shed light on the biochemical effects of *Dialium guineense* extract. The trends observed in lipid modulation and liver enzyme stability indicate a possible functional or therapeutic potential of *Dialium guineense* extract, warranting further long-term investigation.

CONCLUSION

In Wistar rats, the study found that oral administration of *Dialium guineense* extract produced modulating effects on lipid profile and liver function. The extract might have protective qualities by keeping liver enzyme levels—like bilirubin, ALT, AST, and ALP—within normal limits. Additionally, it enhanced lipid metabolism by boosting protective activities and reducing total cholesterol, triglycerides, and LDL levels, suggesting it could be a promising therapeutic option for liver disease and dyslipidemia.

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Contemporary Trends in Chemical Sciences: Integrating Artificial Intelligence, Sustainability, Advanced Materials, and Emerging Technologies

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ABSTRACT

Contemporary chemical sciences are undergoing a profound transformation driven by the convergence of artificial intelligence, sustainability-oriented frameworks, advanced materials design, and emerging technologies. This review provides an integrative overview of the major trends reshaping modern chemistry, highlighting how digital and data-driven approaches are accelerating molecular discovery, materials development, and process optimization. The growing centrality of green and circular chemistry is examined, emphasizing waste valorization, sustainable synthesis, and life cycle-based decision-making as essential components of responsible chemical innovation. Advances in nanochemistry, porous and smart materials, and functional hybrid systems are discussed in relation to their roles in energy, environmental, and biomedical applications. The review further explores progress in energy, electrochemical, and bio-inspired chemistry, underscoring chemistry's contribution to decarbonization, renewable energy integration, and environmentally benign manufacturing pathways. Beyond individual advances, the article critically examines challenges associated with data quality, scalability, reproducibility, interdisciplinary integration, and workforce preparedness. By synthesizing developments across traditionally separate subfields, this review presents a holistic perspective on the evolving landscape of chemical sciences and outlines a forward-looking roadmap for research and innovation. The findings underscore that the future impact of chemistry will increasingly depend on the effective integration of digital intelligence, sustainability principles, and advanced functional materials to address global scientific, industrial, and societal challenges.

Keywords: Artificial intelligence in chemistry, Sustainable and green chemistry, Circular chemistry and waste valorization, Advanced materials and nanochemistry, Energy and electrochemical chemistry, Data-driven chemical discovery, Bio-inspired chemistry

INTRODUCTION

Chemistry has long been recognized as the central science, underpinning advances in materials development, energy technologies, pharmaceuticals, agriculture, and environmental protection. However, the nature of chemical research has changed profoundly over the past two decades. Contemporary chemical sciences are no longer driven solely by incremental experimental discovery but increasingly shaped by the convergence of artificial intelligence (AI), sustainability imperatives, advanced materials design, and emerging digital technologies. This transformation has repositioned chemistry as a highly interdisciplinary and solution-oriented discipline, responding directly to global challenges such as climate change, resource depletion, energy insecurity, and environmental pollution (Anastas & Warner, 1998; Clark & Macquarrie, 2016).



One of the most influential forces reshaping modern chemistry is the rapid integration of artificial intelligence and data-driven methodologies. Machine learning, deep learning, and automated data analytics are now routinely applied to chemical reaction prediction, retrosynthetic planning, catalyst discovery, and materials screening. These tools enable chemists to navigate vast chemical spaces that would be impractical using traditional trial-and-error approaches, significantly reducing cost, time, and resource consumption (Butler *et al.*, 2018; Schneider *et al.*, 2020). The emergence of autonomous laboratories and digital twins further highlights a paradigm shift from intuition-driven experimentation to predictive and adaptive chemical research systems.

In parallel with digitalization, sustainability has become a defining priority of chemical innovation. The principles of green chemistry and sustainable engineering emphasize waste minimization, energy efficiency, renewable feedstocks, and reduced environmental toxicity (Anastas & Eghbali, 2010). These principles are now deeply embedded in both academic research and industrial practice. Increasing attention has been given to circular chemistry approaches, including waste valorization, biomass conversion, and plastic recycling, aimed at closing material loops and reducing the environmental footprint of chemical processes (Sheldon, 2016; Zimmerman *et al.*, 2020). As regulatory pressures and societal expectations grow, sustainability considerations are no longer optional but fundamental to chemical research design and evaluation.

Another critical trend is the rapid advancement of functional and nanostructured materials, which serve as key enablers across energy, environmental, and biomedical applications. Developments in nanochemistry, porous materials such as metal-organic frameworks (MOFs) and covalent organic frameworks (COFs), and smart responsive materials have expanded the functional capabilities of chemical systems beyond conventional limits (Furukawa *et al.*, 2013; Wang *et al.*, 2020). These materials offer tunable physicochemical properties that can be precisely engineered for targeted applications, including catalysis, gas storage, water purification, sensing, and drug delivery.

Closely linked to materials innovation is the growing importance of energy and electrochemical chemistry, particularly in the context of global decarbonization efforts. Advances in batteries, supercapacitors, electrocatalysis, and sustainable fuel production demonstrate chemistry's central role in enabling the energy transition (Seh *et al.*, 2017; Armaroli & Balzani, 2011). At the same time, bio-inspired and biocatalytic approaches are gaining prominence as environmentally benign alternatives to traditional chemical synthesis, further reinforcing the shift toward sustainable and biologically integrated chemical processes (Bornschäuer *et al.*, 2012).

Despite the rapid progress across these domains, much of the existing literature remains fragmented, with reviews typically focusing on isolated subfields such as AI in chemistry, green chemistry, materials science, or energy applications. While these specialized reviews provide valuable depth, they often fail to capture the interconnected nature of contemporary chemical research, where advances in one area increasingly depend on progress in others. There is therefore a clear need for an integrative review that synthesizes these major trends, highlights their points of convergence, and critically examines the challenges and opportunities arising from their integration.



The objective of this review is to provide a comprehensive and integrative overview of contemporary trends in chemical sciences, with particular emphasis on the interplay between artificial intelligence, sustainability, advanced materials, and emerging technologies. Rather than offering an exhaustive account of each subdiscipline, this article focuses on key developments, unifying concepts, and cross-cutting challenges that define the current and future landscape of chemistry. By adopting this holistic perspective, the review aims to inform researchers, educators, and industry practitioners, while offering strategic insights into the directions that are likely to shape chemical sciences in the coming decades.



Figure 1: Convergence in Contemporary Chemical Sciences

Methodology of Literature Selection

A systematic and transparent literature selection strategy was adopted to ensure that this review accurately reflects the current and converging trends in chemical sciences, with emphasis on artificial intelligence, sustainability, advanced materials, and emerging technologies. The methodology was designed to balance breadth and relevance, while maintaining academic rigor and minimizing selection bias.

Data Sources and Search Strategy

The literature search was conducted using major scholarly databases, including Scopus, Web of Science, Google Scholar, and selected publisher platforms (Elsevier, Springer Nature, Wiley, and the American Chemical Society). These databases were chosen due to their extensive coverage of peer-reviewed journals in chemistry and related interdisciplinary fields. Searches were performed using combinations of keywords and Boolean

operators such as “*artificial intelligence in chemistry*,” “*machine learning chemical discovery*,” “*green chemistry*,” “*sustainable chemical processes*,” “*advanced materials*,” “*nanochemistry*,” “*energy chemistry*,” and “*emerging technologies in chemical sciences*.”

To capture interdisciplinary contributions, additional search strings combining multiple themes (e.g., *AI AND sustainable chemistry*, *materials chemistry AND energy applications*) were employed. Reference lists of highly cited articles and recent reviews were also examined to identify relevant studies not captured in the initial database searches.

Inclusion and Exclusion Criteria

To ensure relevance and timeliness, the review primarily focused on literature published between 2015 and 2025, reflecting the period during which significant advances in AI-driven chemistry, sustainability-oriented research, and advanced materials have accelerated. Peer-reviewed



research articles, review papers, and authoritative perspective articles published in English were included.

Publications were excluded if they were (i) conference abstracts without full peer review, (ii) patents, editorials, or opinion pieces lacking substantive scientific analysis, or (iii) studies focused narrowly on technical details without broader implications for contemporary chemical trends. While seminal older works were cited where necessary to provide conceptual foundations, the emphasis remained on recent developments shaping current research directions.

Screening and Thematic Categorization

Following the initial search, titles and abstracts were screened to assess relevance to the core themes of this review. Full texts of selected articles were then examined to ensure conceptual alignment with at least one of the major thematic areas: digital and data-driven chemistry, sustainability and circular chemistry, advanced materials, energy and electrochemical chemistry, or emerging integrative technologies.

Rather than organizing the review strictly by traditional sub-disciplines of chemistry, the selected literature was thematically categorized based on functional relevance and technological convergence. This approach facilitated cross-comparison and synthesis across fields, reflecting the increasingly interdisciplinary nature of modern chemical research (Tranfield *et al.*, 2003).

Limitations of the Review Methodology

Despite efforts to conduct a comprehensive and balanced review, certain limitations are acknowledged. The reliance on English-language publications may exclude relevant research published in other languages. Additionally, rapid

advancements in areas such as AI-assisted chemistry and sustainable materials mean that some very recent developments may not yet be fully represented in the peer-reviewed literature. Nevertheless, by focusing on highly cited and methodologically robust studies, this review aims to provide a reliable and up-to-date synthesis of contemporary trends in chemical sciences.

Table 1 summarizes the major contemporary trends in chemical sciences, highlighting enabling technologies, representative applications, and key challenges (Anastas & Warner, 1998; Butler *et al.*, 2018; Sheldon, 2016).

Table 1: Major Contemporary Trends in Chemical Sciences

Trend Area	Key Enabling Technologies / Concepts	Representative Applications	Key Challenges / Gaps
Artificial Intelligence & Data-Driven Chemistry	Machine learning, deep learning, cheminformatics, autonomous laboratories	Reaction prediction, retrosynthesis, catalyst and materials discovery	Data bias, interpretability, reproducibility
Green & Sustainable Chemistry	Green solvents, catalysis, process intensification	Low-waste synthesis, energy-efficient processes	Scalability, cost, sustainability metrics
Circular Chemistry & Waste Valorization	Chemical recycling, biomass conversion, life cycle assessment	Plastic upcycling, agro-waste valorization	Feedstock variability, infrastructure limitations
Advanced Materials & Nanochemistry	Nanomaterials, MOFs, COFs, smart materials	Energy storage, gas separation, biomedical devices	Stability, recyclability, environmental risks
Energy & Electrochemical Chemistry	Batteries, supercapacitors, electrocatalysis	Renewable energy storage, CO ₂ reduction	Material degradation, resource availability



successfully applied to predict physicochemical properties such as band gaps, solubility, toxicity, and catalytic activity, allowing researchers to pre-screen candidates before experimental validation (Rajan, 2015; Butler *et al.*, 2018). These predictive capabilities are particularly valuable for sustainable chemistry, where minimizing waste and resource consumption is a key objective.

3. Digital and Data-Driven Transformation of Chemistry

The digital transformation of chemistry represents one of the most profound shifts in the history of the discipline. Advances in computational power, data availability, and algorithmic sophistication have enabled the widespread adoption of artificial intelligence (AI), machine learning (ML), and automation across chemical research and development. These tools are redefining how chemical knowledge is generated, validated, and applied, moving the field toward more predictive, efficient, and adaptive research paradigms (Butler *et al.*, 2018; Schneider *et al.*, 2020).

Machine Learning for Chemical Prediction and Design

Machine learning methods have become central to modern chemical prediction tasks, including reaction outcome forecasting, retrosynthetic analysis, and quantitative structure–property and structure–activity relationships (QSPR/QSAR). By learning patterns from large experimental and computational datasets, ML models can predict reaction yields, selectivities, and optimal conditions with increasing accuracy (Segler *et al.*, 2018; Coley *et al.*, 2019). This capability significantly reduces reliance on exhaustive experimental screening and accelerates decision-making in both academic and industrial laboratories.

In molecular and materials design, data-driven approaches enable the rapid exploration of chemical space, which is otherwise astronomically large. ML models have been

AI-Assisted Catalyst and Materials Discovery

Catalysis and materials chemistry have benefited substantially from AI-guided discovery frameworks. High-throughput computational screening combined with ML algorithms enables the identification of promising catalysts and functional materials from vast libraries of hypothetical structures (Nørskov *et al.*, 2011; Jablonka *et al.*, 2020). In materials science, AI-driven models have facilitated the discovery of novel porous materials, battery components, and electrocatalysts with tailored properties for energy and environmental applications.

Beyond prediction, AI is increasingly used to optimize synthesis pathways and processing conditions. Reinforcement learning and generative models can propose new molecular structures or materials compositions that satisfy predefined performance criteria, representing a shift from passive data analysis to active chemical creativity (Gómez-Bombarelli *et al.*, 2018). These approaches highlight the growing role of AI not merely as a support tool, but as an integral partner in chemical innovation.

Automation, Robotics, and Autonomous Laboratories

The integration of AI with laboratory automation has given rise to self-driving or autonomous



laboratories, where robotic platforms conduct experiments, analyze results, and iteratively refine hypotheses with minimal human intervention (MacLeod *et al.*, 2020). Such systems exemplify a closed-loop approach to chemical discovery, combining experimental execution with real-time data analysis and decision-making.

Automation enhances reproducibility, reduces human error, and enables continuous operation, which is particularly advantageous for high-throughput experimentation and materials screening. When coupled with AI algorithms, automated laboratories can rapidly converge on optimal chemical systems, offering a powerful framework for accelerating discovery while reducing resource use (Häse *et al.*, 2019).

Digital Twins and Computational Process Modeling

In parallel with laboratory-scale innovations, digital tools are transforming chemical process design and scale-up. Digital twins—virtual replicas of physical chemical systems—allow researchers and engineers to simulate reactions, processes, and material behavior under varying conditions (Glaessgen & Stargel, 2012). These models facilitate process optimization, safety analysis, and sustainability assessment before implementation in real-world systems.

For industrial chemistry, digital twins and advanced simulations support energy-efficient process intensification and predictive maintenance, aligning closely with sustainability and economic objectives. Their integration with AI further enhances adaptive control and real-time optimization capabilities (Venkatasubramanian, 2019).

Challenges and Ethical Considerations

Despite their transformative potential, digital and data-driven approaches in chemistry face several challenges. The quality, completeness, and bias of training data remain critical concerns, as ML models are inherently limited by the datasets on which they are trained (Hutson, 2019). Issues of model interpretability and transparency also pose barriers to widespread adoption, particularly in safety-critical or regulatory contexts.

Furthermore, the increasing automation of chemical research raises ethical and educational questions regarding data ownership, intellectual property, and workforce skill requirements. Addressing these challenges will require the development of standardized data practices, open and interpretable models, and interdisciplinary training that bridges chemistry, data science, and engineering (Walsh, 2015).

Sustainability and Circular Chemistry

Sustainability has emerged as a central organizing principle in contemporary chemical research, driven by escalating environmental challenges, resource constraints, and societal demand for cleaner technologies. Modern chemistry is increasingly evaluated not only by performance and efficiency but also by its environmental footprint, safety, and long-term viability. Within this context, green chemistry and circular chemistry frameworks provide conceptual and practical foundations for redesigning chemical products and processes in a more sustainable manner (Anastas & Warner, 1998; Sheldon, 2016).

Green Chemistry and Sustainable Process Design

Green chemistry emphasizes the design of chemical processes that reduce or eliminate the



use and generation of hazardous substances while improving energy and material efficiency. Core strategies include the use of benign solvents, renewable feedstocks, catalytic rather than stoichiometric reagents, and process intensification (Anastas & Eghbali, 2010). Advances in catalysis, including heterogeneous, homogeneous, and biocatalytic systems, have played a critical role in improving selectivity and atom economy across a wide range of chemical transformations.

Process optimization using alternative energy inputs—such as microwave irradiation, mechanochemistry, and photochemical activation—has further reduced energy consumption and reaction times in both laboratory and industrial settings (James *et al.*, 2012). These approaches not only align with sustainability objectives but also offer economic advantages by lowering operational costs and improving scalability.

Circular Chemistry and Waste Valorization

Beyond minimizing waste, contemporary chemical research increasingly focuses on closing material loops, a defining characteristic of circular chemistry. This paradigm seeks to transform waste streams into valuable resources, thereby reducing dependence on virgin raw materials and mitigating environmental pollution (Sheldon, 2016; Zimmerman *et al.*, 2020). Chemical recycling of plastics, for example, has gained prominence as an alternative to mechanical recycling, enabling the depolymerization or upcycling of polymers into fuels, monomers, or high-value chemicals (Coates & Getzler, 2020). Similarly, the valorization of agricultural and biomass waste through chemical, thermochemical, and biocatalytic pathways has

attracted significant attention. Lignocellulosic residues, food-processing waste, and agro-industrial by-products are increasingly explored as feedstocks for the production of biofuels, platform chemicals, and functional materials. These strategies support both environmental sustainability and regional economic development, particularly in resource-rich but industrially developing regions (Clark & Deswarthe, 2015).

Life Cycle Assessment and Sustainability Metrics

As sustainability considerations become integral to chemical innovation, life cycle assessment (LCA) has emerged as a critical tool for evaluating the environmental impact of chemical products and processes. LCA provides a quantitative framework for assessing energy use, greenhouse gas emissions, water consumption, and toxicity across the entire life cycle of a chemical system—from raw material extraction to end-of-life disposal (ISO, 2006).

The integration of LCA into chemical research enables more informed decision-making, helping to avoid unintended environmental trade-offs and supporting the design of genuinely sustainable technologies. Increasingly, LCA is being combined with techno-economic analysis and data-driven optimization tools to guide early-stage research toward scalable and environmentally responsible solutions (Hellweg & Milà, 2014).

Policy, Regulation, and Industrial Drivers

Regulatory frameworks and policy initiatives play a significant role in shaping sustainability-oriented chemical research. International agreements on climate change, chemical safety



regulations, and corporate sustainability commitments have accelerated the adoption of green and circular chemistry practices in industry. Concepts such as extended producer responsibility and sustainable procurement are encouraging chemical manufacturers to consider product life cycles and environmental impacts more holistically (Geissdoerfer *et al.*, 2017).

At the same time, sustainability-driven innovation is increasingly recognized as a source of competitive advantage. Companies that integrate green chemistry principles into product development benefit from improved regulatory compliance, reduced environmental risk, and enhanced public trust. As a result, sustainability is no longer viewed as a constraint on chemical innovation but as a catalyst for technological advancement and value creation.

Advanced Materials and Nanochemistry

Advanced materials chemistry occupies a central position in contemporary chemical sciences, serving as a bridge between fundamental molecular design and practical technological applications. The ability to precisely control composition, structure, and functionality at the nano- and molecular scales has enabled the development of materials with unprecedented performance in energy, environmental, electronic, and biomedical systems. These advances are increasingly supported by digital tools and guided by sustainability considerations, reflecting the broader convergence shaping modern chemistry (Butler *et al.*, 2018; Wang *et al.*, 2020).

Nanomaterials and Hybrid Systems

Nanomaterials, including metal and metal-oxide nanoparticles, carbon-based nanostructures, and polymer nanocomposites, have transformed

multiple areas of chemistry due to their high surface area, tunable physicochemical properties, and unique size-dependent phenomena. Applications range from heterogeneous catalysis and energy storage to sensing, environmental remediation, and drug delivery (Whitesides, 2005; Kamat, 2013).

Hybrid materials that integrate organic and inorganic components further expand functional possibilities by combining the structural diversity of organic chemistry with the robustness and electronic properties of inorganic systems. These materials offer enhanced stability, multifunctionality, and processability, making them particularly attractive for sustainable technologies where durability and efficiency are critical (Sanchez *et al.*, 2011).

Porous Framework Materials

Porous materials represent one of the most rapidly evolving classes of advanced materials in chemistry. Metal-organic frameworks (MOFs) and covalent organic frameworks (COFs) are characterized by high surface areas, ordered porosity, and tunable chemical functionality, enabling precise control over adsorption, diffusion, and catalytic behavior (Furukawa *et al.*, 2013; Diercks & Yaghi, 2017).

These materials have been extensively explored for gas storage and separation, carbon capture, catalysis, and water purification. Their modular synthesis allows for rational design tailored to specific applications, while recent advances aim to improve their stability, scalability, and environmental compatibility. The integration of machine learning and high-throughput screening has further accelerated the discovery and optimization of porous materials, highlighting the



synergy between digital tools and materials chemistry (Jablonka *et al.*, 2020).

Smart and Responsive Materials

Smart materials that respond dynamically to external stimuli—such as temperature, light, pH, electric fields, or chemical environments—are gaining increasing attention across chemical research. Stimuli-responsive polymers, self-healing materials, and adaptive surfaces exemplify how chemical systems can be engineered to exhibit autonomous or programmable behavior (Stuart *et al.*, 2010). These materials are particularly relevant in biomedical and environmental applications, including controlled drug release, biosensing, and adaptive filtration systems. Their development requires an interdisciplinary approach that integrates synthetic chemistry, materials science, and systems design, further illustrating the convergence of modern chemical research domains.

Sustainability Considerations in Materials Design

As the production and disposal of advanced materials scale up, sustainability has become an essential consideration in materials chemistry. Traditional synthesis routes for nanomaterials and functional materials often involve hazardous reagents, high energy inputs, or limited recyclability. Consequently, increasing efforts are directed toward green synthesis methods, renewable precursors, and recyclable or biodegradable material systems (Kralisch & Ott, 2007).

Life cycle assessment and environmental impact evaluation are increasingly applied to materials research to ensure that performance gains do not

come at the expense of environmental responsibility. In this regard, the integration of sustainability metrics with AI-driven design tools offers a promising pathway toward the development of materials that are not only high-performing but also environmentally benign.

Energy, Electrochemical, and Bio-Inspired Chemistry

The transition toward sustainable energy systems and environmentally responsible chemical manufacturing has placed energy, electrochemical, and bio-inspired chemistry at the forefront of contemporary research. These interconnected areas highlight chemistry's pivotal role in addressing global energy demand, reducing greenhouse gas emissions, and enabling cleaner production pathways. Advances in materials design, catalysis, and system integration continue to drive innovation across this rapidly evolving landscape (Armaroli & Balzani, 2011; Seh *et al.*, 2017).

Energy Storage and Conversion Materials

Energy storage technologies are critical to the deployment of renewable energy sources and the electrification of transportation and industry. Chemical research has contributed significantly to the development of advanced batteries, supercapacitors, and hybrid energy storage systems with improved energy density, cycle life, and safety. Innovations in electrode materials, electrolytes, and interfacial chemistry have been central to these advances, particularly in lithium-ion, sodium-ion, and emerging multivalent battery systems (Tarascon & Armand, 2001; Goodenough & Kim, 2010).

Beyond storage, chemical approaches to energy conversion—such as photocatalysis and solar fuel



generation—aim to directly harness renewable energy to drive chemical transformations. These strategies exemplify the integration of materials chemistry, photophysics, and catalysis in the pursuit of carbon-neutral energy solutions.

Electrocatalysis and Electrochemical Technologies

Electrochemical processes offer highly controllable and scalable pathways for sustainable chemical transformations. Electrocatalysis plays a central role in reactions relevant to the energy transition, including hydrogen evolution, oxygen evolution, oxygen reduction, and electrochemical carbon dioxide reduction (Seh *et al.*, 2017). Advances in catalyst design, informed by both experimental and computational studies, have improved activity, selectivity, and stability, while reducing reliance on scarce or expensive noble metals.

Electrochemical technologies also extend beyond energy applications into areas such as wastewater treatment, resource recovery, and green chemical synthesis. By coupling renewable electricity with electrochemical reactors, these systems enable decentralized and low-emission chemical production, aligning closely with circular and sustainable chemistry goals (Nørskov *et al.*, 2011).

Bio-Inspired and Biocatalytic Chemistry

Bio-inspired chemistry draws inspiration from natural systems to develop efficient, selective, and environmentally benign chemical processes. Enzymes and whole-cell biocatalysts exhibit remarkable specificity and operate under mild conditions, making them attractive alternatives to traditional chemical catalysts (Bornscheuer *et al.*, 2012). Advances in protein engineering and

synthetic biology have expanded the substrate scope and robustness of biocatalysts, facilitating their application in pharmaceuticals, fine chemicals, and bio-based materials production. Beyond catalysis, bio-inspired principles inform the design of functional materials and systems, such as artificial photosynthesis and biomimetic membranes. These approaches illustrate how insights from biological chemistry can guide the development of sustainable technologies that emulate nature's efficiency and adaptability.

Integration with Sustainability and Digital Tools

Energy, electrochemical, and bio-inspired chemistry increasingly benefit from integration with digital and data-driven tools, including machine learning and multiscale modeling. These approaches accelerate the discovery of high-performance materials and catalysts, optimize reaction conditions, and support the design of scalable energy systems (Butler *et al.*, 2018). When combined with sustainability metrics and life cycle assessment, digital tools enable more holistic evaluation of energy technologies, ensuring that improvements in performance translate into real environmental benefits.

Convergence, Challenges, and Future Roadmap

The defining characteristic of contemporary chemical sciences is not merely the emergence of new tools or subfields, but the convergence of artificial intelligence, sustainability, advanced materials, energy systems, and bio-inspired approaches into an increasingly unified research ecosystem. This convergence is reshaping how chemical knowledge is generated, how technologies are developed, and how chemistry



contributes to societal goals. While these developments offer unprecedented opportunities, they also introduce complex challenges that must be addressed to ensure responsible and impactful progress (Zimmerman *et al.*, 2020; Butler *et al.*, 2018).

Interdisciplinary Integration and Knowledge Convergence

Modern chemical research increasingly transcends traditional disciplinary boundaries. AI-driven modeling informs materials discovery; sustainability metrics guide process design; and advances in materials chemistry enable breakthroughs in energy and environmental applications. This interconnected landscape requires chemists to operate across computational, experimental, and systems-level domains, fostering collaboration between chemists, data scientists, engineers, and environmental scientists (Rajan, 2015).

However, effective integration remains uneven. Differences in data standards, terminology, and research cultures can impede collaboration and limit knowledge transfer. Addressing these barriers will require harmonized data infrastructures, open-access platforms, and interdisciplinary training frameworks that enable seamless communication across fields.

Scalability, Translation, and Industrial Adoption

A persistent challenge in chemical innovation is the translation of laboratory-scale advances into scalable, economically viable technologies. Many high-performing catalysts, materials, and electrochemical systems demonstrate promising results under controlled conditions but face limitations related to cost, durability, resource

availability, and manufacturability when scaled up (Armaroli & Balzani, 2011).

Similarly, AI-driven models and digital tools often struggle to transition from academic proof-of-concept studies to industrial deployment. Limited availability of high-quality proprietary data, concerns over intellectual property, and difficulties integrating AI tools into existing industrial workflows hinder broader adoption (Venkatasubramanian, 2019). Bridging this gap will require closer collaboration between academia and industry, as well as the development of standardized validation protocols and pilot-scale demonstrations.

Data Quality, Ethics, and Reproducibility

As data-driven methods become increasingly embedded in chemical research, data quality and reproducibility emerge as critical concerns. Machine learning models are highly sensitive to the completeness, consistency, and bias of training datasets, raising questions about reliability and generalizability (Hutson, 2019). Inconsistent reporting standards and limited access to negative or failed experimental results further exacerbate these challenges.

Ethical considerations also extend to data ownership, algorithmic transparency, and the potential displacement of traditional research roles through automation. Addressing these issues requires the establishment of open data standards, interpretable modeling approaches, and ethical guidelines that balance innovation with accountability (Walsh, 2015).

Education, Skills, and Workforce Development

The convergence shaping modern chemistry has significant implications for education and workforce development. Future chemists will



require not only strong foundations in chemical theory and experimentation but also competencies in data science, computational modeling, and sustainability assessment. Curricula that integrate these skills are essential for preparing researchers capable of navigating interdisciplinary research environments (Rajan, 2015).

At the same time, lifelong learning and professional retraining will become increasingly important as digital tools and sustainability frameworks evolve. Institutions and professional societies play a critical role in facilitating this transition through updated training programs and cross-disciplinary initiatives.

Future Research Directions and Roadmap

Looking ahead, the future of chemical sciences will be shaped by the deep integration of digital intelligence, sustainable design principles, and advanced functional materials. Short-term priorities include improving data infrastructures, developing interpretable and trustworthy AI models, and embedding sustainability metrics into early-stage chemical design. Medium-term efforts should focus on scalable materials and energy technologies, autonomous experimentation, and circular chemistry platforms that enable closed-loop material flows.

In the long term, chemistry is expected to play a central role in achieving global sustainability and energy goals through the development of carbon-neutral processes, resilient materials, and bio-inspired systems that mimic nature's efficiency. By embracing convergence and addressing the associated challenges, chemical sciences are well positioned to drive transformative innovation and societal impact in the decades ahead.

CONCLUSIONS

This review has examined the contemporary trends shaping chemical sciences, emphasizing the increasing integration of artificial intelligence, sustainability-driven principles, advanced materials, and emerging technologies. Collectively, these developments reflect a fundamental transformation in how chemistry is practiced, evaluated, and applied. Rather than operating as isolated subdisciplines, modern chemical research increasingly functions as an interconnected system in which digital tools, sustainable design strategies, and materials innovation reinforce one another to address pressing global challenges.

Artificial intelligence and data-driven methodologies have emerged as powerful enablers of predictive and efficient chemical research. Their application across molecular design, materials discovery, catalysis, and process optimization has accelerated innovation while reducing experimental cost and resource consumption (Butler *et al.*, 2018; Schneider *et al.*, 2020). However, the full potential of these tools depends on the availability of high-quality data, transparent modeling frameworks, and effective integration with experimental workflows.

Sustainability and circular chemistry now serve as guiding frameworks rather than peripheral considerations in chemical innovation. Green synthesis, waste valorization, and life cycle assessment have become central to the development of environmentally responsible chemical technologies, aligning chemical research with broader societal and regulatory expectations (Anastas & Eghbali, 2010; Sheldon, 2016). The shift toward circular material flows underscores chemistry's critical role in enabling resource efficiency and long-term environmental resilience.



Advances in advanced materials and nanotechnology continue to underpin progress across energy, environmental, and biomedical applications. Porous frameworks, nanostructured systems, and smart materials exemplify how precise chemical design can deliver tailored functionality while supporting sustainable performance goals (Furukawa *et al.*, 2013; Wang *et al.*, 2020). When combined with digital design tools and sustainability metrics, materials chemistry is increasingly positioned to deliver scalable and impactful solutions.

Energy, electrochemical, and bio-inspired chemistry further highlight chemistry's central role in the global transition toward low-carbon energy systems and greener manufacturing pathways. Innovations in energy storage, electrocatalysis, and biocatalytic processes demonstrate the capacity of chemical sciences to contribute directly to decarbonization and sustainable industrial development (Seh *et al.*, 2017; Bornscheuer *et al.*, 2012).

Despite these advances, significant challenges remain. Issues related to scalability, reproducibility, data governance, and workforce training must be addressed to ensure that technological progress translates into real-world impact. Overcoming these barriers will require interdisciplinary collaboration, standardized data practices, and education models that equip future chemists with both traditional chemical expertise and digital literacy.

In conclusion, the future of chemical sciences lies in convergence rather than specialization alone. By integrating artificial intelligence, sustainability principles, advanced materials, and emerging technologies, chemistry is evolving into a more predictive, responsible, and socially responsive discipline. Continued emphasis on

integration, transparency, and sustainability will be essential for ensuring that chemical innovation contributes meaningfully to global scientific, industrial, and societal objectives in the decades ahead.

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Current and Future Sustainable Energy Systems Worldwide: Technologies, System Integration, and Transition Pathways – A Review

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ABSTRACT

The global transition toward sustainable energy systems is central to achieving climate mitigation, energy security, and long-term socioeconomic development goals. While renewable energy technologies such as solar, wind, hydropower, and energy storage have experienced rapid deployment worldwide, their large-scale integration has revealed significant system-level challenges related to variability, storage limitations, infrastructure constraints, and sectoral decarbonization gaps. These challenges indicate that achieving net-zero energy systems requires more than the substitution of fossil fuels with renewable technologies. This review provides a comprehensive, system-level assessment of current and future sustainable energy systems, focusing on technologies, system integration, and transition pathways. Current sustainable energy technologies are reviewed and critically assessed based on their present applications and limitations. Emerging and future technologies—including advanced renewable systems, long-duration energy storage, hydrogen and Power-to-X pathways, firm low-carbon energy options, carbon removal technologies, and smart digital energy systems—are examined as complementary solutions designed to address the structural limitations of existing systems. The study synthesizes recent peer-reviewed literature and authoritative global energy reports to highlight the importance of system integration, including sector coupling, multi-timescale energy storage, digitalization, and coordinated infrastructure planning. The review further emphasizes that energy transition pathways are adaptive, portfolio-based, and region-specific, shaped by resource availability, infrastructure, policy frameworks, and socioeconomic conditions. Overall, the findings demonstrate that sustainable energy transitions are fundamentally system transformations rather than technology substitutions. The review contributes a structured framework for understanding how current and future technologies can be integrated into resilient, reliable, and equitable net-zero energy systems, while identifying key research gaps and priorities for future energy system development.

Keywords: Sustainable energy systems; Renewable energy technologies; Energy system integration; Energy transition pathways; Emerging energy technologies; Long-duration energy storage; Hydrogen energy systems; Power-to-X; Net-zero energy systems; Decarbonization

INTRODUCTION

The global energy system is undergoing a profound transformation driven by the urgent need to address climate change, improve energy security, and support sustainable socioeconomic development. The energy sector is responsible for nearly three-quarters of global greenhouse gas

emissions, placing it at the center of efforts to meet international climate goals, including limiting global warming to 1.5 °C above pre-industrial levels (IPCC, 2023). As a result, accelerating the transition from fossil fuel-based systems to sustainable energy systems has become



a key priority for governments, industries, and research communities worldwide.

In response to this challenge, renewable energy technologies such as solar, wind, hydropower, and bioenergy have expanded rapidly over the past decade, supported by declining costs and policy incentives (IEA, 2024). Energy storage technologies and hydrogen systems are also gaining attention as enablers of flexibility and sector coupling. Despite this progress, the large-scale deployment of these technologies has revealed significant system-level challenges, including variability of renewable generation, limited long-duration energy storage, grid and infrastructure constraints, and persistent difficulties in decarbonizing transport, industry, and heating sectors (IEA, 2023a; IRENA, 2023).

These challenges highlight a critical limitation of many existing studies, which tend to focus on individual technologies in isolation rather than on how technologies interact within complex energy systems. In practice, energy systems consist of interconnected components involving electricity generation, storage, transmission and distribution networks, end-use sectors, and governance frameworks. Without effective system integration, high shares of renewable energy can lead to inefficiencies, increased curtailment, and reliability concerns, even when sufficient generation capacity is available (Lund *et al.*, 2021).

At the same time, a new generation of emerging and future sustainable energy technologies is being developed to address the structural limitations of current systems. These include advanced renewable technologies, long-duration energy storage, hydrogen and Power-to-X pathways, firm low-carbon energy options, carbon removal technologies, and smart digital

energy systems (IEA, 2024; IPCC, 2023). While these technologies hold significant promise, they vary widely in maturity, cost, and system role, and their contributions are best understood as complementary rather than substitutive.

Against this background, there is a growing need for integrated, system-level reviews that connect current technologies, emerging solutions, and transition pathways within a unified analytical framework. Such an approach is essential for understanding how sustainable energy systems can evolve over time toward reliable, resilient, and net-zero configurations, while accounting for regional differences in resources, infrastructure, and socioeconomic conditions (IRENA, 2023).

This review addresses this need by providing a comprehensive assessment of current and future sustainable energy systems worldwide, with a focus on technologies, system integration, and transition pathways. The study reviews key current technologies and their limitations, examines emerging and future solutions designed to overcome these challenges, and synthesizes insights on system integration and adaptive transition pathways. By adopting a system-oriented perspective, this review aims to support researchers, policymakers, and practitioners in designing effective and equitable pathways toward long-term sustainable energy futures.

Review Methodology

This review adopts a structured semi-systematic literature review approach to investigate current knowledge on sustainable energy systems and emerging future energy technologies at the global scale. A semi-systematic methodology is particularly suitable for interdisciplinary research domains such as sustainable energy, where studies span engineering, energy systems analysis,



environmental science, and policy research, and where both conceptual integration and thematic synthesis are required (Snyder, 2019). This approach enables comprehensive coverage of recent developments while allowing flexibility to incorporate diverse perspectives on technology evolution, system integration, and transition pathways.

Literature Search Strategy

The literature search was conducted primarily using the Scopus database, which was selected due to its extensive coverage of peer-reviewed journals in energy, engineering, and sustainability research. To ensure completeness, Web of Science and IEEE Xplore were used as complementary databases, particularly for system-level studies and emerging technology research. Similar multi-database strategies are widely recommended for reviews addressing complex energy systems to reduce database bias and improve coverage (Tranfield *et al.*, 2003; Xiao & Watson, 2019).

Search queries were developed using combinations of keywords related to sustainable energy systems, emerging and future energy technologies, system integration, and energy transition pathways. Representative search terms included “*sustainable energy systems*,” “*future energy technologies*,” “*energy system integration*,” “*net-zero energy systems*,” and “*energy transition pathways*.” Boolean operators, truncation, and field restrictions (title, abstract, and keywords) were applied to refine search results and ensure relevance.

Inclusion and Exclusion Criteria

To ensure the timeliness and relevance of the reviewed literature, this study focused primarily

on peer-reviewed journal articles published between 2019 and 2024, reflecting the rapid evolution of sustainable energy technologies and transition research. Earlier publications were included selectively when they provided foundational system-level frameworks or highly cited conceptual models that remain relevant to current research. Studies were included if they addressed sustainable or low-carbon energy systems, emerging or future energy technologies, system integration, or transition pathways at regional or global scales.

Articles were excluded if they focused exclusively on single technologies without broader system implications, lacked relevance to sustainability or decarbonization objectives, or were non-peer-reviewed. In addition, conference papers and grey literature were generally excluded, except for selected reports from internationally recognized organizations where they provided authoritative insights into global energy trends and transition scenarios (IEA, 2023; IRENA, 2023).

Screening and Selection Process

The initial database search produced a large pool of publications, which were screened through a multi-stage process. First, titles and abstracts were reviewed to eliminate clearly irrelevant studies. Second, full-text screening was conducted to assess alignment with the objectives of this review, particularly the emphasis on current and future sustainable energy systems, system integration, and global transition pathways. The final set of selected articles was analyzed thematically, allowing the identification of dominant research trends, emerging technologies, and key system-level challenges, consistent with established practices for semi-systematic reviews (Snyder, 2019, Xiao & Watson, 2019).



Scope and Limitations

While this review aims to provide a comprehensive and forward-looking synthesis of sustainable energy systems, several limitations are acknowledged. The rapid pace of technological development means that cost estimates, performance metrics, and technology readiness levels for emerging energy technologies may evolve beyond the time frame considered. Furthermore, although the review adopts a global

perspective, it does not attempt to provide exhaustive country-specific policy or market analyses. Instead, the focus is placed on system-level trends, comparative insights, and widely recognized transition frameworks, which are more robust to regional and temporal variability.

Figure 1 provides a conceptual framework summarizing the evolution from current sustainable energy technologies to emerging and future solutions, and their integration into resilient, net-zero energy systems.

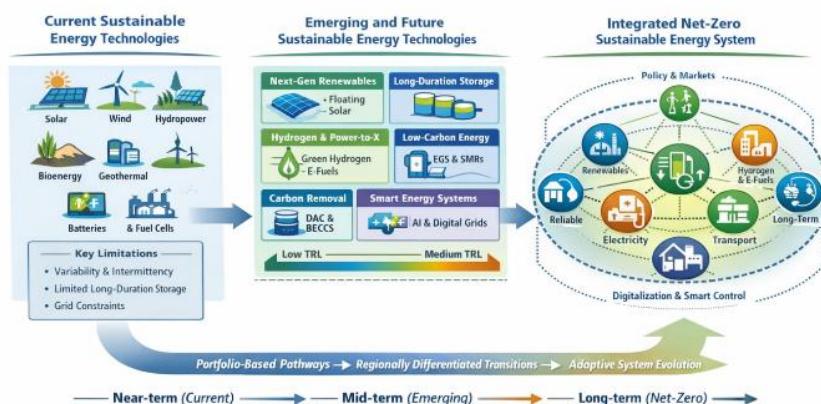


Figure 1: Conceptual framework of current and future sustainable energy systems and transition pathways

Current Sustainable Energy Technologies and Their System-Level Challenges

Current sustainable energy technologies constitute the foundation of ongoing global efforts to decarbonize energy systems. As summarized in Table 1, these technologies are already deployed at various scales and play important roles in electricity generation, energy storage, and sector coupling. However, their large-scale integration into energy systems reveals several technical, economic, and structural challenges that limit their ability to fully replace fossil fuel-based systems on their own.

Solar Energy Systems

Solar energy systems primarily include photovoltaic (PV) and solar thermal technologies that convert sunlight into electricity or heat. Solar PV is widely deployed at both distributed and utility scales due to its rapidly declining costs and modular nature (IEA, 2024). It currently plays a major role in electricity generation in many regions worldwide.

Despite its widespread adoption, solar energy is inherently variable and dependent on daylight and weather conditions. This intermittency creates challenges for grid stability and requires complementary storage or flexible demand solutions. In addition, large-scale deployment



raises concerns related to land use, material supply chains, and end-of-life recycling of PV modules (IRENA, 2023).

Wind Energy Systems

Wind energy systems, including onshore and offshore wind turbines, convert kinetic energy from wind into electricity. Wind power is one of the fastest-growing renewable energy sources and contributes significantly to low-carbon electricity generation in many countries (GWEC, 2023).

However, wind energy output varies with wind availability, leading to temporal and spatial mismatches between supply and demand. Offshore wind faces additional challenges related to high capital costs, complex installation, and grid connection requirements. Public acceptance and environmental concerns also influence deployment in certain regions (IEA, 2024).

Hydropower Systems

Hydropower systems generate electricity by harnessing the potential energy of flowing or stored water. Unlike solar and wind, hydropower can provide dispatchable and flexible power, making it a valuable balancing resource in renewable-dominated systems (IEA, 2023a).

Nevertheless, large hydropower projects can have significant ecological and social impacts, including habitat disruption and community displacement. Climate change-induced variability in water availability also affects hydropower reliability, while opportunities for new large-scale projects are increasingly limited in many regions (IRENA, 2023).

Bioenergy Systems

Bioenergy systems produce electricity, heat, or fuels from biomass resources such as agricultural

residues, forest products, and organic waste. Bioenergy offers dispatchable energy and can support energy storage in the form of biofuels (IPCC, 2023).

The sustainability of bioenergy remains a key concern. Competing land use, lifecycle greenhouse gas emissions, and impacts on food security limit the scale at which bioenergy can be responsibly deployed. As a result, bioenergy is increasingly viewed as a targeted solution rather than a universal replacement for fossil fuels (Searchinger *et al.*, 2020).

Geothermal Energy Systems

Geothermal energy systems utilize heat from the Earth's interior to produce electricity and provide direct heating. Where resources are available, geothermal energy offers reliable, low-carbon baseload power (IEA, 2024).

However, conventional geothermal deployment is geographically constrained and often associated with high upfront exploration costs and geological risks. These limitations restrict its contribution to global energy supply despite its technical advantages (IRENA, 2023).

Battery Energy Storage Systems

Battery energy storage systems, particularly lithium-ion batteries, are widely used for short-duration storage, grid balancing, and integration of variable renewable energy. Batteries play an essential role in stabilizing power systems with high shares of solar and wind generation (IEA, 2023a).

Key challenges include limited storage duration, material supply risks, degradation over time, and recycling constraints. These factors limit the ability of batteries to address long-term or



seasonal energy balancing on their own (IRENA, 2023).

Pumped Hydropower Storage

Pumped hydropower storage stores energy by pumping water to a higher elevation during periods of excess electricity and releasing it to generate power when needed. It currently represents the largest form of grid-scale energy storage globally (IEA, 2023a).

Despite its maturity and long lifetime, pumped hydropower is constrained by geographical suitability, environmental impacts, and lengthy permitting processes. These challenges restrict its expansion in many regions (IEA, 2024).

Hydrogen Energy Systems

Hydrogen energy systems use hydrogen as an energy carrier for storage, transport, and end-use applications. Currently, hydrogen is mainly produced from fossil fuels and used in industrial processes, with growing interest in low-carbon and renewable hydrogen pathways (IEA, 2023b). While hydrogen enables sector coupling and long-term energy storage, present systems face low overall efficiency, high costs, and limited infrastructure. These constraints limit hydrogen's near-term role in energy systems dominated by direct electrification (IRENA, 2023).

Fuel Cell Technologies

Fuel cell technologies convert hydrogen or other fuels directly into electricity and heat through electrochemical processes. Fuel cells are used in niche applications such as backup power, material handling, and demonstration-scale transport systems (IEA, 2023b).

Challenges include high capital costs, durability issues, and reliance on hydrogen supply infrastructure. As a result, fuel cells are currently complementary technologies rather than mainstream power generation solutions (IPCC, 2023).

Synthesis of Current Technology Limitations

In summary, the technologies presented in Table 1 play vital roles in current sustainable energy systems but exhibit structural limitations when deployed at scale. Variability, storage duration constraints, infrastructure requirements, and sustainability concerns collectively limit their ability to fully decarbonize energy systems. These challenges provide the motivation for the emerging and future technologies discussed in the next section.

Table 1: Comparison of Current Sustainable Energy Technologies: Limitations and Proposed Solutions

Technology	Primary Role in Current Energy Systems	Key Limitations	Proposed / Ongoing Solutions
Solar energy (PV & CSP)	Electricity generation	Intermittency and diurnal variability; land use for large-scale PV; reliance on critical materials; end-of-life recycling challenges	Grid-scale and distributed storage; demand-side management; advanced grid integration; material-efficient PV designs; recycling and circular-economy



Technology	Primary Role in Current Energy Systems	Key Limitations	Proposed / Ongoing Solutions
Wind energy (onshore & offshore)	Large-scale low-carbon electricity generation	Variability and site dependence; transmission bottlenecks; environmental and social acceptance issues	approaches (IEA, 2024; Fraunhofer ISE, 2024) Expanded transmission infrastructure; hybrid systems with storage; offshore wind deployment; improved siting and environmental mitigation strategies (GWEC, 2023; REN21, 2023)
Hydropower	Dispatchable renewable electricity and grid flexibility	Ecosystem disruption; social displacement; climate sensitivity to droughts; limited new sites	Modernization of existing plants; pumped hydropower optimization; improved environmental flow management; climate-resilient planning (IEA, 2023a; IPCC, 2023)
Bioenergy / biomass systems	Dispatchable electricity, heat, and transport fuels	Land-use competition; feedstock sustainability concerns; lifecycle emissions uncertainty	Use of residues and wastes; sustainability certification; integration with carbon capture (BECCS); limited, targeted deployment (Searchinger et al., 2020; IEA, 2023b)
Geothermal energy	Baseload electricity and direct heat	Geographical constraints; high upfront costs; exploration risks; induced seismicity	Enhanced geothermal systems (EGS); risk-sharing mechanisms; improved drilling technologies; hybrid geothermal systems (IRENA, 2023; IEA, 2024)
Battery energy storage (mainly Li-ion)	Short-duration storage and grid balancing	Limited duration; material supply risks; degradation and recycling challenges	Alternative chemistries; recycling infrastructure; integration with long-duration storage; improved system design (IEA, 2023a; Sepulveda et al., 2021)
Pumped hydropower storage	Large-scale, long-duration electricity storage	Geographic constraints; environmental impacts; long permitting timelines	Repurposing existing reservoirs; closed-loop systems; hybrid storage portfolios (IEA, 2023a; IRENA, 2023)



Technology	Primary Role in Current Energy Systems	Key Limitations	Proposed / Ongoing Solutions
Hydrogen energy systems	Energy carrier for storage, industry, transport, and power	Low system efficiency; high costs; limited infrastructure; reliance on renewable electricity availability	Cost reductions in electrolysis; hydrogen carriers (ammonia, LOHCs); infrastructure development; sector coupling (IEA, 2023c; IRENA, 2023)
Fuel-cell technologies	Conversion of hydrogen to electricity and heat	High capital costs; durability and material constraints; infrastructure dependence	Materials innovation; scale-up manufacturing; integration with green hydrogen systems (IEA, 2023c; IRENA, 2023)

Emerging and Future Sustainable Energy Technologies

Building on the limitations of current sustainable energy systems discussed in Section 3, this section examines emerging and future sustainable energy technologies that form the basis of Table 2. These technologies are designed to overcome structural challenges such as variability, insufficient long-duration storage, sectoral decarbonization gaps, and system inflexibility. Rather than replacing existing solutions, they are increasingly viewed as complementary components of integrated, low-carbon energy systems.

Perovskite Solar Cells

Perovskite solar cells are next-generation photovoltaic technologies characterized by high power conversion efficiencies and low material requirements. Compared with conventional silicon photovoltaics, perovskite cells offer the potential for higher efficiency and lower manufacturing costs, particularly when deployed in tandem configurations (Green *et al.*, 2023). Their key contribution lies in improving land-use

efficiency and energy yield. However, long-term stability, scalability, and concerns related to lead-based materials remain significant challenges (IEA, 2024).

Tandem Solar Cells

Tandem solar cells combine multiple absorber layers to capture a broader spectrum of sunlight, thereby exceeding the efficiency limits of single-junction solar cells. Silicon–perovskite tandems, in particular, can substantially increase electricity output without increasing land or infrastructure requirements (IRENA, 2023). Despite their promise, challenges persist in manufacturing complexity, durability, and cost-effective large-scale deployment.

Floating Solar Photovoltaics

Floating solar photovoltaic systems are installed on water bodies such as reservoirs and lakes, reducing competition for land and enabling synergies with existing hydropower infrastructure. These systems can improve overall system efficiency by reducing water evaporation and benefiting from natural cooling effects



(IRENA, 2023). However, higher installation costs, environmental impacts on aquatic ecosystems, and long-term structural reliability require further investigation.

Floating Offshore Wind

Floating offshore wind technology enables wind energy deployment in deep-water regions where fixed-bottom turbines are not feasible. This significantly expands the global wind resource base and offers access to stronger and more consistent wind speeds (GWEC, 2023; IEA, 2024). Key challenges include high capital costs, complex installation processes, and the need for dedicated offshore transmission infrastructure.

4.5 Airborne Wind Energy Systems

Airborne wind energy systems use tethered kites or drones to harvest high-altitude wind resources, which are typically stronger and more consistent than surface winds. These systems could reduce material use and expand deployment flexibility compared to conventional turbines (REN21, 2023). However, airborne wind remains at an early development stage, facing regulatory, safety, and reliability challenges.

Long-Duration Energy Storage Technologies

Long-duration energy storage (LDES) technologies enable energy storage over periods ranging from several hours to multiple days or seasons, addressing one of the most critical limitations of renewable-dominated systems. Technologies such as thermal, mechanical, and chemical storage can support system reliability during prolonged periods of low renewable output (Sepulveda et al., 2021; IEA, 2023a). Key barriers include high costs, efficiency losses, and limited large-scale operational experience.

Advanced Battery Technologies

Advanced battery chemistries, including sodium-ion and solid-state batteries, aim to overcome the material, safety, and cost limitations of lithium-ion batteries. These technologies could reduce dependence on critical minerals and improve safety while supporting grid-scale storage applications (IRENA, 2023). However, most remain at pilot or early commercial stages, with manufacturing scalability and long-term performance still uncertain.

Green Hydrogen via Advanced Electrolysis

Green hydrogen is produced through water electrolysis powered by renewable electricity and serves as a versatile energy carrier for storage and sector coupling. Compared with direct electrification, hydrogen enables decarbonization of hard-to-electrify sectors such as steelmaking, chemicals, and long-distance transport (IEA, 2023b). Major challenges include high production costs, efficiency losses, and limited infrastructure availability.

Power-to-X Fuels

Power-to-X technologies convert renewable electricity into synthetic fuels such as ammonia, methanol, and e-fuels. These fuels facilitate long-distance energy transport and seasonal storage, supporting decarbonization of aviation, shipping, and industry (IPCC, 2023). Their main limitations are low overall efficiency and high system costs, restricting their use to sectors with limited alternatives.

Enhanced Geothermal Systems

Enhanced geothermal systems (EGS) expand geothermal energy deployment beyond naturally occurring reservoirs by engineering subsurface



heat extraction. EGS can provide reliable, dispatchable, low-carbon electricity and heat, improving system stability (IEA, 2024). Challenges include high upfront investment costs, geological uncertainty, and risks related to induced seismicity.

Small Modular Nuclear Reactors

Small modular reactors (SMRs) are advanced nuclear technologies designed for flexible deployment and enhanced safety. They offer firm, low-carbon power that can complement variable renewable generation (Sepulveda et al., 2021). However, high capital costs, regulatory complexity, and public acceptance concerns limit their near-term impact.

Bioenergy with Carbon Capture and Storage

Bioenergy with carbon capture and storage (BECCS) combines biomass energy generation with CO₂ capture, enabling net-negative emissions. BECCS can offset residual emissions from other sectors and support climate targets (IPCC, 2023). Sustainability concerns related to biomass sourcing, land use, and lifecycle emissions remain significant challenges.

Direct Air Capture

Direct air capture (DAC) technologies remove carbon dioxide directly from the atmosphere, offering flexibility in location and deployment. DAC plays a potential role in balancing unavoidable emissions and achieving net-negative emissions in the long term (IEA, 2024). However,

current systems are energy-intensive and costly, limiting scalability.

Smart Grids and Digital Energy Systems

Smart grids integrate digital technologies, advanced sensors, and artificial intelligence to optimize energy system operation. These systems improve flexibility, reliability, and efficiency in renewable-dominated energy systems (Lund et al., 2021). Challenges include cybersecurity risks, data governance, and regulatory adaptation.

Fusion Energy

Fusion energy aims to replicate the processes powering the sun to generate large amounts of low-carbon electricity. If successfully commercialized, fusion could provide abundant, reliable energy with minimal fuel constraints (IEA, 2024). Despite recent progress, fusion remains a long-term option facing substantial technical, economic, and temporal challenges.

Synthesis and System-Level Implications

Collectively, the technologies summarized in Table Y demonstrate that future sustainable energy systems will depend on diverse, complementary solutions rather than singular technological breakthroughs. Their successful deployment requires coordinated system integration, infrastructure development, and supportive policy frameworks. These insights form the foundation for the system integration and transition pathways discussed in the next section.

**Table 2: Emerging and Future Sustainable Energy Technologies: Readiness, Challenges, and System Roles**

Technology	Primary Purpose in Future Energy Systems	Indicative TRL	Key Challenges	Expected System-Level Role
Perovskite & tandem solar cells	High-efficiency, low-material solar electricity generation	5–7	Long-term operational stability, large-scale manufacturing, and environmental concerns related to lead-based materials (Green et al., 2023; IEA, 2024)	Increased solar efficiency and reduced land and material intensity in future solar deployment (IEA, 2024)
Floating solar PV	Expansion of solar deployment without land competition	6–8	Structural durability, impacts on aquatic ecosystems, and higher capital costs compared with ground-mounted PV (IRENA, 2023)	Complementary solar capacity near demand centers and hybridization with hydropower systems (IRENA, 2023; IEA, 2024)
Floating offshore wind	Access to deep-water, high-quality wind resources	6–7	High investment costs, complex marine engineering, and grid connection challenges (GWEC, 2023; IEA, 2024)	Large-scale renewable electricity generation in coastal and deep-water regions (IEA, 2024)
Terrestrial wind energy	High-altitude wind energy harvesting	3–5	System reliability, control complexity, safety, and regulatory uncertainty (IRENA, 2023; REN21, 2023)	Long-term niche generation with potentially high capacity factors (IRENA, 2023)
Long-duration energy storage (LDES)	Multi-day to seasonal energy balancing	4–7	High costs, efficiency losses, and lack of market mechanisms supporting long-duration storage (Sepulveda et al., 2021; IEA, 2023a)	Enabling high shares of variable renewables and reducing curtailment in deeply decarbonized systems (IEA, 2023a)
Advanced battery chemistries (e.g., sodium-ion, solid-state)	Safer and lower-cost electrical energy storage	5–7	Manufacturing scale-up, performance validation, and lifecycle sustainability (IEA, 2023a; IRENA, 2023)	Diversification of storage options and reduced dependence on critical minerals (IRENA, 2023)
Green hydrogen (advanced electrolysis)	Energy carrier for long-term storage and sector coupling	6–7	High electrolysis costs, large renewable electricity demand, and efficiency losses across the value chain (IEA, 2023b; IRENA, 2023)	Long-duration storage and decarbonization of industry, transport, and power systems (IEA, 2023b)



Technology	Primary Purpose in Future Energy Systems	Indicative TRL	Key Challenges	Expected System-Level Role
Power-to-X (ammonia, synthetic fuels)	Conversion of renewable electricity into transportable fuels	4–6	Low overall efficiency, infrastructure requirements, and high production costs (IEA, 2023b; IPCC, 2023)	Decarbonization of aviation, shipping, and chemical sectors where electrification is limited (IPCC, 2023)
Enhanced geothermal systems (EGS)	Firm, low-carbon baseload electricity	4–6	Geological uncertainty, induced seismicity risks, and high upfront investment costs (IEA, 2024; IRENA, 2023)	Dispatchable renewable baseload power supporting grid stability (IEA, 2024)
Bioenergy with carbon capture and storage (BECCS)	Net-negative emissions with energy generation	6–7	Biomass sustainability, land-use impacts, and CCS infrastructure availability (IPCC, 2023; IEA, 2023a)	Carbon dioxide removal to offset residual emissions in net-zero pathways (IPCC, 2023)
Direct air capture (DAC)	Removal of CO ₂ directly from the atmosphere	4–6	Very high energy demand, high costs, and limited storage deployment (IPCC, 2023; IEA, 2024)	Long-term climate stabilization and carbon management (IPCC, 2023)
Advanced nuclear (SMRs)	Firm, low-carbon electricity generation	5–7	Capital costs, licensing complexity, and public acceptance (Sepulveda et al., 2021; IEA, 2024)	Firm low-carbon power supporting deep decarbonization scenarios (IEA, 2024)
Fusion energy	Long-term, high-density clean energy	2–4	Technical feasibility, cost, and long development timelines (IEA, 2024; IPCC, 2023)	Potential post-2050 baseload energy source (IEA, 2024)
AI-enabled smart energy systems	Optimization of complex, multi-vector energy systems	6–8	Cybersecurity, data governance, and regulatory adaptation challenges (Lund et al., 2021; IEA, 2023a)	System integration, flexibility, and reliability at high renewable penetration (Lund et al., 2021)

TRL = Technology Readiness Level

System Integration, Transition Pathways, and Key Research Priorities

The transition to sustainable energy systems extends beyond the deployment of individual low-carbon technologies and requires effective system integration and coordinated transition pathways. As highlighted in earlier sections, current and

emerging sustainable energy technologies can only achieve their full potential when they are integrated across energy sectors, infrastructures, and governance frameworks.

System Integration in Sustainable Energy Systems

System integration refers to the coordinated operation of electricity generation, energy



storage, transmission networks, and end-use sectors such as heating, transport, and industry. High shares of variable renewable energy increase the need for flexibility through a combination of energy storage, demand-side management, sector coupling, and grid expansion (Lund *et al.*, 2021; IEA, 2023a). Without such integration, renewable-dominated systems risk increased curtailment, reliability challenges, and higher system costs.

Sector coupling plays a particularly important role by linking electricity with other energy uses through electrification and energy carriers such as hydrogen and synthetic fuels. This approach enables surplus renewable electricity to be utilized more effectively and supports decarbonization of hard-to-electrify sectors (IRENA, 2023). Digitalization, including smart grids and data-driven control systems, further enhances system flexibility and operational efficiency by enabling real-time coordination of increasingly complex energy systems (IEA, 2023a).

Transition Pathways toward Sustainable Energy Futures

Energy transition pathways describe how energy systems evolve over time from fossil-fuel dependence toward low-carbon and net-zero configurations. Most recent global assessments emphasize a phased transition, with rapid deployment of mature renewable technologies and energy efficiency measures in the near term, followed by increasing roles for emerging technologies such as long-duration energy storage, hydrogen systems, firm low-carbon energy sources, and carbon removal technologies in the medium to long term (IPCC, 2023; IEA, 2024).

Importantly, transition pathways are region-specific and influenced by resource availability, infrastructure, economic conditions, and policy priorities. While developed regions often focus on retrofitting existing systems, developing regions face the dual challenge of expanding energy access while avoiding carbon-intensive development pathways (UN DESA, 2023). Consequently, no single transition pathway is universally applicable, reinforcing the need for adaptive and context-dependent strategies.

Key Research and Implementation Priorities

Despite substantial progress, several critical challenges remain that require continued research and policy attention. A major priority is improving understanding of system-level interactions among renewable generation, storage, grids, and sector coupling, particularly at high renewable penetration levels (Lund *et al.*, 2021). In addition, long-duration and seasonal energy storage remains underdeveloped, yet essential for ensuring reliability in renewable-dominated systems (IEA, 2023a).

Other key priorities include addressing the sustainability of materials and supply chains, improving the scalability and cost competitiveness of emerging technologies, and ensuring that energy transitions are socially inclusive and equitable. Policy frameworks and market designs must also evolve to value flexibility, long-term system benefits, and negative-emission technologies, rather than focusing solely on short-term costs (IEA, 2024; IPCC, 2023).

Research Gaps and Future Directions

Despite significant progress in sustainable energy technologies and deployment, several critical



research gaps remain that limit the effectiveness, scalability, and equity of global energy transitions. Addressing these gaps is essential for moving from incremental decarbonization toward fully integrated, resilient, and net-zero energy systems.

A major gap lies in system-level integration research. Much of the existing literature continues to evaluate individual technologies in isolation, while real-world energy systems involve complex interactions among generation, storage, networks, and end-use sectors. Limited attention has been given to how high shares of variable renewable energy affect system stability, flexibility requirements, and infrastructure planning when multiple technologies operate simultaneously (Lund *et al.*, 2021; IEA, 2023a).

Another key gap concerns long-duration and seasonal energy storage. While short-duration battery storage is increasingly deployed, technologies capable of balancing energy supply and demand over days, weeks, or seasons remain underdeveloped. Comparative assessments of long-duration storage options—such as hydrogen, thermal storage, and mechanical systems—are still limited, particularly in terms of system-level cost, efficiency, and environmental impacts (IEA, 2023a; IRENA, 2023).

There is also considerable uncertainty surrounding the scalability and maturity of emerging energy technologies. Many future solutions, including advanced storage systems, green hydrogen pathways, and carbon removal technologies, face challenges related to cost reduction, infrastructure requirements, and real-world performance. More large-scale demonstration projects and harmonized assessments of technology readiness are needed to reduce investment and planning risks (IEA, 2024).

Material supply chains and sustainability represent another important research gap. The rapid expansion of renewable energy and storage technologies increases demand for critical minerals and materials, raising concerns about supply security, environmental impacts, and geopolitical dependence. Greater emphasis is needed on lifecycle assessment, recycling technologies, and circular-economy approaches to ensure long-term sustainability of energy transitions (IEA, 2023b).

In addition, regional and social dimensions of energy transitions remain underexplored. Many global transition scenarios are based on assumptions derived from industrialized regions and may not adequately reflect the realities of developing economies. Research should place greater emphasis on region-specific pathways, decentralized systems, energy access, affordability, and social acceptance to support equitable transitions (UN DESA, 2023; IPCC, 2023).

Finally, gaps persist in policy, market design, and governance research. Existing market structures often undervalue flexibility, long-term system benefits, and negative-emission technologies. Future studies should explore adaptive policy frameworks and market mechanisms that better align technological innovation with system needs and societal goals (IEA, 2024).

In summary, future research on sustainable energy systems should prioritize system-level integration, long-duration storage, technology scalability, sustainable supply chains, regionally differentiated pathways, and adaptive governance. Addressing these interconnected challenges will be critical for translating technological potential into effective, inclusive, and durable global energy transitions.



Summary of Key Research Priorities

In summary, future research on sustainable energy systems should prioritize:

- System-level and cross-sector integration
- Long-duration and seasonal energy balancing
- Scalable deployment of emerging technologies
- Sustainable materials and circular supply chains
- Regionally differentiated and socially inclusive pathways
- Adaptive policy and governance frameworks

Addressing these research gaps will be essential for translating technological potential into effective, equitable, and enduring global energy transitions.

7. Conclusions and Recommendations

7.1 Conclusions

This review has examined current and future sustainable energy systems with a focus on technologies, system integration, and transition pathways. The analysis shows that while significant progress has been made in deploying renewable energy technologies such as solar, wind, hydropower, and energy storage, these technologies alone are insufficient to achieve reliable, resilient, and fully decarbonized energy systems.

A key conclusion is that sustainable energy transitions are fundamentally system-level transformations, rather than simple substitutions of fossil fuels with renewable technologies. The limitations of current systems—such as variability, insufficient long-duration storage, infrastructure constraints, and sectoral

decarbonization gaps—highlight the need for complementary emerging and future technologies. These include advanced renewable technologies, long-duration energy storage, hydrogen and Power-to-X systems, firm low-carbon energy sources, carbon removal technologies, and smart digital energy systems.

The review further demonstrates that system integration—through sector coupling, multi-timescale energy storage, digitalization, and coordinated infrastructure planning—is central to the effective functioning of high-renewable energy systems. Transition pathways toward net-zero energy systems are therefore best understood as adaptive, portfolio-based processes that evolve over time and vary across regions depending on resources, infrastructure, and socioeconomic conditions.

Overall, achieving sustainable and net-zero energy systems requires not only technological innovation but also coordinated system design, supportive policy frameworks, and long-term planning that aligns energy, climate, and development objectives.

Recommendations

Based on the findings of this review, several key recommendations are proposed:

1. Adopt a system-oriented approach in energy planning and research, with greater emphasis on integration across electricity, heat, transport, and industrial sectors rather than isolated technology deployment.
2. Prioritize the development of long-duration and seasonal energy storage, which is essential for balancing high shares of variable renewable energy and ensuring system reliability.



3. Support the responsible scaling of emerging technologies, including hydrogen systems, Power-to-X fuels, firm low-carbon energy, and carbon removal technologies, through targeted research, demonstration projects, and infrastructure investment.
4. Strengthen energy infrastructure and digital systems, including smart grids and advanced control platforms, to enhance flexibility, efficiency, and resilience in future energy systems.
5. Design adaptive policy and market frameworks that value flexibility, long-term system benefits, sustainability, and equity, while accounting for regional differences and development needs.

By implementing these recommendations, policymakers, researchers, and industry stakeholders can better support the transition toward integrated, resilient, and sustainable energy systems capable of meeting global climate and development goals.

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Prevalence of Adenovirus Gastroenteritis Among Children Under Five Attending Faith Mediplex Hospital, Benin City, Edo State, Nigeria.

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ABSTRACT

Adenoviruses are non-enveloped deoxyribonucleic acid (DNA) viruses that cause various illnesses, including gastroenteritis in children. Enteric adenovirus types 40 and 41 are notable causes of acute diarrhoea in children under five years, often leading to dehydration and increased morbidity. This study examined the prevalence, associated risk factors, and clinical characteristics of adenovirus infection among diarrhoeic under-five children at Faith Mediplex Hospital, Benin City. 150 stool samples were tested using immunochromatographic assays (Aria, USA), with supporting demographic and clinical data collected via piloted structured questionnaires. The overall prevalence was 5.3%, highest among infants under a year of age (20.0%), and absent in the 3–4-year age group. There was no statistically significant association between the prevalence of adenovirus infection ($p = 0.863$). While not statistically significant, infection was more frequently observed among children whose fathers had no formal education (22.2%) and mothers with tertiary education (10.7%), suggesting that education alone may not influence infection risk but some other factors. Clinically, blood in stool was the only symptom significantly associated with infection (22.6%, $p = 0.001$), pointing to potential mucosal damage. Other symptoms, such as fever and vomiting, showed no statistically significant link ($p = 0.863$). Moreover, overcrowding was identified as a critical environmental risk factor. Children living in overcrowded households showed a notably higher prevalence of adenovirus infection (13.0%) compared to those from less crowded homes (1.0%), with the difference being statistically significant ($p = 0.017$). This highlights the role of environmental hygiene and close person-to-person contact in facilitating viral transmission and possible infection. Adenovirus is a relevant cause of diarrhoea in young children, particularly in Edo State. Age, overcrowding, and blood in stool were found to be key indicators of infection. The findings from this study highlight the importance of promoting better personal hygiene, increased public awareness, and implementing sensitization programs to support timely diagnosis to help mitigate the impact of adenovirus gastroenteritis among children in Edo State and by implication Nigeria at large.

INTRODUCTION

Adenoviruses, first isolated in 1953, are non-enveloped DNA viruses that can cause various human infections (Bastug *et al.*, 2021). Adenoviruses belonging to the Mastadenovirus

genus in the family Adenoviridae cause various diseases and are prevalent worldwide (De Francesco *et al.*, 2022; Amberg *et al.*, 2020). Other members of Adenoviruses include: Aviadenovirus (Infects Birds), Atadenovirus



(Infects Reptiles, Birds, and Some Mammals), Siadenovirus (Infects Birds and Amphibians) (Kosoltanapiwat *et al.*, 2022). Adenovirus has become a valuable tool in medical research and therapeutic applications because Adenovirus vectors, created by modifying wild-type viruses, are used in gene therapy, cancer treatment, and vaccine development (Davison *et al.*, 2024). These vectors can be engineered to be replication-defective and carry therapeutic genes to specific tissues (Trivedi *et al.*, 2023). Researchers have introduced several modifications to improve vector efficiency, including fiber switching and capsid alterations (Benko *et al.*, 2022). Adenovirus vectors have played crucial roles in advancing cancer therapy, gene therapy, and vaccine development, particularly evident in recent COVID-19 vaccine successes (Trivedi *et al.*, 2023). Despite challenges faced when collecting those samples from infected individuals, ongoing research continues to optimize adenovirus vectors, promising new frontiers in cell and gene therapies (Trivedi *et al.*, 2023).

Gastroenteritis is the inflammation of the stomach and intestines, typically caused by viral, bacterial, or parasitic infections (De Francesco *et al.*, 2021). Gastroenteritis associated with adenovirus is a significant health concern, particularly in children under 5 years old (De Francesco *et al.*, 2021). While adenovirus types 40 and 41 are commonly linked to acute gastroenteritis, other non-enteric types may also play a role (De Francesco *et al.*, 2021). Adenovirus infections can be especially severe in immunocompromised patients, such as those undergoing hematopoietic stem cell transplantation and those with Human Immunovirus (HIV) (Davison *et al.*, 2022). Symptoms typically include vomiting and

abdominal pain. (De Francesco *et al.*, 2022). Accurate diagnosis of adenovirus gastroenteritis may require a broad-range PCR methods to detect all adenovirus types as current techniques may have limitations because of co-infections with other enteric viruses such as rotavirus, norovirus, or astrovirus. (De Francesco *et al.*, 2021).

Adenoviruses (AdVs) are globally distributed DNA viruses that can cause mild to severe infections in humans and animals (Kosoltanapiwat *et al.*, 2022). They can be fatal in immunocompromised individuals with untreated severe cases having mortality rates exceeding 50% (Hasan *et al.*, 2021). Economically, Adenoviruses impact both human health and agriculture, causing significant outbreaks in commercial poultry farming (Ruzlan *et al.*, 2021). Along with other respiratory viruses, Adenoviruses contribute to substantial morbidity, mortality, and economic losses worldwide (Ruzlan *et al.*, 2021)

Adenovirus is a significant cause of diarrhoea among children under five in Nigeria, with prevalence rates ranging from 8.7% to 29% (Nantegae *et al.*, 2022). The incidence of adenovirus 40/41 diarrhoea peaks in children aged 7-15 months, with a substantial burden also observed in infants 0-6 months old (Guga *et al.*, 2022). Risk factors associated with diarrhoeal diseases include age, nutritional status, and water sources (Guga *et al.*, 2022). Exclusive breastfeeding strongly protects against adenovirus 40/41 diarrhoea because breast milk, especially colostrum, is rich in secretory immunoglobulin A (IgA), providing mucosal immunity (Guga *et al.*, 2022). The seasonality of adenovirus infections varies between locations, with some studies reporting higher incidence during the dry season (Benko *et al.*, 2023).



METHODOLOGY

A total of 150 stool samples from Faith Mediplex Hospital, Benin City, (selected for this study), were collected from October 2024 to December 2024. Stool samples were carefully collected in clean, sterile containers that were clearly labeled with each child's Mediplex identification number (ID) and the date inscribed. The collection was done by caregivers or hospital staff, who were given proper direction/instructions on how to collect the samples correctly. All the stool samples were then transported under a cold chain to the Medical Microbiology Laboratory at Igbinedion University Teaching Hospital, Okada, Edo State. Upon receipt of the stool samples at the Microbiology Laboratory, they were allowed to thaw at room temperature before serological screening using immunochromatographic techniques.

RESULTS

Table 1 shows the sociodemographic characteristics of the infant's participants in the study. The majority of participants (55.3%) were aged 1-2 years, followed by 28.0% aged 3-4 years, and 16.7% aged less than a year of age. The mean

age of infant's participants was 2.11 ± 1.12 years. The sample was predominantly female (65.3%), and males 34.7%. Regarding the mothers' educational status, 6.0% had no formal education (the least group), this was closely followed by those with primary education (7.3%), the largest group had secondary education (68.0%) and others with tertiary education (18.7%). Similarly, for fathers' educational status included those with no formal education (6.0%) (least group), primary education (10.7%), most had secondary education (54.7%) (highest group), followed by tertiary education (28.7%). The least category of family with low income was 9.3% followed by those with high income 14.0% and majority of families were in the middle-income category (76.7%). In terms of the number of siblings, 50.7% of participants had 1-2 siblings, 26.0% had 3-4 siblings, and 23.3% had more than 4 siblings. Regarding residence, 56.7% of participants lived in rented accommodations, 27.3% in owned houses, and 16.0% in ancestral houses. The prevalence of adenovirus infection was found to be 5.3% (8/150) as shown in figure 1.

Table 1 Sociodemographic Characteristics of Participants

Variable	Number	Percentage (%) (n=150)
Age		
<1 year	25	16.7
1-2 years	83	55.3
3-4 years	42	28.0
Sex		
Male	52	34.7
Female	98	65.3
Mothers' educational status		
No formal Education	9	6.0
Primary	11	7.3
Secondary	102	68.0
Tertiary	28	18.7



Fathers' educational status

No formal Education	9	6.0
Primary	16	10.7
Secondary	82	54.7
Tertiary	43	28.7

Family's Income

Low	14	9.3
Middle	115	76.7
High	21	14.0

Number of Siblings

1-2	76	50.7
3-4	39	26.0
>4	35	23.3

Mode of Residence

Rented	85	56.7
Owned	41	27.3
Ancestral House	24	16.0

Mean age=2.11±1.12 years.

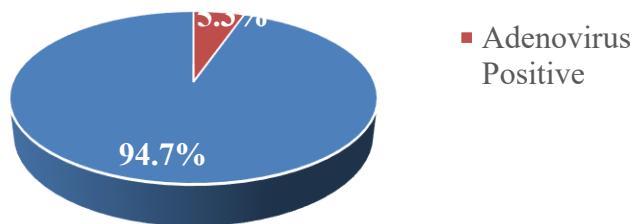


Figure 1 Prevalence of Adenovirus infection among diarrheic under five children attending Faith Mediplex Hospital.

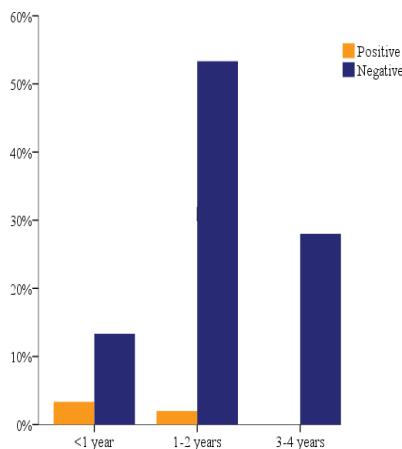


Figure 2 Prevalence of adenovirus infection among different age groups.

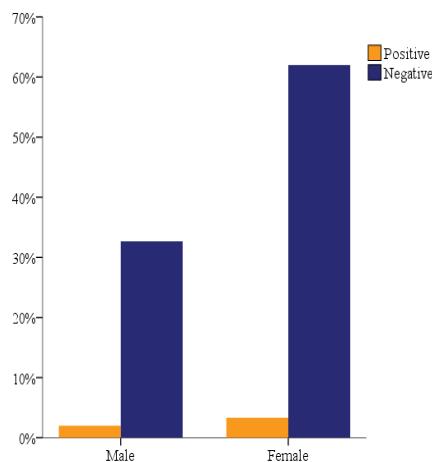


Figure 3 Prevalence of adenovirus infection among diarrheic under five male and female children.

Sociodemographic Parameters and Prevalence of Adenovirus Infection

The relationship between sociodemographic parameters and the prevalence of adenovirus infection was examined among 150 participants. Age was significantly associated with adenovirus infection ($p = 0.001$). Participants under a year of age had the highest prevalence of infection

(20.0%), while those aged 1-2 years had a lower prevalence (3.6%), and no infections were observed in participants aged 3-4 years. Sex did not show a significant relationship with adenovirus infection ($p = 0.863$). Males had a slightly higher infection rate (5.8%) compared to females (5.1%), but this difference was not statistically significant ($OR = 1.139$, 95% CI: 0.261-4.966). Mothers' educational status was not significantly associated with adenovirus infection ($p = 0.420$). However, infection rates varied across educational levels, with those having tertiary education showing a higher infection rate (10.7%) compared to secondary (3.9%), primary (9.1%), and no formal education (0%). Fathers' educational status also did not show a significant relationship with adenovirus infection ($p = 0.132$). Participants whose fathers had no formal education had the highest infection rate (22.2%), while those with secondary education had the lowest rate (3.7%).

Family income was not significantly associated with adenovirus infection ($p = 0.293$). Participants from low-income families had a higher infection rate (14.3%) compared to those from middle (4.3%) and high-income families (4.8%). The number of siblings did not significantly impact the prevalence of adenovirus infection ($p = 0.718$). Participants with 1-2 siblings had an infection rate of 6.6%, compared to 5.1% for those with 3-4 siblings and 2.9% for those with more than 4 siblings. Lastly, the mode of residence was not significantly associated with adenovirus infection ($p = 0.775$). Infection rates were similar across different living situations, with participants living in rented accommodations showing an infection rate of 4.7%, those in owned houses 4.9%, and those in family houses 8.3% (Table 2).

**Table 2: Relationship Between Sociodemographic Parameters and Prevalence of Adenovirus Infection**

Variable	No. Examined (%)	No. Infected (%)	OR	95%CI	p value
Age					
<1 year	25 (16.7)	5 (20.0)			0.001
1-2 years	83 (55.3)	3 (3.6)			
3-4 years	42 (28.0)	0 (0)			
Sex					
Male	52 (34.7)	3 (5.8)	1.139	0.261-4.966	0.863
Female	98 (65.3)	5 (5.1)			
Mothers' educational status					
None	9 (6.0)	0 (0)			0.420
Primary	11 (7.3)	1 (9.1)			
Secondary	102 (68.0)	4 (3.9)			
Tertiary	28 (18.7)	3 (10.7)			
Fathers' educational status					
None	9 (6.0)	2 (22.2)			0.132
Primary	16 (10.7)	1 (6.3)			
Secondary	82 (54.7)	3 (3.7)			
Tertiary	43 (28.7)	2 (4.7)			
Family's Income					
Low	14 (9.3)	2 (14.3)			0.293
Middle	115 (76.7)	5 (4.3)			
High	21 (14.0)	1 (4.8)			
Number of Siblings					
1-2	76 (50.7)	5 (6.6)			0.718
3-4	39 (26.0)	2 (5.1)			
>4	35 (23.3)	1 (2.9)			
Mode of Residence					
Rented	85 (56.7)	4 (4.7)			0.775
Owned	41 (27.3)	2 (4.9)			
Ancestral House	24 (16.0)	2 (8.3)			

P<0.05 represents significance

Prevalence of Adenovirus in Relation to Risk Associated Factors

The analysis of risk factors associated with adenovirus infection revealed significant associations with living conditions and toy-sharing habits. Children living in overcrowded conditions had a significantly higher risk of adenovirus infection (20.7%) compared to those in non-overcrowded conditions (1.7%), with an

odds ratio (OR) of 15.52 (95% CI: 2.947-81.449, p = 0.001). Similarly, toy-sharing was significantly associated with an increased risk of infection, with children who shared toys showing a higher infection rate (16.7%) compared to those who did not (1.8%), with an OR of 11.20 (95% CI: 2.150-58.33, p = 0.001). Access to clean drinking water did not show a significant association with adenovirus infection (p = 0.160). However, children without access to clean drinking water had a higher infection rate (10.7%)



compared to those with access (4.1%), with an OR of 0.356 (95% CI: 0.080-1.588). Proper sanitation practices were also not significantly associated with adenovirus infection ($p = 0.630$), with similar infection rates observed between children in environments with proper sanitation (5.1%) and those without (8.3%), with an OR of 0.588 (95%

CI: 0.066-5.219). Regular handwashing showed no significant association with adenovirus infection, with similar infection rates between those who regularly washed their hands (5.4%) and those who did not (5.3%), and an OR of 1.023 (95% CI: 0.235-4.452, $p = 0.976$) (Table 3).

Table 3: Prevalence of Adenovirus in Relation to Risk Factors.

Variable	No. Examined (%)	No. Infected (%)	OR	95%CI	p value
Living Conditions					
Overcrowded	29 (19.3)	6 (20.7)	15.52	2.947-81.449	0.001
Not Overcrowded	121 (80.7)	2 (1.7)			
Access to Clean Drinking Water					
Yes	122 (81.3)	5 (4.1)	0.356	0.080-1.588	0.160
No	28 (18.7)	3 (10.7)			
Proper Sanitation					
Yes	138 (92.0)	7 (5.1)	0.588	0.066-5.219	0.630
No	12 (8.0)	1 (8.3)			
Regular Handwashing					
Yes	93 (62.0)	5 (5.4)	1.023	0.235-4.452	0.976
No	57 (38.0)	3 (5.3)			
Sharing Toys					
Yes	36 (24.0)	6 (16.7)	11.20	2.150-58.33	0.001
No	114 (76.0)	2 (1.8)			

P<0.05 represents significance

Prevalence of Adenovirus Infection in Relation to Symptoms of Diarrhoea

The analysis of some symptoms associated with diarrhoea revealed significant associations with the prevalence of adenovirus infection. Children with blood in their stool had a significantly higher prevalence of adenovirus infection (22.6%) compared to those without blood in stool (0.8%), with an odds ratio (OR) of 34.417 (95% CI: 4.046-292.74, $p = 0.001$). Fever, vomiting, and

abdominal pain did not show significant associations with adenovirus infection. Specifically, children with fever had a slightly higher infection rate (6.5%) compared to those without fever (4.8%) with an OR of 1.381 (95% CI: 0.316-6.041, $p = 0.667$). Similarly, vomiting (6.7% vs. 5.0%, OR = 1.357, 95% CI: 0.260-7.087, $p = 0.716$) and abdominal pain (6.3% vs. 4.7%, OR = 1.367, 95% CI: 0.329-5.684, $p = 0.666$) were not significantly associated with adenovirus infection (Table 4).

Table 4: Prevalence of Adenovirus in Relation to Symptoms of Diarrhoea.

Variable	No. Examined (%)	No. Infected (%)	OR	95%CI	p value



Fever					
Yes	46 (30.7)	3 (6.5)	1.381	0.316-6.041	0.667
No	104 (69.3)	5 (4.8)			
Vomiting					
Yes	30 (20.0)	2 (6.7)	1.357	0.260-7.087	0.716
No	120 (80.0)	6 (5.0)			
Abdominal Pain					
Yes	64 (42.7)	4 (6.3)	1.367	0.329-5.684	0.666
No	86 (57.3)	4 (4.7)			
Blood in Stool					
Yes	31 (20.7)	7 (22.6)	34.417	4.046-292.74	0.001
No	119 (79.3)	1 (0.8)			

P<0.05 represents significance

DISCUSSION

This study examined the prevalence and risk factors for adenovirus infection in children under 5 years of age who had diarrhea and were receiving treatment at Faith Mediplex Hospital in Benin City, Edo State. In this study, it was observed that Children aged 1–2 years contributed the highest prevalence, accounting for 3.6%, while the lowest positive cases (0.0%) were seen in children between 3- 4 years of age. These findings align closely with existing research by Guga *et al.* (2022) who conducted a cross-sectional study in Northern Nigeria using polymerase chain reaction (PCR)- based diagnostics on 204 stool samples from diarrheic under-five years of age children and found that over 60% of adenovirus-positive cases occurred in children under 2 years, attributing this to immature immune systems development and early exposure to adenovirus contaminated objects. Similarly, Guga *et al.* (2022) examined a total of 150 children under five years of age with diarrhoea in Southwest Nigeria and reported that the majority of adenovirus infections occurred between 6 and 24 months of age, reinforcing that early infancy and toddlerhood are critical risk windows. The findings are further supported by

De Francesco *et al.* (2021) and Trivedi *et al.* (2023), who reported that enteric adenoviruses, particularly types 40 and 41, affect children below the age of two, due to immature immune defenses, lack of prior exposure, and greater environmental contact through hand-to-mouth behaviors which is often displayed by infants.

Considering the prevalence of adenovirus gastroenteritis among genders in this study, 65.3% of children were females and 34.7% were males, showing a female-dominant participant pool. However, adenovirus infection rates were almost evenly distributed between sexes (males 5.8%, females 5.1%), and this difference was not statistically significant ($p = 0.863$). This observation aligns with findings from Nantegie *et al.* (2022) and Ndoboli *et al.* (2023), who found no significant association between gender and adenovirus infection. Globally, (Ndungutse *et al.*, 2022) support the idea that adenovirus infections are not gender-specific, with both male and female children equally susceptible under similar exposure conditions. Random sample variability or behavioral characteristics like boys' propensity for exploratory or high-contact play behaviors, which increases their exposure to contaminated surfaces and objects, could be responsible for the small variation observed in this study. However,



both genders were similarly exposed to transmission vectors, including sharing toys, food, and water in structured situations like homes and clinics where hygiene conditions are often uniform, which could explain the slight differences seen.

This study also assessed parental educational background. To determine the impact on the likelihood of adenovirus infection among diarrheic children under 5 years of age. The analysis revealed no statistically significant correlation between adenovirus infection and either parent's level of education ($p=0.863$). However, the highest infection rate (22.2%) was observed in children whose fathers had no formal education, while among mothers, the highest infection rate (10.7%) occurred in those with tertiary education. These findings, though not statistically significant ($p=0.863$), present a paradoxical trend, suggesting that educational attainment alone may not reliably predict infection risk, especially within the complex socioeconomic dynamics of urban Nigerian settings. Several other studies across Nigeria support this outcome. Offor *et al.* (2022), in a study on diarrhoeal diseases in Southern Nigeria, reported that maternal education level did not significantly reduce infection rates among children under five years of age. The researchers emphasized that although education improves awareness, it does not always lead to improved health outcomes unless accompanied by appropriate behavioral practices, adequate infrastructure, and access to basic services like clean water and sanitation. Similarly, (Shieh *et al.*, 2022) found that socioeconomic conditions often overrode the benefits of education, especially when households face constraints such as overcrowding and inconsistent water supply.

Additionally, this study examined the clinical symptoms of adenovirus gastroenteritis, with a particular focus on markers that could assist in early clinical detection among children under five years of age. Of all the symptoms evaluated, blood in stool emerged as a statistically significant clinical indicator ($p=0.001$), with a prevalence of 22.6% among children presenting with this symptom, compared to 0.8% in those without it. This finding is especially important as adenovirus infections, specifically types 40 and 41, are traditionally associated with watery, non-bloody diarrhoea. However, the data from this study indicated that in certain cases, particularly among infants or immunocompromised children, adenovirus may present more aggressively, potentially causing haemorrhagic enteritis and mucosal injury. This observation is supported by Kajon *et al.* (2022), who noted that while the majority of adenovirus gastroenteritis cases are non-inflammatory, atypical presentations involving bloody diarrhoea have been reported, especially in severe cases of co-infection. Similarly, De Francesco *et al.* (2021) reported that adenoviral cytopathic effects can result in epithelial cell destruction, leading to mucosal ulceration and bleeding in the gastrointestinal tract. These complications are more likely to occur in children with high viral loads or underlying nutritional deficiencies. Furthermore, clinical insight was provided by Kajon *et al.* (2020), whose review of adenoviral pathogenesis emphasized that strong viral replication or co-infection with bacterial pathogens (e.g., *Shigella spp* or *Campylobacter spp*) can exacerbate mucosal inflammation, resulting in ulcerative lesions. These findings offer a plausible explanation for the association between adenovirus and blood in stool observed in this



study, suggesting that these symptoms, although uncommon, should raise a high index of suspicion for viral gastroenteritis, especially when laboratory testing facilities are unavailable.

With due considerations, this study offers a critical perspective on the epidemiology of adenovirus infection in Edo State's children under five years of age. The results highlight the necessity of clinical knowledge for early detection and management of adenoviral infection, as well as focused initiatives targeting environmental cleanliness, hygiene education, and household overcrowding.

CONCLUSION

This study has shown that adenovirus is a notable cause of diarrhoea among children under five years of age in Benin City, Edo State. The infection was most common in younger children and was associated with factors such as poor hygiene, and certain socio-demographic characteristics such as age, parental education level, and gender. Blood in stool was a significant clinical marker of infection in this study. These findings underscore the public health importance of adenoviral gastroenteritis and highlight the need for greater awareness, early detection, and preventive efforts to reduce its burden among vulnerable populations; infants.

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Prevalence of Rotavirus Gastroenteritis Among Children Under Five Attending Eyaen Primary Health Centre, Uhunwode LGA, Edo State, Nigeria.

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ABSTRACT

Rotavirus is still one of the main causes of severe dehydration and acute gastroenteritis in children under five, especially in environments with limited resources. In this study, children who attended Eyaen Primary Health Center in Uhunmwode Local Government Area, Edo State, Nigeria, had their rotavirus infection prevalence, risk factors, and clinical presentation examined. Using a cross-sectional design, stool samples were collected from 150 children under the age of five and tested for rotavirus antigens using Immunochromatographic techniques (Aria, USA), supported by demographic and clinical data gathered through structured, researcher-administered questionnaires. The overall prevalence of rotavirus infection was 8.7%, with the highest rate (17.4%) observed in infants under one year of age. Significant associations were found between infection and risk factors, including age ($p = 0.038$), low family income ($p = 0.005$), poor sanitation ($p = 0.002$), and lack of vaccination ($p = 0.001$). Children from low-income households and those without access to proper sanitation were disproportionately affected. Vaccinated children had significantly lower infection rates (3.0%) compared to their unvaccinated counterparts (20.0%). Although daycare attendance and overcrowded living conditions did not show statistically significant associations, both showed trends toward increased infection risk. Clinical indicators such as the presence of bloody diarrhea ($p = 0.001$) and mucus in stool ($p = 0.001$) were strongly correlated with infection. These findings underscore the urgent need for expanded vaccination coverage, improved sanitation, and targeted community health interventions to reduce rotavirus transmission and its associated burden in vulnerable populations.

Keywords: Rotavirus, Vaccination, Gastroenteritis, prevalence, under five.

INTRODUCTION

Rotavirus is a type of non-enveloped double-stranded RNA (ds RNA) virus that belongs to the Reoviridae family. Since their discovery in the 1970s, human rotaviruses have been primarily linked to infectious gastroenteritis in infants and children worldwide (Burnett *et al.*, 2020). The

virus is considered the most frequent cause of diarrhoea in children under five years old, posing a significant public health challenge. Diarrhoeal illnesses are responsible for over 500,000 childhood deaths annually, highlighting the high morbidity and mortality rates associated with this condition (Omosigho *et al.*, 2024).



Rotaviruses are known for their triple-layered particle structure, which is formed by the arrangement of these segments, which are: VP1, VP2, VP3, VP4, VP6, and VP7, and six nonstructural proteins (NSP1 to NSP6). The capacity of the virus to multiply inside host cells and spread infection is largely dependent on its genome (Patton, 2023). Rotavirus-related deaths disproportionately affect low- and middle-income countries, with eight African nations, including Nigeria, accounting for 60% of these deaths (Pius *et al.*, 2023). In underdeveloped countries, over 75% of children contract rotavirus at least once before their first birthday. This underscores the urgent need for targeted public health interventions, particularly in vulnerable regions with limited access to healthcare, poor sanitation, and low immunization coverage (Pius *et al.*, 2023). In Nigeria, rotavirus continues to be a significant contributor to diarrhoea-related illnesses among children under five. The introduction of rotavirus vaccines has improved disease prevention, yet the burden remains substantial, particularly in states like Edo, where local data is limited (Iyoha & Abiodun, 2015). Understanding the prevalence and impact of rotavirus in Edo State is crucial for developing effective health policies and interventions that aim to reduce child morbidity and mortality (Iyoha & Abiodun, 2015). This study focused on the prevalence of rotavirus gastroenteritis among children under five attending Eyaen Primary Health Centre in Uhunwode Local Government Area, Edo State. By assessing the significance of the problem, this research aimed to contribute to the body of evidence needed to enhance child healthcare services and reduce the

disease burden in the State. One of the main causes of severe gastroenteritis in children worldwide, especially in those under five years, is rotavirus. Diarrhoea, Fever, and vomiting are the hallmarks of gastroenteritis, which is a serious public health issue because it significantly increases child morbidity and death, particularly in low- and middle-income nations, for several reasons such as inadequate access to clean water, severe dehydration, malnutrition, and limited healthcare access (Tale *et al.*, 2016). About 215,000 deaths among children under five are caused by rotavirus alone each year, making up roughly 28% of all diarrhoea-related deaths worldwide (Izevbua *et al.*, 2021). Rotavirus, a member of the *Reoviridae* family, is the etiological agent of rotaviral infections (Omosigho *et al.*, 2024). Its segmented double-stranded RNA genome, coupled with frequent genetic reassortment, contributes to the virus's substantial genetic diversity (Patton, 2022). According to Izevbua *et al.* (2021), rotavirus is primarily transmitted via the fecal-oral route, with contaminated food, water, and surfaces serving as common sources of infection. Although rotavirus vaccines have substantially reduced disease burden in many countries, the prevalence of infection remains high in regions characterized by poor sanitation, limited healthcare access, and low vaccination coverage (Burnett, 2020). This study examined the incidence of rotavirus gastroenteritis in children under five, highlighting the importance of this condition for public health and the necessity of efficient prevention and control strategies.

METHODOLOGY



A total of 150 stool samples were collected. Each specimen was aseptically obtained in a sterile commercial stool container and appropriately labeled with the patient's identification and date of collection by caregivers or hospital staff, following standardized instructions for proper sample collection. All samples were subsequently transported under a maintained cold chain to the Medical Microbiology Laboratory at Igbinedion University Teaching Hospital, Okada, Edo State. Upon receipt of the stool samples at the Microbiology Laboratory, they were allowed to thaw to room temperature before serological testing using immunochromatographic methods was performed.

RESULTS

Table 1 shows the sociodemographic characteristics of the participants (n = 150). The majority of children were aged 3-4 years (36.0%), followed by those aged 1-2 years (33.3%), and those under 1 year (30.7%). The sample included

more females (56.0%) than males (44.0%). Regarding mothers' educational status, most had secondary education (54.0%), followed by tertiary education (26.7%), no formal education (10.0%), and primary education (9.3%). For fathers, 45.3% had secondary education, 36.7% had tertiary education, 14.7% had primary education, and 3.3% had no formal education. In terms of family income, 59.3% of the participants were from middle-income families, 20.7% from low-income families, and 20.0% from high-income families. The number of siblings in the families was evenly distributed, with 34.7% of the participants having 3-4 siblings, another 34.7% having more than 4 siblings, and 30.7% having 1-2 siblings. The mean age of the participants was 1.91 ± 1.26 years. The prevalence of rotavirus gastroenteritis among children under five attending Eyaen primary health centre was found to be 8.7% (13/150), as shown in Figure 1.

Table 1. Sociodemographic Characteristics of Participants

Variable	Number (n=150)	Percentage (%)
Age		
<1 year	46	30.7
1-2 years	50	33.3
3-4 years	54	36.0
Sex		
Male	66	44.0
Female	84	56.0
Mothers' educational status		
No formal	15	10.0
Education		
Primary	14	9.3
Secondary	81	54.0
Tertiary	40	26.7
Fathers' educational status		
No formal	5	3.3
Education		
Primary	22	14.7



Secondary	68	45.3
Tertiary	55	36.7

Family's Income

Low	31	20.7
Middle	89	59.3
High	30	20.0

Number of Siblings

1-2	46	30.7
3-4	52	34.7
>4	52	34.7

Mean age=1.91±1.26 years.

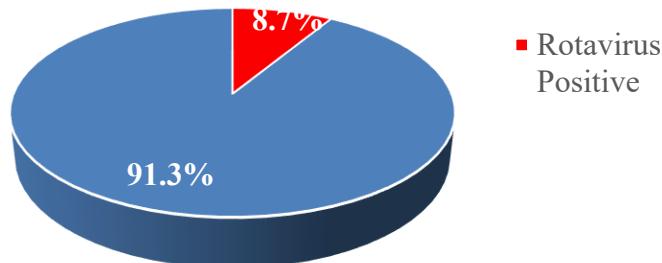


Figure 1: Prevalence of Rotavirus infection among children under five.

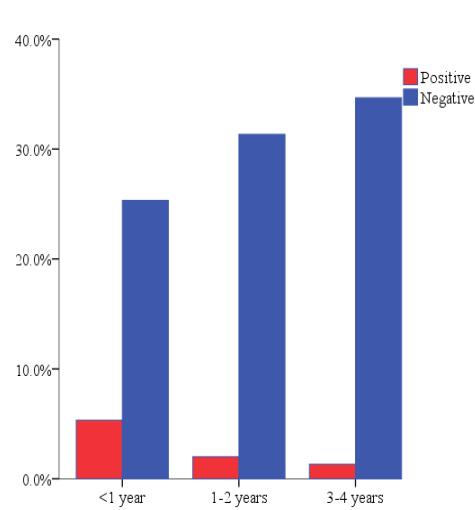


Figure 2. Prevalence of rotavirus infection among age of participants

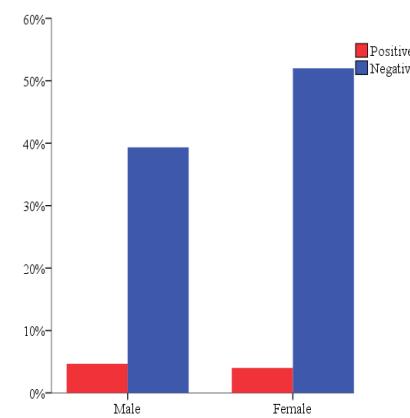


Figure 3. Prevalence of rotavirus infection among male and female participants

Sociodemographic Parameters and Prevalence of Rotavirus Infection



The relationship between sociodemographic parameters and the prevalence of rotavirus infection was analyzed. Age showed a significant association with the prevalence of rotavirus infection ($p = 0.038$) (figure 1), with children under 1 year of age having the highest infection rate (17.4%), compared to those aged 1-2 years (6.0%) and 3-4 years (3.7%) (figure 2). Gender was not significantly associated with rotavirus infection ($p = 0.454$), though males had a slightly higher prevalence (10.6%) than females (7.1%) (figure 3), with an odds ratio (OR) of 1.542 (95% CI: 0.492-4.831). Mothers' educational status was not significantly related to infection rates ($p = 0.872$), with the highest prevalence among

children of mothers with primary education (14.3%). Similarly, fathers' educational status was not significantly associated with infection ($p = 0.619$), with the highest prevalence seen in children of fathers with no formal education (20.0%). Family income demonstrated a significant association with rotavirus infection ($p = 0.005$), with children from low-income households exhibiting the highest infection rate (22.6%). The number of siblings did not show a significant association with the prevalence of infection ($p = 0.941$), with similar rates across different sibling groups (Table 2).

Table 2: Relationship Between Sociodemographic Parameters and Prevalence of Rotavirus Infection

Variable	No. Examined (%)	No. Infected (%)	OR	95%CI	p value
Age					
<1 year	46 (30.7)	8 (17.4)			0.038
1-2 years	50 (33.3)	3 (6.0)			
3-4 years	54 (36.0)	2 (3.7)			
Sex					
Male	66 (44.0)	7 (10.6)	1.542	0.492-4.831	0.454
Female	84 (56.0)	6 (7.1)			
Mothers' educational status					
None	15 (10.0)	1 (6.7)			0.872
Primary	14 (9.3)	2 (14.3)			
Secondary	81 (54.0)	7 (8.6)			
Tertiary	40 (26.7)	3 (7.5)			
Fathers' educational status					
None	5 (3.3)	1 (20.0)			0.619
Primary	22 (14.7)	3 (13.6)			
Secondary	68 (45.3)	5 (7.4)			
Tertiary	55 (36.7)	4 (7.3)			
Family's Income					
Low	31 (20.7)	7 (22.6)			0.005
Middle	89 (59.3)	3 (23.1)			
High	30 (20.0)	3 (23.1)			
Number of Siblings					
1-2	46 (30.7)	4 (8.7)			0.941
3-4	52 (34.7)	5 (9.6)			
>4	52 (34.7)	4 (7.7)			



P<0.05 indicates significance

Risk Factors and Prevalence of Rotavirus Infection

The analysis of risk factors associated with rotavirus positivity revealed significant associations with vaccination status and proper sanitation. Children who were vaccinated had a significantly lower risk of rotavirus infection (3.0%) compared to those who were not vaccinated (20.0%), with an odds ratio (OR) of 0.124 (95% CI: 0.032-0.473, $p = 0.001$). Proper sanitation was also significantly associated with a reduced risk of rotavirus infection, with children living in environments with proper sanitation showing a lower infection rate (3.2%) compared to those without proper sanitation (17.5%), with an OR of 0.157 (95% CI: 0.041-0.597, $p = 0.002$).

Daycare attendance and overcrowded living conditions were not significantly associated with rotavirus infection. However, children in daycare had a higher infection rate (12.3%) compared to those not in daycare (5.2%), with an OR of 2.566 (95% CI: 0.754-8.734, $p = 0.121$). Similarly, children living in overcrowded households had a higher rotavirus infection rate (15.2%) than those in non-overcrowded settings (5.8%), although this association did not reach statistical significance (OR = 2.932, 95% CI: 0.927-9.276; $p = 0.058$). Conversely, access to clean water showed no significant relationship with infection status ($p = 0.771$), as infection rates were similar among households with and without clean water (8.3% vs. 9.8%) (Table 3).

Table 3: Risk Factors and Rotavirus Positivity.

Variable	No. Examined (%)	No. Infected (%)	OR	95%CI	p value
Vaccination					
Yes	100 (66.7)	3 (3.0)	0.124	0.032-0.473	0.001
No	50 (33.3)	10 (20.0)			
Daycare					
Yes	73 (48.7)	9 (12.3)	2.566	0.754-8.734	0.121
No	77 (51.3)	4 (5.2)			
Living conditions					
Overcrowded	46 (30.7)	7 (15.2)	2.932	0.927-9.276	0.058
Not	104 (69.3)	6 (5.8)			
Clean water					
Yes	109 (72.7)	9 (8.3)	0.833	0.242-2.867	0.771
No	41 (27.3)	4 (9.8)			
Proper Sanitation					
Yes	93 (62.0)	3 (3.2)	0.157	0.041-0.597	0.002
No	57 (38.0)	10 (17.5)			



P<0.05 indicates significance

Symptoms of Gastroenteritis and Prevalence of Rotavirus Infection

The analysis of symptoms of gastroenteritis and their association with rotavirus prevalence revealed significant relationships for diarrhea and the presence of blood or mucus in stool. Children with diarrhea had a significantly higher prevalence of rotavirus infection (25.0%) compared to those without diarrhea (3.5%), with an odds ratio (OR) of 9.167 (95% CI: 1.624-32.019, p = 0.001). Similarly, the presence of blood or mucus in stool was significantly associated with rotavirus infection, with a prevalence of 33.3% in affected children

compared to 2.5% in those without this symptom, and an OR of 19.50 (95% CI: 4.932-77.094, p = 0.001). Other symptoms, including vomiting, abdominal pain, fever, and dehydration, did not show statistically significant associations with rotavirus infection. Specifically, vomiting was associated with a higher infection rate (15.4%) compared to no vomiting (7.3%), but this difference was not statistically significant (OR = 2.323, 95% CI: 0.657-8.215, p = 0.181). Abdominal pain was not significantly related to infection (OR = 0.851, 95% CI: 0.265-2.736, p = 0.787), nor was fever (OR = 0.506, 95% CI: 0.133-1.924, p = 0.310) or dehydration (OR = 0.648, 95% CI: 0.136-3.086, p = 0.584) (Table 4).

Table 4: Symptoms of Gastroenteritis and Prevalence of Rotavirus.

Variable	No. Examined (%)	No. Infected (%)	OR	95%CI	p value
Diarrhoea					
Yes	36 (24.0)	9 (25.0)	9.167	1.624-32.019	0.001
No	114 (76.0)	4 (3.5)			
Vomiting					
Yes	26 (17.3)	4 (15.4)	2.323	0.657-8.215	0.181
No	124 (82.7)	9 (7.3)			
Abdominal Pain					
Yes	63 (42.0)	5 (7.9)	0.851	0.265-2.736	0.787
No	87 (58.0)	8 (9.2)			
Fever					
Yes	54 (36.0)	3 (5.6)	0.506	0.133-1.924	0.310
No	96 (64.0)	10 (10.4)			
Dehydration					
Yes	32 (21.3)	2 (6.3)	0.648	0.136-3.086	0.584
No	118 (78.7)	11 (9.3)			
Blood/Mucus in Stool					
Yes	30 (20.0)	10 (33.3)	19.50	4.932-77.094	0.001
No	120 (80.0)	3 (2.5)			

P<0.05 indicates significance

DISCUSSION

This study investigated the prevalence and associated risk factors of rotavirus gastroenteritis among children under five years attending Eyaen Primary Health Centre, Edo State. The overall

prevalence of rotavirus infection in this study was 8.7%, which is considerably lower than the 28.7% reported by Izevbuwa *et al.* (2023) in Ilorin, Kwara State. This discrepancy may be explained by variations in geographic and environmental



factors, seasonal differences in sample collection, and differences in the characteristics of the study populations. In the present study, the highest prevalence was recorded among children younger than one year (17.4%), consistent with the findings of Izevbhuwa *et al.* (2023), who reported that infants under three years of age experience more frequent diarrheal episodes due to heightened exposure and immature immune systems. The significant association observed between age and rotavirus infection rate in this present study ($p = 0.038$) reinforces the well-documented vulnerability of younger children, particularly infants and toddlers, to rotavirus gastroenteritis. This trend is consistent with findings by Jonesteller *et al.* (2017), who analyzed global surveillance data and reported that children under five years of age accounted for over 90% of rotavirus-related deaths, with the highest incidence among those aged 6 to 24 months. Similarly, Troeger *et al.* (2018), in their Global Burden of Disease (GBD) study, estimated that rotavirus caused approximately 128,500 deaths in children under five in 2016, with an incidence rate of 18,700 per 100,000 population in infants, markedly higher than the findings in older children. These global patterns confirm the age-specific susceptibility identified in this study and highlight the critical importance of early-life preventive interventions, such as timely vaccination. Regarding sex distribution, although a higher infection rate was observed among males (10.6%) compared to females (7.1%), the difference was not statistically significant ($p = 0.454$), suggesting that gender may not be a primary determinant of rotavirus infection. This observation aligns with the findings of Lanata *et al.* (2013), who conducted a systematic review and reported that while male children often

exhibited slightly higher rotavirus positivity rates, the pooled odds ratio across multiple studies was only 1.12 (95% CI: 1.01–1.25), indicating a marginal and inconsistent sex-related difference. Furthermore, Bányai *et al.* (2012), analyzing global rotavirus strain data, also noted a male predominance in infections, but without consistent statistical significance across regions, implying that biological sex alone is insufficient to explain infection patterns and that other environmental and behavioral factors likely play a more substantial role. In this study, family income was significantly associated with rotavirus infection ($p = 0.005$), with children from low-income households showing the highest infection rate (22.6%). This is consistent with several Nigerian studies linking low socioeconomic status to higher rotavirus prevalence. For instance, Tagbo *et al.* (2014), found that children from low-income families in Enugu had a significantly higher infection rate (31%) compared to those from higher-income households. Similarly, Ekanem *et al.* (2017), in Calabar, observed that children from families with poor income and education backgrounds were more likely to suffer from rotavirus-associated diarrhea. They reported a statistically significant correlation between low maternal income and infection risk ($p < 0.01$). Additionally, Akinyemi *et al.* (2015), in Ibadan found rotavirus prevalence to be concentrated in overcrowded, low-income areas lacking basic sanitation, reporting infection rates above 25% in such environments. These data demonstrate how poverty may increase exposure due to factors such as insufficient water supply, poor hygiene, and restricted access to timely healthcare. These findings further emphasize the critical need for equal access to vaccination and sanitary infrastructure in economically disadvantaged



Nigerian areas. Vaccination demonstrated a strong protective effect against rotavirus infection in this study, with infection rates significantly lower among vaccinated children (3.0%) compared to unvaccinated children (20.0%) ($p = 0.001$). This protective trend is consistent with numerous studies confirming the effectiveness of rotavirus vaccines in reducing disease burden. For instance, *Armah et al.* (2016), in a post-introduction study conducted in Ghana, found a 65% reduction in rotavirus hospitalization among vaccinated children under five, while *Lessem et al.* (2014), reported a 79% decline in rotavirus-related hospitalizations in countries that introduced the vaccine into their national immunization programs. In Nigeria, similar outcomes have been observed. According to *Tagbo et al.* (2020), analysis of data from the WHO Rotavirus Surveillance Network revealed a notable reduction in rotavirus detection rates, decreasing from 42% during the pre-vaccine period to 18% after the introduction of the vaccine at selected surveillance sites. *Akinloye et al.* (2018) observed a substantially lower rotavirus infection rate among vaccinated infants in southwestern Nigeria, with vaccine effectiveness exceeding 60% against moderate-to-severe diarrhea. These findings highlight the pivotal role of rotavirus vaccination in resource-limited settings and emphasize the necessity of enhancing vaccine coverage and public health measures to further mitigate transmission. Proper sanitation was also significantly associated with a reduced risk of rotavirus infection in this study. Children living in environments with access to proper sanitation had a much lower infection rate (3.2%) compared to those without adequate sanitation (17.5%) ($p = 0.002$). This observed link reinforces the importance of clean-living environments in

preventing the spread of rotavirus and other diarrheal diseases. Similar patterns have been observed in other studies. For instance, *Omatola et al.* (2020) found that children from households in North Central Nigeria with poor sanitation were significantly more likely to contract rotavirus, with infection rates more than double those in households with clean water and improved toilet facilities. Likewise, *Omotola et al.* (2016), in Osun State, highlighted that open defecation, lack of handwashing facilities, and shared latrines contributed substantially to rotavirus transmission, especially among children under five. Globally, the role of sanitation in rotavirus prevention has been well documented. *Walker et al.* (2011), in their global analysis of child health risk factors, emphasized that improvements in water, sanitation, and hygiene (WASH) could prevent a significant proportion of diarrheal illnesses. Nonetheless, in areas where vaccination is available, poor sanitation can undermine its impact by maintaining high levels of environmental contamination. Together, these findings highlight that improving sanitation is not just a supplementary measure; it is a critical part of an integrated strategy to reduce rotavirus infections, especially in low-resource settings. In this study, the presence of blood or mucus in stool was significantly associated with rotavirus infection ($p = 0.001$), with a prevalence of 33.3% among affected children compared to 2.5% in those without these symptoms. This finding underscores the potential of clinical indicators, such as mucoid or bloody diarrhea, to signal more severe or complicated cases of rotavirus infection. Similar associations have been documented in other studies; for instance, *Akinyemi et al.* (2015) in Ibadan, Nigeria, reported that children with mucus in their stool were significantly more likely



to test positive for rotavirus, suggesting a link between viral infection and gastrointestinal mucosal irritation. Although rotavirus infection is generally characterized by watery, non-bloody diarrhea, previous studies, including Bishop *et al.* (1973) and Paulke-Korinek *et al.* (2010), have documented the occurrence of mucoid stools, particularly in moderate to severe cases. Tagbo *et al.* (2014) also found a small percentage of rotavirus-positive cases presenting with blood-streaked stools, suggesting that while uncommon, such symptoms should not rule out rotavirus in clinical diagnosis. In this study, daycare attendance and overcrowded living conditions were not significantly associated with rotavirus infection ($p = 0.121$); however, both factors showed trends toward higher infection rates. Children attending daycare had an infection rate of 12.3%, compared to 5.2% among those who stayed at home. Similarly, children living in overcrowded households had a higher infection rate of 15.2% versus 5.8% among those in less crowded homes ($p = 0.058$). Despite the fact that these results were not statistically significant, they suggest possible associations that are consistent with findings from other research. For instance, Parashar *et al.* (2003) noted that daycare centers often serve as hotspots for rotavirus transmission due to close contact among children, shared toys, and challenges in maintaining strict hygiene. In Nigeria, Omatola *et al.* (2020) reported higher rotavirus infection rates among children attending early childcare facilities, though statistical significance varied by region and sample size. Likewise, Nwachukwu *et al.* (2015) observed a similar trend in southeastern Nigeria, where children in crowded living environments defined as more than three persons per room had increased exposure to enteric pathogens, including

rotavirus. While overcrowding and daycare exposure alone may not always reach statistical thresholds, their contribution to overall transmission risk especially in combination with poor sanitation and limited hand hygiene has been widely acknowledged (Dennehy, 2008). These environmental and behavioral factors can amplify the spread of rotavirus in communal and low-resource settings, underscoring the need for improved hygiene practices in both households and childcare centers. The prevalence of rotavirus infection was similar among individuals with access to clean water (8.3%) and those without clean water (9.8%), with no statistically significant difference ($p = 0.771$). This suggests that access to clean water alone may not have a strong direct correlation with rotavirus infection rates. While this might seem counterintuitive, it aligns with findings from other studies indicating that rotavirus is primarily transmitted through person-to-person contact rather than through water, distinguishing it from many other enteric pathogens. For example, Parashar *et al.* (2003) and Mwenda *et al.* (2010) both emphasized that even if water quality is crucial for general health, rotavirus tends to spread more through fecal-oral transmission in close-contact settings such as households, daycares, and hospitals, often independent of water source or quality. In Nigeria, Akinyemi *et al.* (2015) similarly reported that while poor water access increased the risk for bacterial diarrheal infections, it had a weaker association with rotavirus, which was more influenced by hygiene practices and environmental sanitation. Additionally, from WHO and UNICEF reports have highlighted that even in communities with improved water sources, contamination at the point of use (e.g., unclean storage containers or lack of



handwashing) can invalidate the protective effects of clean water. This reiterates the idea that clean water must be coupled with proper hygiene, safe food handling, and sanitation practices to effectively reduce rotavirus transmission. Children who presented with fever had a lower rotavirus infection rate (5.6%) compared to those without fever (10.4%), though the difference was not statistically significant ($p = 0.310$). This pattern suggests that fever may not be a reliable distinguishing symptom for rotavirus infection. Rotavirus infection often presents with fever, but it is not a common symptom in all cases. For example, Tagbo *et al.* (2014), in a multicenter Nigerian surveillance study, reported that although fever was frequently observed in cases of acute gastroenteritis, it was not significantly more prevalent among rotavirus-positive children compared with those who tested negative. Similarly, Dennehy (2008) noted that while fever may accompany rotavirus infection, particularly in severe cases, its absence does not exclude the infection, nor does its presence definitively confirm it. Akinyemi *et al.* (2015), also reported a mixed pattern of fever in rotavirus cases in southwestern Nigeria, with some rotavirus-positive children showing no fever at all. This variability supports the idea that fever alone is not a strong clinical indicator for rotavirus and that diagnosis should rely on a broader range of symptoms, such as diarrhea frequency, vomiting, and stool characteristics, alongside laboratory confirmation. Finally, these findings highlight the necessity of integrated public health approaches that incorporate vaccination with long-term advancements in WASH (water, sanitation, and hygiene) practices. To further reduce rotavirus morbidity and death in susceptible groups,

community-level interventions and ongoing surveillance are crucial.

CONCLUSION

This study offers critical insight into the prevalence and associated risk factors of rotavirus gastroenteritis among children under five in Eyaen Primary Health Centre, Uhunmwode LGA, Edo State. The observed 8.7% prevalence indicates that rotavirus remains a significant contributor to childhood diarrheal illness in the region. Key risk factors such as low family income, poor sanitation, lack of vaccination, and young age were significantly associated with higher infection rates, emphasizing the vulnerability of children in under-resourced communities. While some factors, like daycare attendance and overcrowding, did not reach statistical significance, they showed concerning trends that align with broader transmission dynamics. These findings underscore the urgent need to enhance routine rotavirus immunization, strengthen water, sanitation, and hygiene (WASH) infrastructure, and improve caregiver education on hygiene and early childhood health. Addressing these risk factors through integrated public health strategies can significantly reduce the burden of rotavirus infections and prevent associated complications such as dehydration and hospitalizations. Ultimately, this study reinforces the value of preventive measures particularly vaccination and improved living conditions as essential tools for reducing the incidence of rotavirus gastroenteritis and improving child health outcomes in Nigeria.

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Detection of *Klebsiella variicola*, *Escherichia coli* and *Providentia staurti* in sachet water sold in Okada metropolis, Ovia North LGA, Edo state Nigeria

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Abstract:

Availability and access to safe drinking water is very important for public health development. Although water is essential, it is the most common way for infectious diseases to spread. Sachet water from 4 different brands were collected during products distribution in Okada town. Four (4) sachets were randomly collected from each brand to make a total of 16 sachet, and labelled A-D. They were observed for cloudiness or particles, taste, color and odor. Colony forming unit counts and coliform count/100ml present in the water of sample A and B have average count of 1.5×10^4 cfu/ml and 1.8×10^4 cfu/ml respectively. Sample B had the highest colony count and coliform count among the selected samples with 1.8×10^4 cfu/ml and 3.0×10^3 CC/100ml respectively. All the sample water A-D were prospect of acid forming organisms. Morphological and Microscopy revealed the presence of gram-negative rods. Molecular analysis using 16S rRNA amplification revealed the presence w1= *Klebsiella* sp, w2= *Escherichia coli* and w3= *Providencia* sp., contaminating the sachet water A -D. Sequencing and blasting computed using Jukes-Cantor method showed isolate W1 within the *Klebsiella* sp and revealed a closely relatedness to *Klebsiella variicola*, W2 and W3 were closely related to *Escherichia coli* and *Providencia staurti*, than other *Escherichia* respectively. *Klebsiella variicola*, showed strong resistance to all the antibiotics except for Ciprofloxacin. While *Providencia staurti* showed resistance to all antibiotics. The public health impacts of drinking contaminated water cannot be over emphasized, especially in this part of Edo state.

Keywords: Sachet-water, molecular analysis, resistance, organism

INTRODUCTION:

Packaged drinking water refers to water packaged in cans, plastic bags and pouches specifically for drinking purposes. Safe drinking water and income is more common in countries where economic and social conditions are poor. However, many studies have shown that packaged drinking water is unsafe to drink due to the presence of viruses (Ahmed *et al.*, 2013; Onuguh, *et al.*, 2025). Access to safe drinking water is very important for health and development (Adetunde *et al.*, 2014). Due to water scarcity and the government's inability to provide adequate drinking water, most small water producers package drinking water in bags and sell it to the factory (Thliza *et al.*, 2015). These are small nylon

bags containing 0.5 liters of electrically heated water and sealed at both ends as reported Adegoke *et al.*, in Onuguh, *et al.*, (2025). This is believed to be cheaper and less expensive than bottled water and also safer, cleaner and better than hand-packed, hand-tied water in polyethylene bags that was previously widely marketed (Oyedele *et al.*, 2010; Akinde *et al.*, 2010). As a result, bottled water has become the most used water by both the rich and the poor (Akinde *et al.*, 2011; Onuguh, *et al.*, 2025).

Although water is essential for health, it is the most common way for many infectious diseases to spread. Therefore, it is important to verify the quality of water before drinking and to ensure that the water we drink

is safe. Safe drinking water is defined as water whose microbial, chemical and physical properties meet the standards of the International Water Health Organization (World Health Organization,2005). From a microbiological perspective, drinking water must be free of all kinds of viruses and opportunistic microbes. Although there are many microorganisms in water that may be harmful to health, such as *Salmonella*, *Shigella*, coliforms and mycobacteria, coliforms are used to evaluate water quality (Shiaka, *et al.*,2020). Coliforms are Gram-negative bacteria that grow in a high salinity environment and can ferment lactose at 35-37°C, producing acids, gases and aldehydes within 24-48 hours. It is a weak oxidizer and does not form traces. Microorganisms in water can cause various diseases such as typhoid, cholera, diarrhea, dysentery and hepatitis (Shiaka, *et al.*,2020; Onuguh, *et al.*,2025). According to the World Health Organization (2005), insecurity is a major problem and pollution of water resources is an ongoing problem in the world. 1 billion people worldwide depend on safe drinking water from lakes, rivers and open sources. Most of these are located in Asia (20%) and sub-Saharan Africa (42%) (Ribeiro *et al.*, 2006). The use of these unhealthy resources helps explain why 90% of human infections in underdeveloped countries are caused by waterborne diseases (WHO,2005). According to the World Health Organization, up to 80% of diseases worldwide can be caused by poor sanitation, pollution or lack of water. For this reason, water must be purified and disinfected before it can be used as drinking water.

According to Oyedele *et al.*,(2010) waterborne diseases are one of the major health issues in developing countries such as Nigeria. Most bottled drinking water manufacturers in Nigeria mainly

obtain their raw water from sources such as local, municipal tap water or well water, and therefore fail to meet set standards due to lack of drinking water technology (Oluyege *et al.*, 2014).

Studies by Parashare *et al.*,2003; Banu and Menakulu,2010; Ibimesim,2014; Akindé *et al.*,2011; Thliza *et al.*,2015; Opara and Ennodine,2014; Maduka *et al.*,2014), and currently by Shiaka, *et al.*,(2020) and Onuguh, *et al.*,(2025), have studied the quality of sachet and bottled water in Nigeria and have discovered that water is the most common route of transmission for many infectious diseases. Thus, they resolved that ensuring water quality is imperative. Based on these reports the study aims at specifically screening, isolating and using molecularly characterization to detect the presence of bacterial isolates associated with sachet and bottled water sold in Okada, Edo State.

METHODS:

Study Design

This research was conducted in Okada town situated in Ovia North East Area of Edo State Nigeria. Okada is a Sub Urban Community with an area of about 2,301km² and an estimated population of about 155,000 (One Hundred and fifty-five thousand) people as at the 2006 census. The people rely on water from sources such as boreholes, bottled and sachet water, and ponds for their daily use. Okada is also a town where the Premier Private University (Igbinedion University Okada) is located.

Sample collection and Labelling

In a sample of 80 sachet water from 4 different brands (a bag contains 20 sachets per brand). A total of 16 sachet were randomly collected from the bags (16 sachets from 4 brands). The samples were collected



from different local water vendors in Okada town for analysis. Samples collected were coded to aid the identification through Alphabets A-D. the 16-sachet water were used for the analysis.

Physico-chemical analysis

Turbidity/Clarity, Traceability, parameters and Organoleptic determination

Water samples were observed for any sign of cloudiness or particles. The taste of the water samples was observed as well as color, odor of the water samples as described by Shiaka, *et al.*,(2020).

Test for odour: A wide mouthed glass bottles were rinsed with 4M Hydrochloric Acid (HCl) and then cleaned with distilled water. The bottles were half-filled with each sample about 50 mls of the water, stoppered and were shaken vigorously for 2 to 3 seconds. The stoppers were then removed and observed for odour using the nostril (Shiaka, *et al.*, 2020).

Test for taste: The test were carried out according to Shiaka, *et al.*,(2020).An aliquot each of the water samples (1ml) were taken and the list of a 5(five) human panel tasted it by dropping the samples of water each in their mouth. The taste were immediately recorded accordingly. The procedures were repeated until the whole samples were tasted.

Test for color: The sachet and bottle water samples each were poured into clean grease free beaker and were viewed using a bench-top multipurpose photometer. This is done using distilled water for the blank, and calibrate the spectro-photometer to zero as described by Shiaka, *et al.*,(2020), before placing the water sample on the cuvette ,and then inserting into the spectro-photometer for results. This is based

on American Public Health Standard method (APHA).

External features of the various packages were assessed such as NAFDAC number, date of manufacture, expiry date and batch number. These are requirement for products that meet standards as spelt out by the National Agency for food and Drug Administration and Control (NAFDAC).

Determination of pH

The pH of the water samples was determined using a compact pH meter. The pH meter was standardized with pH7 and pH4 buffer solutions in other to adjust the device. The pH probe electrode ball is then immersed in the beaker containing the sachet water sample and the measurement was taken (infiteck, USA, PH-B200/PHB200EM). The values were expressed in mean and standard deviations. The turbidity and organoleptic assay were described based on visibility.

Enumeration of bacteria and molecular identification

An aliquot (1 ml) of each water sample (A-D) was aseptically inoculated on each of the sterilized agar (Plate count agar for AMC and MacConkey agar (Biotech, England) using the pour plate method. They were inoculated in duplicate and incubated at 37°C for 24 hours. The plates were carefully observed for microbial growth and distinct colonies enumerated and expressed in CFU/mL. The isolates were further identified using 16SrRNA sequencing after the gene was purified on agar gel as (Winsley, *et al.*,2012). The 16S rRNA primer was used to locate the internal transcribed spacer gene (Winsley, *et al.*,2012).

Antibiogram test of the isolates

The organisms were screened for antimicrobial activity and were carried out by the single disc agar diffusion method as described in Onyenwe, *et al.*(2011). Using sterile pipette, 0.1ml of 10^{-2} dilution of an overnight broth culture of each test bacterium we analyzed. Selected antibiotics discs from different groups of antibiotics such as the Cephalosporin, Penicillin, Fluoroquinolones and Aminoglycosides were used for the test.

Data analysis

The packaged water/ bottled water statistical analysis were carried out using simple descriptive data analysis like mean and standard deviation.

RESULTS

Summary of Physicochemical properties of the water samples

The pH of the samples ranged from 6.06 – 6.90 for the entire sample analyzed, all the sample were void of particles and their color were clear, tasteless and odorless (Table 1).

Table1: Physico-chemical properties of the isolates

10 Sample Source	PH	Temp (C)	Particles	Colour	Odor	Taste
A	$6.09 \pm 0.$	36.00 ± 0.18	Absents	Clear	Absent	Absent
B	6.06 ± 0.16	36.00 ± 0.15	Absents	Clear	Absent	Absent
C	6.50 ± 0.04	35.00 ± 0.11	Absents	Clear	Absent	Absent
D	6.10 ± 0.23	35.00 ± 0.18	Absents	Clear	Absent	Absent

pH – Concentration of acidity or alkalinity; **Temp**- Temperature

Presumptive and microbial counts analysis

The colony forming unit counts and coliform count/100ml present in the water of samples A and B have average count of 1.5×10^4 cfu/ml and 1.8×10^4 cfu/ml respectively as shown in Table 2. Sample B had the highest colony count and coliform count among the selected samples with 1.8×10^4 cfu/ml and

3.0×10^3 CC/100ml respectively. Also, all isolates were Gram-negative rod-shaped organisms. Some had smooth, moist or glistering colonies and mucoid as seen in samples A and B while other colonies were grayish white and non-sporing as seen in samples B and C (see tables 3 and 4). Only sample D produced gas. Samples A and B show the prospect of acid forming microorganisms which reflects in the chemical features of the water by having the mean lowest pH of the samples.

Table 2; Traceability parameters of the water samples

Sample source	GP	AP	Colony count(cfu/ml) mean	Coliform count(cfu/ml) mean
A	Absent	Present	1.5×10^4	3.0×10^3
B	Absent	Present	1.8×10^4	3.6×10^3
C	Absent	Absent	1.02×10^4	2.0×10^3
D	Present	Absent	1.4×10^4	2.8×10^3

N&A – Name and address; cL–Centilitre; Reg. No – NAFDAC registration number; D.O.M – Date of Manufacturing date D.O.E –Date of Expiring ; N. Val –Nutritional value, A =Absent, P = Present

Table 3: Presumptive and microbial counts analysis

Samples						
Source	N&A	Volume (cL)	D.O.M	D.O.E	Batch	N. val
A	P	75	A	A	A	A
B	P	60	A	A	A	A
C	P	60	A	A	A	A
D	P	60	A	A	A	A

GP – Gas Production; AP – Acid production

Table 4: Morphological/Microscopic and Molecular Identification of Isolates

Morphological/Microscopic Examination	Molecular identification based on 16SrRNA	Samples Source
Gram-negative mucoid and non- motile rods Gram negative rods, Greyish-white rods on agar Gram-negative and mucoid shape rods Gram negative circular rods, Greyish-white rods on agar	<i>Klebsiella variicolla</i> , <i>E. coli</i> , <i>Providencia stuartii</i>	A and B (n=8)
	<i>Providencia stuartii</i> <i>E. coli</i> , <i>Klebsiella variicolla</i>	C and D (n=8)

Molecular identification

PCR Amplification of 16S rRNA

The result of 16S rRNA amplification showed the bands in lane 1= b1, while that of lane 2= L Molecular ladder of 100 bp (base pairs), Lane 3 = b2, and the lane 4= b3 shown Figure 1.

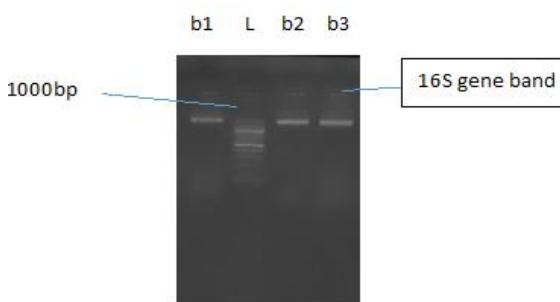


Figure 1: Agarose gel electrophoresis of the 16S rRNA gene of some selected bacterial isolates. Lanes b1, b2, 3 represent the 16SrRNA gene bands (1500bp), lane L represents the 1000bp molecular ladder of sample A and B

16s rRNA sequence of the isolate

The 16s rRNA sequence from the isolate produced an exact match during the mega blast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolate W1 had 100% similarity to other species. The evolutionary distances computed using the Jukes-Cantor method showed isolate W1(c1and b1) within the *Klebsiella* sp and revealed a closely relatedness to *Klebsiella variicola*, W2(b2and c2) and W3(b3and c3) were closely related to *Escherichia coli* and *Providencia staurti* than other *Escherichia* respectively (Fig. 2 and Fig. 3) (Winsley *et al.*,2012).

Note: All the organism recovered in the 4 **water samples** brands (n=16), labelled A-D were later labelled as b1, b2 b3 for A and B, while sample C-D were labelled as c1,c2,c3 respectively for PCR and molecular analysis.

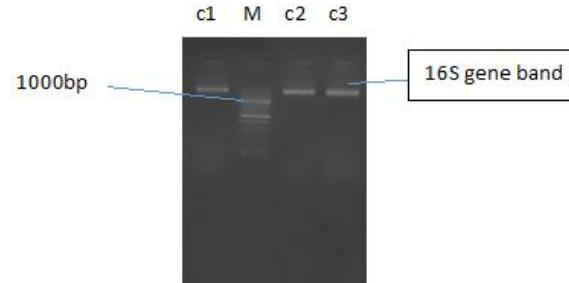


Figure2: Phylogenetic tree showing the evolutionary distance between the bacterial isolates w1(c1 and b1), w2(b2 and c2) and w3(b3 and b3) represent the 16SrRNA gene bands (1500bp), lane M represents the 1000bp molecular ladder of sample C and D.

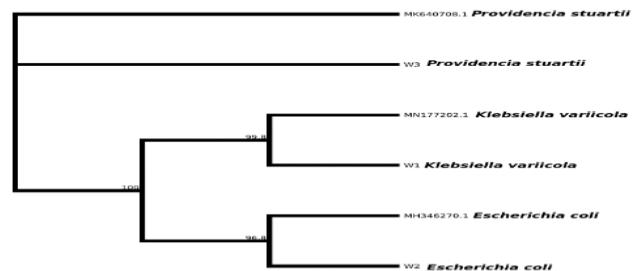


Figure3: Phylogenetic tree showing the evolutionary distance between the bacterial isolates w1(c1 and b1), w2(b2 and c2) and w3(b3 and b3)

Table 5: Antibiogram test of the isolates detected

Antibiotics	w1	w2	w3
CAZ	R	R	R
CRX	R	R	R
GEN	R	R	30mm
CXM	R	R	R
OFL	R	30mm	R
AUG	R	R	R
NIT	R	R	R
CPR	25mm	20mm	R

R – Resistant, S - Susceptible; CAZ – Ceftazidime, CRX – Cefuroxime; GEN – Gentamycin; CXM – Cefixime; OFL – Ofloxacin; AUG- Augmentin (Clavulanic acid &Amoxicillin); NIT – Nitrofurantoin; CPR – Ciprofloxacin; 1-*Klebsiella variicola*; 2-*Escherichia coli*; 3-*Providencia staurti*

Table 6: Phenotypic resistance pattern of isolates

ORGANISM	NUMBER OF ANTIBIOTIC	RESISTANCE PHENOTYPIC PATTERN
<i>Klebsiella varicola</i>	5	CAZ,CRX,CXM,AUG,NIT
<i>E. coli</i>	5	CAZ,CRX,CXM,AUG, NIT
<i>Providencia stuartii</i>	8	CAZ,CRX,CXM,AUG,NI T,GEN, OFL,CPR

CAZ – Cefazidime, CRX – Cefuroxime; GEN – Gentamycin; CXM – Cefixime; OFL – Ofloxacin; AUG – Augmentine, CPR – Ciprofloxacin, NIT – Nitrofurantoin

Antibiogram test of the isolates

The analysis of the antibiogram in Table 5 and 6 respectively shows the susceptibility/resistant pattern and Phenotypic resistance of the microorganisms found in 4 brands of the water samples. The result indicates that the isolates were strongly resistant to the various antibiotic drugs used against them. *Klebsiella variicola* showed strong resistance to all the antibiotics used except for Ciprofloxacin, *E. coli* showed susceptible features to Gentamycin, Ofloxacin and Ciprofloxacin; while, *Providencia stuartii* showed resistance to all antibiotics used against it.

Discussion:

According to the World Health Organization, diarrheal diseases constitute 4.1% of the world's total daily burden and affect 8 million people annually. It is estimated that 88% of this burden is caused by inadequate water, sanitation and hygiene (WHO, 2005). 50% (8/16) of the water samples analyzed in this study had a drinking water pH lower than WHO 6.5- 8.5. These sachet water has passed basic water tests and is tasteless, odorless, colorless and does not contain particles. This demonstrates that water purification standards that meet WHO standards are of good practice. In this study, the presence of

organisms indicates that the water is contaminated with pollutants, while their absence indicates the general safety of the water. Although, coliform organisms are not always directly related to contamination or the presence of contamination in drinking water, coliform testing is still useful in monitoring the microbial quality of drinking water (Magda *et al.*, 2008; Shiaka, *et.al.*, 2020; Onuguh, *et al.*, 2025). The analysis also found that the presence of these organisms in water samples may be due to inefficient or ineffective treatment methods. Therefore, appropriate treatment methods must be used to obtain safe and clean packaged water (Oyedele, *et al.*, 2010; Shiaka, *et. al.*, 2020). Coliforms are not pathogenic organism, rather they are organism found in the intestinal tract of warm-blooded animals; therefore it is considered an indicator of fecal contamination. Its presence is therefore indicative of contamination by human waste or animals. As reported by Shiaka, *et al.*, (2020) and Onuguh, *et al.*, (2025), the presence of *E. coli* in water is almost always associated with recent pollution and is indicative of organisms selected for this purpose. In this study, sachet water samples were analyzed for microbial quality and antibiotic resistance among isolated bacteria, with the aim of raising awareness about the public health risks of drinking this type of water. Although some of the samples B and C showed an average value of more than 100 cfu / ml, it was still less than 100 cfu / ml. According to WHO,(2005) guidelines, no *Escherichia coli* or any coliform bacteria should be present in a 100 ml samples of water, This is contrary to what we found in this study as shown in the results of the molecular analysis (Fig,1 and 2), and it is indicative of possible sachet water contamination. Thus, most of the sachet water sampled were unsafe for human consumption,

according to Shiaka *et al.*,(2020), other bacteria, such as *Klebsiella variicola* isolated from the water samples, may have entered the water during packaging or processing because the organisms are part of the normal flora of human skin or through sewage (Hunter, 1993). However, the presence of organisms in drinking water is of great importance for public health (Shiaka, *et al.*,2020). Antibiotic test results showed high antibiotic resistance in bacteria isolated from packaged water. The presence of antibiotic-resistant bacteria in drinking water is important because of the risk of developing more antibiotic-resistant organisms in humans. The prevalence of resistant organisms has created a serious problem for physicians. Nevertheless, this study provides guidelines for antibiotic-resistant bacteria that can manifest on individuals as result of drinking contaminated sachet / bottled water. Drinking water containing antibiotic-resistant organisms may prolong the treatment of waterborne diseases. The molecular results of this study showed the presence of *Klebsiella varicolla*, *Providencia staurtii* and *Escherichia coli* in water samples. Previous studies in other parts of the country have shown that similar bacteria characterize water quality (Shiaka, *et al.*,2020; Oludairo *et al.*,2015), but not *Providencia staurtii*, with 4.5% of samples containing the total coliform and 2.3% had feces (Shiaka, *et al.*,2020). Another study on packaged drinking water in Ibadan, Nigeria, found that higher proportion of bagged water was statistically better than bottled water (Ajayi *et al.*,2008). Less than 7.0% of water pollution occurs after production, while 40 to 45% of the product were detected among markets and road transporters (Omalu *et al.*,2010). The results of this study revealed the presence of some microorganisms in sachet water samples which pose

a public health concern. Access to good drinking water has been reported to be on the number six of the sustainable development goals (SDGs) according to Adewale, *et al.*,(2023), but until now, is still quite unfortunate that access to portable drinking water is still an issue, especially in this part of Okada, Benin city in Nigeria, and its also of global concern.

Conclusion:

It has been observed that many people are engaged in the production and sale of bottled water as a source of income. Therefore, health authorities need to ensure that producers comply with government regulations, as some of this sachet / bottled water may be produced without hygiene and therefore may not meet the legal standards or requirements of the World Health Organization. Therefore, the public health impacts of drinking contaminated water in this part of the country cannot be over emphasized based on the results found in this study. Additionally, lack of knowledge about clean sachet water storage for as long as a years from the time of production, and high ambient temperature may be the reasons for the high bacterial contamination of sachet or bottled water in Okada City. It is well known that sachet water should be treated before human consumption and microbiological tests should be performed regularly to prevent waterborne diseases from sachet water.

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Conflicts on interest

Authors declare no conflict of interest

Informed consent

Ethical approval number was issued to this study;

IUO/Ethics/016/22



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